Waste-derived Technosols for the treatment of soil and water affected by potentially harmful elements

Antonio Aguilar-Garrido

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PhD THESIS

Waste-derived Technosols for the treatment of soil and water affected by potentially harmful elements

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Tecnosoles derivados de residuos para el tratamiento de suelos y aguas afectados por elementos potencialmente contaminantes

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PROGRAMA DE DOCTORADO EN CIENCIAS DE LA TIERRA

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Memoria de Tesis Doctoral que presenta D. Antonio Aguilar-Garrido para optar al Grado de Doctor con mención internacional por la Universidad de Granada.

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A patch of bare polluted soil surrounded by reclaimed areas in the Guadiamar Green Corridor

Seville, Spain

May 2018

Francisco José Martín Peinado

SUMMARY

SUMMARY

The global expansion of the mining industry, in particular sulphide mining, raises serious health and environmental concerns as a major source of potentially harmful elements (PHEs: As, Cd, Cu, Pb, Sb and Zn) released into the environment. Such concerns motivate the search for strategies that reconcile the production of mining resources with the protection of ecosystems, in order to avoid cases such as the Aznalcóllar mining accident, which has caused residual soil pollution and ecosystem degradation in the Guadiamar Green Corridor (GGC) (Iberian Pyrite Belt, SW Spain) that still persists more than 25 years later. On the other hand, the increase in waste production in industrialised countries leads to the proposal of circular economy and zero waste strategies as essential for the ecosystem preservation. In this context, to respond to these two challenges, this PhD thesis comprehensively evaluates the effectiveness of some waste-derived Technosols in the remediation of different environments degraded by sulphide mining, with high levels of PHEs: (i) artificial acid mine drainage, (ii) polluted soils, and (iii) gossan waste.

In **chapter 3**, we assessed the acid neutralisation capacity and the removal effectiveness of PHEs of ten inorganic and organic wastes from the main waste-generating activities (urban, mining, and agro-industrial) through their application in a control treatment of an artificial acid mine drainage (AMD). In this way, we can select those wastes that can be used in the production of the Technosols of this PhD thesis. The wastes selected are those with the highest PHEs removal rates and which meet the necessary conditions to cope with pollution by favouring acidity neutralisation and the immbolisation of PHEs, such as carbonate character (carbonated waste from peat bog [CW] and marble cutting sluge [MS]), richness in organic matter (vermicompost from garden waste [VC], composted solid olive-mill by-product [OL], composted sewage sludge [WS]) and iron oxyhydroxides (iron oxyhydroxide sludge [IO]). Carbonated wastes provide pH buffering capacity, organic wastes favour the recovery of biological activity and contribute to soil aggregation, and iron-rich wastes promote the retention capacity of anionic PHEs (i.e. As, Sb). The combination of these wastes with polluted soil to form efficient Technosols for AMD control can be a viable and effective ecotechnology, as they have a very high acidity neutralisation capacity, a strong capacity to immobilise PHEs, and a variable critical load of PHEs, as discussed in **chapter 5**. However, the

responses of the different Technosols indicate that results may differ depending on the dose, type, and combination of waste used; therefore, the final application must be adjusted to solve this problem in each specific situation. Furthermore, given that the presence of a continuous AMD can pose a medium to long term risk due to the saturation of the capacity to retain certain PHEs and the loss of efficacy as a protective barrier over time, if Technosols applied in AMD control, they must be monitored regularly and corrective measures proposed to maintain their functionality.

In **chapter 4**, we characterised the six Technosols produced from polluted soil (PS) and a combination of the aforementioned wastes (T1, T2, T3, T4, T5, and T6) and evaluated their effectivennes in the remediation of PHE-polluted soils at physicochemical, biological, and ecotoxicological levels and a microcosm scale (in growth chamber conditions). After two months of surface application of Technosols on polluted soils, we analysed the soil properties, PHEs concentration in total, soluble, and bioavailable fractions, soil enzymatic activities, and growth responses of *Trifolium campestre* Schreb. and *Lactuca sativa* L. in both Technosols and underlying polluted soils. All Technosols improved the unfavourable conditions of PS by neutralising acidity, increasing organic carbon content, reducing the mobility of most PHEs, and stimulating both the soil enzymatic activities and growth of *T. campestre* and *L. sativa*. Thus, we conclude that these Technosols constitute a potential solution for the remediation of persistently polluted soils that should be applied in large-scale and long-term interventions to reinforce their feasibility as a cost-effective ecotechnology.

Prior to field installation, to gain a detailed understanding of how PHE-polluted soil causes phytotoxicity, as well as the role of Technosols in mitigating these effects, in **chapter 6** we focused on the comprehensive assessment of ecotoxicity through physiological parameters related to phytotoxicity in *L. sativa*, such as plant growth, photosynthetic capacity, oxidative stress, and antioxidant defence. In this chapter, the evaluation of phytotoxicity reduction is limited to Technosols T4, T5, and T6. The plants of *L. sativa* grown on T6 and T4 showed a higher growth rate (+90% and +41%, respectively) than PS, whereas no increase occurred in T5. This lower growth of *L. sativa* in PS and T5 may be due to the higher toxicity resulting from the presence of PHEs. However, lower oxidative stress and impact on net photosynthetic rate was observed in all Technosols compared to PS (+344% in T6, +157% in T4 and +194% in T5). Thus, this ecotechnology can be a potential solution for the remediation of polluted soils, as a widely used plant such as lettuce can be grown on them without experiencing toxicity; although the effectiveness of Technosols depends largely on their components, as some, such as T6, greatly improve conditions while others, such as T5, may not bring similar benefits.

After evaluating in **chapters 4**, **5**, and **6** the effectiveness of Technosols in the remediation of soils and water affected by PHEs at laboratory and microcosm level; the next step is the application under real conditions of the two best performing Technosols (**T4** and **T6**). This chapter presents the results of the field application of these Technosols on unvegetated patches of polluted soils in the GGC. In particular, this chapter comprehensively evaluates the effectiveness of *in situ* remediation of polluted soils of Technosols T4 and T6 by analysing the changes that occurred in: i) soil physicochemical properties, ii) PHEs solubility, iii) soil enzymatic activity, iv) soil microbial composition and diversity, and v) vegetation recovery. The two selected Technosols remediated the adverse state of PS, both by their integration into the Technosols and by the effect of their surface application, neutralising acidity, boosting organic carbon levels, minimising the mobility of PHEs, and stimulating soil enzymatic functions. The progressive increase in soil enzyme activity implies an improvement of the microbial community. In addition, this increased microbial diversity favours greater connectivity between microbial species, indicating an acceleration of ecological interactions as evidenced by the increasing presence of plant growth-promoting microorganisms in soils over time, which positively influenced the establishment of a biodiverse and functional vegetation cover with autochthonous plants. Therefore, the integration of Technosols composed of the polluted soil itslef results in a substantial improvement of soil quality together with the recovery of its ecosystem functions, creating a favourable environment for vegetation growth while mitigating the risks associated with PHEs in polluted soils.

Finally, in **chapter 8**, we focused on the remediation of mining tailings, such as gossan waste, which is one of the main sources of pollution in sulphide mining. Specifically, the aim of this chapter is to evaluate the potential of a ecotechnology

in mining reclamation that combines the crop of the widely tolerant multifunctional legume, *Lablab purpureus* (L.) Sweet, with two designed Technosols derived from mixing gossan waste (GW) with a combination of organic/inorganic wastes from local industries (TC50 and TC75). In particular, the purpose is to promote favourable conditions in the gossan waste from the São Domingos mine for optimal growth of *L. purpureus*, as well as to evaluate the safety of this plant for animal consumption. Both Technosols improved physicochemical conditions, nutritional status, and microbiological activity, and reduced the bioavailability of most PHE (except As) of GW. *L. purpureus* thrived in both Technosols and showed PHEs accumulation mainly in roots, with PHEs concentrations in shoots that are safe for cattle and sheep consumption. Thus, this is a potential plant that, in conjunction with Technosols, constitutes a potential integrated biotechnology approach for the conversion of marginal lands, such as abandoned mining areas, into food production areas under the condition of exhaustive quality and food safety controls.

In this PhD thesis, we have deepened the knowledge of Technosols applied to real situations, and we have seen that they produce a significant improvement in severely degraded areas affected by PHE-pollution, while we have found some drawbacks or potential risks that need to be further investigated and carefully managed for each specific situation. Furthermore, this thesis can serve as a basis for many future lines of research (e.g. ecotoxicity studies, studies on the improvement of physical properties, long-term monitoring of Technosols applied in the GGC, phytoremediation with species of interest in the region, designed Technosols for salinity problems, potential of Technosols for reconversion in production areas).

RESUMEN

RESUMEN

La expansión global de la industria minera, en particular de la minería de sulfuros, suscita serias preocupaciones sanitarias y ambientales por ser una fuente importante de emisión de elementos potencialmente contaminantes (EPCs: As, Cd, Cu, Pb, Sb y Zn). Tales preocupaciones motivan la búsqueda de estrategias que concilien la explotación minera con la protección de los ecosistemas, a fin de evitar casos como el desastre de Aznalcóllar, que provocó una contaminación residual del suelo y una degradación de los ecosistemas en el Corredor Verde del Guadiamar (CVG) (Faja Pirítica Ibérica, SO España) que persiste tras más de 25 años. Por otro lado, la creciente generación de residuos en países industrializados lleva a plantear estrategias de economía circular y residuo cero como imprescindibles para la preservación del ecosistema. En este contexto, para responder a estos dos retos, esta tesis doctoral evalúa de forma exhaustiva la eficacia de algunos Tecnosoles derivados de residuos en la remediación de diferentes ambientes degradados por la minería de sulfuros, con altos niveles de EPCs: (i) drenaje ácido de minas, (ii) suelos contaminados, y (iii) residuos de gossan.

En el **capítulo 3**, evaluamos la capacidad amortiguadora y la eficacia de retención de EPCs de diez residuos inorgánicos y orgánicos procedentes de las principales actividades productoras de residuos (urbanas, mineras y agroindustriales) mediante su aplicación en un tratamiento de control de un drenaje ácido de minas artificial (DAM). De esta forma, podemos seleccionar aquellos residuos que pueden ser utilizados en la producción de los Tecnosoles de esta tesis doctoral. Los residuos seleccionados son aquellos que presentan las mayores tasas de retención de EPCs y que reúnen las condiciones necesarias para hacer frente a la contaminación favoreciendo la neutralización de la acidez y la inmbolización de los EPCs, como el carácter carbonatado (residuos carbonatados de turbera [CW] y lodos de corte de mármol [MS]), la riqueza en materia orgánica (vermicompost de residuos de jardinería [VC], subproducto sólido de almazara compostado [OL], lodos de depuradora compostados [WS]) y en oxihidróxidos de hierro (lodos de oxihidróxido de hierro [IO]). Los residuos carbonatados proporcionan capacidad amortiguadora del pH, los orgánicos favorecen la recuperación de la actividad biológica y contribuyen a la agregación del suelo, y los residuos ricos en hierro promueven la capacidad de retención de EPCs aniónicos como As y Sb. La combinación de estos residuos con el suelo contaminado para formar Tecnosoles

eficientes para el control de DAM puede ser una ecotecnología viable y eficaz, ya que tienen una capacidad de amortiguadora del pH muy elevada, una gran capacidad de inmovilización de EPCs y una carga crítica variable de EPCs, como se discute en el **capítulo 5**. Sin embargo, las respuestas de los distintos Tecnosoles indican que los resultados pueden diferir en función de la dosis, el tipo y la combinación de residuos, por lo que la aplicación final debe ajustarse para resolver este problema en cada situación concreta. Además, como la exposición a DAM puede suponer un riesgo a medio y largo plazo debido a la saturación de la capacidad de retención de ciertos EPCs y a la pérdida de eficacia como barrera protectora con el tiempo, si se aplican Tecnosoles en el control de DAM, éstos se deben monitorizar y aplicar medidas correctoras para mantener su funcionalidad.

En el **capítulo 4**, caracterizamos los seis Tecnosoles producidos a partir del suelo contaminado (PS) y una combinación de los residuos (T1, T2, T3, T4, T5 y T6) y evaluamos su eficacia en la remediación de suelos contaminados con EPCs a nivel fisicoquímico, biológico y ecotoxicológico y a escala de microcosmos (en condiciones de cámara de crecimiento). Tras dos meses de aplicación superficial de los Tecnosoles sobre los suelos contaminados, analizamos las propiedades del suelo, la concentración totales, solubles y biodisponibles de EPCs, las actividades enzimáticas del suelo y las respuestas de crecimiento de *Trifolium campestre* Schreb. y *Lactuca sativa* L. tanto en los Tecnosoles como en los suelos contaminados subyacentes. Todos los Tecnosoles mejoraron las condiciones desfavorables de PS neutralizando la acidez, aumentando el contenido de carbono orgánico, reduciendo la movilidad de la mayoría de los EPCs y estimulando tanto las actividades enzimáticas del suelo como el crecimiento de *T. campestre* y *L. sativa*. Así pues, concluimos que estos Tecnosoles constituyen una solución potencial para la remediación de suelos contaminados que debería aplicarse en intervenciones a gran escala y a largo plazo para reforzar su viabilidad como ecotecnología rentable.

Antes de instalarlos en campo, para obtener una comprensión detallada de cómo el suelo contaminado causa fitotoxicidad, así como el papel de los Tecnosoles mitigando estos efectos, en el **capítulo 6** nos centramos en la evaluación exhaustiva de la ecotoxicidad a través de parámetros fisiológicos relacionados con la fitotoxicidad en *L. sativa*, como el crecimiento, la capacidad fotosintética, el estrés oxidativo y la defensa antioxidante. La evaluación de la reducción de la fitotoxicidad se limita a T4, T5 y T6. Las plantas de *L. sativa* cultivadas en T6 y T4 mostraron un crecimiento superior al de PS (+90% y +41%, respectivamente), mientras que en T5 no se produjo ningún aumento. Este menor crecimiento en PS y T5 puede deberse a la mayor toxicidad por EPCs. Sin embargo, se observó un menor estrés oxidativo e impacto en la fotosíntesis en todos los Tecnosoles respecto PS (+344% en T6, +157% en T4 y +194% en T5). Así pues, esta ecotecnología puede ser una solución para remediar suelos contaminados, ya que una planta ampliamente utilizada como la lechuga puede cultivarse en ellos sin experimentar toxicidad; aunque la eficacia de los Tecnosoles depende en gran medida de sus componentes, ya que algunos como T6 mejoran enormemente las condiciones, mientras que otros como T5 pueden no aportar beneficios similares.

Tras evaluar en los **capítulos 4**, **5** y **6** la eficacia de los Tecnosoles en la remediación de suelos y aguas afectados por EPCs a nivel de laboratorio y microcosmos; el siguiente paso es la aplicación en condiciones reales de los dos Tecnosoles con mejores resultados (**T4** y **T6**). En el **capítulo 7** se presentan los resultados de la aplicación sobre el terreno de estos Tecnosoles en suelos contaminados sin vegetación del CVG. En particular, se evalúa exhaustivamente la eficacia de la remediación *in situ* de los suelos contaminados de T4 y T6 mediante el análisis de los cambios producidos en: i) propiedades fisicoquímicas del suelo, ii) solubilidad de los EPCs, iii) actividad enzimática del suelo, iv) composición y diversidad microbiana del suelo, y v) recuperación de la vegetación. Los dos Tecnosoles remediaron el estado adverso de los suelos contaminados, tanto por su integración en los Tecnosoles como por el efecto de su aplicación superficial, neutralizando la acidez, aumentando el nivel de carbono orgánico, minimizando la movilidad de los EPCs y estimulando la actividad enzimática del suelo. Además, este aumento de la diversidad microbiana favorece una mayor conectividad entre las especies microbianas, lo que indica una aceleración de las interacciones ecológicas, como demuestra la presencia creciente de microorganismos promotores del crecimiento vegetal, influendo positivamente en el establecimiento de una cubierta vegetal biodiversa y funcional con plantas autóctonas. Por tanto, la integración de Tecnosoles compuestos por el suelo contaminado resulta en una mejora sustancial de la calidad del suelo junto con la recuperación de sus funciones ecosistémicas, creando un entorno favorable para el crecimiento de la vegetación a la vez que se mitigan los riesgos asociados a los EPCs en suelos contaminados.

Por último, en el **capítulo 8**, nos centramos en la remediación de estériles mineros, como los residuos de gossan, que constituyen una de las principales fuentes de contaminación en la minería de sulfuros. En concreto, el objetivo es evaluar el potencial de una ecotecnología en la recuperación minera que combina el cultivo de la leguminosa multifuncional ampliamente tolerante, *Lablab purpureus* (L.) Sweet, con dos Tecnosoles compuestos de residuos de gossan (GW) junto a residuos orgánicos e inorgánicos de industrias locales (TC50 y TC75). En particular, el objetivo es promover condiciones favorables en los residuos de gossan de la mina de São Domingos para el crecimiento óptimo de *L. purpureus*, así como evaluar la seguridad de esta planta para el consumo animal. Ambos Tecnosoles mejoraron las condiciones fisicoquímicas, el estado nutricional y la actividad microbiológica, y redujeron la biodisponibilidad de la mayoría de los EPCs (excepto As). Las plantas de *L. purpureus* prosperaron en ambos Tecnosoles sin signos de toxicidad y acumulando los EPCs sobre todo en las raíces, con concentraciones de EPCs en los brotes aptas para el consumo de ganado vacuno y ovino. Por tanto, se trata de una planta potencial que, junto con los Tecnosoles, constituye un enfoque biotecnológico integrado potencial para la conversión de tierras marginales, como las zonas mineras abandonadas, en zonas de producción bajo la condición de controles exhaustivos de calidad y seguridad alimentaria.

En esta tesis doctoral, hemos profundizado en el conocimiento de los Tecnosoles aplicados a situaciones reales, y hemos visto que producen una mejora significativa en áreas severamente degradadas y afectadas por la contaminación por EPCs, al tiempo que hemos encontrado algunos inconvenientes o riesgos potenciales que necesitan ser investigados más a fondo y gestionados cuidadosamente para cada situación específica. Además, esta tesis puede servir de base para muchas líneas de investigación futuras (por ejemplo, estudios de ecotoxicidad, estudios sobre la mejora de las propiedades físicas, seguimiento a largo plazo de los Tecnosoles aplicados en el CVG, fitorremediación con especies de interés en la región, Tecnosoles diseñados para problemas de salinidad, potencial de los Tecnosoles para la reconversión en zonas de producción).

Close-up of the old pyrite tailings from the Aznalcóllar mining accident (1998) over soils at Vado de Quema (near Doñana Natural Park)

Seville, Spain

February 2023

Antonio Aguilar Garrido

CHAPTER 1

INTRODUCTION

1.1. SOIL DEGRADATION

1.1.1. SOIL'S ROLE IN ECOSYSTEMS AND ITS DEGRADATION

Soil is one of the key components of the life cycle in nature, along with water and air, operating at the interface between the lithosphere, biosphere, hydrosphere, and atmosphere (Smith et al. 2021). Soil plays a fundamental role in the functioning of ecosystems, as it is one of the main providers of ecosystem functions and services, and is home to about 25% of the Earth's biodiversity (FAO et al. 2020). Soil also occupies a basic role for humans, as human survival is linked to the maintenance of its productivity (Adhikari and Hartemink 2016), with soils providing humans with 98.8% of our food (Kopittke et al. 2019). Furthermore, soil has a greater buffering capacity against degradation processes than other ecosystem compartments, but the intensive and unsustainable soil use by humans is causing severe degradation of many terrestrial ecosystems worldwide over time (Kopittke et al. 2019; Khan et al. 2021). In fact, 33% of the Earth's soils are already degraded and more than 90% could become degraded by 2050 (FAO and ITPS 2015; IPBES 2018).

The Food and Agriculture Organization of the United Nations (FAO) defines soil degradation as "a change in the soil health status resulting in a diminished capacity of the ecosystem to provide goods and services for its beneficiaries. Degraded soils have a health status such that, they do not provide the normal goods and services of the particular soil in its ecosystem." [1]. Soil degradation processes are driven or intensified by human activity, caused mainly by: (i) agricultural and industrial pollution; (ii) loss of arable land due to urban sprawl, overgrazing, and unsustainable agricultural practises; and (iii) long-term climate change (Maximillian et al. 2019). Thus, major threats to its ecological functions include erosion, organic matter depletion, compaction, salinisation, landslides, pollution, sealing and biodiversity loss (Montanarella 2007).

1.1.2. SOIL POLLUTION BY POTENTIALLY HARMFUL ELEMENTS

The increasing incidence of soil pollution in recent decades, with more than 10 million polluted sites worldwide (He et al. 2015), represents one of the major

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[1] https://www.fao.org/soils-portal/soil-degradation-restoration/en/ (accessed 15/11/2023)

problems for the environment, human health, and food security in the modern era (Li and Yao 2023). Over centuries, especially since the 19th century, with the exponential demographic and urban growth, anthropogenic activities have led to the accumulation of pollutants in soils that, in many cases, can generate a risk of environmental toxicity and, thus, a threat to living organisms (Rodríguez-Eugenio et al. 2018). The main anthropogenic sources of soil pollution are related to industrial (e.g. mining), urban, and agricultural activities, and most of the world's polluted soils contain complex mixtures of both organic and inorganic pollutants. Among them, metals and metalloids, hereafter referred to as potentially harmful elements (PHEs), according to Bini and Bech (2014), are of great concern and are considered one of the most problematic pollutants. These elements can persist in soils for long periods of time (Azhar et al. 2022) as they bind strongly to soil components and cannot be degraded. Their potential bioavailability in soils can lead to a cumulative effect on organisms; representing the main source of global environmental pollution with harmful implications also for human health (Turdi and Yang 2016).

In fact, half of the polluted sites worldwide are affected by PHEs (Khan et al. 2021), and in Europe, they are responsible for 37% of soil pollution (Dağhan and Öztürk 2015). In this sense, about 2.8 million potential PHEs-affected sites are estimated to exist in the European Union (EU) countries alone (EEA 2019), 19% of China's agricultural soils contain high PHEs concentrations (Hou et al. 2020), and in the USA, around 6000 km^2 are polluted by PHEs (Raffa et al. 2021). Specifically, in Spain, a preliminary study identified 4532 potentially polluted soils before the 2000s (BOE 1995), of which only 270 were remediated by investing about 170 M € from public funds (MARM 2007); however, in a recent and exhaustive study, in Andalusia alone, nearly 27,000 potentially polluted sites have been inventoried (BOJA 2018). Indeed, the global economic impact of soil pollution is remarkable, with an estimated cost of more than US$ 10 billion annually (He et al. 2015). Thus, integrated efforts are urgently needed to address the environmental and economic challenges posed by soil pollution on a global scale.

Metals and metalloids are released from different anthropogenic activities, but mining activities are one of the most worrying sources (Rodríguez Martín et al. 2015). Mining and processing of metal ores activities frequently result in the release of PHEs, such as heavy metals (i.e. Cd, Cu, Pb and Zn) and metalloids (i.e. As, Sb), which can accumulate and mobilise within the soil-plant-water system, resulting in potential adverse effects not only for the environment, but also for public health over a long period of time (Burgos et al. 2008a; Song et al. 2009). Thus, the great expansion of the global mining industry due to the need for strategic elements for energy and digital transition (e.g. critical metals, rare earth elements, platinum-group elements, technology-critical elements) raises serious health and environmental concerns (Grande et al. 2018; Watari et al. 2021; Tao et al. 2022); many of these concerns are related to the large amount of waste generated (over 100 billion tonnes per year) (Tayebi-Khorami et al. 2019). In particular, sulphide mining is one of the main suppliers of these elements (Vaughan 2005), and consequently, is one of the major sources of PHEs pollution. The intense extractive activity of sulphide ores generates various types of wastes (i.e. gossan waste: gossan materials mixed with host rocks, sulphide waste: crushed pyrite and smelter ash); leading to the generation of acid mine drainage (AMD) from the oxidation of these sulphide materials or acidic waters during ore concentration. Indeed, AMD is among the major environmental concerns related to mining activity that can affect soils, surface water, and groundwater both in mining areas and in large surrounding areas, being also a problem of great temporary impact, as its effects can last for decades or centuries (Sánchez España et al. 2005; Naidu et al. 2019).

1.1.3. SCENARIO OF THE IBERIAN PYRITE BELT

A clear example of this problem is found in the Iberian Pyrite Belt (IPB) (SE Portugal and SW Spain), one of the largest metallogenetic provinces of massive sulphides worldwide, where large-scale mining activities date back to the 19th century and the earliest activities to the 3rd millennium BC (Nocete et al. 2014). Today, many of them have already ceased their activity (e.g. São Domingos, Caveira, Aljustrel, San Miguel, Concepcion), leaving a large legacy of mining wastes (i.e. enormous sulphide-bearing waste-rock piles, tailings, and flooded pits) (Mourinha et al. 2022). However, with the increase in demand and mineral prices, a significant number of mines are still active (e.g. Neves-Corvo, Lousal, Aguas Teñidas, Las Cruces, Riotinto, Sotiel Coronada) and/or in the process of reopening (e.g. Tharsis, Aznalcóllar-Los Frailes), as it is estimated that up to 400 Mt of ore remain to be extracted. Therefore, the existing operational mines, the historical waste associated with abandoned mines, and the reopening of mines in the IPB constitute a potential source of pollution, being the IPB representative of the main concerns related to other sulphide mines located around the world.

In this context, the Aznalcóllar disaster occurred in this region, which is considered the second largest mining spill in the world and the largest reported in Europe up to the date of the accident (Ayala-Carcedo 2004; Nikolic et al. 2011). On April 25, 1998, a rupture in the tailings pond dam of the Aznalcóllar pyrite mine discharged approximately 36 x 10^5 m^3 of acidic waters and 9 x 10^5 m^3 of toxic tailings with high concentrations of sulphides and several PHEs (i.e. Zn, Cu, Cd, As, Pb, Tl) into the Agrio and Guadiamar river basins, resulting in severe pollution of soils and surface waters by PHEs (Figure 1.1).

Figure 1.1. *Rupture of the Aznalcóllar tailing pond dam and release of toxic tailings and acidic waters to the Agrio river. Extracted from El Confidencial* [2].

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[2] https://www.elconfidencial.com/espana/andalucia/2018-04-25/aznalcollar-desastre-pudo-evitarse-ecologistas-alerta_1554645/ (accessed 05/12/2023)

Given the magnitude of the accident and the high degree of soil pollution, the Regional Government of Andalusia invested an enormous amount of human, technical and economic resources in the recovery of the area between 1998 and 2001, which was finally declared a Protected Area, currently known as the Guadiamar Green Corridor (GGC) (Consejería de Medio Ambiente 2003). To a large extent, the total and bioavailable concentrations of many PHEs have been decreased as a consequence of the remediation actions applied and the effect produced by ageing to stabilise the affected area, with recent studies in the area focusing mainly on those PHEs that persist in higher concentrations such as Pb, As, Zn, Cu and Cd (Madejón et al. 2006b; Martín Peinado et al. 2015; Romero-Freire et al. 2016). However, the persistence of residual pollution in certain areas of the GGC more than 25 years later despite massive decontamination efforts undertaken (Pastor-Jáuregui et al. 2022), such as that shown in Figure 1.2, poses a long-term toxic risk to living organisms in the area (Romero-Freire et al. 2016; García-Carmona et al. 2017; Paniagua-López et al. 2023).

Figure 1.2. *Polluted soils in the experimental plot "El Vicario" located in the GGC (Seville, Spain) more than 25 years after the Aznalcóllar disaster.*

This residual pollution is evidenced by these patches of unvegetated soil randomly distributed in the first 18 km downstream of the GGC, which represent about 7% of the affected area (Martín Peinado et al. 2015). These patches of very heterogeneous size (between 2 and 200 m^2) are characterised by high PHEs concentrations and unfavourable physicochemical properties (i.e. acidic pH, low organic matter content) (Sierra Aragón et al. 2019). Thus, they can act as a source of diffuse pollution that needs to be addressed (Martín Peinado et al. 2015); since according to García-Carmona et al. (2017), these bare soil patches pose an environmental risk, assessed by risk quotients (EPA 2007) for total concentrations of As, Cu, and Pb, and for Cu and Zn when considering water-soluble concentrations.

1.2. NEED FOR ECOLOGICAL RESTORATION

The ecological restoration of these degraded environments is clearly necessary to mitigate the adverse impacts caused by PHEs incorporated into ecosystems (Mahar et al. 2016). When addressing the disturbances caused by mining, terms like remediation, reclamation, rehabilitation, and restoration are often used interchangeably in scientific literature or in government reports and policy documents (Lima et al. 2016). However, they denote distinct phases of the ecological restoration process (Finger et al. 2007), as defined below:

- ☐ **Remediation:** Physical, chemical, or biological actions to remove pollutants with the aim of reducing and managing the risks posed by polluted sites to living organisms (Lima et al. 2016). In essence, remediation is the cleanup of polluted areas (Finger et al. 2007).

- ☐ **Reclamation:** The process of converting degraded environments into areas suitable for cultivation or other human uses (Gerwing et al. 2022). This term is commonly used when the remediation target is mined land, where the focus is on geotechnical stabilisation and revegetation to improve the landscape and return it to a regionally defined useful purpose (Finger et al. 2007).

- ☐ **Rehabilitation:** Management actions aimed at restoring a level of ecosystem functioning in degraded sites, where the goal is to maintain the provision of ecosystem services rather than to establish the biodiversity and integrity of a native reference ecosystem (Gerwing et al. 2022).

- ☐ **Restoration:** The process of returning an ecosystem to a close approximation of its historical or pre-disturbance condition to the disturbance (National Research Council 1992).

The most frequently used terms in scientific literature are restoration and remediation (Lima et al. 2016). In this thesis, soil remediation is predominantly used, although environmental rehabilitation may occasionally be mentioned, as in these cases, it goes beyond the clean-up of the degraded environments.

In this sense, the environmental rehabilitation must be holistic and include the development of ecosystem services, biodiversity enhancement, and soil and water conservation (Gebremeskel et al. 2018). Therefore, soil remediation constitute a vital process within environmental rehabilitation, as soil plays a key role in triggering processes that lead to the recovery of ecosystem structure and function (Bünemann et al. 2018; Bhaduri et al. 2022). Soil underpins a wide variety of ecological processes (e.g. pollutant removal, nutrient cycling, reservoir of biodiversity, carbon sequestration, plant growth and productivity, water quality control), so its health condition significantly influences the overall well-being of an environment.

1.2.1. CONVENTIONAL SOIL REMEDIATION TECHNIQUES

In recent decades, many *ex situ* and *in situ* techniques have been developed to restore, remediate, reclaim, excavate, or clean-up polluted soils by PHEs (Ok et al. 2020; Palansooriya et al. 2020; Xu et al. 2021). *Ex situ* techniques are easier to control and more effective in the short term, but require excavation and removal of affected soils, resulting in a greater impact on an already disturbed environment, as well as health risks, waste production, and higher costs (Azubuike et al. 2016). Therefore, they are a less realistic and viable option for large extensions of polluted soils (Burgos et al. 2008b). In contrast, *in situ* methods, although often considered less effective, are usually more economical and tend to preserve the natural structure and functions of soils, thus providing a more eco-efficient solution (Liu et al. 2018). Conventional remediation technologies are generally based on physical, chemical, and biological approaches, which are often combined to clean polluted soils to an acceptable and safe level (Khalid et al. 2017); and the following can be highlighted: physical (soil replacement, soil isolation, vitrification, electrokinetic remediation, thermal desorption), chemical (soil washing, chemical stabilisation, redox transformation, treatment with nanoparticles), and biological (phytoremediation, microbial remediation) (Khalid et al. 2017; Khan et al. 2021; Azhar et al. 2022; Sánchez-Castro et al. 2023). These treatment methods have been widely used in most industrial-scale technical decontamination applications. However, despite their effectiveness, these procedures have certain drawbacks such as limited efficiency, high costs, poor field applicability, and the generation of potentially harmful wastes. For example, the cost and duration of soil remediation, although dependent on technique and site, have been estimated to be at up to US$ 500 t^{-1} soil and 15 years (He et al. 2015). These limitations can be mitigated by applying integrated strategies that have proven to be more effective for PHEs immobilisation (Raffa et al. 2021; Aparicio et al. 2022; Kumar et al. 2023). Basically, an integrated remediation process is the combination of two or more remediation techniques that not only increase the efficiency and removal rate, but also remove additional pollutants along with PHEs from polluted soils (Kumar et al. 2023).

1.2.2. ASSISTED NATURAL REMEDIATION BY WASTES

Sustainable strategies based on the use of plants and/or soil additives *in situ* have also been used to remediate PHE-polluted soils (Adriano et al. 2004; Kumpiene et al. 2008). These pollutants can be retained in soil by sorption, precipitation, and complexation reactions that occur naturally in soils, reducing their mobility and bioavailability (Xiong et al. 2015). This process is called natural remediation, which in dry climates may be too slow, but adding some wastes and by-products as amendments this process can be enhanced, giving rise to the so-called assisted natural remediation technique, which contrasts with most classical remediation techniques that drastically alter soil properties (Adriano et al. 2004). The use of wastes as amendments in polluted soils to immobilise PHEs within the soil matrix not only meets the objective of restoring soil quality and promoting the recovery of soil biota from microorganisms to plants (Lombi et al. 2002; Adriano et al. 2004; Kumpiene et al. 2019), but also contribute to the waste revalorisation. Thus, the assisted natural remediation using wastes as soil amendments constitutes an environmentally friendly, cost-effective and feasible solution (Rodríguez-Jordá et al. 2012) in line with zero-waste principles (Greyson 2007).

In the case of remediation of PHE-polluted soils, the application of a wide variety of amendments has been shown to be effective in immobilising PHEs at the field scale because of the physicochemical changes that occurred in soil after their application (Adriano et al. 2004; Aguilar et al. 2007; Clemente et al. 2015; Xiong et al. 2015). These include organic amendments such as processed animal wastes, compost/plant residues, biosolids/sewage sludge, and biochar, as well as inorganic amendments like clay minerals, coal fly ash, industrial wastes, liming materials, metal oxides/red mud, and phosphates (Palansooriya et al. 2020).

1.2.3. WASTE-DERIVED TECHNOSOLS

In general, the application of amendments leads to a decrease in PHEs mobility and an improvement in the physicochemical and biological characteristics for plants and soil microbiota growth (Santos et al. 2018). However, the impact of some amendments may be weak in the long term (Macías 2004; Macías et al. 2011), requiring successive applications that increase remediation costs (Pérez-De-Mora et al. 2011). Furthermore, the integrated application of appropriate amendments with contrasting properties is recommended to maximise their use efficiency (Rodríguez-Vila et al. 2014; Oldfield et al. 2018). In this sense, different and complementary wastes can be used to produce a specific and designed soil (Technosol, IUSS Working Group WRB 2022) that promotes different biogeochemical and edaphic processes, leading to an improvement of soil properties and a more persistent decrease in the mobility and availability of PHEs (Macías et al. 2011; Santos et al. 2019).

This emerging ecotechnology is based on the principles of pedo-engineering, sustainability, and circular economy. They consist of human-made soils specifically designed for a particular environmental problem (called Technosol "a la carte" or tailor-made Technosol, Macías 2004), whose properties and pedogenesis are characterised by their technical origin (containing ≥ 20% artefacts by volume) IUSS Working Group WRB 2022). Indeed, most Technosols applied in soil remediation programmes are waste-based, as this ecotechnology also tackles the unsustainable waste generation. Apart from improving basic soil functions, Technosols promote various biogeochemical and edaphic processes (i.e. acid neutralisation, decrease sulphide oxidation, PHEs immobilisation, enhancement of soil fertility, stimulation of soil biological activity) because of the complementarity of their components, contributing to the medium- and long-term sustainability of the remediation process (Macías et al. 2007; Santos et al. 2016a). The efficacy of this ecotechnology for the remediation of mining areas (i.e. soils, tailings, and leachates) was demonstrated in previous studies for several Technosols and climate regions, both in microcosm studies under controlled conditions (Rodríguez-Vila et al. 2016; Santos et al. 2016a; Jordán et al. 2017; Asemaninejad et al. 2018; Asensio et al. 2019) and in large-scale interventions (Asensio et al. 2013; Santos et al. 2019; Ruiz et al. 2020; Arán et al. 2022; Queiroz et al. 2022; Walmsley et al. 2022).

This PhD thesis was conceived in this line and aimed at studying the potential use of different waste-derived Technosols specifically designed for the remediation of sites affected by sulphide mining activity, including both persistently polluted soils such as the bare areas of the GGC, as well as mining waste and acidic mine waters that may constitute the pollutant source. In particular, this thesis explored the combined use of Technosols and the development of autochthonous plants to enhance and accelerate remediation processes compared to the use of only one of these technologies (Santos et al. 2014b).

1.3. COMPREHENSIVE SOIL REMEDIATION ASSESSMENT

A comprehensive understanding of the evolution and effectiveness of remediation techniques applied to polluted soils requires a holistic approach. In contrast, many studies on remediation of polluted soils have been limited to assessing the effectiveness of the applied ecotechnology mainly by changes in soil properties and mobility/bioavailability of PHEs. To a large extent, the mobility and toxicity of PHEs in soils are strongly conditioned by certain properties, such as pH, organic carbon, and carbonate content, among others (Kabata-Pendias 2010; García-Carmona et al. 2017). However, the interaction between PHEs, soil matrix, and organisms, as well as the resulting effects, cannot be assessed only through a physicochemical study (Leitgib et al. 2007; ISO 17402 2008). Therefore, many other studies complement these analyses with different approaches such as microbial community characterisation, ecotoxicity and phytotoxicity tests that can be very useful by providing valuable information from other scientific disciplines, which is a maxim of this PhD thesis. Likewise, most studies on Technosols focus on analysing the changes in the Technosols themselves and the surface effects, but do not include information on the underlying polluted soils. To the best of our knowledge, this is the one of the first studies to consider this holistic approach and involve changes in both Technosols and underlying polluted soils.

1.3.1. IMPORTANCE OF PHEs MOBILITY AND BIOAVAILABILITY

Pollution by PHEs is usually determined in terms of the total PHEs concentration in soils; indeed, most guideline values in global regulatory legislation are stated as total concentrations (Paniagua-López et al. 2023). For example, the Andalusian government, where the polluted soils object of this thesis are located, established that the intervention values for Cu, Zn, As, Pb, and Cd are 595, 10,000, 36, 275, and 25 mg kg^{-1}, respectively (BOJA 2015). However, there is still no clear consensus for determining the guideline values for PHEs in soils, so they differ between countries and regions (Table 1.1). In Spain, intervention values for some PHEs vary more than 10-fold depending on the regional government. This is the case with the legislation in Galicia (NW Spain), where the guideline values for Cu, Zn, and Cd are 50, 300 and 2 mg kg^{-1}, respectively, more than 10 times lower than the Andalusian regulatory values (DOG 2009). For other countries, the values differ even more; for example, in China, the risk detection value for Cd is 0.2 mg kg^{-1} (MEEPRC 2018), or in Canada, the intervention value for As is 12 mg kg^{-1} (CCME 2007). Therefore, the risk of pollution may be overestimated or underestimated when studies are based on total concentrations only, as it is highly dependent on local regulations.

Furthermore, the toxicity of PHE in soil is mainly controlled by its mobility and availability rather than by its total concentration; therefore, PHE fractionation in soil is critical when investigating pollution risk (Giannakis et al. 2021). For example, PHEs that are encapsulated, insoluble, or tightly bound to solids may not be prone to biological absorption or cause a biological response, whereas those that are dissolved may be readily available (Kuppusamy et al. 2017). In this regard, the determination of water-soluble and bioavailable forms of PHEs is widely accepted in the study of soil pollution for its ease and affordability. Water-soluble forms constitute the portion of PHE that can be readily absorbed, subsequently reaching endpoints where they might pose a threat to target organs of living organisms (Bagherifam et al. 2019). The bioavailable fraction can be extracted using different reagents; one of the most used is ethylenediaminetetraacetic acid (EDTA), which mainly extracts the PHE contained in the non-silicate-bound soil phases (carbonates and Fe-/Al- oxides), which is indicative of the relatively long-term availability of PHE (Labanowski et al. 2008; Nenova et al. 2018). Thus, both the soluble and bioavailable fractions constitute the part of PHE in soil that can be taken up by organisms and thus cause damage to the ecosystem and/or enter the trophic chain (Son et al. 2019). Also, the application of Technosols is not expected to alter the total PHEs content, as their influence lies mainly on soluble and bioavailable forms. Therefore, it is important that these PHEs fractions are determined before and after remediation, and that regulations are based on them and not only on total contents.

Table 1.1. *Guideline values of main PHEs in mg kg^{-1} to declare a soil as potentially polluted by legislations of different countries.*

PHEs	**Andalusia (Spain) [1]**	**Galicia (Spain) [2]**	**China [3]**	**Canada [4]**	**Portugal [5]**
As	36	50	15	12	11
Cd	25	2 (1)	0.2	1.4	1
Cu	595	50	35	63	62
Pb	275	100 (80)	35	70	45
Zn	10,000	300 (200)	100	200	290

[1] *Generic Reference Value (NGR) for trace elements in Andalusia* (BOJA 2015). [2] *Generic Reference Value (NXR) for contaminants in Galicia* (DOG 2009). [3] *Environmental quality standards for soils (HM) in China* (MEEPRC 2018). [4] *Canadian Soil Quality Guidelines for different PHEs.* (CCME 2007). [5] *Reference values for the main soil pollutants in Portugal* (APA 2019). *The value presented is the most restrictive by land use: Andalusia - other uses (generally, agricultural), Galicia the same (in brackets ecosystem protection value), China – Level I soils, Canada - agricultural and/or residential/parkland use, and Portugal - agricultural. Table extracted from* Paniagua-López et al. (2023).

1.3.2. INFLUENCE OF SOIL PHYSICOCHEMICAL PROPERTIES

Potentially harmful elements can be involved in a series of complex physicochemical and biological interactions largely influenced by soil properties and components that affect their speciation and bioavailability (Sheppard and Evenden 1988). In this sense, the intended effect of Technosols application in polluted soils is that their constituent amendments can reduce the mobility, bioavailability, and toxicity of PHEs in soil through several mechanisms such as precipitation, complexation, redox reactions, ion exchange, and electrostatic interaction. Thus, certain soil physicochemical properties listed below are important factors governing the effectiveness of Technosols in the immobilisation of PHEs in soils.

- ☐ **pH**: Due to competitive ion sorption, precipitation-dissolution reactions, and pH-dependent complexation of PHEs (Romero-Freire 2015), soil solution pH controls the immobilisation of most PHEs by its influence on the electrical charge of the colloidal soil fraction (e.g. organic matter, clay) (Roberts et al. 2005). At alkaline pH, the colloidal soil fraction is more negatively charged and thus PHEs cations are removed from solution and bound to colloids (Russell and Wild 1988). In addition, the solubility of metal salts is often pH-dependent, causing higher levels of cationic PHEs in soil solution at low pH. In general, maximum retention of cationic PHEs (i.e. Cd, Pb, Ni, Cu, Zn) occurs at pH > 7, while that of anionic PHEs (i.e. As, Sb, V) is at pH < 7 (Draszawka-Bołzan 2017).
- ☐ Nature, content, and quality of **organic matter (OM)**: The amount and type of organic matter added as an amendment also affects the solubility of PHEs by sorption and complexation processes (Romero-Freire et al. 2015). The importance of organic carbon (OC) as the main component for PHEs adsorption and soil recovery has long been recognised (Alloway 2012), but there are controversial results depending on PHE and soil properties such as pH (García-Carmona et al. 2017). Moreover, in certain situations, increasing OC through organic amendment not only does not remove PHE, but increases its bioavailability and thus the potential risk of toxicity (Huang et al. 2016).
- ☐ **Calcium carbonate ($CaCO_3$)**: The presence of carbonates in soil is not only essential for their ability to neutralise acidity (Cravotta and Trahan 1999; García-Carmona et al. 2017), but also for their potential to immobilise certain PHEs (i.e. Pb, Cd, Ni, As) by precipitation and co-precipitation mechanisms (Goldberg and Glaubig 1988; Mourid 2014; Aguilar-Garrido et al. 2022a).
- ☐ **Clay minerals**: Soil texture, which reflects the size distribution of its constituent mineral particles, has a high impact on PHEs mobility, mainly by fine particles such as oxides and clays. In particular, clays are the most important adsorption arrsurface for PHEs, as they constitute an important part of the colloidal

fraction of the soil together with soil organic matter (SOM). Thus, the more clay in soil, the greater the adsorption of cationic PHEs (Seraj and Rahman 2018).

- **Cation exchange capacity (CEC)**: It measures the number of negatively charged adsorption sites, mainly conditioned by SOM and clay contents, as well as the presence of other adsorption phases such as Fe and Mn oxyhydroxides and soil pH (Romero-Freire 2015). Thus, the higher the CEC, the more available positions to which cationic PHEs can adsorb, reducing their availability IN the short term; anyway, adsorption is a reversible process, so PHEs retained in the exchangeable complex can be available at the medium term (Campillo-Cora et al. 2020).
- **Fe oxy(hydr)oxides**: They are important mineral sorbents for PHEs in soils. In particular, these minerals are important exchangeable soil particles with a high affinity for anionic PHEs (i.e. As, Sb), leading to their adsorption or coprecipitation (García et al. 2009). The degree of crystallinity of Fe oxy(hydr)oxides can affect their adsorption capacity, with the amorphous (non-crystalline) forms having the highest binding capacity (Yang et al. 2019).

1.3.3. CRITICAL LOAD OF TECHNOSOLS

Soils are capable of attenuating the mobility, bioavailability, and potential toxicity of most PHEs through, processes such as filtration, neutralisation, adsorption, and precipitation, preventing the entry of these pollutants into more sensitive compartments such as water or trophic chain. But this buffering capacity is limited, and when it is exceeded, the "chemical time bomb" phenomenon can occur, in which the soil ceases to function as an ecosystem protector and becomes even a source of pollution (Stigliani 1991; Díez et al. 2009). Therefore, it is important to consider the critical load of soils, which represents "the maximum quantity of a given contaminant that can be supplied to a soil without causing chemical changes leading to long-term harmful effects on ecosystem structure and function" (Hettelingh et al. 1991). This capacity to retain PHEs depends on the composition and properties of soils (Alloway 2012). For this reason, defining the critical load of Technosols is crucial for this PhD thesis (**chapter 5**), as it is directly related to the long-term capacity of Technosols to cope with residual pollution.

1.3.4. TOXICITY OF PHEs TO LIVING ORGANISMS

In the EU context, to protect human health and terrestrial, aquatic, and atmospheric ecosystems, the ecotoxicological risk or damage under pollution conditions must be assessed to remediate or decide on risk reduction measures (De Bruijn et al. 2002; van Gestel 2012). This environmental risk assessment of polluted soils is carried out through an integrated approach that combines the use of chemical methods, toxicological bioassays, and ecological studies for the characterisation of the risk and associated effects (Jensen and Mesman 2006).

The study of the behaviour of living organisms by toxicity bioassays is essential for proper environmental risk assessment and to ensure the effectiveness remediation techniques (Song et al. 2006). Since the mere determination of PHEs content, both total and available forms, provides an idea of the degree of decontamination that is not sufficient, it becomes imperative to determine the potential effect that PHEs may have on organisms exposed to them (Ojuederie and Babalola 2017). To this end, different national and international organisations (e.g. Organization for Economic Co-operation and Development [OECD], International Organization for Standardization [ISO], Environmental Protection Agency [EPA]) have developed a series of standardised toxicity tests with the most important groups of soil organisms (i.e. plants, invertebrates, microorganisms).

The effects of PHEs on organisms vary greatly due to different uptake pathways; for example, they can reduce microbial biomass (Shukurov et al. 2014), affect the diversity of soil communities (Stefanowicz et al. 2008), and act on various soil microbial processes, thereby altering nutrient cycling and the ability to perform key ecological functions (e.g. mineralisation of organic compounds and synthesis of organic substances) (Giller et al. 1998; Moreno et al. 2009). Therefore, a battery of tests with representative species from different taxonomic groups is desirable to evaluate the toxic effects due to the high variability when comparing different bioassays to study the reduction of soil toxicity (Romero-Freire et al. 2018). Moreover, it is recommended that the toxicity assessment takes into account the different exposure routes, thus, the selected set of bioassays should jointly consider exposure to both solid and liquid phases (e.g. soil extract, leachate, pore water) of soils (Rodriguez-Ruiz et al. 2014).

In this PhD thesis, the potential toxicity after remediation with Technosols is evaluated by a total of four different well-established ecotoxicological tests using different organisms and soil fractions. In the soil-solid fraction, toxicity was determined by plants such as *Trifolium campestre* Schreb. (**chapter 4**), *Lactuca sativa* L. (**chapter 6**), and *Lablab purpureus* (L.) Sweet (**chapter 8**); in the soil-water fraction, it was used *L. sativa* (**chapter 4**). Specifically, in **chapter 6**, the on-soil *L. sativa* growth test aims to comprehensively assess the phytotoxicity caused by PHEs, including the study of plant growth, photosynthetic capacity, oxidative stress, and antioxidant defence.

1.3.5. VEGETATION RECOVERY

Vegetation also plays an important role in the remediation processes of polluted areas; indeed, its presence or absence usually acts as an indicator of the degree of soil pollution (Paniagua-López et al. 2024). Hence, assisted natural remediation by native vegetation that spontaneously colonises polluted areas is an important factor to consider when assessing the success of remediation actions (Álvarez-Rogel et al. 2021), as they can act as nurse plants and thus facilitate the growth of less stress-tolerant species (Navarro-Cano et al. 2018). The growth of plant cover is highly relevant, as it favours the improvement of physicochemical and biological conditions of these degraded soils, for instance, by increasing SOM content and nutrient availability, preventing the dispersion of PHEs in the ecosystem, and even, avoiding soil erosion (Madejón et al. 2006b; Pérez-de-Mora et al. 2006; Bolan et al. 2011). For this reason, in **chapter 7**, which covers the *in situ* remediation of polluted soils in the GGC using two Technosols produced in this thesis, the recovery of natural vegetation is monitored.

1.3.6. IMPORTANCE OF NATIVE MICROBIOLOGY ON SOIL HEALTH

Microorganisms are an essential part of living soil and extremely important for soil health because they exert a vital role in terrestrial ecosystem processes, especially in biogeochemical cycles (Doran and Zeiss 2000). When the equilibrium of the soil system is disturbed by environmental perturbations, they are stressed and respond more rapidly to these changes than other higher organisms (Giller et al. 1998; Nielsen and Winding 2002; Zhang et al. 2022a). Consequently, soil microbial community characterisation is increasingly applied to determine the response of soils to environmental stresses and disturbances (e.g. PHEs pollution) and, is therefore commonly used as an indicator of ecosystem recovery (Costantini et al. 2016; Muñoz-Rojas et al. 2016b; Schloter et al. 2018). Changes in microbial community composition (abundance, diversity and richness) following remediation actions may be an indicator of the recovery of soil ecosystem functions (Muñoz-Rojas 2018).

In recent years, molecular biological methods, such as amplicon sequencing of evolutionary biomarkers like 16S rRNA, have advances our understanding of the microbial composition, which would otherwise be limited to those cultivable under laboratory conditions. Several techniques are now available to determine soil microbial characteristics, including biogeochemical and physiological approaches (e.g. substrate-induced respiration), metabolic assessments (e.g. enzymatic activities), and more recently, molecular analysis such as the examination of soil-extracted nucleic acid sequences (DNA, RNA) (Muñoz-Rojas et al. 2016a).

For example, soil enzymes are one of the most active organic components in rhizosphere with a great control over the soil's potential to sustain biochemical processes essential for maintaining soil fertility (Kumar et al. 2013; Liao et al. 2023). Soil enzyme is a general term for both intracellular and extracellular enzymes produced by soil microorganisms, plant roots and other biological cells (e.g. animals, earthworms, ants) in soils (Burns et al. 2013). They effectively promote the metabolic process of soil and play an important role in the biogeochemical cycles (i.e. C, N, P, S) and the energy flow process of the soil ecosystem. Thus, their activity reflects the intensity and direction of various biochemical processes in soil, and thus also soil health (Liao et al. 2023). In addition, soil enzyme activity is very sensitive to environmental stress (e.g. high PHEs concentrations, drought) responding rapidly to induced changes (Dick 1997; Rao et al. 2014) so they are a good indicator to monitor soil remediation effectiveness since they reflect the soil functional diversity, changes in the microbial community composition, and microbial status (Kumar et al. 2013). In this context, the determination of enzymatic activities can also be considered as toxicity bioassays assessing the effect of PHEs on soil microorganisms. For this reason, the effectiveness of Technosols-mediated remediation process was also assessed by determining different soil enzyme activities in **chapters 4**, **7** and **8**. The enzymes used for this purpose include dehydrogenase, cellulase, β-glucosidase, protease, urease, and acid phosphatase, which have been widely used to assess the remediation of degraded environments (e.g. polluted soils, mining waste) in short-medium term (de Varennes et al. 2010; Santos et al. 2016a).

On the other hand, in **chapter 7**, which covers the *in situ* remediation of the polluted soils in the GGC using two Technosols produced in this thesis, the focus is on the analysis of the alterations on the composition and diversity of the microbial communities. Apart from the study of soil enzymatic activities, the degree of recovery of the microbial community in polluted soils is assessed by examining the DNA extracted from soil and the sequencing of amplicons of the V3/V4 region of 16S rDNA.

1.4. AIM AND OUTLINE OF THIS THESIS

It is hypothesised that by producing Technosols from the integrated application of organic and inorganic amendments with contrasting properties, the effectiveness of PHEs neutralisation and the improvement of physicochemical and biological characteristics for plants and soil microbiota growth in polluted areas can be maximised and prolonged over time. Thus, waste-derived Technosols specifically designed to have the appropriate mineralogical composition and biogeochemical properties to address this problem may constitute an effective remediation ecotechnology.

Therefore, the main objective of this PhD thesis is to assess the effectiveness of different waste-derived Technosols in the remediation of degraded environments by sulphide mining (i.e. soils, tailings and water) with high levels of potentially harmful elements (PHEs).

From this general objective, three different sections are distinguished according to the target of remediation, each with its own specific objectives, with a multidisciplinary approach and at different scales. The results of these sections are summarised, discussed, and integrated in the general discussion of this PhD thesis (**Chapter 9**).

1.4.1. TREATMENT OF PHEs-BEARING WATER

In sulphide mining, one of the main sources of pollution that can affect soils, surface water, and groundwater corresponds to the acid mine drainage and acidic water, which are characterised by their extremely acidic nature, high salinity due to the presence of sulphates and sulphosalts, and high PHEs concentrations. For this reason, part of the thesis has focused on the treatment of water polluted with different PHEs using an AMD simulated in the laboratory.

- ☐ **Chapter 3.** The specific objective in this chapter is to evaluate the acid neutralisation capacity and the removal effectiveness of PHEs present in AMD of ten different inorganic and organic wastes from the main waste-generating activities (urban, mining, and agro-industrial activities). In this way, this study makes it possible to screen the best performing wastes to be used in the production of the Technosols conceived in this thesis.

- ☐ **Chapter 5.** As in the previous chapter, the aim is to evaluate the acid neutralisation capacity and the removal effectiveness of PHEs present in AMD of the six Technosols designed in the thesis for their application in the treatment of both water and soil affected by PHEs. Hence, this study is also valuable to determine the critical load of these Technosols, which is a very relevant aspect of the thesis.

1.4.2. TREATMENT OF POLLUTED SOILS

Soils polluted by PHEs due to sulphide mining are the main remadiation target of this thesis. Specifically, we have used the soils affected by residual pollution located in the unvegetated patches of the GGC (Seville, Spain) (Figure 1.2), an area that constitutes a large and widely used open-air laboratory with a large list of previous research studies that would be of great help to have a complete and exhaustive knowledge of our own results.

- ☐ **Chapter 4.** The specific objective of this chapter is to assess, at physicochemical, biological, and ecotoxicological levels, the effectiveness of six Technosols produced from polluted soils and a mixture of organic and inorganic wastes in the remediation of PHE-polluted soils at a microcosm scale (in growth chamber conditions).

- ☐ **Chapter 6.** This chapter focuses on the analysis of phytotoxicity reduction using *L. sativa* as a bioindicator in some of these Technosols. Thus, the evaluation of ecotoxicity is more complete by considering physiological parameters related to phytotoxicity in lettuce plants, such as plant growth, photosynthetic capacity, oxidative stress and antioxidant defence.
- ☐ **Chapter 7.** After having evaluated the efficacy of the six Technosols at the laboratory/microcosm level in the previous chapters, the next step is the application of the two best performing Technosols in a real situation, in one of the unvegetated patches of the GGC. The specific objective of this chapter is to evaluate the effectiveness of *in situ* remediation of polluted soils through the application of two Technosols; by analysing the changes that occurred in: i) soil properties, ii) PHEs solubility, iii) soil enzymatic activity, iv) soil microbial composition and diversity, and v) vegetation recovery.

1.4.3. TREATMENT OF MINING WASTE

On the other hand, although it is not included in the thesis title, another remediation target is mining waste, since it may in fact constitute the primary source of pollution in sulphide mining.

- ☐ **Chapter 9.** The central objective of this chapter is to evaluate the potential in mining reclamation of a green biotechnology that combines the widely tolerant multifunctional legume, *Lablab purpureus* (L.) Sweet, with two designed Technosols derived from mixing gossan waste with a combination of organic/inorganic wastes from local industries (urban gardening services, quarries, cafes, and breweries). Specifically, the purpose is to promote favourable conditions in the gossan waste for optimal growth of *L. purpureus* (e.g. improve physicochemical properties and microbiological activity, and decrease PHEs bioavailability), as well as to evaluate the safety of this plant for animal consumption.

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September 2021

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CHAPTER 2

MATERIALS AND METHODS

2.1. STUDY AREA

This thesis is developed from materials related to the Iberian Pyrite Belt (IPB), located in the SW of the Iberian Peninsula with an extension 230 km long and 40 km wide, covering the districts of Beja, Setúbal and Faro in Portugal and the provinces of Huelva and Seville in Spain (Figure 2.1). Within this metallogenic zone, two study areas are selected, one related to the Aznalcóllar mine (★) and the other to the São Domingos mine (★).

The IPB was formed 350 M years ago by active hydrothermal volcanism, which resulted in the formation of a volcano-sedimentary complex that constitutes one of the most important volcanogenic massive sulphide districts worldwide, with original reserves of about 2000 Mt (Tornos et al. 2008; Almodóvar et al. 2019). These massive sulphide ores consist mainly of pyrite (FeS_2), about 95%, with variable amounts of chalcopyrite ($CuFeS_2$), sphalerite (ZnS), galena (PbS) and arsenopyrite (FeAsS), and about 5% of Cu-rich minerals (Solomon et al. 2004; Chopin and Alloway 2007); containing estimated reserves of 14.6 Mt Cu, 13.0 Mt Pb, 34.9 Mt Zn, 46,100 t Ag, 880 t Au and significant amounts of other metals, in particular Sn (Leistel et al. 1997).

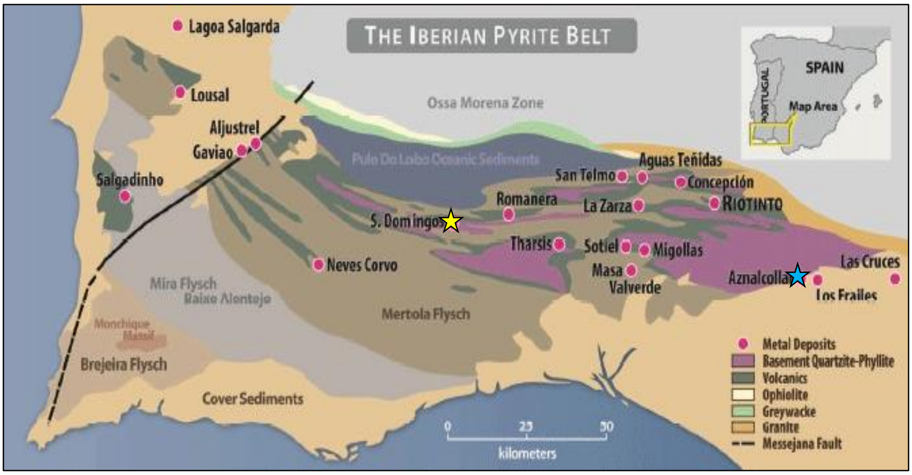

Figure 2.1. *Location and geology of the Iberian Pyrite Belt, including the most important massive sulphur deposits in Spain and Portugal. Extracted from Fundación Descubre* [3].

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[3] https://idescubre.fundaciondescubre.es/noticias/demuestran-la-almeja-asiatica-detecta-estado-contaminacion-aguas-acidas/ (accessed 21/11/2023)

2.1.1. GUADIAMAR GREEN CORRIDOR

Most of the thesis (**chapters 4**, **6** and **7**) has focused on the area affected by the Aznalcóllar mining spill in the GGC (SW Spain); specifically in the sector closest to the old tailing pond of the Aznalcóllar pyrite mine, where the presence of persistent PHE-polluted soils is frequent (Figure 2.2). This area is located in the province of Seville (37°00'–37°30' N, 6°10'–6°20' W) and covers an area of 45 km long and 0.5 km wide with an irregular shape on both sides of the fluvial plains of the Agrio and Guadiamar rivers.

This region of the Guadiamar river valley is formed by sedimentary materials from the Neogene to the Quaternary; specifically, the sector where this study area is located consists of alluvial deposits, including two younger fluvial terraces of Holocene age and an older one of Pliocene age. These alluvial deposits are composed of silty-sandy materials with gravels dominated by quartzite, shales, and schists, with a base of Miocene coarse blue marls (Manzano et al. 2000; Salvany et al. 2001). Thus, the main soil types in the area are associated with the fluvial dynamics of the Agrio and Guadiamar rivers, namely Fluvisols and Regosols, according to the World Reference Base (IUSS Working Group WRB 2022), or Entisols, based on Soil Taxonomy - USDA (Soil Survey Staff 2014). In the sector closest to the Aznalcóllar mine, the soils in the vicinity of the riverbed are eutric Fluvisols, and outside the floodplain are eutric Regosols. These are soils with poorly developed profiles, relatively high fertility, and anthropic influences (Cabrera et al. 1999). In all cases, soils have neutral pH (6.5-7.5), OM content between 0.8 and 2.9%, low $CaCO_3$ content (< 1%), and texture ranging from clay-loam to sandy-loam with a high gravel content (> 10%) (Simón et al. 1998; Ordóñez et al. 2003). Regarding the edaphic profile, the temperature regime is thermic and the moisture regime is Xeric (Soil Survey Staff 2014).

The climate of the zone is semi-arid Mediterranean (mesothermal semiarid – Thornthwaite classification) influenced by its location in the Guadalquivir Depression. It is characterised by cold, wet winters and hot, dry summers (> six months of intense droughts). The average annual temperature is 18.3 °C, and the annual rainfall is 490 mm, with an evapotranspiration coefficient of 920 mm due to annual insolation above 2800 h per year, based on the last 20 years (2003–2023).

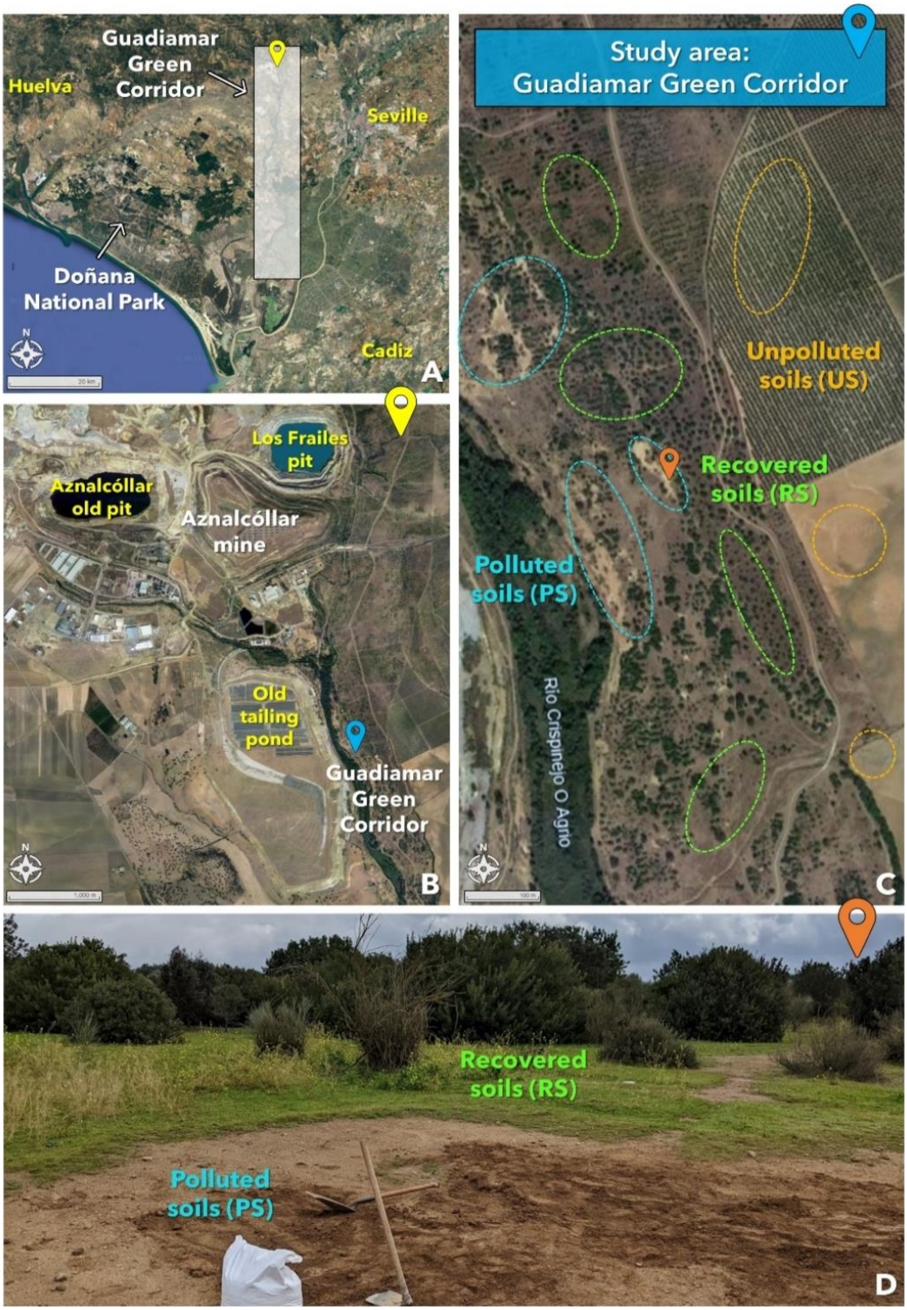

Figure 2.2. *Location of the GGC (A), specific study area (B), aerial image with different soil samples (polluted soil [PS], recovered soil [RS] and unpolluted soil [US]) (C), and detail of one of the bare soil patches (D). A, B and C obtained from Google Earth.*

The floodplains of the Guadiamar and Agrio rivers remain mostly covered by crops and orchards in the non-affected area; but the affected area, now declared protected area after the remediation actions conducted, is characterised by riparian vegetation near the riverbed (e.g. *Fraxinus angustifolia* Vahl, *Populus alba* L., *Populus nigra* L., *Ulmus minor* Mill, *Salix purpurea* L., *Salix atrocinerea* Brot), Mediterranean forest in the peripheral areas (e.g. *Quercus ilex* L., *Quercus suber* L., *Pyrus bougaeana* Decne, *Retama sphaerocarpa* (L.) Boiss, *Daphne gnidium* L., *Thymus mastichina* L.) and annual grasslands filling the gaps in this open forest (Martín Peinado 2001; García Robles 2020). Specifically, García-Carmona et al. (2019a) studied the vegetation present in the residually polluted soils and surrounding reclaimed areas, identifying a total of 27 species. In the polluted area only *Lamarckia aurea* (L.) Moench and *Spergularia rubra* (L.) J. Presl & C. Presl appear with a very low cover (~4%), in the transition zone *L. aurea* was dominant (80%) in association with other species (e.g. *Anthemis arvensis* (L.), *Hypochaeris glabra* (L.), *S. rubra*, *Vulpia membranacea* (L.) Dumort); while in the recovered area *L. aurea* is hardly present and is replaced by *Trifolium campestre* Schreb. with 70% of cover, together with *Leontodon longirostris* (Finch & P.D. Sell) Talavera, *Avena sterilis* (L.), *Chrysanthemum coronarium* (L.) and *Anagallis arvensis* (L.).

2.1.2. SÃO DOMINGOS MINE

In this thesis, a separate chapter (**chapter 8**) was devoted to the abandoned mining area of São Domingos (Beja, SE Portugal), one of the most emblematic Portuguese massive sulphide deposits. This mine is located in the northern sector of the IPB (37°40′28″ N, 7°30′01″ W), about 5 km from the Spanish border (Figure 2.3). São Domingos mine is now abandoned, having ceased activity in the 1960s, but it is considered one of the historical mining centres with known activity since pre-Roman times, with extraction of Au, Ag, and Cu (Álvarez-Valero et al. 2008).

This region consists of a single vertical mass of chalcopyrite associated with sphalerite and galena; like many other volcanogenic massive sulphide mineralisations at IPB. The large area covered by the mine is the result of the extraction of more than 25 Mt of ore (Cu concentrate production) and consequent production of enormous waste dumps, mainly from 1857 to 1966, of which 9.9 Mt of cupriferous pyrite (Gaspar 1998; Anawar et al. 2011).

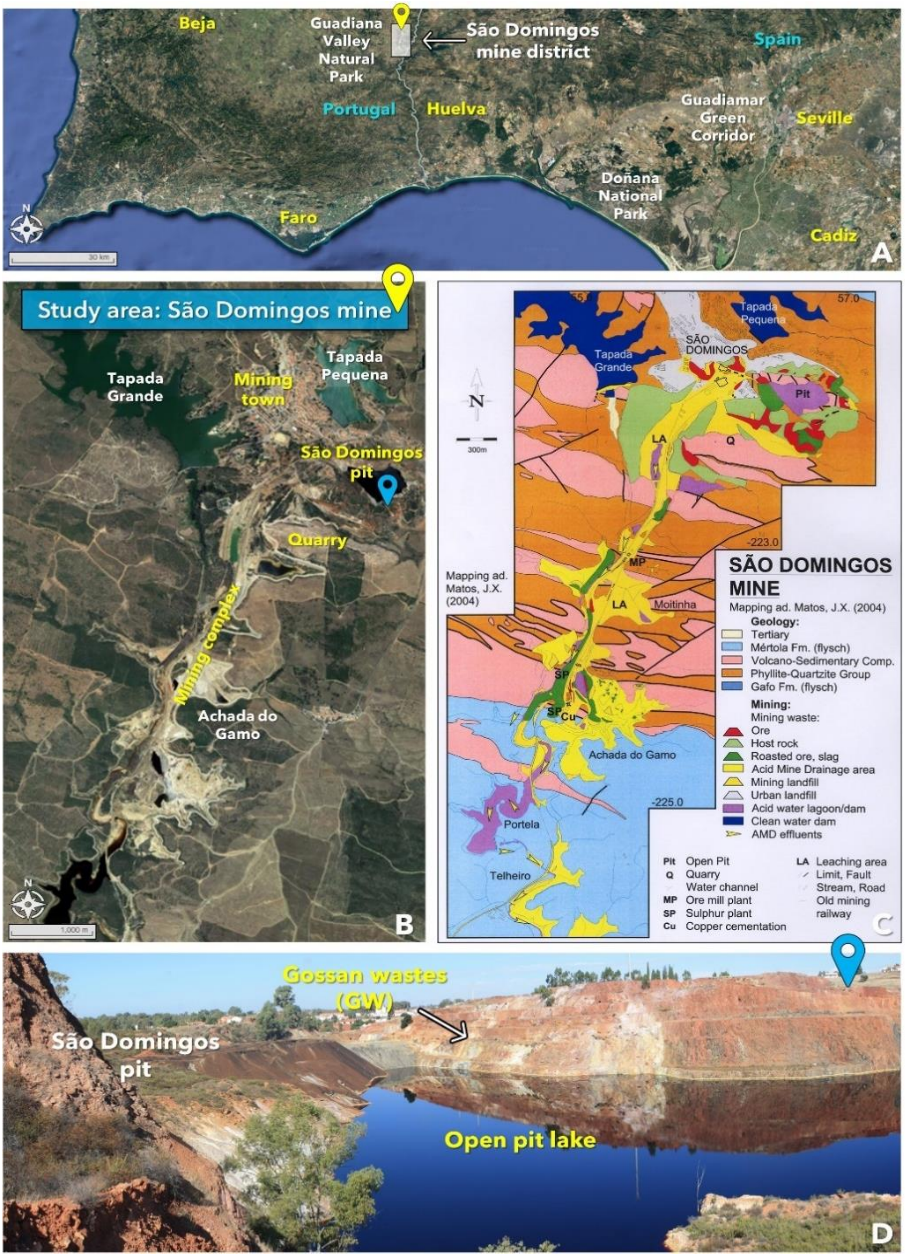

Figure 2.3. *Location (A), aerial image (B) and geological and mining map of São Domingos mine (C), and details of the gossan wastes (D). A and B obtained from Google Earth, C from* Abreu et al. (2010), *and D from Proyecto GEO_FPI* [4].

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[4] https://geo-fpi.igme.es/es/default.htm *(accessed 27/12/2023)*

The large accumulation of mining waste during the various exploitation periods, with approximately 5 Mt irregularly scattered throughout the mine area, represents a high environmental risk (Álvarez-Valero et al. 2008; Pérez-López et al. 2008), and has only enabled the development of incipient soils (Santos et al. 2016b). These soils are classified as Spolic Technosol Toxic (IUSS Working Group WRB 2022) because they have developed on mixtures, in varying proportions, of different mining wastes and host rocks. Alongside these, there are also some soils developed on schists and greywackes (Lithic Leptosol; IUSS Working Group WRB 2022), which are influenced by adjacent tailings and/or AMD.

The climate of the area is typically Mediterranean (mesothermal semiarid – Thornthwaite classification), with two distinct seasons: a moderately cold and wet period from November to March and, a dry and hot period from May to September. The average annual temperature is 17.6 °C, and annual precipitation is 559 mm, with hot summers (30 °C) and cold winters (12 °C) (Abreu et al. 2008).

The plant community of the São Domingos mine comprises 24 identified plant species (Freitas et al. 2004) with different adaptation strategies to PHEs pollution, including trees, shrubs, pastures, and semi-aquatic plants. The tree species present are *Quercus ilex* L. subsp. *balota* (Desf.) Samp., *Eucalyptus* spp., and *Pinus* spp. Among the dominant shrubs are *Cistus* spp., *Lavandula stoechas* L. subsp. *pedunculata* (Miller) Samp. and Rozeira, and *Genista hirsuta* Vahl. The semi-aquatic community includes *Juncus efusus* L., *Juncus conglomeratus* L., and *Scirpus holoschoenus* L. In addition, two endemic species of the genus *Erica* are present, which have the capacity to grow in PHEs-enriched and acidic soils: *Erica andevalensis* Cabezudo & Rivera, and *Erica australis* L. (Abreu et al. 2008; Márquez-García et al. 2012).

2.2. MATERIALS

2.2.1. ARTIFICIAL ACID MINE DRAINAGE

For **chapters 3** and **5**, an ***artificial AMD*** was prepared in the laboratory following a method-based on the oxidation of pyritic tailings with hydrogen peroxide (H_2O_2) (Simón et al. 2005). In detail, this pollutant solution used as AMD was prepared by progressive addition of 1 l H_2O_2 (33%) + 1 l H_2O to pyrite tailings

(~40 g in chapter 3, ~20 g in chapter 5) and after three days, the solution was extracted by discarding the precipitated sediment. The pyrite tailing used comes from the Aznalcóllar mine, and belongs to the toxic tailings discharged in the Aznalcóllar disaster in 1998 (Simón et al. 1999; Aguilar et al. 2003; Nikolic et al. 2011). The PHEs concentrations in these toxic tailings immediately after the accident were measured in previous studies (López-Pamo et al. 1999; Simón et al. 1999) and listed in Table S2.1. The artificial AMD obtained have an extremely acid pH (2.2-2.9), high electrical conductivity (3.7-7.2 dS m^{-1}) and very high concentrations of soluble PHEs (see section 3.2.1 and section 5.2.1 for detailed information on artificial AMD characteristics).

2.2.2. SOILS FROM THE GUADIAMAR GREEN CORRIDOR

In this study area, three different soil samples were used to properly assess the effectiveness of the remediation process in **chapters 4**, **6** and **7** (see Figure 2.2):

- ☐ ***Polluted soils (PS):*** These correspond to patches of soil without vegetation located in the sector closest to the tailing pond of the Aznalcóllar mine that ruptured in 1998, mainly in the first 12 km downstream from the pond. In these soils, with unfavourable physicochemical conditions (i.e. acidic pH, low organic matter content), high concentrations of PHEs are still present and may pose a toxicological risk. Thus, they are the main target areas for remediation in this thesis.

- ☐ ***Recovered soils (RS):*** These are soils that were affected by the spill, but are now considered recovered as a result of the remediation actions applied. They are adjacent to the unvegetated soil patches but the vegetation established on them is highly developed. Hence, these soils have been selected as reference soils with a non-hazardous degree of pollution, because although PHEs concentrations are higher than before the spill, the remediation and phytostabilisation actions have effectively contributed to reduce toxicity.

- ☐ ***Unpolluted soils (US):*** They correspond to soils not affected by the spillage but which are located in a former fluvial terrace near the studied area. Therefore, these soils are considered as the reference for the unaffected soils of the GGC.

For each soil (PS, RS and US), a composite sample was collected from five different specific locations, taken at a depth of 0-10 cm using an Edelman auger, as pollutants concentrate at this depth (Simón et al. 2001). Previously, the different sampling points for each soil were identified using satellite imagery following the methodology described in García-Carmona et al. (2017) (Figure 2.2).

2.2.3. GOSSAN WASTES FROM SÄO DOMINGOS MINE

At the São Domingos mine, two main groups of wastes were distinguished: mining waste piled in dumps (gossan waste and disseminated sulphide country rocks) and industrial waste from ore processing operations (slags, iron oxides, smelter ashes, pyrite-rich dumps, leaching tank rejects, and industrial dumps) containing high amounts of PHEs (in tonnes, Fe: 172,514, S: 10,564, Pb: 6644, Zn: 2610, Mn: 1126, Cu: 1032, Cr: 183, As: 109, Sb: 34, and Cd: 0.9) that could be released into the environment, constituting a major source of pollution (Pérez-López et al. 2008).

In **chapter 8**, we worked with the ***gossan waste (GW)*** from this abandoned mine (Figure 2.3), considered as the fourth most hazardous waste produced in this exploitation and the third in terms of volume/mass (with 1.7 hm^3 and 4.1 Mt) (Pérez-López et al. 2008). Thus, the selection of this GW can be considered as a reference model for the study of remediation solutions for sulphide mining areas.

2.2.4. WASTE MATERIALS

In this thesis, as the aim is to evaluate the potential of different waste-derived Technosols, a large variety of wastes from common activities in urban, mining, and agro-industrial environments is used. Two main groups will be distinguished: (i) those used in the production of Technosols together with polluted soils from the GGC (**chapter 4**, **5**, **6** and **7**) after characterisation, testing and selection in **chapter 3**, and (ii) those used in the Technosols derived from gossan waste of the São Domingos mine (**chapter 8**).

In the first group, 10 waste materials (four inorganics and six organics) available in the case study region (SE Spain) were selected because the proximity of these materials is a key factor in the cost-effectiveness of the remediation treatments applied (Figure 2.4). Inorganic wastes are of mining origin and include:

i) ***dry sludge rich in iron oxyhydroxides (IO)*** from the Alquife iron mine (E Granada), ii) ***dry sludge from marble cutting and polishing (MS)*** in the Macael quarries (NW Almería), iii) ***carbonated waste (CW)*** from the Agia peat bog mining at Padul (SE Granada), and iv) ***gypsum mining spoil (GS)*** of the Escúzar quarries (SW Granada). Organic wastes come from both urban and agro-industrial activities. Those of urban origin are: i) ***composted sewage sludge (WS)*** from Almería, Granada, and Malaga produced in Santa Fe (W Granada), ii) ***bio-stabilised material (BM)*** from the municipal solid waste plant (Ecocentral Granada) in Alhendin (W Granada), and iii) ***vermicompost from pruning and gardening waste (VC)*** of the Faculty of Sciences in Granada city; those of agro-industrial origin are: i and ii) ***two composted solid olive-mill by-products*** (***OW***: irrigated with drinking water, ***OL***: irrigated with liquid olive-mill waste) produced in Loja (W Granada), one of the most olive-growing regions of Andalusia, hence of the world, and iii) ***compost from agricultural greenhouse waste (AW)*** of Almeria and Granada made in a greenhouse waste treatment plant in Motril (S Granada).

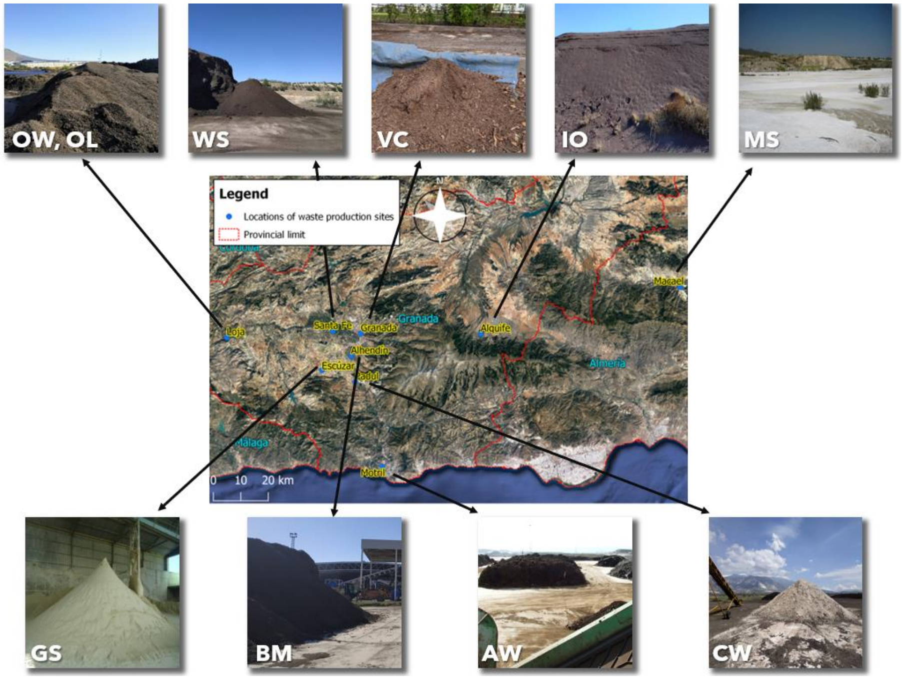

Figure 2.4. *Location of waste production sites in SE Spain. Satellite image generated using QGIS 3.20 Odense software and orthophotography provided by OpenStreetMap.*

In **chapter 3**, the above wastes are characterised including the evaluation of their acid neutralisation capacity and PHEs removal efficiency in an artificial AMD, so that the most efficient wastes can be selected for the production of Technosols.

In the second group, a total of five wastes (one inorganic and four organic) were chosen, which, like the previous ones, are close to the study area, in this case southern Portugal, and can be found almost anywhere. The only inorganic waste used consists of ***limestone rock waste*** (Ø < 5 mm) from a local quarry, while the organics include: i) ***biomass pruning*** from surrounding municipalities, ii and iii) ***sludge and waste kieselguhr from breweries*** provided by Sociedade Central de Cervejas e Bebidas, S.A., and iv) ***coffee grounds*** from local coffee shops. Characterisation of these wastes can be found in (Cortinhas et al. 2020, 2021).

2.2.5. TECHNOSOLS

On the one hand, within the context of the GGC study area, ***six Technosols (T1, T2, T3, T4, T5, and T6)*** were designed and produced by mixing the polluted soil (PS) with a mixture of amendments in the proportions given in Figure 2.5. These amendments consisted of organic and inorganic wastes from mining (iron oxyhydroxide-rich sludge [IO], carbonated waste from peat extraction [CW], and marble cutting and polishing sludge [MS]), agro-industry (solid olive mill by-product [OL]), and urban activity (composted sewage sludge [WS], vermicompost from pruning and gardening [VC]).

The selection of these wastes is based on the needs of Technosols to cope with pollution; carbonated wastes (CW and MS) provide pH buffering capacity, organic wastes (OL , WS, and VC) support the recovery of biological activity and contribute to soil aggregation, and iron-rich wastes (IO) promote the retention capacity of anionic PHEs (i.e. As, Sb). The proportions of wastes in Technosols are based on the actual doses applied in the field during the remediation actions previously undertaken (Pastor-Jáuregui et al. 2021). For the production of Technosols, wastes were dried, sieved to < 4 mm, and manually mixed with the dry polluted soil (total fraction) in plastic trays. Technosols were incubated for two months at room temperature (20–25 °C) with periodic watering and aeration (every three days) to maintain 70% field capacity.

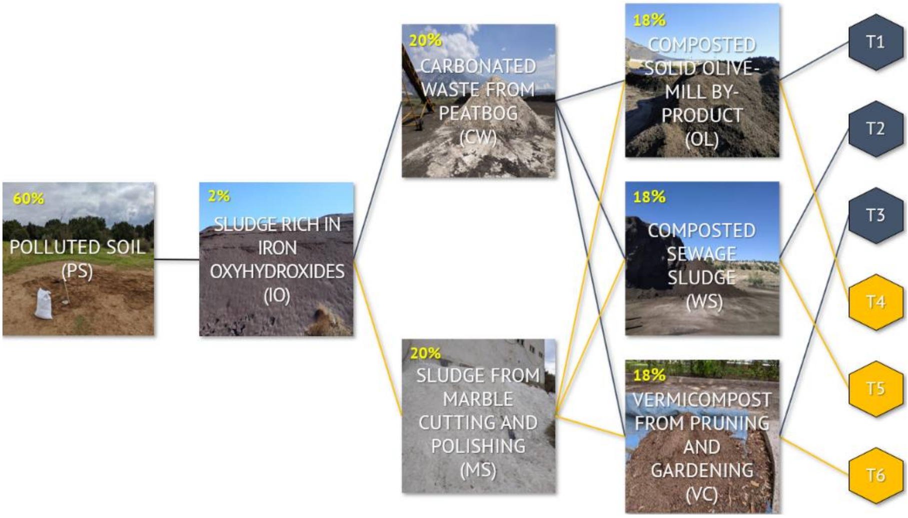

Figure 2.5. *Composition of Technosols used in the GGC study area (T1, T2, T3, T4, T5, and T6): % of polluted soil and of each waste.*

On the other hand, within the context of the São Domingos mine study area, ***two Technosols (TC50 and TC75)*** were designed and produced by combining gossan waste (GW) with a waste mixture at a ratio of 50 and 75 g kg^{-1}, respectively. This waste mixture comprised biomass pruning, limestone rock waste from a quarry (Ø < 5 mm), coffee grounds, sludge and waste kieselguhr from breweries in the following proportions at 25:20:20:25:10. After manually mixing all components of the Technosols, they were incubated at 75% of water-holding capacity and room temperature (20–25 °C) for 30 days. The incubation period of both the abovementioned and these Technosols is considered necessary for the stabilisation of biogeochemical processes.

2.3. METHODS

2.3.1. LABORATY TESTS

2.3.1.1. GENERAL CHARACTERISATION

Throughout the thesis, the following set of variables listed in Table 2.1, comprising physicochemical properties, biological parameters and PHEs concentrations in different fractions, have been measured in the different materials (i.e. artificial AMD, soils, wastes, and Technosols).

Table 2.1. *Variables measured in the different chapters of this thesis, together with the measurement methodology and instruments used.*

Variable	Methodology	Instrument	Chapters
pH	Water extraction (1:2.5 m:V)	914 pH/conductometer (Metrohm AG, Herisau, Switzerland)	3, 4, 5, 6, 7, 8
EC	Water extraction (1:5 m:V) * 1:2.5	Eutech CON700 conductometer (Oakton Instruments, Vernon Hills, IL, USA)	3, 4, 5, 6, 7, 8*
OC	Wet oxidation (Tyurin 1951)	Bürette Digital Brand (BRAND GMBH + CO KG, Wertheim, Germany)	3, 4, 6, 7
	Wet oxidation (Walkley and Black 1934)	Automatic titrator 876 Dosimat plus (Metrohm, Herisau, Switzerland)	8
$CaCO_3$	Volumetric gases (Barahona 1984)	Modified Bernard calcimeter	3, 4, 6, 7
CEC	Ammonium/sodium acetate method (SCS - USDA 1972)	Atomic absorption spectrometry (AAS): VARIAN SpectrAA 220FS (Varian Associates, Palo Alto, California, USA)	3
		Inductively coupled plasma optical emission spectrometry (ICP-OES): PerkinElmer Avio 500 (PerkinElmer, Inc., Waltham, MA, USA)	6
Ca, Mg, Na, K	Ammonium/sodium acetate method (SCS - USDA 1972)	AAS: VARIAN SpectrAA 220FS	3
		ICP-OES: PerkinElmer Avio 500	6
	Extraction method of Lakanen and Erviö (1971)	AAS: VARIAN SpectrAA 220FS	8
Fe, Mn, Zn, Cu	Extraction method of Lakanen and Erviö (1971)	AAS: VARIAN SpectrAA 220FS	8
C_T	Dry combustion	Elemental analyser LECO TruSpec CN (St. Joseph, MI, USA)	3, 4
N_T	Dry combustion	Elemental analyser LECO TruSpec CN	3, 4
	Method of Kjeldahl (1883)	Kjeltec 8200 Auto-analyser (Foss, Hillerød, Denmark)	8
Fe_T	Acid digestion *Wastes: Inorganic - HNO_3 + HF 3:1 V:V, Organic - HNO_3 + H_2O_2 1:1 V:V*	Mars XP1500 Plus microwave (CEM Corporation, Matthews, CN, USA) ICP-OES: PerkinElmer Avio 500	3
P_A	Sodium carbonate extraction (Olsen and Sommers 1982)	Helios γ UV–vis spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA)	3
	NH_4-lactate/acetic acid extraction (Egnér et al. 1960)	UV-Visible T60 spectrophotometer (PG Instruments Ltd., United Kingdom)	8
K_A	NH_4-lactate/acetic acid extraction (Egnér et al. 1960)	UV-Visible T60 spectrophotometer	8
BR	Determination of CO_2 flux (ISO 17155 2002)	Microbiological analyser µ-Trac 4200 SY-LAB model (SY-LAB Geräte GmbH, Neupurkersdorf, Austria)	3

Continued on next page

Variable	Methodology	Instrument	Chapters
Total (T), water-soluble (W), and EDTA-extracted bioavailable (E) PHEs concentrations			
T	Acid digestion *Wastes: Inorganic - HNO_3 + HF 3:1 V:V, Organic - HNO_3 + H_2O_2 1:1 V:V*	Mars XP1500 Plus microwave ICP-OES: PerkinElmer Avio 500	3
	X-ray fluorescence (XRF)	Portable NITON XL3t-980 GOLDD+ analyser (Thermo Fisher Scientific, Waltham, MA, USA)	4, 7
	Acid digestion *Soils and Technosols: aqua regia (HNO_3 + HCl 3:1 V:V)*	Mars XP1500 Plus microwave Inductively coupled plasma mass spectrometry (ICP-MS): PerkinElmer NexION 300D spectrometer (PerkinElmer, Inc., Waltham, MA, USA)	6
	Acid digestion *Gossan waste: aqua regia* (Activation Laboratories Ltd. 2005a)	ICP-MS (Activation Laboratories Ltd. 2005b; 2005c)	8
W	Water extract (1:5 m:V) (Sposito et al. 1982)	ICP-MS: PerkinElmer NexIO 300D ICP-OES: PerkinElmer Avio 500	3, 4, 6 5, 7
E	EDTA extraction (1:10 m:V) (Quevauviller et al. 1998)	ICP-MS: PerkinElmer NexION 300D	4
	Rhizosphere-based method (Feng et al. 2005)	ICP-MS (Activation Laboratories Ltd. 2005b; 2005c)	8
Soil enzymatic activities			
DHA	Tetrazolium chloride reduction method (Tabatabai 1994)	UV-Visible T60 spectrophotometer Helios γ UV–vis spectrophotometer	4, 8 7
BGA EC 3.2.1.21	*p*-nitrophenol hydrolysis from *p*-nitrophenyl-β-glucopyranoside (Eivazi and Tabatabai 1988)	UV-Visible T60 spectrophotometer	4, 8
CLA EC 3.2.1.4	Glucose formation from Avicel (Hope and Burns 1987)	UV-Visible T60 spectrophotometer Helios γ UV–vis spectrophotometer	4, 8 7
PA EC 3.4	Amino acid formation from sodium caseinate (Ladd and Butler 1972)	UV-Visible T60 spectrophotometer Helios γ UV–vis spectrophotometer	8 7
UA EC 3.5.1.5	Colourimetry of ammonium formation from urea (Kandeler and Gerber 1988)	UV-Visible T60 spectrophotometer	8
APA EC 3.1.3.2	*p*-nitrophenol hydrolysis from *p*-nitrophenyl phosphate (Eivazi and Tabatabai 1977)	UV-Visible T60 spectrophotometer Helios γ UV–vis spectrophotometer	4, 8 7

EC – Electrical conductivity, OC – Organic carbon content, $CaCO_3$ – Calcium carbonate content, CEC – Cation exchange capacity, Ca, Mg, Na, K – Exchangeable bases, Fe, Mn, Zn, Cu – Micronutrients, C_T, N_T, Fe_T – Total concentrations of C, N and Fe, P_A, K_A – Assimilable/Extractable concentrations of P and K, BR – Basal respiration rate, DHA – Dehydrogenase activity, BGA – β-glucosidase activity, CLA – Cellulase activity, PA – Protease activity, UA – Urease activity, APA - Acid phosphate activity.

2.3.1.2. AMD TREATMENT

To determine the acid neutralisation capacity and PHEs removal effectiveness of wastes (**chapter 3**) and Technosols (**chapter 5**), two treatments of acid mine drainage (AMD) were performed in which artificial AMD (see section 2.2.1) was added to these materials.

The experience of **chapter 3** was made by the addition of 50 ml of artificial AMD to 10 g of each waste in triplicate (*n = 3*). This mixture was kept in contact by stirring for 24 h, centrifugated at 3500 rpm for 15 min, and filtered (Filter-Lab n°1250, pore size: 10-13 µm), separating the waste material (solid phase) from the leachate (liquid phase). In the leachate, which is the AMD treated, $pH_{(L)}$, $EC_{(L)}$, and PHEs concentrations in solution by ICP-MS were determined. Prior to this, the following waste characterisation in triplicate (*n = 3*) was carried out: pH, EC, OC, $CaCO_3$, CEC and exchangeable bases (Ca, Mg, Na, K), N_T, C_T, Fe_T, P_A, BR, and total concentrations of PHEs (i.e. As, Cd, Co, Cr, Cu, Fe, Ni, Pb, Sb, V, and Zn).

For **chapter 5**, as we also want to determine the critical load of Technosols, the procedure was slightly different. In addition to Technosols (T1, T2, T3, T4, T5, and T6), there were two controls: polluted soil (PS) and unpolluted soil (US). In this case, increasing volumes of artificial AMD were added to 1 g of each soil sample in triplicate (*n = 3*). The following volumes based on Díez et al. (2009) depending on soil properties (i.e. texture, pH, $CaCO_3$) were used: i) 2, 6, 10, 15, and 20 ml for Technosols, ii) 1, 2, 4, 6, and 10 ml for US, and iii) 1, 1.5, 2, 3, and 5 ml for PS. The procedure was the same as in chapter 3, but keeping the mixture in contact for 72 h instead of 24 h and measuring PHEs concentrations in solution by ICP-OES.

Note: In chapter 5, as the acidity buffering capacity of soils is expressed as the amount of added acidity (mmol H^+) per kg soil needed to decrease one pH unit (Bennardi et al. 2018), the volume of added AMD was represented as the added acidity. So, as pH of AMD was 2.22, it corresponds to an acidity of: $10^{-2.22} = 0.006026$ mmol H^+ ml^{-1}. Therefore, adding 1 ml of AMD to the soil would add 0.006026 mmol H^+. Finally, the added acidity was expressed in mmol H^+ kg $soil^{-1}$, so for every 1 ml of AMD added, an acidity of 6.03 mmol H^+ kg $soil^{-1}$ is added. Thus, the added acidity was as follows: i) 12.05, 36.15, 60.26, 90.38, and 120.51 mmol H^+ kg $soil^{-1}$ for Technosols, ii) 6.03, 12.05, 24.10, 36.15, and 60.26 mmol H^+ kg $soil^{-1}$ for US, and iii) 6.03, 9.04, 12.05, 18.08, and 30.13 mmol H^+ kg $soil^{-1}$ for PS.

2.3.2. GROWTH CHAMBER AND GREENHOUSE EXPERIMENTS

2.3.2.1. REMEDIATION OF POLLUTED SOILS WITH TECHNOSOLS

In **chapter 4**, the following experiment was performed under growth chamber conditions to determine the effectiveness of the six Technosols produced from PS at the physicochemical, biological, and ecotoxicological levels.

Experimental set-up

The assay included two controls (polluted soil [PS] and unpolluted soil [US]) and six treatments consisting of the application of Technosols (T1–T6) on top of polluted soil (R1–R6) (Figure 2.6) to obtain composite samples (T1R1–T6R6) to study the effect of Technosols on polluted soil. Each substrate had six replicates (*n* = 6), and each of these replicates consisted of three alveoli of approximately 30 cm^3 in volume (analytical replicates). All substrates were incubated at 70% of field capacity and at room temperature for one week.

In this experiment, to evaluate the reduction in toxicity, a bioassay in the soil-solid fraction with *Trifolium campestre* Schreb. (big hop clover, dicotyledonous, Fabaceae) was implemented. For this, each replicate of all substrates was sown with 15 seeds of *T. campestre* (5 per alveolus).

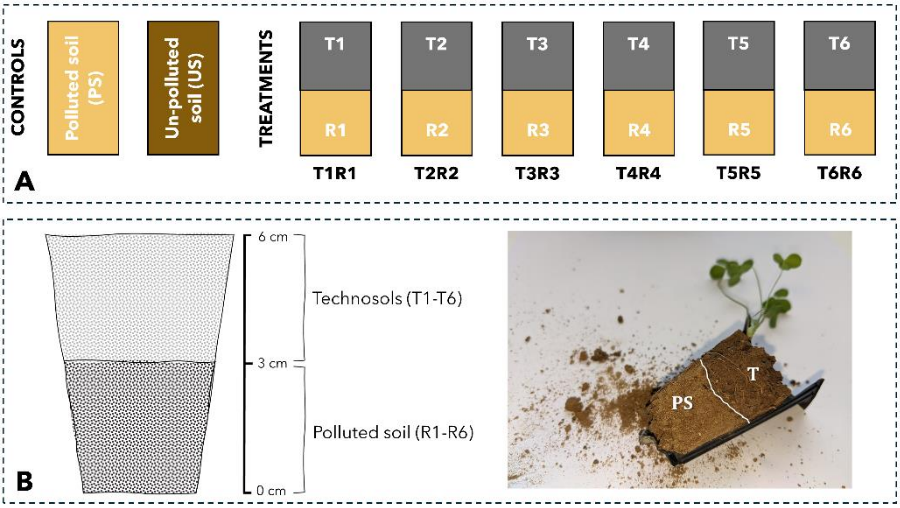

Figure 2.6. *Diagram of the experimental design (A) and close-up of the remediation treatments of polluted soils by surface application of Technosols (T1R1 - T6R6) (B).*

Plant analysis

The development of *T. campestre* plants was monitored for two months under controlled conditions and regular watering in growth chambers. Seed germination and mortality of *T. campestre* were examined weekly. At the end of the assay, the survival and soil plant analysis development (SPAD) index were measured using a SPAD-502 Plus chlorophyll meter (Konica Minolta Holdings, Inc., Tokyo, Japan). Three leaves from each plant were selected to measure SPAD. Each SPAD value obtained was the mean of four readings (two on each side of the leaf midrib). The SPAD-502 was calibrated using the reading checker supplied by the manufacturer. The dry biomass of the entire plant was also determined. For this purpose, after harvesting, plant samples were washed with tap water and then with distilled water. After washing, plants were sonicated in distilled water for 30 min. Finally, they were weighed after drying in an oven at 60 °C for 96 h.

Soil analysis

After two months of *T. campestre* growth, samples were collected from PS, US, and both the part of Technosols (T1–T6) and underlying polluted soils (R1–R6). In these samples, the following variables were measured: pH, EC, OC, $CaCO_3$, N_T, C_T, and the concentrations of some PHEs (As, Cd, Cu, Pb, Sb, and Zn) in the total, water-soluble, and EDTA-extracted bioavailable fractions.

In addition, for the ecotoxicological risk assessment, a liquid-phase toxicity bioassay was performed using *Lactuca sativa* L. (lettuce, dicotyledonous, Asteraceae) (EPA 1996). In Petri dishes, a filter paper with 5 ml of soil/water extract (1:5) was incubated with 20 seeds per replicate ($n = 6$) at 25 ± 1 °C for five days. As a control, the same procedure was conducted with distilled water in triplicate. After this period, the number of germinated seeds (SG) and the lengths of developing roots were recorded. The percentage of root elongation (RE) was calculated in relation to control samples with distilled water, with values from 0% (no elongation, maximum toxicity) to 100% (same elongation as control, no toxicity). Values above 100% indicate overstimulation (hormesis). In the solid phase, several soil enzymatic activities were also analysed as biological parameters to evaluate the remediation process, namely dehydrogenase, β-glucosidase, cellulase, and acid phosphatase.

2.3.2.2. PHYTOTOXICITY REDUCTION OF POLLUTED SOILS USING TECHNOSOLS

To focus on the evaluation of phytotoxicity reduction with Technosols, the following assay was carried out in **chapter 6** using *L. sativa* as a bioindicator.

Experimental set-up and sampling

The assay consisted of three treatments and two controls placed in eight pots of 1.6 dm^3 volume per substrate. The substrates were (i) recovered soil (RS), (ii) polluted soil (PS), (iii) Technosol T4, (iv) Technosol T5, and (v) Technosol T6. The selection of these three Technosols and not all is because the only difference between them (T1 vs. T4, T2 vs. T5, and T3 vs. T6) is the carbonate waste used; MS being preferred over CW for its greater ubiquity and magnitude, and because in **chapter 4**, no major differences were observed between using one or the other.

One seedling of *L. sativa,* cv. Phillipus per pot and substrate was planted. These seedlings were obtained from seed germination and growth for 35 days in cell flats (90 cm^3) filled with perlite placed on benches in an experimental greenhouse in southern Spain (Saliplant S.L., Motril, Granada). After transplanting, seedlings were transferred to a growth chamber under controlled environmental conditions: relative humidity 60-80%, temperature 25/15 °C, photoperiod 16/8-h (day/night), 350 $\mu mol\ m^{-2}\ s^{-1}$ photosynthetically active radiation (PAR) measured with a 190 SB quantum sensor (LI-COR Inc., Lincoln, NE, USA). Plants were watered by capillary irrigation in lightweight polypropylene trays with 3 L. Seven days after transplantation, acute phytotoxic effects began to be observed in some substrates, thus ten days later, all *L. sativa* plants were collected. Full expanded leaves and roots were washed with distilled water, dried on filter paper, and weighed to obtain fresh weight (*FW*). Half of the roots and leaves of each substrate were pooled into a composite sample and subsequently frozen at -40 °C for biochemical characterisation, and the other half of plant material in a different composite sample was lyophilised to obtain dry weight (*DW*) and analyse PHEs concentrations. The experimental design consisted of a randomised complete block with five substrates (RS, PS, T4, T5, and T6), four plants grouped in a composite sample per substrate and three replications each. The different analyses were performed in triplicate (n = 9).

Soil analysis

A composite sample per substrate was obtained from 100 g aliquots of soil from each pot. From this sample, three replicates per substrate (RS, PS, T4, T5, and T6) were taken and subsequently air-dried at room temperature, homogenised, sieved at 2 mm, and a small fraction finely ground for the following characterisation in triplicate ($n = 9$): pH, EC, OC, $CaCO_3$, CEC, extractable Na, and concentrations of some PHEs (As, Cd, Cu, Fe, Pb, and Zn) in the total and water-soluble fractions.

Plant analysis

Relative growth rate (RGR)

To determine the RGR, leaves of eight *L. sativa* seedlings from the Saliplant S.L. nursery were lyophilised before starting the assay (T_i) to record dry weight (*DW*). After 17 days of growth (T_f), *DW* was also determined. Thus, RGR can be calculated from the increase in leaf *DW* from the beginning to the end of cultivation using the following equation: $RGR = (\ln DW_f - \ln DW_i)/(T)$, where T is the assay duration (17 days).

PHEs concentrations

The composite sample of the lyophilised plants, separated into aerial part and roots, was ground, and then three replicates per substrate were taken for the determination of some PHEs (As, Cd, Cu, Fe, Pb, and Zn) in their tissues by ICP-MS after acid digestion in *aqua regia* in triplicate ($n = 9$).

Leaf gas exchange parameters

Leaf gas exchange parameters were recorded with an infrared gas analyser LICOR 6800 Portable Photosynthesis System (IRGA: LI-COR Inc., Lincoln, NE, USA). Intermediate leaves of three plants for each substrate were placed in a leaf cuvette set with optimal growth conditions. The instrument was heated for 30 min and calibrated before the measurements. Standard optimum cuvette conditions of 400 $\mu mol\ mol^{-1}$ CO_2 concentration, 500 $\mu mol\ m^2\ s^{-1}$ PAR, 70% relative humidity, and room temperature. Net photosynthesis rate (ACO_2), transpiration rate (E), stomatal conductance (g_s), and intercellular CO_2 (C_i) were recorded. Instantaneous water use efficiency (WUE) was calculated as the A/E quotient. Data were analysed using the "Photosyn Assistant" software.

Oxidative stress indicators and antioxidant activity

The malondialdehyde (MDA) concentration was determined according to Fu and Huang (2001) by measuring the absorbance at 532 nm. The non-specific absorbance value at 600 nm was obtained to correct for turbidity. The MDA concentration was calculated using 155 mM^{-1} cm^{-1} as extinction coefficient. Proline (Pro) concentration was measured using an adaptation of the method of Bieleski and Turner (1966) by measuring absorbance at 515 nm.

Two reactive oxygen species (ROS) were also determined: hydrogen peroxide (H_2O_2) and superoxide ion (O_2^-). The H_2O_2 content of the leaf samples was determined by colorimetry according to Junglee et al. (2014), based on a reaction with potassium iodide and an absorbance reading at 350 nm. For O_2^- content, the method of Kubiś (2008) was followed, where the ability of extracts to reduce nitroblue tetrazolium (NBT) was determined by measuring absorbance at 580 nm.

Superoxide dismutase (SOD; EC 1.15.1.1) activity was determined by inhibition of photochemical NBT reduction according to Giannopolitis and Ries (1977) and Beyer and Fridovich (1987), with modifications by Yu et al. (1998). One unit of SOD was defined as the amount of enzyme required to cause 50% inhibition of NBT reduction as monitored at 560 nm. Catalase (CAT; EC 1.11.1.6) activity was determined by Nakano and Asada (1981) and Rao et al. (1997), based on the measurement of H_2O_2 consumption at 240 nm. Protein concentration of extracts was measured by Bradford (1976), using bovine serum albumin as standard.

Ascorbate-glutathione cycle

Total glutathione (GSH Total) and oxidised glutathione (GSH Oxid) were determined according to Noctor and Foyer (1998). Reduced glutathione (GSH Red) content was estimated from the difference between total and oxidised glutathione. The concentrations of total ascorbate (AsA Total) and its reduced form (AsA Red) were determined by Law et al. (1983). Ascorbate peroxidase (APX; EC 1.11.1.11) and glutathione reductase (GR; EC 1.6.4.1) were determined by Rao et al. (1996). For APX activity, the disappearance of H_2O_2 was measured at 290 nm, and for GR activity, the oxidation of NADPH at 340 nm. Protein concentration of extracts was measured by Bradford (1976), using bovine serum albumin as standard.

All biochemical parameters analysed in this and the previous section were performed using an Infinite 200 Nanoquat spectrophotometer (Tecan, Switzerland).

2.3.2.3. RECLAMATION OF GOSSAN WASTES

In **chapter 8**, the following greenhouse trial was conducted to assess the potential of the integrated biotechnological approach based on Technosols and cultivation of a multipurpose drought-resistant legume, *Lablab purpureus* (L.) Sweet (hyacinth bean, dicotyledonous, Fabaceae), for the reclamation of gossan wastes.

Experimental set-up and sampling

The assay consisted of two treatments and one control arranged in six pots of 10 dm^3 volume per substrate ($n = 6$). The substrates were (i) gossan waste (GW) as a control, hereafter GW base (GW_b), and as treatments (ii) Technosol 50 (TC50) and (iii) Technosol 75 (TC75).

After one month of incubation, four seeds of *L. purpureus*, cv. Rongai inoculated with *Bradyrhizobium* sp. (strain CB1024) obtained from an Australian seed supplier (Gold Coast Agribusiness Pty. Ltd., Banora Point, NSW, Australia), were sown per pot and substrate. On sowing day, a composite sample (five subsamples) of substrate per pot was collected, hereafter referred to as initial GW (GW_i), initial TC50 ($TC50_i$), and initial TC75 ($TC75_i$). After 174 days from sowing, plants (shoot and root) were collected, weighed (dry biomass), and measured (length root and shoot). This growing period is based on the premise that 130 days after germination *L. purpureus* is at maximum vegetative growth (corresponding to three trifoliated leaves) (Murphy and Colucci 1999); therefore, it is extended to 174 days, when the long stems start to develop, to avoid root strangulation in pots. Subsequently, a composite sample (five subsamples) of substrate per pot was collected, hereafter called, final GW (GW_f), final TC50 ($TC50_f$), and final TC75 ($TC75_f$). The assay was perfomed in a greenhouse under controlled aeration conditions with the substrata kept at 75% of water-holding capacity. After sampling, an aliquot of each soil sample was stored at 4 °C in sterile, opaque, cold-preserved bottles for analysis of soil enzymatic activity. The remaining part of the sampled soils was air-dried at room temperature, homogenised, and sieved to 2 mm for physicochemical characterisation and determination of PHEs concentrations.

Soil analysis

In the air-dried soil samples of all substrates (GW_{b}, GW_{i}, $TC50_{i}$, $TC75_{i}$, GW_{f}, $TC50_{f}$ and $TC75_{f}$), the following physicochemical characterisation was performed according to Póvoas and Barral (1992): pH, EC, OC, N_T, P_A, K_A, CEC, and total concentrations of macro- (Ca, Mg, Na, K) and micronutrients (Fe, Mn, Zn and Cu). In addition, total concentrations of PHEs (As, Cd, Cr, Cu, Hg, Mn, Ni, Pb, and Zn) were measured in GW_{b}, as well as bioavailable concentrations of these same PHEs in GW_{i}, $TC50_{i}$, $TC75_{i}$, GW_{f}, $TC50_{f}$ and $TC75_{f}$. Several enzymatic activities (total fraction) were also analysed on substrates (GW_{i}, $TC50_{i}$, $TC75_{i}$, GW_{f}, $TC50_{f}$, $TC75_{f}$), namely, dehydrogenase, β-glucosidase, cellulase, acid phosphatase, protease, and urease.

Plant analysis

Plant shoot and root samples were washed with tap water followed by distilled water; roots were also sonicated (after washing) in distilled water in an ultrasound bath for 30 min. Shoots and roots were dried at 40 °C, weighed for dry weight and finally homogenised into a fine powder. The total concentrations of some PHEs (As, Cd, Cr, Cu, Hg, Mn, Ni, Pb, and Zn) and nutrients (Fe, Ca, Mg, Na, K) in shoot and root samples from each substrate was measured by ICP-MS after digestion in *aqua regia* (Activation Laboratories Ltd. 2005b; 2005c).

To evaluate the plant behaviour in the different substrates, the following coefficients were calculated:

- ☐ Biological absorption coefficient = $[\text{Root }(x)]_{(y)}/[\text{PHE bioavailable }(x)]_{(y)}$
- ☐ Translocation coefficient = $[\text{shoots }(x)]_{(y)}/[\text{roots }(x)]_{(y)}$
- ☐ Soil-plant transfer coefficient = $[\text{shoots }(x)]_{(y)}/[\text{PHE bioavailable }(x)]_{(y)}$

Where (x) corresponds to the concentration of a specific PHE present in (y) that corresponds to GW_{f}, $TC50_{f}$, or TC75*f*.

Plants with a translocation coefficient lower than one are considered non-accumulators (McGrath and Zhao 2003; Bu-Olayan and Thomas 2009), while the soil-plant transfer coefficient is higher than one and with a lack of phytotoxicity signs, indicates the level of plant tolerance for PHE (Abreu et al. 2008).

2.3.3. FIELD EXPERIENCE

In **chapter 7**, the following pilot assay was implemented at the experimental plot "El Vicario" (37°26'14" N, 6°13'04" W) in the GGC (Seville, Spain) to test the effectiveness of *in situ* remediation of these polluted soils (see Figure 1.2) through the application of two waste-derived Technosols.

Experimental setup

The experimental plot "El Vicario" (Figure 2.7) does not correspond exactly to the area described for the sampling of soils from GGC used in the thesis (see section 2.2.2), but is located less than 7 km downstream of the latter. In this case, Technosols T4 and T6 were installed, as they were the most effective in previous experiences (see **chapters 4**, **5** and **6**). Both Technosols were prepared in different piles on site by mixing all constituent materials in the proportions specified in section 2.2.5. These piles were incubated for two months with periodic watering and turning to maintain 70% of field capacity and adequate aeration.

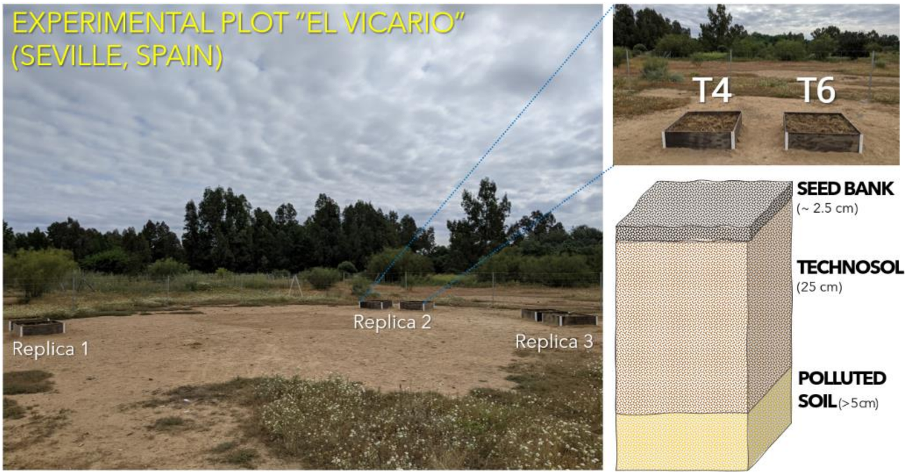

Figure 2.7. *View of the experimental plot "El Vicario" in the GGC and the scheme of the combined remediation system for polluted soils.*

The combined remediation system consisted of spreading Technosols over polluted soil in experimental plots of 1 m^2 (n = 3) with a thickness of 25 cm. On top of these, a 2.5 cm thick layer of nearby recovered soil was placed as seed bank (SB) to promote the growth of natural vegetation (Figure 2.7). Composite samples were collected with a half-round auger from each Technosol (T4 and T6; depth: < 25 cm) and from the underlying polluted soil (T4-PS and T6-PS; depth: 25- 30cm) at three different times: 0 (Apr. 2021), 6 (Oct. 2021), and 12 (Apr. 2022) months after installation. An aliquot of each soil sample was stored at 4°C in sterile, opaque, cold-preserved bottles for microbial analysis. The remaining part of the sampled soils was air-dried at room temperature, homogenised, sieved to 2 mm, and a fraction was finely ground for analysis of some soil properties and PHEs concentrations.

Soil analysis

The following variables were measured in soil samples (T4, T4-PS, T6, and T6-PS): pH, EC, OC, $CaCO_3$, and concentrations of PHEs of interest (As, Cu, Pb, and Zn) in the total and water-soluble fractions. Several enzymatic activities (total fraction) were also analysed: dehydrogenase, cellulase, acid phosphatase, and protease.

Vegetation growth analysis

Vegetation within the plots was monitored by assessing cover, plant species richness, and the Shannon-Wiener index (H'; Shannon, 1948) for each Technosol. For this purpose, a 0.5 m^2 grid divided into 100 cells was randomly placed and replicated three times per plot. Total cover was calculated as the percentage of the total area covered by all plant species present; and richness as the number of species found per grid.

Microbial analysis

DNA extraction and amplicon sequencing

DNA extraction from soil samples (T4, T4-PS, T6 and T6-PS) and recovered soil used as seed bank (SB) was performed using the FastDNA™ Spin Kit for Soil (MP Biomedicals, Germany) with the FastPrep-24™ Classic bead beating system (MP Biomedicals) on 0.5 g of homogenised soil. Sequencing of amplicons from V3/V4 region of 16S rDNA was performed by the Genomic Unit of the Instituto de Parasitología y Biomedicina ''López-Neyra" (CSIC, Granada, Spain). Briefly, DNA concentration was measured using Qubit® 3.0 Fluorometer (Invitrogen, Carlsbad, CA, USA) and normalised to the same concentration. A 2-step PCR was performed on DNA samples. Primers 16S_ProV3V4_forward (5'-CCTACGGGNBGCASCAG-3') and 16S_ProV3V4_reverse (5′- GACTACNVGGGTATCTAATCC-3′), each with the adaptor (5´-CTGTCTCTTATACACATCT-3´), were used to amplify the V3/V4 region with the following cycling conditions: 95°C 3 min; (95°C 30s; 55°C 30s; 72°C 30s) x 25 cycles; 72°C 5 min; 4°C hold. A second PCR step was performed to index the samples using Nextera XT Index Kit v2, for further 8 cycles. Bidirectional sequencing was performed on MiSeq™ System (Illumina, 2 x 275 cycles) (Takahashi et al. 2014).

Sequence processing

After obtaining demultiplexed sequences obtained from the MiSeq™ System, the data were subjected to extensive processing using QIIME2 v2019.10 (Quantitative Insights In Microbial Ecology, Bolyen et al. 2019). Primers were removed using Cutadapt (Martin 2011), pair-end reads were joined using VSEARCH (maxdiff=20, Rognes et al. 2016), and denoised with DADA2 (Callahan et al. 2016) using a quality threshold of 20, and truncating the length at 500bp. The dataset has 967,207 reads, with a mean depth of 15,600 reads per sample.

16S rDNA sequence accession number

The 16S rDNA sequences are available in the Genbank-NCBI Sequence Read Archive (SRA) (https://www.ncbi.nlm.nih.gov/sra/), BioProject: PRJNA1029985.

Statistical analysis

Microbiological sequencing data were analysed according to Pesciaroli et al. (2021). Briefly, for non-metric multi-dimensional scaling (NMDS) and analysis of similarities (ANOSIM) based on Bray-Curtis distance, we used Primer 6 software on ASVs (Amplicon Sequence Variant). The R packages Pheatmap (Kolde 2015) and RColorBrewer using R 2.7.2 (www.r-project.org) were used to draw the heatmap, applying Euclidean distance clustering to show similarity patterns on taxonomic units. Taxa clustered to species level (97% similarity with Greengenes database v.13.5. trained classifier with V3/V4 primers above) were labelled 's_' before their taxonomic name. Unclassified clustered taxa to species level were labelled by their last known taxon, g_genus, f_family, o_order, c_class, p_phylum, and k_kingdom.

Microbial communities were assessed using diversity indices (Shannon, Simpson and Chao1) at the ASV level with R packages vegan (Oksanen et al. 2017) and fossil (Vavrek 2011). The Shannon index measures species richness and evenness, the Simpson index measures dominance of individual species within mixed populations (a value of 1-D = 0 represents complete dominance within the sample), and the Chao1 index is an abundance-based coverage estimator. These indices provide an overview of the structure, richness, evenness and dominance of the microbial community in the samples studied.

Microbial pairwise interactions in soil were assessed by consensus networks using MetaMIS software (Shaw et al. 2016). The analysis used relative abundance data with default settings (High: > 0.01, Rare: < 0.001; P (concordant pairs) = 0.90). Consensus networks highlight interactions with more consensus directions, providing insights into relationships between highly abundant families and rare/low-abundance microbes. The minimum model for consensus networks was used for each network. Visualisation and analysis of the networks were conducted using Cytoscape (Shannon et al. 2003).

2.4. DATA ANALYSIS

2.4.1. QUALITY CONTROL

The precision and accuracy of XRF, ICP-OES, and ICP-MS methods were tested via the analysis of the following certified reference materials and procedural blanks.

- ☐ X-ray fluorescence (XRF): NITON XL3t-980 GOLDD+
    - Loamy clay soil (CRM 052-050) (RT-Corporation Limited, Salisbury, UK): Table S2.2
- ☐ Inductively coupled plasma optical emission spectrometry (ICP-OES): PerkinElmer Avio 500
    - Lichen BCR® - 482 (EC-JRC-IRMM, Geel, Belgium): Table S2.3
    - Loamy clay soil (CRM 052-050): Table S2.4
- ☐ Inductively coupled plasma mass spectrometry (ICP-MS): PerkinElmer NexION 300D spectrometer
    - Lichen BCR® - 482: Table S2.5
    - Loamy clay soil (CRM 052-050): Table S2.6

These analyses gave satisfactory results, as in most cases the measured values were within the prediction interval of the certified value and/or the average recovery of the elements was adequate. On the other hand, in **chapter 8**, the quality control of the analyses was performed according to the standards of the certified laboratory (ISO/IEC 17025 2017) - Activation Laboratories Lda.

In addition, all calibration standards were prepared from ICP single-element standard solutions (Merck, Darmstadt, Germany) after dilution with 10 % HNO_3. Moreover, quality control of the remaining analyses was carried out by technical replicates, use of certified standard solutions and method reagent blanks.

2.4.2. STATISTICAL ANALYSIS

This section contains different statistical analyses that have been generically used throughout the thesis, which vary according to the casuistry of each chapter.

Basic statistics

Prior to data processing, a preliminary analysis of the descriptive statistics (mean, standard deviation [SD]) was carried out, as well as the testing of certain assumptions (normal distribution and homoscedasticity). To check whether the data follow a normal distribution, the Kolmogorov-Smirnov or Shapiro-Wilk tests were used, and to check the homogeneity of variances, the Levene's test was applied.

Comparison of means

In cases where the sample size was small (Theodorsson-Norheim 1986), and the normality and/or homogeneity of variances were not met, even after log-transforming the variables, non-parametric tests were performed to identify significant differences in the different variables. For the comparison of means, Kruskal-Wallis and Dunn tests ($p < 0.05$) were applied in **chapters 3** and **7**, and Kruskal–Wallis and Mann–Whitney U test ($p < 0.05$) in **chapters 4** and **5**.

On the other hand, in **chapter 6**, differences between means were compared using a simple ANOVA and Fisher's Least Significant Difference (LSD) test. Similarly, in **chapter 8**, as these assumptions were met, the data were analysed by a one-way ANOVA and Tukey's post hoc test ($p < 0.05$).

All these analyses were made with a confidence level of 95%.

Correlations and principal component analysis

Significant bivariate Spearman's correlations were performed in **chapter 3** to analyse the influence of waste properties on their capacity of acid neutralisation and removal of PHEs in artificial AMD.

To determine the relationship between the different study variables, a principal component analysis (PCA) was performed in **chapters 4** and **7**. In **chapter 4**, PCA was performed using Varimax rotation with Kaiser normalisation including soil properties and total, water-soluble, and EDTA-extracted bioavailable PHEs concentrations, soil enzymatic activities, and endpoints calculated from *T. campestre* and *L. sativa* bioassays. While, in **chapter 7**, the RStudio software packages "FactoMineR" and "factoextra" were used to study the relationship between soil properties, total and water-soluble PHEs content, and soil enzyme activity in polluted soils and Technosols.

Statistical packages

Throughout the thesis, the following statistical packages were used: RStudio software V. 2022.02.3 "Prairie Trillium" and V. 2023.06.0 "Mountain Hydrangea" (RStudio Inc., 250 Northern Ave, Boston, USA) (**chapter 3** and **7**, respectively), SPSS v. 23.0 software (SPSS Inc., Chicago, IL, USA) (**chapters 4** and **5**), Statgraphics Centurion 16.1.03 software (Statgraphics Technologies, Inc., The Plains, VA, USA) (**chapter 6**), and GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego, CA, USA) (**chapter 8**).

Acidic mine waters in "Los Frailes" mining open pit, Aznalcóllar mine

Seville, Spain

September 2019

Antonio Aguilar Garrido

CHAPTER 3

REMEDIATION POTENTIAL OF MINING, AGRO-INDUSTRIAL, AND URBAN WASTES AGAINST ACID MINE DRAINAGE

Aguilar-Garrido, A., Paniagua-López, M., Sierra-Aragón, M., Martínez Garzón, F. J., Martín-Peinado, F. J. (2023). Remediation potential of mining, agro-industrial, and urban wastes against acid mine drainage. ***Scientific Reports***, 13, 12120. DOI: 10.1038/s41598-023-39266-4

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ABSTRACT

Acid mine drainage (AMD) poses serious consequences for human health and ecosystems. Novel strategies for its treatment involve the use of waste. This chapter evaluates the remediation potential of wastes from urban, mining, and agro-industrial activities to address acidity and high concentrations of potentially harmful elements (PHEs) in AMD. Samples of these waste products were spiked with artificially prepared AMD, and then pH, electrical conductivity (EC), and PHEs concentrations in the leachates were measured. The artificial AMD obtained through the oxidation of Aznalcóllar's tailing showed an ultra-acid character (pH - 2.89 ± 0.03) and extremely high electrical conductivity (EC - 3.76 ± 0.14 dS m^{-1}). Moreover, most PHEs concentrations were above the maximum regulatory levels in natural and irrigation waters. Wastes studied had a very high acid neutralising capacity and a strong capacity to immobilise PHEs. Inorganic wastes, together with vermicompost from pruning, reduced most PHEs concentrations by over 95%, whereas organic wastes retained between 50% and 95%. Thus, various urban, mining, and agro-industrial wastes have a high potential to be used in the treatment of AMD. This study provides valuable input for the development of new ecotechnologies based on the combination of wastes (e.g. Technosols, permeable reactive barriers) to remediate degraded environments.

3.1. BACKGROUND

The generation of acid mine drainage (AMD) and acidic mine waters in sulphide mining constitute one of the main sources of pollution that can affect soils, surface water, and groundwater. These are particularly problematic because of their scale, both spatially and temporally, as they can affect mining areas and their surroundings over large kilometres for decades or centuries (Naidu et al. 2019). The discharge of untreated AMD exerts negative effects on the environment and living organisms because of its extremely acidic nature, high salinity, and elevated concentrations of several PHEs (Kefeni et al. 2017). In aquatic ecosystems, it is responsible for the entry of PHEs, alteration of water chemistry and nutrient cycles, decrease in oxygen available to organisms, and precipitation of metals (Fe and Al hydroxides), among others. In general, water quality is affected, causing direct toxicity to organisms and rendering it unsuitable for domestic, agricultural, and industrial uses (Hogsden and Harding 2012; Evans et al. 2015; Dhir 2018). In terrestrial ecosystems, AMD can lead to vegetation damage (e.g. phytotoxicity, photosynthesis inhibition) and soil pollution, and consequently accelerate biodiversity loss (Dhir 2018). Moreover, AMD can have serious consequences on human health, such as nervous system damage, cancers, and mental retardation in children (Simate and Ndlovu 2014). Thus, AMD treatment is essential for preserving the health of humans and ecosystems.

Numerous technological solutions involving chemical, physical, and/or biological processes (e.g. oxidation, (bio)reduction, (bio)sorption, ion exchange, complexation, precipitation, dilution, alkaline generation) are available for AMD treatment (Tapia et al. 2019). But these techniques are often costly and limited in the field and over time, even compromising the economic viability of entire mining projects due to large capital investment in materials handling, equipment and maintenance (Skousen et al. 2019). Thus, it is necessary to advance AMD remediation strategies that lead to improved, cost-effective, and environmentally friendly methods (Kefeni et al. 2017). Recently, promising methods have focused on the use of low-cost amendments; for example, employing wastes from several human activities to tackle the negative impacts of AMD and connect with the circular economy strategy (Lèbre et al. 2017; Tayebi-Khorami et al. 2019). In this sense, some research has explored the use of end-of-life materials to control and treat AMD. For instance, wastes from steelmaking processes (slag materials) and gas treatment at a thermal power plant (fly ash and gypsum) removed As, Hg, Pb, Zn, Cd, Cu, and Ni from AMD at the abandoned "La Soterraña" mercury mine (Spain) (Ayala and Fernández 2020). Another example was the use of alkaline waste material from an alumina refining industry as an alternative to neutralise AMD from the Mount Morgan mine (Australia), since they significantly buffer the acid pH and reduce Al, Cu, Fe, Ni, and Zn levels (Kaur et al. 2018).

The unsustainable amount of waste generated today is also a main concern. For example, in 2020, a total of 2135 Mt of waste was generated in EU by all economic activities and households, of which, mining activities, together with wastewater treatment, agriculture, forestry and fishing, and households contributed almost 45% (Eurostat 2023). In 2022, the world's cities were estimated to generate 2240 Mt of municipal solid waste (World Bank Group 2022), with differences between geographical areas (in kg yr^{-1} ca^{-1}; 800 in USA (EPA 2022), 657 in Australia (Hla and Roberts 2015), 505 in EU (Eurostat 2020), 368 in Brazil (Alfaia et al. 2017), 277 in China (Zhu et al. 2021), and 168 in India (Sharholy et al. 2008)). Mining waste quantities are even greater in mass, with global solid waste generation from mineral and metal production estimated at over 100,000 Mt per year (Tayebi-Khorami et al. 2019). In Europe, 500 Mt of mining and quarrying wastes were generated in 2020 (23% of all waste produced in EU) (Eurostat 2023). Agro-industry, also essential in the primary sector, generates 140,000 Mt of waste each year worldwide: mainly maize stalks, straw, sugarcane leavings, bagasse, manure from cattle, poultry, and pigs, forestry residues, and garden pruning (UNEP 2015). The main global crops (wheat, maize, rice, soybean, barley, rapeseed, sugarcane and sugar beet) produced generates almost 3300 Mt waste, where China, USA, India and Europe are among the biggest producers (with 716, 682, 605, and 580 Mt, respectively) (Tripathi et al. 2019). Other example comes from our study area, Andalusia (Spain), where the production of olive oil creates 6 Mt of waste every year (AGAPA 2015).

Most of the literature published to date on waste-based ecotechnologies for AMD remediation has focused on industrial and mining waste, with little attention devoted to the use of other wastes such as agro-industrial or urban waste. For this reason, this chapter assesses the remediation potential of various inorganic and organic wastes coming from the main waste-generating activities (urban, mining and agro-industrial) (see section 2.2.4) to cope with the acidity and PHE concentrations of an artificial AMD (see section 2.2.1). For detailed information on the methodology followed based on previous studies (Simón et al. 2005; Aguilar-Garrido et al. 2022a), see section 2.3.1.2. Specifically, the **objective** is to evaluate the acid neutralisation capacity and removal effectiveness of PHEs from 10 different organic and inorganic wastes to contribute to the implementation of new ecotechnologies for the control of AMD in a circular economy scenario. Hence, this study can help to select those wastes with the best performance in neutralising PHEs pollution, so that they can be used in the production of the Technosols of this thesis.

3.2. RESULTS

3.2.1. CHARACTERISATION OF WASTE MATERIALS AND ACID MINE DRAINAGE

All inorganic wastes were characterised by neutral to moderately alkaline pH (7.3-8.3), low organic carbon content (< 1.3% OC), low total nitrogen concentration (< 0.1% N_T), and moderate to low cation-exchange capacity (CEC < 15 $cmol^+ kg^{-1}$) (Table 3.1). However, they differed in terms of other properties. Dry sludge rich in iron oxyhydroxides (IO) was dominated by iron (Fe_T ~ 87%), had moderate to low calcium carbonate content (~ 13% $CaCO_3$) and very low EC (< 0.04 dS m^{-1}). Dry marble sludge (MS) and carbonated waste (CW) had low total iron (< 0.3%), very high $CaCO_3$ content (> 90%) and high EC (> 1 dS m^{-1}). Gypsum spoil (GS) had moderate to low total iron (~ 1%), moderately high $CaCO_3$ content (~ 23%) and very high EC (> 2.9 dS m^{-1}). The only inorganic waste that showed an assimilable phosphorus content (P_A ~ 470 mg kg^{-1}) above detection limit was CW.

Organic wastes showed significant differences in relation to the inorganic wastes, mainly due the higher content of OC, CEC, exchangeable bases, total N, and assimilable P (Table 3.1). Otherwise, differences between the organic wastes were also important. Organic carbon ranged between 10.5% in vermicompost of pruning and gardening waste (VC) and 28% in bio-stabilised material of municipal

Table 3.1. *Main properties of all waste materials (IO, MS, CW, GS, WS, BM, VC, OW, OL, AW) (mean ± SD, n = 3).*

Waste	**pH** **(H_2O)**	**EC** (dS m^{-1})	**OC** (%)	**$CaCO_3$** (%)	**CEC** (cmol$^+$ kg^{-1})	**Ca^{2+}** (cmol$^+$ kg^{-1})	**Mg^{2+}** (cmol$^+$ kg^{-1})	**Na^+** (cmol$^+$ kg^{-1})	**K^+** (cmol$^+$ kg^{-1})	**N_T** (%)	**C_T** (%)	**Fe_T** (%)	**P_A** (mg kg^{-1})	**BR** (µg CO_2 day^{-1} kg^{-1})
IO	7.27 ± 0.08 b	0.04 ± 0.01 a	bdl	13.08 ± 0.22 b	6.34 ± 0.38 a	4.44 ± 0.39 a	bdl	1.11 ± 0.03 ab	0.79 ± 0.01 a	bdl	1.55 ± 0.01 a	86.59 ± 0.88 b	bdl	45.91 ± 4.42 a
MS	8.27 ± 0.13 e	1.13 ± 0.08 ab	0.16 ± 0.11 a	99.99 ± 0.01 f	9.34 ± 4.76 ab	5.59 ± 4.60 a	1.44 ± 0.27 a	1.48 ± 0.13 b	0.84 ± 0.02 a	bdl	11.81 ± 0.05 b	0.17 ± 0.01 a	bdl	29.46 ± 0.20 a
CW	7.83 ± 0.04 d	2.81 ± 0.03 bc	1.34 ± 0.03 a	93.12 ± 0.72 e	14.70 ± 0.50 b	2.75 ± 0.80 a	9.92 ± 0.67 cd	1.15 ± 0.04 ab	0.88 ± 0.01 a	0.07 ± 0.02 a	12.51 ± 0.03 b	0.28 ± 0.00 a	470.83 ± 6.16 e	124.05 ± 66.27 b
GS	7.53 ± 0.17 c	2.91 ± 0.05 bc	0.18 ± 0.03 a	23.84 ± 0.57 cd	8.48 ± 1.52 ab	7.18 ± 1.54 ab	0.88 ± 0.13 a	0.0030 ± 0.0004 a	0.39 ± 0.04 a	bdl	3.04 ± 0.03 a	1.01 ± 0.02 a	bdl	54.77 ± 26.30 a
WS	7.16 ± 0.02 b	10.13 ± 0.69 e	21.95 ± 2.91 bc	10.77 ± 0.92 ab	55.11 ± 5.81 d	24.94 ± 1.07 d	9.02 ± 0.71 c	5.67 ± 0.31 c	6.35 ± 0.19 b	3.13 ± 0.09 e	23.37 ± 0.35 c	2.01 ± 0.09 a	401.53 ± 14.07 cd	13.95 ± 1.73 a
BM	6.48 ± 0.01 a	12.37 ± 0.38 f	28.23 ± 0.80 d	7.74 ± 0.64 a	40.49 ± 0.83 c	3.81 ± 2.28 a	4.51 ± 1.47 b	18.85 ± 1.16 f	13.32 ± 0.83 c	1.55 ± 0.05 cd	29.45 ± 0.19 cd	0.47 ± 0.09 a	134.21 ± 6.99 a	40.75 ± 2.87 a
VC	7.35 ± 0.08 bc	0.39 ± 0.07 a	10.50 ± 4.08 b	24.87 ± 1.30 d	35.83 ± 2.16 c	23.25 ± 2.00 d	8.36 ± 0.24 c	1.20 ± 0.02 ab	3.02 ± 0.04 ab	0.64 ± 0.02 b	12.44 ± 0.18 b	0.98 ± 0.13 a	226.94 ± 37.65 ab	82.96 ± 9.36 ab
OW	8.57 ± 0.06 f	2.57 ± 0.90 bc	23.28 ± 5.34 bc	22.62 ± 1.45 cd	80.90 ± 2.28 e	15.91 ± 1.73 c	7.57 ± 1.30 c	6.02 ± 0.11 c	51.41 ± 0.77 d	1.74 ± 0.02 d	25.94 ± 0.24 cd	0.65 ± 0.00 a	263.89 ± 32.58 b	47.75 ± 2.79 a
OL	8.63 ± 0.03 f	3.67 ± 1.86 c	28.08 ± 1.76 d	20.96 ± 1.25 c	90.72 ± 3.57 f	6.54 ± 2.00 a	2.22 ± 0.13 ab	8.81 ± 0.03 d	73.15 ± 1.75 e	1.63 ± 0.28 cd	30.60 ± 6.90 d	0.62 ± 0.00 a	318.70 ± 98.25 bc	70.70 ± 32.73 ab
AW	9.52 ± 0.03 g	7.39 ± 0.59 d	14.17 ± 7.12 b	12.25 ± 1.89 b	43.48 ± 0.60 c	12.85 ± 0.82 bc	11.80 ± 1.54 d	12.80 ± 0.50 e	6.03 ± 2.70 b	1.36 ± 0.03 c	15.64 ± 0.22 b	0.64 ± 0.08 a	402.73 ± 18.64 cd	49.88 ± 2.76 a

IO – Dry sludge rich in iron oxyhydroxides, MS – Dry marble sludge, CW – Carbonated waste of a peat exploitation, GS – Gypsum mining spoil, WS – Composted sewage sludge, BM – Bio-stabilised material of municipal solid waste, VC – Vermicompost from pruning and gardening, OW – Composted solid olive-mill by-product irrigated with drinking water, OL – Composted solid olive-mill by-product irrigated with leachates of the olive-mill, AW – Composted greenhouse plant waste, EC – Electrical conductivity, OC - Organic carbon, $CaCO_3$ - Calcium carbonate, CEC - Cation exchange capacity, N_T/C_T - Total concentrations of N and C, P_A – Assimilable phosphorus, BR – Basal respiration rate, bdl – below detection limit. Letters represent significant differences among waste materials (Kruskal-Wallis and Dunn tests, $p < 0.05$).

solid waste (BM) and composted solid olive-mill irrigated with olive leachate (OL); CEC varied between 36 $cmol^+ kg^{-1}$ in VC and 91 $cmol^+ kg^{-1}$ in OL; N_T was between 0.6% in VC and 3.1% in composted sewage sludge (WS); and P_A ranged between 134 mg kg^{-1} in BM and 403 mg kg^{-1} in compost of agricultural greenhouse (GA). For the other properties, no significant differences were observed with respect to the inorganic wastes, although there were significant differences among the organic wastes. In this way, pH ranged from 6.5 in BM and 9.5 in AW; EC was low for VC (< 0.4 dS m^{-1}), very high for composted solid olive-mill irrigated with water (OW) and OL (2-4 dS m^{-1}), and extremely high (> 7 dS m^{-1}) for the rest; and $CaCO_3$ was also detected in all cases, ranging from 7.7% in BM to 24.9% in VC. Basal respiration (BR) presented a wide range of values without significant differences between inorganic and organic wastes, with a maximum of 124 μg CO_2 day^{-1} kg^{-1} in CW and a minimum of 14 in WS μg CO_2 day^{-1} kg^{-1}.

The total concentrations of PHEs showed significant differences among the wastes analysed (Table 3.2). However, in general, there were no marked differences between organic and inorganic wastes, although the concentrations of Cr, Cu, Ni, and Zn were usually higher in organic wastes than in inorganic ones. Within the inorganic wastes, IO and GS had the highest concentrations of most PHEs, especially IO, with concentrations of As, Pb, and Sb close to 24, 29, and 21 mg kg^{-1}, respectively. Otherwise, MS presented very low concentrations for As, Pb, V, and Zn; while CW showed very low concentrations for Co, Cr, Ni, Sb, and V. The organic wastes presented low concentrations of As, Cd, Co, and Sb, with values below 5.3, 2, 5, and 0.4 mg kg^{-1}, respectively. There were differences in Pb between wastes, ranging from 2.4 mg kg^{-1} in OW to 52.6 mg kg^{-1} in BM; while V ranged between 12 mg kg^{-1} in BM and 27 mg kg^{-1} in WS. The elements with higher concentrations in relation to inorganic wastes also presented significant differences between organics; Cr ranged between 16 mg kg^{-1} in OW and 45 mg kg^{-1} in BM; Cu varied between 25 mg kg^{-1} in VC and 365 mg kg^{-1} in AW; Ni was between 9.7 mg kg^{-1} in OW and 21.5 mg kg^{-1} in BM; and Zn oscillated between 49 mg kg^{-1} in OW and 517 mg kg^{-1} in WS.

Table 3.2. *Total concentrations of main PHEs in waste materials (IO, MS, CW, GS, WS, BM, VC, OW, OL, AW) in* $mg\ kg^{-1}$ *(mean ± SD, n = 3).*

Waste	**As**	**Cd**	**Co**	**Cr**	**Cu**	**Ni**	**Pb**	**Sb**	**V**	**Zn**
IO	23.92 ± 1.98 d	0.03 ± 0.02 ab	3.82 ± 0.52 cd	6.18 ± 0.56 ab	5.80 ± 0.28 a	6.96 ± 0.61abc	28.56 ± 2.94 c	20.85 ± 0.90 c	43.10 ± 2.88 d	25.86 ± 1.68 a
MS	0.77 ± 0.17 a	0.15 ± 0.02 abc	1.21 ± 0.04 ab	5.35 ± 0.18 a	4.01 ± 0.03 a	2.94 ± 0.09 ab	2.44 ± 0.35 a	0.33 ± 0.09 a	5.39 ± 0.31 ab	5.45 ± 0.80 a
CW	1.51 ± 0.37 a	0.26 ± 0.04 abc	0.15 ± 0.05 a	2.31 ± 0.47 a	13.78 ± 2.08 a	0.68 ± 0.37 a	9.01 ± 1.15 b	0.13 ± 0.33 a	2.58 ± 0.44 a	22.50 ± 1.89 a
GS	4.14 ± 0.25 bc	0.04 ± 0.05 ab	8.22 ± 1.84 e	11.46 ± 2.57 ab	11.27 ± 0.71 a	14.92 ± 3.38 de	3.23 ± 1.34 a	2.61 ± 0.48 b	25.64 ± 0.93 c	23.47 ± 5.19 a
WS	5.26 ± 1.76 c	0.72 ± 0.18 bc	3.88 ± 0.71 cd	33.61 ± 9.35 c	211.83 ± 9.52 c	20.75 ± 2.07 e	39.79 ± 5.15 d	bdl	26.79 ± 3.00 c	517.19 ± 67.54 d
BM	1.80 ± 0.17 ab	1.88 ± 0.37 d	2.37 ± 0.35 bc	45.07 ± 8.12 d	135.13 ± 24.11b	21.51 ± 4.52 e	52.61 ± 9.55 e	0.31 ± 0.10 a	11.63 ± 2.12 b	339.71 ± 68.83 c
VC	3.22 ± 0.42 abc	0.19 ± 0.04 abc	2.83 ± 0.36 bcd	24.05 ± 4.37 bc	25.13 ± 2.60 a	13.20 ± 2.12 cd	24.09 ± 2.71 c	bdl	23.50 ± 3.44 c	156.08 ± 24.23 b
OW	1.18 ± 0.39 a	0.09 ± 0.04 abc	3.89 ± 0.72 cd	16.04 ± 3.42 abc	111.34 ± 14.22 b	9.71 ± 1.91 bcd	2.44 ± 0.47 a	bdl	20.40 ± 3.52 c	48.77 ± 9.04 a
OL	2.40 ± 0.73 ab	0.06 ± 0.04 ab	4.96 ± 1.02 d	16.29 ± 3.35 abc	147.08 ± 26.73 b	12.27 ± 2.44 cd	3.54 ± 0.84 a	0.27 ± 0.06 a	25.10 ± 4.51 c	62.86 ± 11.38 a
AW	4.20 ± 1.74 bc	0.92 ± 0.85 c	2.67 ± 1.32 bcd	40.00 ± 16.23 cd	365.35 ± 38.10 d	12.41 ± 4.98 cd	10.33 ± 0.47 b	bdl	12.49 ± 5.49 b	190.05 ± 13.55 b

IO – Dry sludge rich in iron oxyhydroxides, MS – Dry marble sludge, CW – Carbonated waste of a peat exploitation, GS – Gypsum mining spoil, WS – Composted sewage sludge, BM – Bio-stabilised material of municipal solid waste, VC – Vermicompost from pruning and gardening, OW – Composted solid olive-mill by-product irrigated with drinking water, OL – Composted solid olive-mill by-product irrigated with leachates of the olive-mill, AW – Composted greenhouse plant waste, bdl – below detection limit. Letters represent significant differences among waste materials (Kruskal-Wallis and Dunn tests, $p < 0.05$*).*

The artificial AMD prepared by oxidation of the toxic tailings from Aznalcóllar accident showed both the typical ultra-acid character (pH$_{(L)}$ – 2.89 ± 0.03) and the extremely high EC$_{(L)}$ (3.76 ± 0.14 dS m^{-1}). Moreover, most PHEs were present in high concentrations in AMD (Table 3.3). Below 100 µg l^{-1} were Ba, Be, In, Mo, Sc, Th, Tl, U, V and Y; between 100 and 500 µg l^{-1} were Bi, Cd, Co, Cr, Ni and Sn; from 500 and 1000 µg l^{-1} were Pb and Sb; and over 1000 µg l^{-1} were As, Cu, Mn and Zn.

Table 3.3. *Comparison of PHEs concentrations in artificial AMD (mean ± SD, n = 3) with maximum regulatory levels in natural and reclaimed water.*

PHEs	**AMD concentration** (µg l^{-1})	**Regulatory level** (µg l^{-1})		
		Natural water in Spain [1]	**Reclaimed water for irrigation**	
			Spain [2]	**USA [3]**
As	**2859.70 ± 270.16**	50	100	100
Ba	34.27 ± 9.83	-	-	-
Be	3.19 ± 0.36	-	100	100
Bi	180.20 ± 10.98	-		-
Cd	**452.02 ± 5.82**	-	10	10
Co	**434.61 ± 8.20**	-	50	50
Cr	**351.89 ± 5.66**	50	100	100
Cu	**6238.22 ± 67.11**	120	200	200
In	31.56 ± 0.38	-	-	-
Mn	**12,937.64 ± 216.04**	-	200	200
Mo	6.33 ± 0.30	-	10	10
Ni	197.82 ± 1.89	-	200	200
Pb	597.69 ± 81.54	-	-	5000
Sb	817.85 ± 32.29	-	-	-
Sc	29.93 ± 0.19	-	-	-
Sn	443.91 ± 32.71	-	-	-
Th	8.25 ± 1.43	-	-	-
Tl	25.00 ± 2.19	-	-	
U	14.55 ± 0.68	-		-
V	54.92 ± 9.45	-	100	100
Y	39.86 ± 0.29	-	-	-
Zn	**32,208.45 ± 495.51**	500	-	2000

[1] *Normas de calidad ambiental (NCA): Concentration of a PHE in water that must not be exceeded to protect human health and environment set by Spanish legislation in Annex V - RD 817/2015* (BOE 2015).

[2] *Maximum admissible value (VMA): The highest level of a PHE that is allowed in reclaimed water used for irrigation established by the Spanish legislation* (BOE 2007).

[3] *Recommended water quality criteria for irrigation: The highest level of a PHE that is allowed in reclaimed water used for irrigation established by the United States Environmental Protection Agency* (EPA 2012).

3.2.2. ACID MINE DRAINAGE TREATMENT USING WASTE MATERIALS

All leachates obtained after waste treatment showed a $pH_{(L)}$ close to slightly acidic–neutral values (6.0-7.3), although there were statistically significant differences among the wastes (Figure 3.1A). Changes in $EC_{(L)}$ due to waste treatment were quite heterogeneous among the waste materials used ($EC_{(L)}$: 2-24 dS m^{-1}). Some of them (IO, MS, GS, and VC) reduced the $EC_{(L)}$ of the AMD; however, other wastes caused a significant increase in $EC_{(L)}$ (AW, WS, BM, OW, and OL) between 2- and 6-fold the EC measured in artificial AMD (Figure 3.1B).

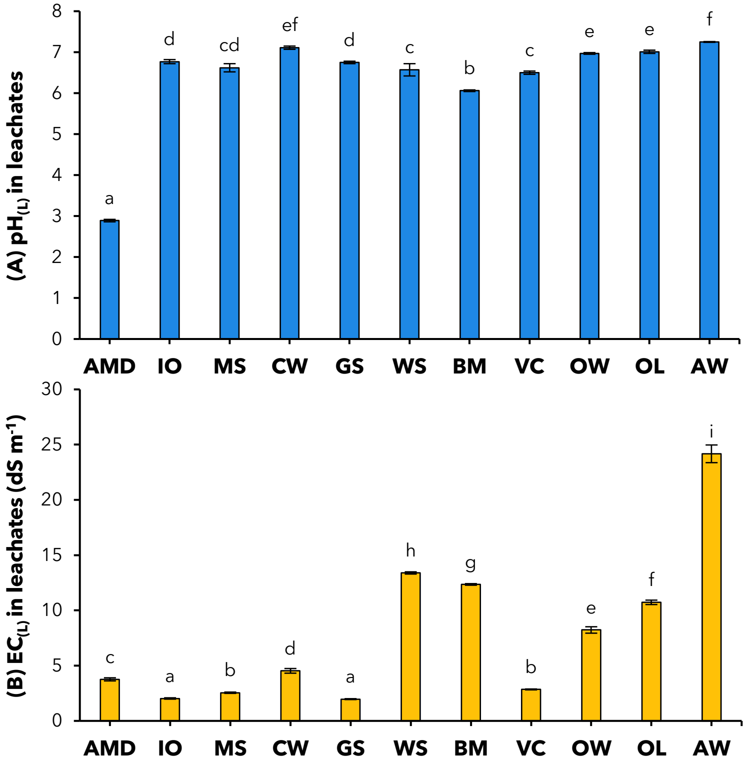

Figure 3.1. *Variation of pH (A) and EC (B) in the resulting leachates after the treatment of artificial AMD by different waste materials (n = 3). AMD – Acid mine drainage, IO - Dry sludge rich in iron oxyhydroxides, MS – Dry sludge from marble cutting and polishing, CW – Carbonated waste from peat bog mining, GS – Gypsum mining spoil, WS – Composted sewage sludge, BM – Bio-stabilised material from municipal solid waste, VC – Vermicompost from pruning and gardening waste, OW – Composted solid olive-mill by-product irrigated with drinking water, OL – Composted solid olive-mill by-product irrigated with liquid olive-mill wate, AW – Composted from agricultural greenhouse waste. Letters represent significant differences among waste materials (Kruskal-Wallis and Dunn tests, p < 0.05).*

Table 3.4. *Retention effectiveness of several PHEs of waste materials (IO, MS, CW, GS, WS, BM, VC, OW, OL, AW) in % (mean ± SD, n = 3).*

PHEs	**IO**	**MS**	**CW**	**GS**	**WS**	**BM**	**VC**	**OW**	**OL**	**AW**
As	99.96 ± 0.01 e	99.53 ± 0.27 e	99.74 ± 0.07 e	99.87 ± 0.04 e	69.56 ± 6.98 b	46.95 ± 3.49 a	99.67 ± 0.01 e	88.13 ± 3.68 d	81.72 ± 3.04 cd	77.20 ± 2.92 bc
Ba		40.27 ± 4.17 b	33.81 ± 36.49 ab	0.09 ± 9.27 a						
Be	100.00 ± 0.00 e	99.95 ± 0.09 e	100.00 ± 0.00 e	99.63 ± 0.18 e	90.80 ± 2.04 d	70.53 ± 2.32 b	99.74 ± 0.45 e	80.77 ± 4.47 c	62.80 ± 4.60 a	80.46 ± 1.92 c
Bi	99.55 ± 0.24 d	96.82 ± 1.43 cd	97.93 ± 0.66 d	98.68 ± 0.18 d	80.87 ± 8.10 b	70.54 ± 2.20 a	99.76 ± 0.05 d	88.79 ± 2.27 cd	92.16 ± 0.14 cd	97.33 ± 0.49 d
Cd	98.91 ± 0.04 ef	99.28 ± 0.02 ef	99.68 ± 0.02 f	95.82 ± 0.51 c	97.44 ± 0.30 d	75.12 ± 0.73 a	98.54 ± 0.07 df	98.26 ± 0.29 de	94.85 ± 0.30 c	89.65 ± 0.72 b
Co	98.80 ± 0.02 f	64.25 ± 0.69 b	95.49 ± 0.22 f	38.20 ± 6.54 a	83.57 ± 0.84 cd	39.48 ± 2.45 a	95.17 ± 0.59 f	93.35 ± 0.33 ef	86.96 ± 0.47 de	79.95 ± 2.37 c
Cr	100.00 ± 0.00 b	100.00 ± 0.00 b	100.00 ± 0.00 b	100.00 ± 0.00 b	83.52 ± 2.65 b	16.57 ± 4.36 a	100.00 ± 0.00 b	87.72 ± 3.60 b	85.88 ± 1.63 b	
Cu	99.93 ± 0.01 e	99.46 ± 0.03 e	98.45 ± 0.01 e	99.56 ± 0.05 e	92.49 ± 0.38 c	67.12 ± 0.90 a	99.82 ± 0.01 e	95.73 ± 0.48 d	90.04 ± 0.60 c	78.79 ± 2.42 b
In	100.00 ± 0.00 d	99.94 ± 0.07 d	100.00 ± 0.00 d	100.00 ± 0.00 d	91.86 ± 1.65 ab	91.39 ± 0.31 a	100.00 ± 0.00 d	94.38 ± 1.61 c	93.81 ± 0.58 bc	92.73 ± 0.84 abc
Mn	98.02 ± 0.16 b	78.43 ± 0.30 a	94.28 ± 0.30 b		73.12 ± 11.73 a		71.64 ± 2.50 a	75.19 ± 0.67 a	70.43 ± 1.03 a	69.54 ± 2.83 a
Mo	3.45 ± 81.16 a	73.25 ± 2.85 b								
Ni	85.30 ± 0.16 e	62.98 ± 0.76 c	74.74 ± 0.54 d		19.98 ± 17.38 a		87.42 ± 1.24 e	69.88 ± 0.94 cd	47.79 ± 1.65 b	47.76 ± 6.51 b

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PHEs	IO	MS	CW	GS	WS	BM	VC	OW	OL	AW
Pb	100.00 ± 0.00 c	99.97 ± 0.05 c	100.00 ± 0.00 c	100.00 ± 0.00 c	93.19 ± 4.65 b	82.18 ± 1.30 a	99.93 ± 0.01 c	94.51 ± 1.83 b	90.90 ± 1.46 b	91.41 ± 1.38 b
Sb	99.33 ± 0.03 d	98.55 ± 0.06 d	98.13 ± 0.10 d	98.27 ± 0.14 d	62.07 ± 6.33 b	40.90 ± 3.51 a	98.27 ± 0.12 d	85.24 ± 2.59 c	84.31 ± 0.30 c	85.30 ± 1.91 c
Sc	91.83 ± 0.09 d	97.35 ± 0.43 d	95.55 ± 0.13 d	95.68 ± 0.21 d	77.58 ± 4.79 c	50.15 ± 2.12 a	90.34 ± 0.76 d	67.70 ± 5.87 b	71.76 ± 1.01 bc	78.65 ± 2.47 c
Sn	100.00 ± 0.00 c	99.38 ± 0.54 c	100.00 ± 0.00 c	100.00 ± 0.00 c	43.34 ± 22.57 a		99.70 ± 0.42 c	83.22 ± 4.71 b	87.11 ± 0.91 b	90.10 ± 2.49 b
Th	98.95 ± 0.51 d	95.88 ± 0.92 d	95.51 ± 1.03 d	97.33 ± 0.28 d	61.90 ± 8.95 c	16.58 ± 2.04 a	99.03 ± 0.21 d	56.40 ± 7.41 c	51.68 ± 1.85 bc	43.92 ± 5.20 b
Tl	95.86 ± 0.03 f	60.10 ± 0.77 a	91.47 ± 0.16 ef	85.72 ± 1.47 c	90.98 ± 2.91 de	75.13 ± 0.68 b	91.70 ± 0.02 ef	86.42 ± 0.28 cd	83.24 ± 0.72 c	78.07 ± 3.96 b
U	80.20 ± 1.00 b	94.28 ± 0.17 c		91.96 ± 1.02 c	66.31 ± 7.12 a	79.39 ± 0.94 b	94.28 ± 1.37 c	74.45 ± 1.54 ab	71.68 ± 0.74 a	
V	100.00 ± 0.00 b	100.00 ± 0.00 b	100.00 ± 0.00 b	100.00 ± 0.00 b			100.00 ± 0.00 b	22.11 ± 20.57 a		
Y	99.93 ± 0.11 d	99.92 ± 0.06 d	99.87 ± 0.22 d	99.83 ± 0.08 d	79.26 ± 10.56 c	34.75 ± 2.33 a	99.67 ± 0.06 d	81.85 ± 3.88 c	59.57 ± 2.14 b	71.51 ± 0.99 c
Zn	99.22 ± 0.05 ef	93.63 ± 0.13 cd	99.86 ± 0.01 f	92.87 ± 0.64 bc	95.17 ± 0.83 d	64.01 ± 1.57 a	97.92 ± 0.19 e	97.96 ± 0.39 ef	95.09 ± 0.36 d	91.09 ± 0.74 b

IO - Dry sludge rich in iron oxyhydroxides, MS – Dry sludge from marble cutting and polishing, CW – Carbonated waste from peat bog mining, GS – Gypsum mining spoil, WS – Composted sewage sludge, BM – Bio-stabilised material from municipal solid waste, VC – Vermicompost from pruning and gardening waste, OW – Composted solid olive-mill by-product irrigated with drinking water, OL – Composted solid olive-mill by-product irrigated with liquid olive-mill wate, AW – Composted from agricultural greenhouse waste. Letters represent significant differences among different waste materials for a same element (Kruskal-Wallis and Dunn tests, $p < 0.05$). Very high retention > 95% (green), High retention > 50% (yellow), Low retention < 50% (red), No retention (white).

Most PHEs concentrations in the soluble fraction decreased significantly after treatment with wastes (Table S3.1), although there were large differences in removal effectiveness between organic and inorganic wastes. Inorganic wastes showed a higher removal effectiveness of PHEs than organic wastes, excluding VC, which had similar removal rates to inorganic wastes (Table 3.4). For the main PHEs (i.e. As, Cd, Cr, Cu, Pb, Sb, and Zn), the retention rate of all tested inorganic wastes (IO, MS, CW, GS) as well as VC was above 95% in most cases and close to 100% for many of them (Table 3.4). Thus, reducing the concentration of these elements to values below the regulatory levels in most cases. Similarly, the retention rate of other uncommon PHEs such as In, Sc, Sn, Th, Tl, V, and Y was also outstanding, almost 100% in all inorganic wastes and VC (Table 3.4). Furthermore, there were other less significant PHEs for which the variability in retention rate was very high, such as Ba, Be, Bi, Co, Mn, Mo, Ni, and U . Among the inorganic wastes, dry sludge rich in iron oxyhydroxides (IO) had the highest capacity to retain PHEs, followed by wastes with a high calcium carbonate content (MS: dry marble sludge, CW: carbonated waste from a peat exploitation). Gypsum spoil (GS) was not effective for Ba, Co, Mn, Mo, and Ni retention, but for other PHEs, it was as effective as the other inorganic wastes. On the other hand, most organic wastes demonstrated an overall good removal effectivity for these PHEs, although lower than that for inorganic wastes, with the exception of VC. The wastes with the lowest retention capacity for most PHEs were BM and AW.

3.3. DISCUSSION

The physical, chemical, and biological characteristics of the waste materials reflect considerable differences in their composition. There are wastes with a strong carbonate character (CW and MS), others that are highly organic (VC, AW, OL, WS, OW, and BM), and also a waste with high iron oxyhydroxide content (IO). These characteristics are selected because of their important role in the immobilisation of PHEs and the acid neutralisation (Merdy et al. 2009; Bolan et al. 2014; Aguilar-Garrido et al. 2022a). For example, organic matter has a high affinity for some PHEs due to the presence of ligands or functional groups (Harter and Naidu 1995), in the following order: $Cu^{2+} > Hg^{2+} > Cd^{2+} > Fe^{2+} > Pb^{2+} > Ni^{2+} > Co^{2+} > Mn^{2+} > Zn^{2+} > As^{5+} > As^{3+}$ (Takamatsu et al. 1983; Adriano 2001). Thus, organic matter together with total humic extract and humic and fulvic acids provide an important content of reactive colloidal fractions that allow the complexation of the different chemical forms of PHEs (Davis 1984; Zhang et al. 2020). Carbonates also exert a strong control over pH, which is considered a key property in controlling the immobilisation of most PHEs because of their influence on the electrical charge of colloidal components (Roberts et al. 2005). In addition, it is a key component to neutralise acid solutions (Simón et al. 2005). Likewise, iron oxyhydroxides content is another constituent to consider for the retention of some PHEs, especially As, for which they exert a strong control on speciation and bioavailability (Bissen and Frimmel 2003; Juhasz et al. 2003). In fact, the results of the AMD treatment test indicate that many wastes tested show considerable acid neutralisation and PHEs immobilisation capacity.

The concentration of most PHEs in AMD was very high, exceeding the guideline values established by different laws for As, Cd, Co, Cr, Cu, Mn, and Zn: i) environmental quality standard for surface water in Spain (BOE 2015), ii) legal regime for the reuse of treated water for irrigation in Spain (BOE 2007), and iii) guidelines for water reuse in USA (EPA 2012) (Table 3.3). The highest concentrations were found for As, Cd, Cu, and Zn, exceeding about 29-, 45-, 31-, and 16-fold the guideline values for reuse of reclaimed water for irrigation according to Spanish legislation and the United States Environmental Protection Agency (BOE 2007; EPA 2012); besides, other PHEs such as Co, Cr, Cu, and Mn were also considered relevant in relation to their high concentrations exceeding these regulatory levels. Other elements like Pb, Sb, and Tl presented potentially concerning concentrations, although their guideline values are not included in the previous references. In addition, most of the PHEs in this artificial AMD were at much higher concentrations than those found in the acidic water discharged in the Aznalcóllar mine accident (Simón et al. 1999), as well as the concentrations in AMD generated in metal mines in Australia (Lottermoser 2010) or in other mining areas around the world (Kefeni et al. 2017). Thus, the results in this study can be extrapolated to most acid mine water treatment situations around the world; moreover, the use of the wastes tested in this study to treat real AMD worldwide would most likely produce a better quality treated water than that achieved for the artificial AMD used in this study.

The treatment of AMD with wastes has been effective in neutralising the acidity in all cases. The pH in treated water increases from pH < 3 to values above 6 and close to neutrality depending on the waste used. In this sense, although the role of carbonates in AMD neutralisation has already been widely demonstrated (Maree and du Plessis 1994; Heviánková et al. 2013), no statistical correlation was found between pH in leachate ($pH_{(L)}$) and the $CaCO_3$ concentration in different wastes (Table S3.2). Nevertheless, carbonates are not the only buffering components controlling pH; other constituents in the wastes (e.g. organic matter, exchangeable bases, Fe and Al oxides, silicates) influence this capacity (Nawaz et al. 2010; Bennardi et al. 2018). Likewise, the concentrations of several PHEs in AMD after treatment with wastes were significantly reduced. Indeed, the removal efficiencies of PHEs obtained from these wastes have been much higher than those achieved in other studies (Zhang 2011; Kefeni et al. 2017; Dhir 2018). Among the wastes used, inorganic wastes were much more effective in retaining PHEs than organic ones. The decreasing order of effectiveness was as follows: IO > CW ≥ MS ≥ VC > GS > OW > OL > WS > AW > BM; where wastes rich in iron oxyhydroxides and carbonates are more effective than wastes rich in organic matter in the retention of PHEs. The removal rates for wastes dominated by carbonates (CW and MS) or iron oxyhydroxides (IO) are above 95% for most PHEs present in AMD, whereas for organic wastes, the removal rate was below 95% in most cases, with values as low as 15% in the case of bio-stabilised material of municipal solid waste (BM). In other studies, for similar wastes, the removal rates achieved were similar or even lower. For example, water filters partly made of iron-rich materials achieved removal rates of 50% for As (Casentini et al. 2019). However, other studies that also explored the As retention capacity of water filters with iron oxide-rich materials reached rates of 90% (Nguyen et al. 2009) and 99% (Nighojkar et al. 2019). The latter study concerned not only filters made from iron-rich waste, but also marble slurry filters for which the As removal rate is 95% (Nighojkar et al. 2019). Furthermore, the success of these materials is not limited to As; for example, along with near 100% As retention in groundwater affected by an abandoned gold mine when treated with various mixtures composed of organic carbon, zero-valent iron, and limestone, a strong decrease in the concentration of Al, Cd, Co, Cu, and Ni has been demonstrated (Angai et al. 2022); although the concentrations of these elements in the groundwater are much lower than in our study. On the other hand, although less studied, the capacity of some organic wastes has also been assessed; for example, it has been reported a 70% reduction of some PHEs (Al, As, Cd, Cu, Fe, Ni, Mn, Pb, and Zn) present in sulphide mine leachates by the addition of aqueous organic wastes from domestic wastewater (Tapia et al. 2019). Agricultural wastes have also been used to remove pollutants; for example, solid-olive mill by-products have a great capacity to remove Cr, Mn, Cu, Zn, Ni, and Pb from mining wastewater (Fernández-González et al. 2018). Similarly, there is an extensive list of agricultural wastes (agave, bananas, wheat, rice, citrus fruits) that have been used for the immobilisation of different PHEs (Cd, Pb, and Zn) with uncertain results (Xu and Mckay 2017). Particularly noteworthy is the case of vermicompost (VC), which shows retention rates of PHEs close to those of carbonated and iron-rich wastes. This may be due to the higher content of $CaCO_3$ and Fe_T compared to other organic wastes, and, to a lesser extent, its considerable high OC content. In this sense, vermicompost can be a very effective material for the treatment of AMD. Carrillo-González et al. (2022) using vermicompost and other agricultural by-products (sheep, cow, and rabbit manure) for AMD treatment reported retention rates of 90% for As, Cd, Cu, and Zn in AMD. Similarly, gypsum spoil (GS) also has a high retention capacity for PHEs similar to that of other inorganic wastes, although for some, such as Ni and Co, it was very low. The high retention capacity of GS is related to its high $CaCO_3$ and Fe_T contents.

Equally, it should not be overlooked that the PHEs content in some wastes may pose a potential risk. In relation to the initial concentration of PHEs in the wastes, IO and GS presented slightly high concentrations of As, Pb, and Sb. However, they do not exceed the guideline values to declare a soil polluted according to the regional regulations (BOJA 2015) or the maximum levels that a compound must have in order to be used as a fertiliser product in Spain (BOE 2013). The remaining inorganic wastes have low concentrations of most PHEs. The same applies to organic wastes, although some of them show high concentrations of certain PHEs (Cr, Cu, V, and Zn), they do not exceed the guideline values. In particular, the organic wastes with the highest concentrations are compost from greenhouse waste (AW), composted sewage sludge (WS), and bio-stabilised material from municipal solid waste treatment (BM); which are also the wastes with the lowest retention capacity. The presence of PHEs in waste related to urban activities is common (Shaheen et al. 2017; Palansooriya et al. 2020), although in our case, they do not exceed the guidelines values and therefore pose a low risk of PHEs pollution. In any case, concern should be raised about their use due to the very high salinity reflected in their high EC values. In fact, most organic wastes, except VC, cause an increase in EC in the leachates resulting from the treatment with respect to AMD.

The main PHEs (i.e. As, Cd, Cr, Cu, Pb, and Zn) have been successfully removed (close to 100%) from artificial AMD by waste treatment. Especially inorganic wastes and vermicompost have the highest capacity, leaving the concentrations of most of them in the treated water below the regulatory levels for irrigation and surface water in Spain (BOE 2007; EPA 2012; BOE 2015). In contrast, in the treatment with the organic wastes, although significantly reduced the PHEs concentrations, the values were above the regulatory levels in most cases. However, the retention of other less studied PHEs such as In, Sc, Sn, Th, Tl, V, and Y is also remarkable. Promising results are obtained for specific elements, as in the case of V, where previous studies with commercial iron products and a ferric residue from groundwater treatment obtained 85% of removal of this element from mining water (Zhang et al. 2022b), compared to values close to 100% removal in our study for inorganic and vermicompost wastes. Thallium is another highly toxic element and quite understudied (Liu et al. 2019); and the treatment and removal in wastewater is one of the major challenges in the coming years (Zhao et al. 2020). In our study, the removal rate of Tl in AMD is above 75% for all wastes analysed and for some wastes such as IO, CW, WS and VC above 90%, whereas in other studies included in Liu et al. (2019), the reduction of Tl in wastewaters after treatment with lime is between 21% and 49%. Antimony is also considered a concern element due to the potential toxicity in surface and groundwater; and the use of commercial coagulants such as iron salts have proven to be effective in remediating Sb-polluted waters; in this case, the ferric chloride coagulant presented removal rates higher than 80% across a broad pH range (Inam et al. 2019). The efficiency of Sb removal from AMD in our study is higher than 95% for inorganic and vermicompost wastes, which shows the high potential application of the wastes that we have analysed.

Nowadays, many elements are expected to be in high demand to achieve energy transition, so mining is an essential activity revived. The production and availability of technology-critical elements is also a current concern. In this scenario, a global increase in the pollution of the environment by PHEs is foreseen in the short term, together with the production of waste related to different human activities. This study is framed by both problems (increased input of pollutants into the environment and increased production of waste), so the promising results obtained can contribute to environmental protection and human safety.

3.4. CONCLUSIONS

This chapter tests the effectiveness of various wastes as a potential treatment of AMD to promote mine restoration and environmental protection through the sustainable management of urban, mining, and agro-industrial wastes in a circular economy scenario. Our results conclude that the waste materials studied have a very high acid neutralising capacity and a strong capacity to retain PHEs. Inorganic wastes, together with vermicompost from pruning and gardening, reduced by more than 95% the concentrations of most PHEs in a highly polluted artificial AMD, while organic wastes retained between 50% and 95%. The potential effectiveness followed this order: IO > CW ≥ MS ≥ VC > GS > OW > OL > WS > AW > BM. Thus, several mining, urban, and agro-industrial wastes could be recovered for AMD treatment. The use of these wastes showed promising results to be applied in the decontamination of polluted waters and as a control technique on tailing deposits to prevent AMD generation. This study is the first step in the development of green technologies based on the combination of different wastes with contrasting characteristics to create solutions (e.g. Technosols, permeable reactive barriers) with a higher capacity to retain a greater variety of PHEs and reduce acidity in polluted environments. The use of waste to remediate AMD will decrease the cost of water treatment. This is especially relevant for the remediation of areas with historical or abandoned mines, where the decrease in cost by replacing commonly used and expensive reagents with worthless waste will increase the affordability of water treatments. Nevertheless, additional site-specific studies should be conducted to include the cost of waste transport and to evaluate the *in situ* effectiveness of waste combinations under real field conditions.


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CHAPTER 4

WASTE-DERIVED TECHNOSOLS FOR THE REMEDIATION OF PHE-POLLUTED SOIL: A MICROCOSM ASSAY

Aguilar-Garrido, A., Romero-Freire, A., Paniagua-López, M., Martínez-Garzón, F. J., Martín-Peinado, F. J., Sierra-Aragón, M. (2023). Technosols derived from mining, urban, and agro-industrial waste for the remediation of metal(loid)-polluted soils: a microcosm assay. ***Toxics***, 11(10), 854.
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CHAPTER 4: MICROCOSM ASSAY OF POLLUTED SOILS REMEDIATION BY WASTE-DERIVED TECHNOSOLS

ABSTRACT

This chapter evaluated the effectiveness of six Technosols designed for the remediation of polluted soils (PS) by potentially harmful elements (PHEs) at the physicochemical, biological, and ecotoxicological levels at the microcosm scale. Technosols T1–T6 were prepared by combining PS with a mix of organic and inorganic wastes from mining, urban, and agro-industrial activities. After two months of surface application of Technosols on polluted soils, we analysed the soil properties, PHEs concentration in total, soluble, and bioavailable fractions, soil enzymatic activities, and growth responses of *Trifolium campestre* Schreb. and *Lactuca sativa* L. in both Technosols and underlying polluted soils. All Technosols improved the unfavourable conditions of PS by neutralising acidity, increasing OC content, reducing the mobility of most PHEs, and stimulating both the soil enzymatic activities and growth of *T. campestre* and *L. sativa*. The composition of organic waste used in Technosols strongly conditioned the changes induced in polluted soils; in this sense, Technosols composed of pruning and gardening vermicompost (T3 and T6) showed greater reductions in toxicity and plant growth than other Technosols composed with different organic wastes. Thus, these Technosols constitute a potential solution for the remediation of persistently polluted soils that should be applied in large-scale and long-term interventions to reinforce their feasibility as a cost-effective ecotechnology.

CHAPTER 4: MICROCOSM ASSAY OF POLLUTED SOILS REMEDIATION BY WASTE-DERIVED TECHNOSOLS

4.1. BACKGROUND

The effects of polluting accidents, such as the Aznalcóllar mine spill, can be prolonged over time. The consequences of this tragic environmental disaster still persist in the GGC after more than 25 years (Pastor-Jáuregui et al. 2022), marked by the presence of bare soil patches with high PHEs concentrations and unfavourable physicochemical properties (i.e. acidic pH, low OM content) (Sierra Aragón et al. 2019) (see Figure 1.2); thereby posing a long-term toxic risk to living organisms (Paniagua-López et al. 2023). Therefore, remediation of these persistently polluted areas requires specific interventions to reduce their potential toxic risk. Recently, the ecotechnology of waste-derived Technosols is becoming one of the most applied remediation techniques, with strong scientific support for its effectiveness (for more information, see section 1.2.3). However, not many studies simultaneously examine alterations in physical, chemical, and biological properties, which are essential to understand the evolution of remediation techniques applied to polluted soils from a holistic approach. Similarly, most research on Technosols focus on analysing changes within Technosols and surface effects, but do not include information on the underlying polluted soils. To our knowledge, this chapter is one of the first to adopt a holistic approach and include the study of changes in both the Technosols and the underlying polluted soils.

Therefore, the specific **objective** of this chapter is to evaluate, on a physicochemical, biological, and ecotoxicological levels and on a microcosm scale (under growth chamber conditions), the effectiveness of six Technosols (T1, T2, T3, T4, T5, and T6) in the remediation of polluted soils; analysing both the effects on the Technosols and on the underlying polluted soils. For detailed on the experimental design and analyses applied in this experience, see section 2.3.2.1.

Technosols are composed of polluted soil and a mixture of organic and inorganic wastes in the proportions given in Table 4.1. The wastes selected were those with the highest removal rates of PHEs in an artificial AMD (see **chapter 3**), and which presented the necessary conditions to favour the immobilisation of PHEs and acidity neutralisation, such as carbonate character (CW: carbonated peat bog waste, MS: marble cutting and polishing sludge), richness in organic matter (OL: composted solid olive-mill by-product, WS: composted sewage sludge, VC:

vermicompost from pruning and gardening waste), and iron (IO: iron oxyhydroxide-rich sludge). For more information on Technosols production, see section 2.2.5.

Table 4.1. *Composition of Technosols: % of polluted soil and each waste.*

Technosols	PS	IO	CW	MS	OL	WS	VC
T1	60	2	20	-	18	-	-
T2	60	2	20	-	-	18	-
T3	60	2	20	-	-	-	18
T4	60	2	-	20	18	-	-
T5	60	2	-	20	-	18	-
T6	60	2	-	20	-	-	18

Polluted soil (PS), iron oxyhydroxide-rich sludge (IO), carbonated waste (CW), marble sludge (MS), solid olive mill by-product (OL), composted sewage sludge (WS), vermicompost from gardening waste (VC).

4.2. RESULTS

4.2.1. SOIL PROPERTIES AND PHEs CONCENTRATIONS

The PHE-polluted soil (PS) had an extremely acidic pH, a very high EC, and a very low $CaCO_3$ content. This soil also showed low potential fertility, manifested by a low OC content, which was five times lower than that in the reference unpolluted soil (US), as well as a low total N content (Table 4.2). Furthermore, it showed high total concentrations of some PHEs (mg kg^{-1}; As: 346; Pb: 640) compared to the low concentrations in US (mg kg^{-1}; As: 25; Pb: 79) (Table 4.3). Nevertheless, considering the solubility and bioavailability of PHEs, As and Pb were not of critical concern, as water-soluble (W) and EDTA-bioavailable (E) concentrations were similar to those in US (Pb_E was even lower). In contrast, Cd, Cu, and Zn occurred in very high concentrations in water soluble fraction (µg kg^{-1}; Cd_W: 214; Cu_W: 941; Zn_W: 11,992), namely 227, 64, and 570 times higher than in US (Table 4.3). Additionally, none of the PHEs considered were in high amounts in EDTA-extracted bioavailable fraction with concentrations similar to those in US, except for Cu and Zn, slightly higher.

The properties of Technosols (T1–T6) were significantly better than those of PS, although there were certain differences between them (Table 4.2). All Technosols had a pH between slightly (T2, T5, and T6) and moderately alkaline (T1, T3, and T4) due to the moderately high $CaCO_3$ content present (Table S4.1). The very high EC in PS decreased slightly in Technosols but did not reach the very low values in US. Thus, EC remained considerably high in Technosols (> 1.2 dS m^{-1}). The organic carbon content increased relative to PS by almost 14-fold in T1, 16-fold in T2, 6.5-fold in T3, 13.5-fold in T4, 14.5-fold in T5, and 5-fold in T6. Thus, Technosols made of sewage sludge (T2 and T5) had the highest OC contents, and those composed of vermicompost from pruning and gardening (T3 and T6) had the lowest with values ranging between 2.15% and 6.15%. High concentrations of total N were also found in Technosols. The concentration of N_T in T1 and T4 (composed of solid olive mill by-product) and in T2 and T5 (composed of sewage sludge) was significantly higher than in US, whereas in T3 and T6 (composed of vermicompost from pruning and gardening) there were no significant differences compared to US.

Table 4.2. *Physicochemical properties of polluted soil (PS), unpolluted soil (US), and six designed Technosols (T1–T6) (mean ± SD; n = 6).*

	PS	**US**	***Technosols***					
			T1	**T2**	**T3**	**T4**	**T5**	**T6**
pH (H_2O)	3.53 ± 0.03 a	6.91 ± 0.08 b	8.19 ± 0.04 e	7.83 ± 0.02 c	8.10 ± 0.03 d	8.01 ± 0.05 d	7.79 ± 0.01 c	7.78 ± 0.04 c
EC (dS m^{-1})	2.77 ± 0.12 d	0.05 ± 0.01 a	2.09 ± 0.15 c	1.37 ± 0.16 b	1.25 ± 0.22 b	2.18 ± 0.25 c	1.62 ± 0.25 b	1.42 ± 0.19 b
OC (%)	0.42 ± 0.11 a	2.24 ± 0.14 bc	5.97 ± 0.15 d	6.61 ± 0.31 e	2.74 ± 0.18 c	5.67 ± 0.33 d	6.15 ± 0.37 de	2.15 ± 0.12 b
$CaCO_3$ (%)	0.39 ± 0.07 a	2.66 ± 0.05 b	27.22 ± 4.48 c	26.11 ± 1.48 c	26.87 ± 0.97 c	27.57 ± 2.03 c	26.85 ± 4.31 c	25.92 ± 2.59 c
N_T (%)	0.11 ± 0.01 a	0.20 ± 0.02 bc	0.52 ± 0.01 d	0.84 ± 0.04 e	0.22 ± 0.02 c	0.51 ± 0.03 d	0.90 ± 0.03 f	0.14 ± 0.03 ab
C_T (%)	0.48 ± 0.02 a	2.50 ± 0.19 b	8.28 ± 0.13 d	8.40 ± 0.16 d	5.20 ± 0.20 c	8.66 ± 0.29 d	8.71 ± 0.19 d	5.02 ± 0.07 c

EC – electrical conductivity; OC – organic carbon; $CaCO_3$ – calcium carbonate; N_T/C_T – total N and C. Letters represent significant differences among treatments (Kruskal–Wallis and Mann–Whitney U tests; $p < 0.05$).

The solubility and bioavailability of PHEs in Technosols strongly changed in relation to PS (Table 4.3). The very high water-soluble concentrations of Cd, Cu, and Zn in PS strongly decreased in all Technosols up to values roughly close to those in US. In particular, Cd_W diminished by more than 99%, Cu_W by 92 to 98%, and Zn_W by more than 99% relative to PS. In contrast, As, Pb, and Sb had low mobility and bioavailability in PS, while in Technosols significantly increased, although with differences among Technosols. The soluble and bioavailable As contents increased

Table 4.3. *Total, water-soluble, and EDTA-extracted bioavailable concentrations of PHEs in polluted soil (PS), unpolluted soil (US), and six designed Technosols (T1–T6) (mean ± SD; n = 6).*

		PS	**US**	***Technosols***					
				T1	**T2**	**T3**	**T4**	**T5**	**T6**
Total mg kg^{-1}	**As**	345.66 ± 25.29 f	25.02 ± 2.71 a	215.73 ± 6.12 b	242.76 ± 4.25 c	300.88 ± 10.03 e	250.33 ± 3.60 cd	205.49 ± 2.87 b	268.79 ± 4.45 d
	Cu	106.80 ± 5.24 c	37.72 ± 2.00 a	72.01 ± 2.68 b	109.76 ± 12.08 c	73.93 ± 3.60 b	72.91 ± 4.21 b	101.43 ± 3.91 c	64.09 ± 1.65 b
	Pb	640.17 ± 48.69 e	79.17 ± 10.76 a	382.61 ± 8.80 b	431.95 ± 12.92 bc	511.50 ± 6.16 d	428.24 ± 6.45 bc	366.31 ± 2.40 b	474.56 ± 5.40 cd
	Sb	20.83 ± 5.10 a	bdl	21.91 ± 4.18 a	23.10 ± 5.32 a	19.68 ± 5.75 a	19.92 ± 4.72 a	23.61 ± 2.73 a	23.06 ± 6.12 a
	Zn	185.09 ± 6.46 d	138.13 ± 1.54 c	106.05 ± 5.86 a	303.55 ± 1.34 f	144.66 ± 2.96 c	111.91 ± 0.33 a	266.93 ± 2.15 e	124.32 ± 3.02 b
Water-soluble µg kg^{-1}	**As**	8.91 ± 1.41 a	4.50 ± 2.86 a	47.51 ± 8.45 c	37.00 ± 2.43 bc	71.01 ± 2.78 d	67.40 ± 15.99 d	39.47 ± 4.34 bc	26.54 ± 0.34 b
	Cd	213.89 ± 42.71 b	0.94 ± 0.63 a	0.59 ± 0.05 a	1.02 ± 0.02 a	0.43 ± 0.06 a	1.26 ± 0.53 a	0.69 ± 0.09 a	0.25 ± 0.04 a
	Cu	940.79 ± 105.12 d	14.60 ± 4.10 a	37.45 ± 3.70 a	76.56 ± 2.20 a	24.24 ± 5.16 a	75.46 ± 20.51 a	85.80 ± 9.55 a	14.43 ± 0.92 a
	Pb	bdl	bdl	3.05 ± 2.63 a	8.20 ± 2.77 ab	bdl	9.60 ± 5.78 b	15.97 ± 1.80 c	bdl
	Sb	27.01 ± 1.65 a	19.69 ± 5.17 a	308.80 ± 4.68 d	224.38 ± 2.82 b	395.43 ± 17.06 e	262.03 ± 20.88 c	204.34 ± 25.10 b	317.70 ± 14.77 d
	Zn	11,992.27 ± 2953.45 b	21.04 ± 11.47 a	14.39 ± 3.56 a	36.25 ± 3.32 a	3.83 ± 0.90 a	39.07 ± 20.96 a	51.21 ± 4.86 a	4.54 ± 1.06 a
EDTA-extracted mg kg^{-1}	**As**	0.18 ± 0.03 b	0.11 ± 0.02 a	0.98 ± 0.05 g	0.57 ± 0.01 e	0.91 ± 0.02 f	0.40 ± 0.07 d	0.34 ± 0.01 d	0.26 ± <0.01 c
	Cd	0.30 ± 0.09 b	0.32 ± 0.10 b	0.09 ± <0.01 a	0.10 ± <0.01 a	0.10 ± <0.01 a	0.11 ± <0.01 a	0.11 ± <0.01 a	0.11 ± 0.01 a
	Cu	5.16 ± 0.37 d	2.09 ± 0.07 c	2.09 ± 0.02 c	1.65 ± 0.01 b	1.38 ± 0.03 a	2.39 ± 0.24 c	2.03 ± 0.08 c	1.74 ± 0.06 b
	Pb	0.01 ± <0.01 a	6.56 ± 0.18 c	0.39 ± 0.14 b	0.61 ± <0.01 b	0.09 ± 0.01 a	0.82 ± 0.43 b	0.68 ± 0.02 b	0.09 ± <0.01 a
	Sb	0.13 ± 0.02 a	0.22 ± 0.02 b	1.35 ± 0.02 h	0.88 ± 0.01 e	1.17 ± 0.02 g	0.78 ± 0.06 d	0.60 ± 0.02 c	0.95 ± 0.03 f
	Zn	12.99 ± 7.05 bc	7.70 ± 3.64 b	2.09 ± 0.02 a	7.11 ± 0.12 b	2.54 ± 0.03 a	1.79 ± 0.02 a	8.80 ± 0.21 b	2.12 ± 0.04 a

Cd_T: < 10 mg kg^{-1} (detection limit XRF). Letters represent significant differences among treatments (Kruskal-Wallis and Mann-Whitney U tests; $p < 0.05$).

by 3–8 times and 1.4–5.4 times compared to PS, respectively. The soluble Pb, from being in PS below the detection limit of ICP-MS, increased to concentrations up to 3 and 15 µg kg^{-1} in T1, T2, T4, and T5; however, it remained at undetectable concentrations in T3 and T6. The bioavailable fraction of Pb was similar to the soluble fraction, increasing significantly in T1, T2, T4, and T5 with respect to PS, while T3 and T6 showed values similar to PS. Particularly notable was the increase in the solubility and bioavailability of Sb, as Sb_W increased from 7.5-fold to 14.5-fold, and Sb_E increased from 4.5-fold to 10-fold in Technosols with respect to PS.

The unfavourable initial conditions of polluted soils improved with the surface application of Technosols (Table 4.4). pH increased in all underlying polluted soils (R1–R6), but none reached neutral pH values. Technosols T1, T2, T4, and T6 were the most effective buffers for the extreme acidity of PS to slightly acidic values in R1, R2, R4, and R6. As for EC, it remained at values similar to those of PS. The OC content also improved because of Technosols, except in R5 and R6, which remained similar to PS. In R1, R2, and R4, it almost doubled, and in R3, it also increased, but to a lesser extent. Similarly, $CaCO_3$ also increased with respect to PS, especially when treated with marble sludge-derived Technosols (R4, R5, and R6); in Technosols composed of carbonated waste, it also increased (except R1, where it remained constant), but was not statistically significant. Total C content increased in direct relation to increases in OC and $CaCO_3$, while total N remained unchanged.

The effect of Technosol application was not reflected in the total PHEs concentrations in underlying polluted soils (R1–R6) but was visible in their solubility and bioavailability (Table 4.5). Especially noteworthy were the large reductions in Cd, Cu, and Zn solubility, as well as the significant increase in the solubility of Sb. However, the effects on bioavailability were not significant for most PHEs. The soluble As concentration decreased in all underlying soils compared to PS, reaching in R2, R3, R4, and R5 values very similar to US. The bioavailable As showed no significant differences between the treatments and PS except for R2 and R5, where it almost doubled its concentration. Technosol treatments of polluted soils were very effective for Cd, Cu, and Zn, reducing their solubility. There was also a significant reduction in the bioavailable fraction of these three PHEs in the treatments, which was more effective in the case of Zn, where the reduction exceeded 50% in all cases. The opposite was observed for Sb in the underlying polluted soils, where both the soluble and bioavailable fractions increased significantly with respect to PS. However, the increase in the soluble Sb was more pronounced than for the bioavailable fraction, exceeding 3–7 times the soluble concentration in PS, whereas the increase in the bioavailable fraction was not more than twice of that in PS. The soluble Pb concentrations were below the detection limit, and in the bioavailable fraction in PS, it was very low (0.01 mg kg^{-1}), although with Technosols treatment, it increased slightly but without statistical significance.

Table 4.4. *Physicochemical properties of polluted soil (PS), unpolluted soil (US), and polluted soils treated (covered) with each Technosol (R1–R6) (mean ± SD; n = 6).*

	PS	**US**	***Polluted soils treated with each Technosol***					
			R1	**R2**	**R3**	**R4**	**R5**	**R6**
pH (H_2O)	3.53 ± 0.03 a	6.91 ± 0.08 e	6.51 ± 0.21 d	6.41 ± 0.18 d	5.47 ± 0.47 b	6.09 ± 0.21 cd	5.90 ± 0.35 bc	6.08 ± 0.25 cd
EC (dS m^{-1})	2.70 ± 0.27 bcd	0.05 ± 0.01 a	2.72 ± 0.07 cd	2.49 ± 0.08 b	2.54 ± 0.03 bc	2.72 ± 0.05 cd	2.59 ± 0.12 bcd	2.78 ± 0.14 d
OC (%)	0.42 ± 0.11 a	2.24 ± 0.14 c	1.03 ± 0.25 b	0.98 ± 0.12 b	0.77 ± 0.09 b	0.96 ± 0.07 b	0.62 ± 0.26 ab	0.51 ± 0.05 a
$CaCO_3$ (%)	0.39 ± 0.07 a	2.66 ± 0.05 c	0.40 ± 0.08 a	0.58 ± 0.26 a	0.66 ± 0.15 a	1.19 ± 0.25 b	1.18 ± 0.22 b	1.26 ± 0.29 b
N_T (%)	0.11 ± 0.01 a	0.20 ± 0.02 b	0.10 ± 0.01 a	0.11 ± 0.01 a	0.09 ± 0.01 a	0.11 ± 0.05 a	0.13 ± 0.01 a	0.10 ± 0.01 a
C_T (%)	0.48 ± 0.11 a	2.50 ± 0.19 e	1.22 ± 0.12 d	1.18 ± 0.05 cd	0.98 ± 0.04 bc	1.36 ± 0.06 cd	0.90 ± 0.12 d	0.72 ± 0.05 bc

EC – electrical conductivity; OC – organic carbon; $CaCO_3$ – calcium carbonate; N_T/C_T – total N and C. Letters represent significant differences among treatments (Kruskal-Wallis and Mann-Whitney U tests; $p < 0.05$).

4.2.2. GERMINATION AND GROWTH OF TRIFOLIUM CAMPESTRE

Treatment of PHE-polluted soil with Technosols (T1R1–T6R6) stimulated the growth of *T. campestre*, compared to no germination of this plant when sown in polluted soil (PS) (Figure 4.1). However, not all Technosols exhibited the same response. The germination rate in treatments with vermicompost-based Technosols (T3R3 and T6R6) was 50%, similar to that in US (57%), whereas in the others, it was much lower (T1R1: 18%; T2R2: 19%; T4R4: 17%; T5R5: 21%). The survival rate, expressed as the number of *T. campestre* plants established after 10 weeks, showed a similar trend to the germination rate, but with lower values, as some of the germinated seeds died during this time (Figures 4.1 and 4.2).

Table 4.5. *Total, water-soluble, and EDTA-extracted bioavailable concentrations of PHEs in polluted soil (PS), unpolluted soil (US), and polluted soils treated (covered) with each Technosol (R1–R6) (mean ± SD; n = 6).*

		PS	**US**	***Polluted soils treated with each Technosol***					
				R1	**R2**	**R3**	**R4**	**R5**	**R6**
Total mg kg^{-1}	**As**	345.66 ± 25.29 b	25.02 ± 2.71 a	346.58 ± 13.46 b	359.32 ± 13.24 b	361.52 ± 7.39 b	345.69 ± 38.61 b	356.70 ± 10.15 b	347.20 ± 16.39 b
	Cu	106.80 ± 5.24 b	37.72 ± 2.00 a	101.06 ± 3.58 b	105.77 ± 5.10 b	105.48 ± 11.42 b	100.88 ± 13.43 b	101.59 ± 8.69 b	105.93 ± 8.28 b
	Pb	640.17 ± 48.69 b	79.17 ± 10.76 a	606.36 ± 15.42 b	615.74 ± 34.93 b	628.73 ± 16.95 b	597.00 ± 57.24 b	612.49 ± 17.27 b	596.33 ± 24.51 b
	Sb	20.83 ± 5.10 a	bdl	24.11 ± 3.81 a	21.30 ± 6.32 a	19.98 ± 7.75 a	19.49 ± 6.57 a	22.16 ± 3.72 a	22.78 ± 5.40 a
	Zn	185.09 ± 6.46 b	138.13 ± 1.54 a	172.37 ± 7.15 b	183.44 ± 5.95 b	184.38 ± 6.27 b	186.71 ± 8.09 b	183.12 ± 3.80 b	177.49 ± 4.97 b
Water-soluble µg kg^{-1}	**As**	8.91 ± 1.41 a	4.50 ± 2.86 a	7.39 ± 2.16 a	4.84 ± 0.51 a	4.87 ± 1.56 a	4.42 ± 1.45 a	4.51 ± 0.73 a	5.15 ± 2.68 a
	Cd	213.89 ± 42.71 c	0.94 ± 0.63 a	3.07 ± 1.55 a	11.09 ± 1.50 ab	37.58 ± 9.91 b	7.43 ± 2.41 a	6.67 ± 2.81 a	6.35 ± 2.45 a
	Cu	940.79 ± 105.12 c	14.60 ± 4.10 a	73.25 ± 8.76 ab	127.01 ± 14.35 b	52.76 ± 10.53 ab	39.65 ± 1.29 ab	114.72 ± 13.68 b	34.36 ± 7.31 ab
	Sb	27.01 ± 1.65 a	19.69 ± 5.17 a	188.50 ± 50.49 c	105.24 ± 22.39 b	76.07 ± 23.53 ab	89.48 ± 19.78 b	99.24 ± 24.69 b	93.91 ± 4.95 b
	Zn	11,992.3 ± 1652.7 b	21.04 ± 6.96 a	44.60 ± 32.18 a	425.03 ± 229.73 a	1766.10 ± 802.83 a	214.53 ± 116.85 a	186.36 ± 94.10 a	60.55 ± 26.82 a
EDTA-extracted mg kg^{-1}	**As**	0.18 ± 0.03 bc	0.11 ± 0.02 a	0.22 ± 0.02 c	0.37 ± 0.04 d	0.21 ± <0.01 c	0.14 ± 0.01 ab	0.34 ± 0.05 d	0.14 ± 0.01 ab
	Cd	0.30 ± 0.09 bc	0.32 ± 0.10 c	0.14 ± 0.04 a	0.16 ± 0.02 ab	0.18 ± 0.01 ab	0.13 ± 0.01 a	0.13 ± 0.02 a	0.20 ± 0.03 abc
	Cu	5.16 ± 0.37 c	2.09 ± 0.07 a	3.62 ± 0.20 b	3.70 ± 0.71 b	4.20 ± 0.06 b	4.14 ± 0.12 b	3.31 ± 0.07 b	3.63 ± 0.46 b
	Pb	0.01 ± <0.01 a	6.56 ± 0.18 b	0.02 ± <0.01 a	0.08 ± 0.03 a	0.02 ± <0.01 a	0.03 ± 0.01 a	0.13 ± 0.04 a	0.03 ± <0.01 a
	Sb	0.13 ± 0.02 a	0.22 ± 0.02 b	0.26 ± 0.03 b	0.24 ± 0.02 b	0.20 ± 0.02 b	0.22 ± 0.01 b	0.23 ± 0.04 b	0.20 ± 0.03 b
	Zn	12.99 ± 4.63 b	7.70 ± 3.64 ab	4.16 ± 1.70 a	5.09 ± 0.89 a	5.14 ± 0.45 a	3.05 ± 0.38 a	3.66 ± 0.78 a	5.23 ± 0.85 a

Cd$_T$: < 10 mg kg^{-1} (detection limit XRF), Pb$_W$: below detection limit (bdl). Letters represent significant differences among treatments (Kruskal–Wallis and Mann–Whitney U tests; $p < 0.05$).

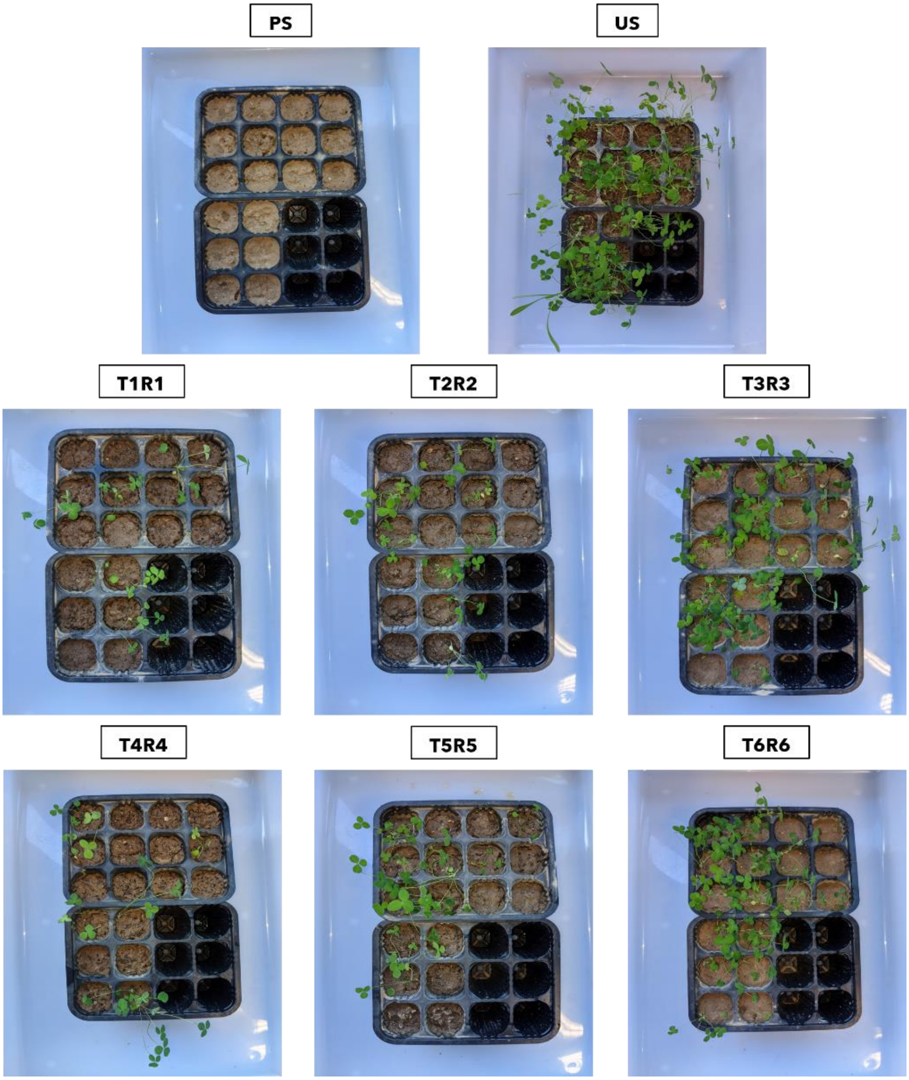

Figure 4.1. *General appearance of T. campestre plants after two months of growth in polluted soil (PS), unpolluted soil (US), and treatments consisting of the application of each Technosol on top of polluted soil (T1R1–T6R6) (n = 6).*

The dry biomass of *T. campestre* in soil treatments showed differences with respect to US, which had the highest mean weight (Figure 4.2). Among the treatments, the highest biomass production was in T3R3 and T6R6, reaching about half of that in US. In the remaining treatments (T1R1, T2R2, T4R4, and T5R5), the biomass production was significantly lower with no statistical differences among them. The soil plant analysis development (SPAD) index of *T. campestre* was higher in all treatments than in US, although with a slight variability among them (Figure 4.2); indeed, only in T1R1 and T3R3 it was statistically higher to US.

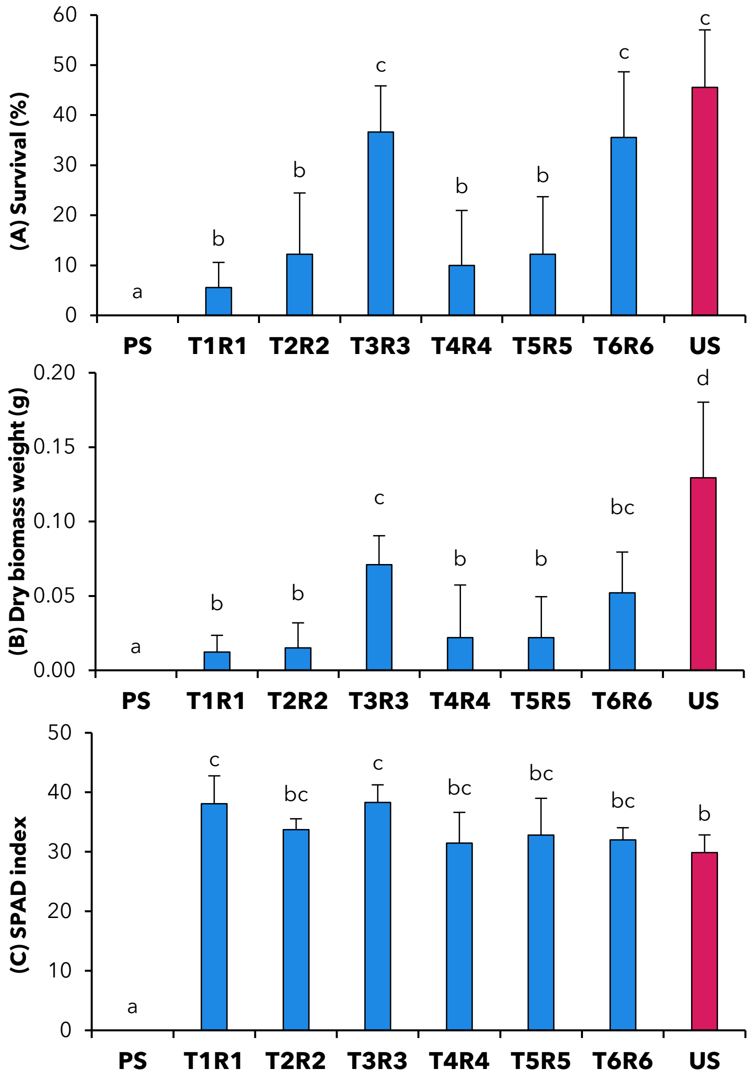

Figure 4.2. *Survival (A), dry biomass weight (B), and soil plant analysis development (SPAD) index (C) of T. campestre plants grown in polluted soil (PS), unpolluted soil (US), and treatments consisting of the application of each Technosol on top of polluted soil (T1R1–T6R6) (n = 6).* Letters represent significant differences among treatments (Kruskal–Wallis and Mann–Whitney U tests; $p < 0.05$).

4.2.3. TOXICITY BIOASSAY WITH LACTUCA SATIVA

The germination rate of *L. sativa* seeds from PS was significantly lower (<50%) than in US (>90%). Likewise, root elongation in PS was also much lower than that in US by about 7.5 times. Both low germination and elongation of *L. sativa* in PS were positively stimulated in Technosols (T1–T6). The germination rate in T2 and T5 remained at values close to those in PS, whereas in the other Technosols, it improved to rates similar to those in US. However, these differences among Technosols were not observed for elongation, which was enhanced in all, reaching, in most of them, the elongation in US. Indeed, although not statistically significant, the elongation rates in T1 and T4 were slightly higher than that in US (Figure 4.3).

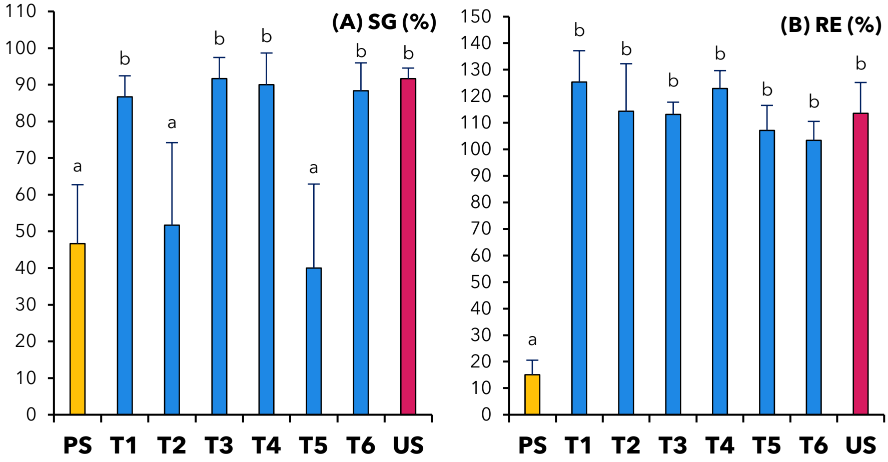

Figure 4.3. *Seed germination [SG] (A) and root elongation [RE] (B) of L. sativa in polluted soil (PS), unpolluted soil (US), and six designed Technosols (T1–T6) (n = 6).* Letters represent significant differences among treatments (Kruskal–Wallis and Mann–Whitney U tests; $p < 0.05$).

Likewise, in the underlying polluted soils (R1–R6), both the germination and elongation rates of *L. sativa* were improved (Figure 4.4). Germination rates ranged from 63% to 92%, with the lowest germination rates in R5, and the highest in R2. The increase in elongation was even more considerable, with an elongation in all of them similar to that in the distilled water control (100%). The maximum elongation was found in R1, which was even higher than that in US, and the minimum elongation was found in R3, although this minimum was at least six times higher than that in PS.

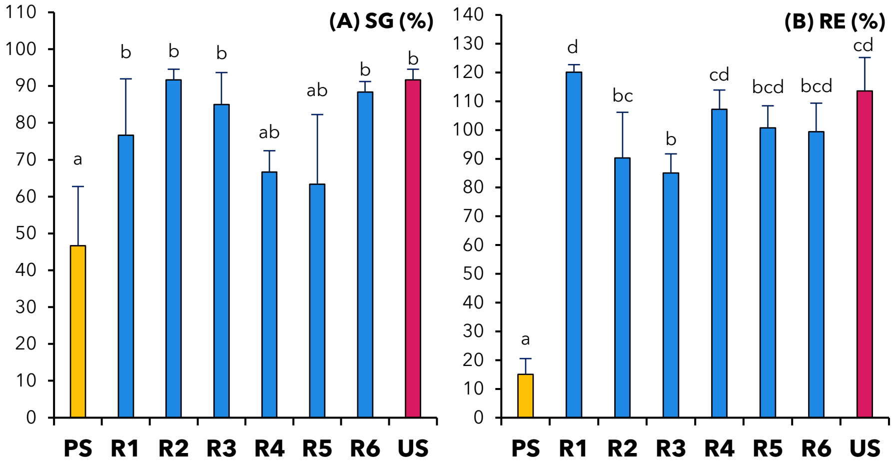

Figure 4.4. *Seed germination [SG] (A) and root elongation [RE] (B) of L. sativa in polluted soil (PS), unpolluted soil (US), and polluted soils treated by surface application of each Technosol (R1–R6) (n = 6).* Letters represent significant differences among treatments (Kruskal–Wallis and Mann–Whitney U tests; $p < 0.05$).

4.2.4. SOIL ENZYMATIC ACTIVITIES

The PHE-polluted soil showed low microbiological activity as assessed via enzymatic activities (Figure 4.5). Dehydrogenase activity, which is widely used as an index of the overall microbial activity, was very low in PS, while in US, it was 23 times higher. C-cycle-related enzymes (β-glucosidase and cellulase) were negligible in PS, as opposed to having almost 100-fold higher values for β-glucosidase and 15-fold higher values for cellulase in US. The acid phosphatase activity was also reduced by half in PS compared to US.

Technosols improved soil properties in relation to PS, notably the increase in OC, which can stimulate soil microbiological activity. The increase in dehydrogenase activity was pronounced, especially in Technosols composed of sewage sludge (T2 and T5) and those composed of solid olive mill by-product (T1 and T4), reaching values above 100 μg TPF g^{-1} in 16 h^{-1}, while in vermicompost-derived Technosols (T3 and T6), values were about 27 and 11 μg TPF g^{-1} in 16 h^{-1}, respectively. Regarding the rest of the enzymatic activities, improvements were also observed in Technosols with respect to PS, but with a large variability among them. In contrast to dehydrogenase activity, the highest β-glucosidase value was found in

Technosols composed of vermicompost (T3 and T6, 0.8–0.9 p-nitrophenol g^{-1} h^{-1}), whereas it was less than half in the rest. As for cellulase, the variability was so high in all cases that there were no significant differences among Technosols. However, T4 had the lowest activity, whereas T2 had the highest (0.23 vs. 0.69 µmol glucose g^{-1} 16 h^{-1}, respectively). Acid phosphatase activity was more than double in US than in PS, and in T1, T4, and T6 was even higher than in US.

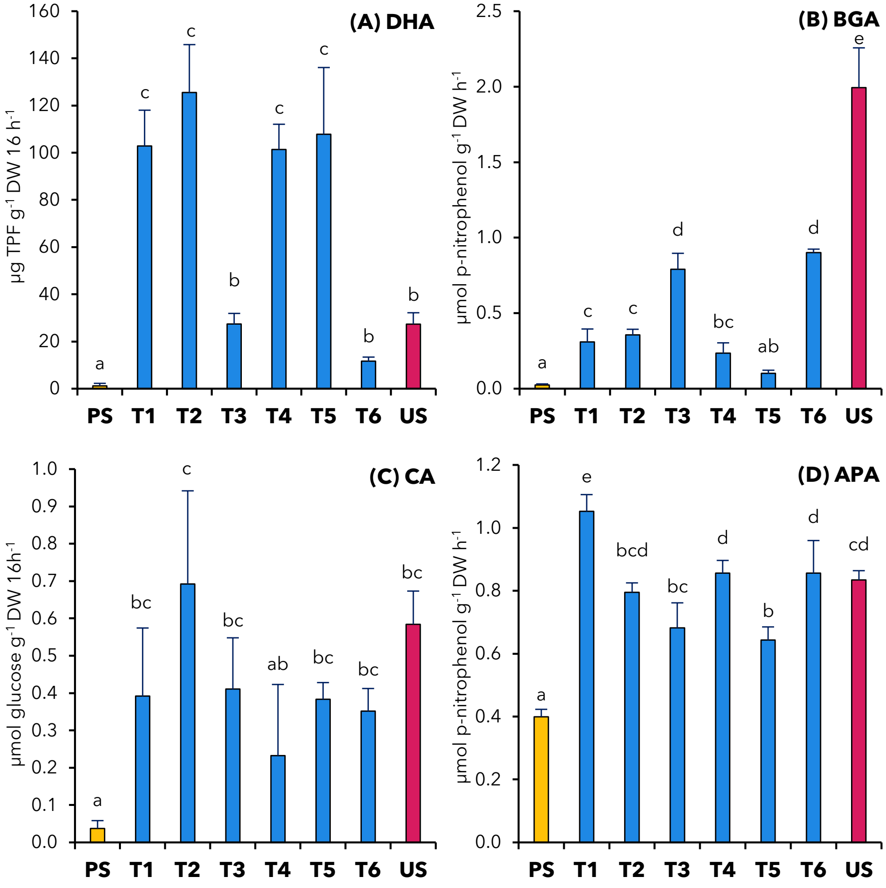

Figure 4.5. *Enzymatic activities [DHA: dehydrogenase (A), BGA: β-glucosidase (B), CA: cellulase (C), APA: acid phosphatase (D)] in polluted soil (PS), unpolluted soil (US), and six designed Technosols (T1-T6) (n = 6).* Letters represent significant differences among treatments (Kruskal–Wallis and Mann–Whitney U tests; $p < 0.05$).

In contrast to Technosols, microbiological activity was not recovered in underlying polluted soils, showing a marked decrease in dehydrogenase activity compared to Technosols (Figure 4.6). Little or no significant changes occurred in any of the studied enzymes (dehydrogenase, β-glucosidase, acid phosphatase, and cellulase) in treated soils (R1–R6) compared to the baseline conditions of PS. The same pattern of variability of dehydrogenase and β-glucosidase activities in Technosols (T1–T6) was repeated in underlying polluted soil (R1–R6). The lowest dehydrogenase and the highest β-glucosidase values were measured in R3 and R6, although these differences were not statistically significant in either case.

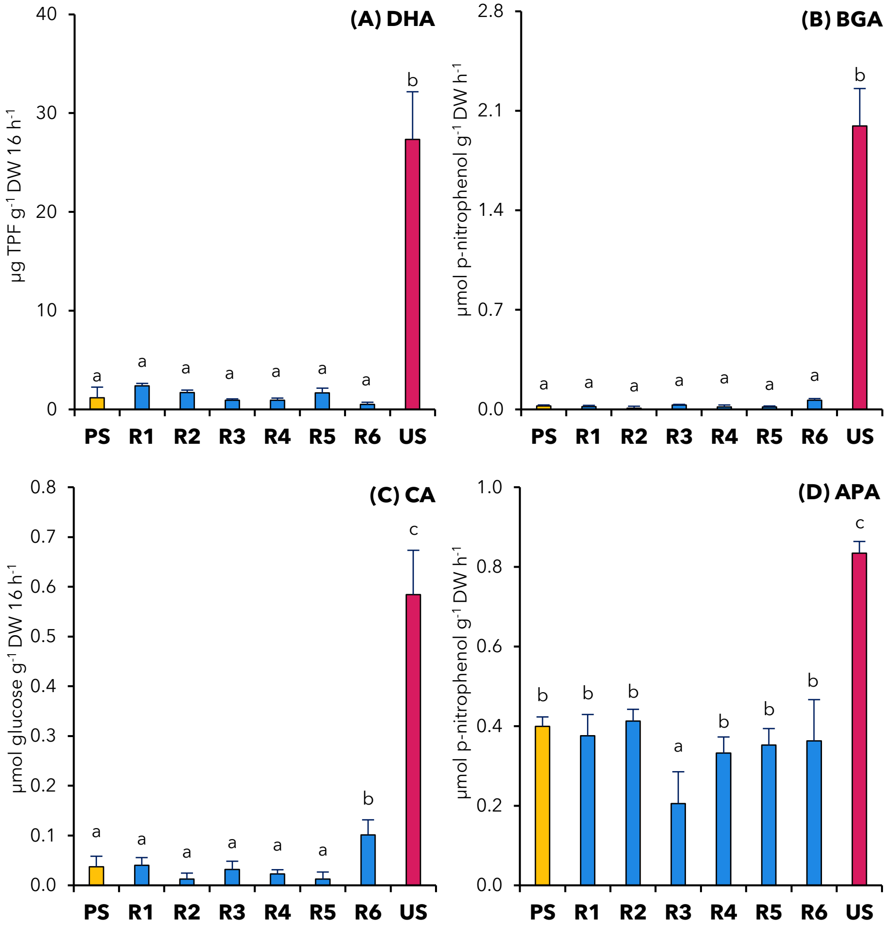

Figure 4.6. *Enzymatic activities [DHA: dehydrogenase (A), BGA: β-glucosidase (B), CA: cellulase (C), APA: acid phosphatase (D)] in polluted soil (PS), unpolluted soil (US), and polluted soils treated by surface application of each Technosol (R1–R6) (n = 6).* Letters represent significant differences among treatments (Kruskal-Wallis and Mann-Whitney U tests; $p < 0.05$).

4.3. DISCUSSION

Total soil concentrations of As and Pb in polluted soil exceed the regulatory levels in Andalusia (Spain) of 36 mg kg^{-1} and 275 mg kg^{-1} by 9.6 and 2.3 times (BOJA 2015), respectively. However, these total concentrations do not raise much concern because the risk of leaching of these two elements is very low due to their low solubility (García-Carmona et al. 2019a) and low precipitation in the area (mean annual precipitation of 490 mm). On the contrary, Cd, Cu, and Zn, with total concentrations not exceeding the regulatory values, showed concentrations high enough to potentially cause toxicity (Guo et al. 2011; Noulas et al. 2018; Dutta et al. 2021), especially in the water-soluble fraction, which is the most readily accessible to living soil organisms. The bioavailable fraction corresponds to the PHEs contained in the non-silicate-bound soil phases (carbonates and Fe-/Al- oxides), reflecting their availability both in the short-term and relatively long-term (Nenova et al. 2018). Thus, both the soluble and bioavailable fractions constitute the part of PHEs in soil that can be transferred to organisms and thus cause damage to the ecosystem and/or enter the trophic chain (Son et al. 2019).

A principal component analysis was conducted to determine the ecotoxicological implications of the changes caused in PHE-polluted soil after treatment with Technosols. This analysis generated three components that grouped all considered variables (soil properties, PHEs concentrations in different fractions, soil enzymatic activities, and bioassay endpoints of *T. campestre* and *L. sativa*), explaining 81.44% of the variance (Table S4.1). Component 1 explained 39.76% of the variance and included variables related to potential toxicity (mainly pH, $CaCO_3$ content, and water-soluble and EDTA-bioavailable concentrations of most PHEs) along with root elongation of *L. sativa* (RE). A direct (positive) relationship between pH and RE, as well as an inverse (negative) relationship between pH and solubility and bioavailability of most PHEs (with the exception of As and Sb) were observed. Component 2, explaining 25.85% of the variance, grouped EC with total concentrations of most PHEs and water-soluble concentrations of Cd, Cu, and Zn in a direct relationship; in contrast, EC was inversely related to C-cycle enzymes (β-glucosidase and cellulase), biomass and survival of *T. campestre*, and root elongation of *L. sativa*. Component 3 explained 15.83% of the variance and showed that soil OC is directly related to total N and C, and also to dehydrogenase activity.

In this sense, although the total concentrations of PHEs decreased significantly in Technosols due to the dilution effect, they still exceeded the regulatory levels for As and Pb. However, the solubility and bioavailability of PHEs strongly changed in Technosols (T1–T6) and underlying polluted soils (R1–R6). The high acidity of polluted soil, caused by the formation of sulphuric acid in the soil matrix from the oxidation of remaining pyritic sludge (Simón et al. 2005), was neutralised in Technosols via the reaction with carbonates provided by carbonate-rich wastes (MS and CW) (Cravotta and Trahan 1999). In underlying polluted soils, the leachates coming from Technosols increased pH by 2 to 3 units in relation to PS, raising the values to near neutrality. The oxidation of pyritic sludge also leads to the formation of soluble sulphates that increase EC in PS (Martín et al. 2008). The increases in acidity and salinity due to the oxidation of sulphides are correlated with the higher solubility of more mobile PHEs such as Cd, Cu, and Zn (Martínez and Motto 2000; García et al. 2009; Ivezić et al. 2012; Romero-Freire et al. 2016) with respect to the less soluble PHEs (As, Pb, and Sb) (Table S4.1). In this sense, the most mobile PHEs are significantly reduced in both Technosols and underlying polluted soils with respect to PS, but in the underlying polluted soil, these soluble concentrations are higher due to potential leaching effects, coinciding with previous observations under natural conditions (Simón et al. 2008).

In contrast, other less soluble PHEs, like As and Sb, have different behaviours. Arsenic can be desorbed by the competition between arsenates (AsO_4^{3-}) and organic matter for the adsorption positions of iron oxides (Redman et al. 2002; Fleming et al. 2013); thus, the As resolubilisation that occurred in Technosols with a significantly higher OC content was not produced in underlying polluted soils. In addition, the influence of pH on As mobility is a key factor. In our case, a lower adsorption of As was observed when the pH increased, and this was attributable to the more negatively charged arsenate species repulsing the anion exchange sites (Simón et al. 2005). Otherwise, under the irrigation conditions used in our experiment, a reductive dissolution of iron oxides (e.g. ferrihydrite) may result in the substantial mobilisation of As in a pH range between 6 and 8 (Burton et al. 2011).

Similar to As, Sb solubility is also strongly controlled by pH and soil organic matter (SOM) content (Table S4.1), so Sb solubilisation/desorption with decreasing acidity and increasing SOM content may be related to competition in the formation of Fe-SOM-Sb complexes (Nakamaru and Martín Peinado 2017).

Likewise, both in Technosols (T1–T6) and underlying polluted soils (R1–R6), decreases in Cd, Cu, and Zn bioavailable fractions were observed in relation to PS, possibly due to the increase in pH and the presence of $CaCO_3$ leached from carbonated wastes (CW and MS). These elements in acidic soils are usually adsorbed in non-specific forms and, therefore, in reversible processes, while in neutral or alkaline soil, they can be specifically adsorbed or even occluded by iron oxides and hydroxides (Reed and Martens 1996; Bradl 2004), or co-precipitated as an iron-sulphate and hydroxysulfate complex (Simón et al. 2010), which are considered as less reversible forms. However, As and Sb bioavailable fractions were not reduced with respect to initial polluted soil, neither in Technosols nor in underlying polluted soils. This is possibly related to the formation of organic–metallic complexes, which could lead to an increase in the availability of these PHEs when pH rises (Wang and Mulligan 2009; Nakamaru and Martín Peinado 2017).

The microcosm assay with *Trifolium campestre* Schreb. evaluated the toxic effect directly from the soil fraction (Graziano et al. 2022). This plant was selected for its capacity to cope with soil pollution and its spontaneous presence in the study area of the GGC (García-Carmona et al. 2019a). Based on this assay, it is inferred that the use of Technosols on polluted soils (T1R1–T6R6) can also be considered to decrease the ecotoxicological risk in the area, as it allows for optimal development of *T. campestre*. Growth stimulation of *T. campestre* was significant in all treatments, with a high survival rate, biomass, and SPAD index compared to PS. These results are consistent with those reported for other plant species (e.g., *Eucalyptus globulus* Labill., *Cistus ladanifer* L., *Dactylis glomerata* L., *E. australis*, and *Lablab purpureus* (L.) Sweet) grown in different Technosols composed of mining waste/polluted soils, either in field or greenhouse assays (de Varennes et al. 2010; Macías et al. 2011; Santos et al. 2014b; Aguilar-Garrido et al. 2023b). Plant growth can be related to the improvement of some soil properties (i.e. pH neutralisation and increases in OC, $CaCO_3$, and N_T), microbiological activity, and decreases in the solubility and bioavailability of most PHEs both in Technosols (T1–T6) and in underlying polluted soil (R1–R6) (Santos et al. 2019). However, treatments T3R3 and T6R6, in which Technosols composed of pruning and gardening vermicompost were used, showed the best performance, indicating that the nature of organic waste influences the capacity to develop a vegetation cover (Larney and Angers 2012; Khan et al. 2018; Soria et al. 2021).

In general, all treatments had a similar response in the toxicity bioassay with *L. sativa*, with SG and RE values similar to those of US (except RE at T2 and T5). The higher toxicity at T2 and T5, represented by a low germination, may be related to the type of organic waste used; in this case, sewage sludge, despite being widely used as an amendment, usually contains a complex mixture of pollutants (e.g. heavy metals and emerging organic pollutants such as pharmaceuticals) that can be harmful to organisms (da Silva Souza et al. 2020).

The effectiveness of the remediation process was also assessed by determining soil enzymatic activities, which are widely used as biological indicators that reflect soil functional diversity, changes in microbial community composition, and microbial status (Kumar et al. 2013). In this sense, Technosols improved soil quality and largely neutralised PHE pollution, strongly stimulating microbiological activity, indicating the good performance of all microbial communities involved in organic matter degradation, mineralisation processes, and nutrient cycling (Arán et al. 2022); however, this improvement in enzymatic activity did not occur in underlying polluted soils, indicating that the changes in some physicochemical properties and the time elapsed during the experiment were not sufficient to promote microbial activity in treated soils.

The addition of organic and inorganic amendments to degraded environments such as these polluted soils leads to a boost in soil microorganism activity, which is evaluated via dehydrogenase activity (Santos et al. 2014b). In this sense, the increase in OC content in Technosols was much greater than that in underlying polluted soils, which is directly related to dehydrogenase activity (Table S4.1). This is in agreement with several studies (Kumar et al. 2013; Santos et al. 2014b; Santos et al. 2016a) that reported that an increase in OC content due to organic amendment addition is a key driver of microbiological activity. However, it is not only the OC content that determines the biological activity but also the different nature of organic matter (Pascual et al. 1998; Kizilkaya 2008; Zhang et al. 2021). Thus, while T3 and T6 were the Technosols with the lowest OC contents and dehydrogenase activities, they had the highest β-glucosidase activity of all tested Technosols, which may be because the pruning and gardening vermicompost has a higher proportion of more easily decomposable compounds (e.g. hemicellulose and cellulose) than more resistant ones (e.g. lignin) compared to the other organic wastes used (Hadas et al. 2004).

4.4. CONCLUSIONS

Soils with persistent pollution in the GGC (more than 25 years after the Aznalcóllar mine accident) constitute degraded environments characterised by high acidity and salinity, low fertility, and high concentrations of PHEs in total, soluble, and bioavailable fractions. These conditions cause the absence of vegetation and low soil microbiological activity. Thus, this study can provide valuable information for the assessment of remediation solutions for PHE-polluted soils, based on the ecotechnology of Technosols, and then serve as a model for consultation.

The six Technosols constructed by mixing polluted soil with a combination of organic and inorganic wastes from local industries (mines, urban gardening services, wastewater treatment services, and olive mills) were effective in soil remediation over the time span tested (two months) under growth chamber conditions. In general, all Technosols improved the unfavourable conditions of polluted soils (by neutralising acidity and increasing OC), reduced the solubility/bioavailability of most PHEs (with the exception of As and Sb, and Pb in some Technosols), and strongly stimulated soil enzymatic activity and the growth of *T. campestre* and *L. sativa* by reducing potential toxicity. In particular, Technosols composed of pruning and gardening vermicompost (T3 and T6) showed the best overall response, whereas those composed of sewage sludge (T2 and T5) showed the worst performance, especially in terms of toxicity reduction, as evaluated using bioassays with *T. campestre* and *L. sativa*. This highlights the importance of the organic matter nature in the organic wastes used in Technosols, as it greatly influences the improvement in soil conditions, PHEs mobility, microbiological activity, and, thus, its capacity to develop a vegetation cover. Technosols also improved the physicochemical properties and reduced the mobility of most PHEs in the underlying polluted soils, although the biological activity evaluated via enzymatic activity was not significantly modified over the time of the experiment. Thus, it was shown that these Technosols can constitute a potential solution for the remediation of persistent polluted soils; nevertheless, they should be applied and assessed in large-scale and long-term interventions to reinforce their feasibility as a cost-effective ecotechnology.


WARNING - DO NOT DEFEAT INTERLOCKS
ATTENTION - NE PAS TRABIQUER LA SERRURE DE SÉCURITÉ
PLASMA N0810275
SHEAR GAS N0810277
Only use
Glassware
NEB

Detail of the detectors, torch nebuliser, and peristaltic pump of PerkinElmer Avio® 500 ICP-OES, Department of Soil Science - UGR

Granada, Spain

February 2024

Antonio Aguilar Garrido

CHAPTER 5

ASSESSMENT OF THE CRITICAL LOAD OF WASTE-DERIVED TECHNOSOLS FOR ACID MINE DRAINAGE CONTROL

Aguilar-Garrido A., Mérida-Sánchez, L., Martín-Peinado, F. J. (no date). Engineered soils constructed from polluted soils and non-hazardous wastes for the control of acid mine drainage. (In preparation to submit to JCR journals)

ABSTRACT

The generation of acid mine drainage (AMD) is a potentially serious environmental problem that needs to be adequately addressed. Novel strategies for its treatment involve the use of waste. In this line, this study evaluates the potential of ecotechnology based on Technosols composed of polluted soil and various non-hazardous wastes, specifically designed for the remediation of water affected by acidity and the presence of PHEs. Samples of six Technosols (T1, T2, T3, T4, T5, and T6), along with polluted soil (PS) and unpolluted soil (US) as controls, were spiked with increasing volumes of an artificial AMD (i.e. to increasing acidity and PHEs concentrations). The pH and PHEs concentrations in the leachates – treated AMD - were then measured to determine acid neutralisation capacity, retention effectiveness of PHEs, and critical load of these soils. The artificial AMD obtained from oxidation of tailings from the Aznalcóllar accident showed an ultra-acid character (pH ~ 2.2), extremely high electrical conductivity (EC > 7 dS m^{-1}) and high concentrations of PHEs (i.e. As, Cd, Cu and Zn) exceeding the maximum regulatory levels in natural and irrigation waters. Retention effectiveness for As, Cd, and Cu of all Technosols were above 95% for all increasing volumes of AMD exposed, and for Zn it remained at these rates until exposure to very high acidity, where retention dropped slightly to 85-90%. Otherwise, US showed a higher retention of PHEs than PS but lower than Technosols (with the exception of As). In relation to the critical load of PHEs, all Technosols presented a very high value for As and high for Zn, although for Cd and Cu were more variable, with T3 and T6 presenting values above US, while in the other Technosols, some values are greater than PS but lower than US. Therefore, the studied Technosols have a very high acidity neutralisation capacity, a strong capacity to immobilise PHEs, and a variable critical load of PHEs. In this sense, the presence of PHEs with variable chemical behaviour must be controlled, as the presence of a continuous AMD can pose a medium to long-term risk due to the saturation of its capacity to retain certain PHEs and the loss of effectiveness as a protective barrier over time; so Technosols used in the control of AMD must be monitored regularly and corrective measures proposed to maintain their functionality over time.

5.1. BACKGROUND

As already stated in the background of **chapter 3** (see section 3.1), the generation of acid mine drainage (AMD) can constitute a major environmental problem that needs to be adequately addressed. In this context, to advance remediation strategies for AMD that lead to improved, cost-effective, and eco-friendly solutions (Kefeni et al. 2017), the most promising methods have focused on the use of low-cost amendments, e.g., using waste from different human activities, to address the negative impacts of AMD and connect with the circular economy strategy (Lèbre et al. 2017; Tayebi-Khorami et al. 2019). For this reason, **chapter 3** assessed the potential use of inorganic and organic wastes from the main waste-generating activities (urban, mining, and agro-industrial) for treating AMD.

In this chapter, we take a step further with the use of the ecotechnology based on Technosols composed of polluted soil and various non-hazardous wastes, which have been specifically designed and produced for the remediation of both soils and water affected by acidity and the presence of PHEs (see section 1.2.3). The different Technosols (T1, T2, T3, T4, T5, and T6), as well as polluted soil (PS) and unpolluted soil (US), were exposed to increasing volumes of an artificial AMD, i.e., to increasing acidity and PHEs concentrations (see section 2.2.1 for details), in order to fulfil the specific **objective** of assessing the capacity to neutralise acidity and retain PHEs of the six Technosols designed in the thesis. In this way, the critical load of Technosols is also assessed, which represents "the maximum quantity of a given contaminant that can be supplied to a soil without causing chemical changes leading to long-term harmful effects on ecosystem structure and function" (Hettelingh et al. 1991) and is relevant for the evaluation of the long-term capacity of Technosols to cope with pollution.

For detailed information on the methodology applied according to previous studies (Simón et al. 2005; Aguilar-Garrido et al. 2022a), consult section 2.3.1.2. Details on the production of these Technosols can also be found in section 2.2.5. Furthermore, a characterisation including soil properties and concentrations of PHEs in the different fractions (total, soluble and bioavailable) of both Technosols and control soils (polluted and unpolluted) is available in section 4.2.1.

5.2. RESULTS AND DISCUSSION

5.2.1. CHARACTERISATION OF ACID MINE DRAINAGE

The artificial AMD prepared by oxidation of the toxic tailing dumped in the Aznalcóllar mine accident showed both an ultra-acidic pH$_{(L)}$ (2.22 ± < 0.01) and an extremely high EC$_{(L)}$ (7.24 ± 0.04 dS m^{-1}), together with high concentrations of several PHEs (Table 5.1); typical characteristics of all AMD worldwide (Akcil and Koldas 2006). In this artificial AMD, of the PHEs measured by ICP-OES, it was found that As, Cu, Fe, and Zn were above 10 mg l^{-1}, and below were Cd, Pb, Sb and V; exceeding the guideline values established by different legislations for As, Cd, Cu, V and Zn (Table 5.1).

Table 5.1. *Comparison of PHEs concentrations in the artificial AMD (mean ± SD, n = 3) with maximum regulatory levels in natural and reclaimed water and with PHEs levels in the acidic mine water discharged in the Aznalcóllar disaster in mg l^{-1}.*

PHEs	**AMD concentration** (mg l^{-1})	**Regulatory level** (mg l^{-1})			**Concentration in acidic mine water of Aznalcóllar spill** [4] (mg l^{-1})
		Natural water in Spain [1]	**Reclaimed water for irrigation** **Spain** [2]	**Reclaimed water for irrigation** **USA** [3]	
As	**26.07 ± 2.37**	0.05	0.1	0.1	2.32 ± 1.34 (1.74 – 4.30) *
Cd	**0.70 ± 0.01**	-	0.01	0.01	0.65 ± 0.06 (0.64 – 0.75)
Cu	**16.44 ± 1.54**	0.12	0.2	0.2	0.30 ± 0.08 (0.24 – 0.40)
Fe	1512.80 ± 137.61	-	-	-	-
Pb	1.98 ± 0.02	-	-	5	2.15 ± 0.29 (2.05 - 2.60)
Sb	0.65 ± 0.01	-	-	-	2.51 ± 0.30 (2.40 – 2.96) *
V	**0.34 ± < 0.01**	-	0.1	0.1	0.32 ± 0.11 (0.28 – 0.49) *
Zn	**71.70 ± 6.52**	0.5	-	2	72.12 ± 1.81 (71.38 – 4.81)

[1] *Normas de calidad ambiental (NCA): Concentration of a PHE in water that must not be exceeded to protect human health and environment set by Spanish legislation in Annex V - RD 817/2015* (BOE 2015).

[2] *Maximum admissible value (VMA): The highest level of a PHE that is allowed in reclaimed water used for irrigation established by the Spanish legislation* (BOE 2007).

[3] *Recommended water quality criteria for irrigation: The highest level of a PHE that is allowed in reclaimed water used for irrigation established by the United States Environmental Protection Agency* (EPA 2012).

[4] *Concentrations of PHEs in the acidic mine water discharged in the Aznalcóllar disaster: median ± SD (minimum – maximum)* (Simón et al. 1999). ** in µg l^{-1}.*

Specifically, the concentrations of As, Cu, and Zn were 520, 137, and 143 times higher than the Spanish regulatory limits in natural surface waters to protect human health and the environment (BOE 2015). Similarly, compared to the maximum permissible levels in reclaimed water for irrigation purposes established by both Spanish legislation (BOE 2007) and the US Environmental Protection Agency (EPA 2012), the concentrations of As, Cd, Cu, V, and Zn exceeded them by 260, 70, 137, 3.4, and 35.8, respectively. On the other hand, in this artificial AMD, most of the PHEs were found at similar (Cd, Pb and Zn) and even much higher concentrations (As: 11,2237-fold, Cu: 55-fold, Sb: 259-fold, V: 106-fold) than those found in the acidic mine water discharged into the Agrio and Guadiamar river basins in the Aznalcóllar mine accident (Simón et al. 1999), as well as the concentrations in different AMD generated in similar sulphide mining areas around the world (Lottermoser 2010; Kefeni et al. 2017).

5.2.2. ACID NEUTRALISATION CAPACITY OF TECHNOSOLS

From the pH variations in the resulting leachates – AMD treated – after processing with Technosols, a sharp decrease (about 1.4-1.7 units pH) was observed in all Technosols with the first exposure to AMD, at an acidity of approximately 12 mmol H^+ kg^{-1}. Nevertheless, as the added acidity increased, $pH_{(L)}$ in leachates stabilised at slightly acidic values ($pH_{(L)}$ 6.1-6.4) in T1, T2 and T3 and at moderately acidic values ($pH_{(L)}$ 5.8-6) in T4, T5 and T6 (Figure 5.1 and Table S5.1); the only difference between these two groups of Technosols was the carbonate amendment used (T1, T2, T3: carbonated waste from peat bog mining [CW]; T4, T5, T6: sludge from marble cutting and polishing [MS]). Technosols before exposure to AMD had slightly–moderately alkaline values (pH 7.7-8.2) (Table 4.1), contrary to the extreme acidity (pH 3.5) of the polluted soil (PS), due to the reaction with carbonates provided by the carbonate-rich wastes (MS and CW) that confer to Technosols the pH buffering capacity (Cravotta and Trahan 1999; Heviánková et al. 2013). However, carbonates are not the only buffering components controlling pH; other constituents in Technosols (e.g. organic matter, exchangeable bases, Fe and Al oxy(hydr)oxides, silicates) have a relevant influence on this capacity (Nawaz et al. 2010; Bennardi et al. 2018). In this sense, CW actually constitutes a soil horizon containing, in addition to carbonates, silicates, organic matter, and Fe and Al oxy(hydr)oxides, and therefore has a higher pH buffering capacity than MS, which constitutes a waste and contains only carbonates (~99.9% $CaCO_3$) (Table 3.1); then T4, T5, and T6 have lower reserves to buffer pH than T1, T2, and T3.

After the sharp drop in $pH_{(L)}$ with the first contact with AMD, Technosols regulate $pH_{(L)}$ with silicates as the main buffering agents between pH 6 and 7.8 (Nawaz et al. 2010). However, as more acidity is added, these are destroyed due to the aggressiveness of AMD, reducing the buffering potential; between pH 5.5 and 6, exchangeable bases and organic matter are involved in maintaining $pH_{(L)}$ stable at 6 (Nawaz et al. 2010; Bennardi et al. 2018). Although $pH_{(L)}$ remained fairly stable, there was a slight decreasing trend until the addition of 90.38 mmol H^+ kg^{-1}, at which point there was a significant increase in $pH_{(L)}$ between 0.15 and 0.50 units in all Technosols leachates (Figure 5.1). This pH increase could be due to the formation of gypsum ($CaSO_4 \cdot 2\ H_2O$); since, when $CaCO_3$ is dissolved by the degradation resulting from AMD attack, Ca^{2+} is released, which sequesters H^+ from the medium, forming gypsum, which, being a sulphate, increases the pH regulation capacity in acid soils (Macías et al. 2005). This phenomenon was also studied in Martín et al. (2008), where this process was observed in the same pH range in soils attacked by an acid solution in the laboratory. Finally, when 120.51 mmol H^+ kg^{-1} was added, a significant reduction in pH was observed, although of a lesser magnitude than the previous increase.

As for polluted soil, the first acid solution added had a lower concentration (6.02 mmol H^+ kg^{-1}), due to the lower buffer capacity of this soil (Simón et al. 2005), and a severe decrease in $pH_{(L)}$ from 3.5 to 2.5 was observed, which continued to fall to $pH_{(L)}$ 2.2 with successive increases in the acidity added (Figure 5.1). For such low pH values, the soil constituents involved in acidity buffering are Fe and Al oxy(hydr)oxides. Therefore, as acidity continues to be added, these minerals are depleted, the maximum degree of soil degradation is reached, and the pH buffering capacity is completely lost (Nawaz et al. 2010). The same one-unit drop in $pH_{(L)}$ from neutral pH 6.9 (Table 4.1) occurred when unpolluted soil was exposed to AMD (6.02 mmol H^+ kg^{-1}), which managed to remain almost stable at a slightly acid $pH_{(L)}$ of 6 with higher acidities (12.05 and 24.10 mmol H^+ kg^{-1}). However, when 36.15 mmol H^+ kg^{-1} acidity were added, another decrease to strongly acid $pH_{(L)}$ 5.5 was

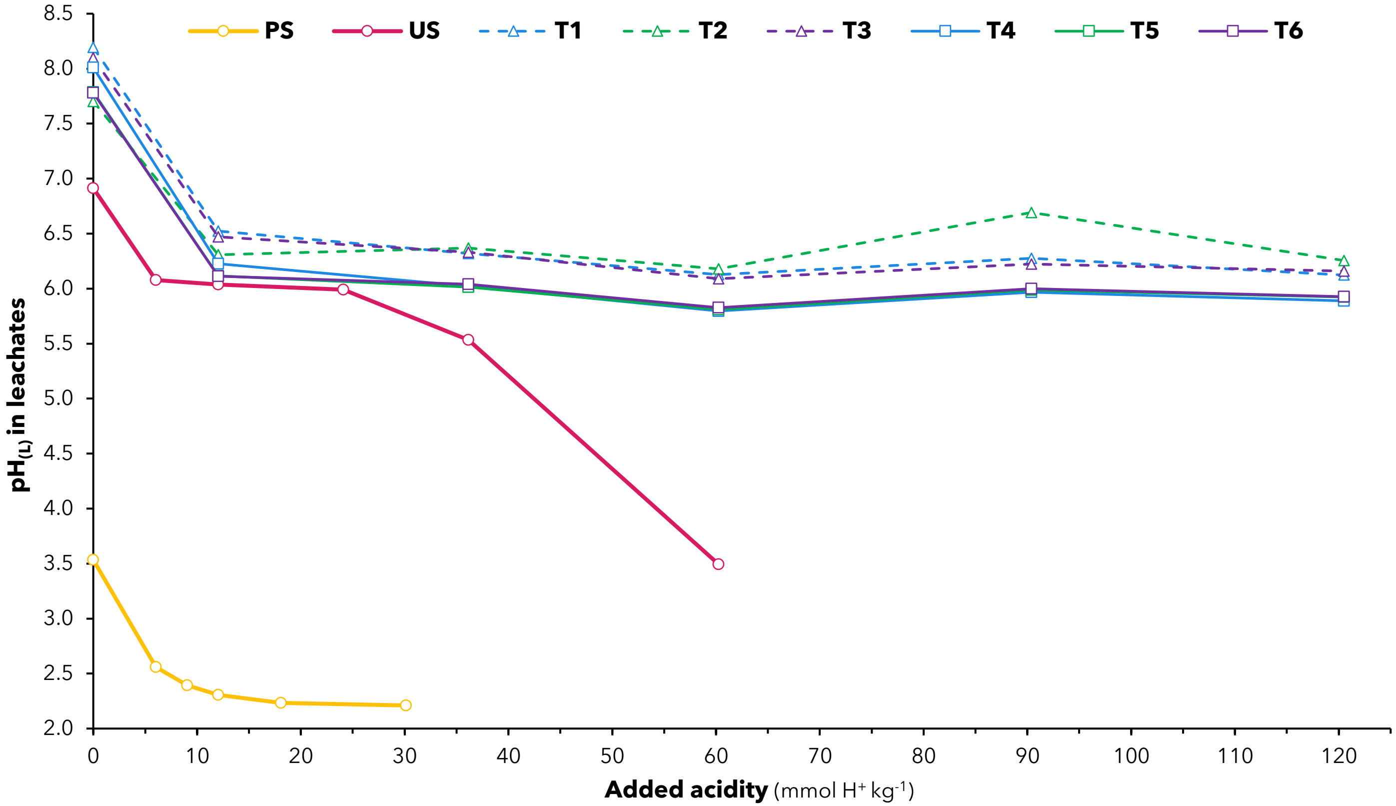

Figure 5.1. *Variation of pH in the resulting leachates after treatment of artificial AMD (as a function of increased added acidity) by polluted soil (PS), unpolluted soil (US), and Technosols (T1-T6) (n = 3).* Detailed pH values (mean ± SD) represented in this figure are given in *Table S5.1*.

observed and an even sharper drop to extreme acidity at $pH_{(L)}$ 3.5 when 60.26 mmol H^+ kg^{-1} were added (Figure 5.1). This shows that this soil non-affected by the Aznalcóllar mining accident has a certain pH buffering capacity, contrary to the polluted soil, maintaining an acceptable pH for ecosystems when exposed to low-medium acidity through the regulating role of carbonates and exchangeable bases present in this soil (Tables 4.1 and 6.1) in the pH range from 5.6 to 6. However, if this soil is exposed to higher acidity, this potential is reduced due to the dissolution of carbonates and removal of exchangeable bases; from this point onward, the buffering reserves of soils that act are Fe and Al oxy(hydr)oxides, which are less effective than carbonates and exchangeable bases (Nawaz et al. 2010), leading to extreme degradation of the soil.

5.2.3. PHEs RETENTION CAPACITY OF TECHNOSOLS

Although the concentrations of several PHEs (i.e. As, Cd, Cu, Pb, Sb, V, Zn) in the artificial AMD were high (Table 5.1), to assess the retention capacity of PHEs of the different Technosols (T1, T2, T3, T4, T5 and T6) compared to polluted (PS) and unpolluted soil (US), in this study we limited the analysis to those PHEs most problematic due to their demonstrated high toxicity (Nriagu et al. 2007; Guo et al. 2011; Bini and Bech 2014; Noulas et al. 2018; Dutta et al. 2021) and elevated levels compared to different regulatory levels and/or concentrations in other AMD (Table 5.1), namely As, Cd, Cu, and Zn. In this sense, the concentrations of As, Cd, Cu, and Zn in the resulting leachates after treatment with Technosols – AMD treated - decreased significantly compared to AMD concentrations (Table S5.2), with all Technosols exhibiting a retention effectiveness above 85% for As, Cd, Cu, and Zn for all increasing volumes of AMD exposed (i.e. added acidities) (Table 5.2). In particular, the retention effectiveness for As, Cd, and Cu were above 95% in all cases and close to 99-100% in most cases. In the case of Zn, the retention rates dropped below 95% efficiency for some acid additions. In Technosols composed of carbonated waste from peat bog mining [CW] (T1, T2, and T3), they only dropped for the highest added acidity (120.51 mmol H^+ kg^{-1}) and remained at values close to 95%. In contrast, in Technosols made of sludge from marble cutting and polishing [MS] (T4, T5, and T6), they dropped from half of the acidity (60.26 mmol H^+ kg^{-1}) and down to values below 90% (except in T5, which remained close to 95%).

Table 5.2. *Retention effectiveness of As, Cd, Cu, and Zn of polluted soil (PS), unpolluted soil (US), and Technosols (T1-T6) in % (mean ± SD, n = 3).*

		Retention rate in artificial AMD (%)			
	Added acidity (mmol H^+ kg^{-1})	**As**	**Cd**	**Cu**	**Zn**
PS	*6.03*	99.41 ± 0.02 d	42.91 ± 1.39 ab	-91.58 ± 4.74 ab	-31.90 ± 2.37 a
	9.04	98.13 ± 0.33 d	48.16 ± 1.06 b	-100.84 ± 3.94 ab	-20.22 ± 3.26 ab
	12.05	93.33 ± 1.70 c	40.91 ± 6.11 a	-116.28 ± 20.73 a	-30.85 ± 11.90 a
	18.08	81.04 ± 0.28 b	40.53 ± 0.43 a	-86.25 ± 6.83 b	-17.34 ± 2.87 ab
	30.13	65.85 ± 0.98 a	36.37 ± 0.92 a	-59.43 ± 3.37 c	-9.21 ± 2.31 b
US	*6.03*	99.80 ± 0.02	99.79 ± 0.01 c	99.63 ± 0.11 b	99.43 ± 0.11 c
	12.05	99.52 ± 0.45	99.05 ± 0.59 c	99.71 ± 0.01 b	97.68 ± 0.61 c
	24.10	99.94 ± 0.11	98.65 ± 0.65 c	99.74 ±0.05 b	96.54 ± 0.42 c
	36.15	99.76 ± 0.13	94.32 ± 0.69 b	99.45 ± 0.04 b	81.79 ± 1.28 b
	60.26	100.00 ± 0.00	69.21 ± 1.80 a	62.22 ± 17.92 a	14.05 ± 4.48 a
T1	*12.05*	99.87 ± 0.09	99.67 ± 0.22 c	97.72 ± 0.50	99.45 ± 0.06 e
	36.15	99.90 ± 0.07	99.82 ± 0.16 c	98.02 ± 0.07	98.60 ± 0.05 d
	60.26	99.88 ± 0.05	99.52 ± 0.04 bc	97.82 ± 0.14	96.52 ± 0.06 c
	90.38	99.93 ± 0.04	99.28 ± 0.08 b	98.30 ± 0.04	96.07 ± 0.25 b
	120.51	99.83 ± 0.04	98.81 ± 0.12 a	98.13 ± 0.07	93.04 ± 0.16 a
T2	*12.05*	98.98 ± 0.44 a	99.03 ± 0.34	95.03 ± 0.39 ab	98.39 ± 0.11 c
	36.15	99.72 ± 0.13 b	99.42 ± 0.22	96.02 ± 0.04 b	98.20 ± 0.07 c
	60.26	99.90 ± 0.08 b	99.36 ± 0.19	95.41 ± 0.15 a	96.55 ± 0.01 b
	90.38	99.91 ± 0.07 b	99.34 ± 0.20	96.51 ± 0.07 c	98.17 ± 0.84 c
	120.51	99.84 ± 0.05 b	98.92 ± 0.15	96.33 ± 0.12 c	94.58 ± 0.16 a
T3	*12.05*	99.70 ± 0.07 a	99.38 ± 0.21 ab	99.03 ± 0.05 c	99.70 ± 0.07 b
	36.15	99.83 ± 0.10 ab	99.75 ± 0.17 b	99.15 ± 0.11 c	98.78 ± 0.06 b
	60.26	99.92 ± 0.09 b	99.30 ± 0.08 ab	98.56 ± 0.06 a	95.96 ± 0.25 a
	90.38	99.99 ± 0.01 b	99.12 ± 0.12 a	98.75 ± 0.00 b	95.81 ± 0.10 a
	120.51	99.86 ± 0.06 ab	98.85 ±0.35 a	98.50 ± 0.06 a	94.52 ± 1.44 a

Continued on next page

		Retention rate in artificial AMD (%)			
	Added acidity (mmol H^+ kg^{-1})	**As**	**Cd**	**Cu**	**Zn**
T4	*12.05*	98.57 ± 0.89 a	98.59 ± 0.76 b	96.11 ± 0.20 a	98.04 ± 0.16 d
	36.15	99.65 ± 0.14 b	99.04 ± 0.44 b	98.39 ± 0.22 b	95.63 ± 0.26 c
	60.26	99.89 ± 0.06 b	98.24 ± 0.06 a	98.84 ± 0.05 c	88.78 ± 0.18 b
	90.38	99.88 ± 0.05 b	98.20 ± 0.13 a	99.27 ± 0.03 d	90.10 ± 1.68 b
	120.51	99.86 ± 0.06 b	97.39 ± 0.03 a	99.21 ± 0.01 d	86.11 ± 0.39 a
T5	*12.05*	97.82 ± 2.58	97.99 ± 1.91	97.12 ± 1.29 a	98.53 ± 1.07 c
	36.15	99.76 ± 0.03	99.46 ± 0.12	98.72 ± 0.07 b	98.10 ± 0.10 c
	60.26	99.87 ± 0.09	99.06 ± 0.13	98.86 ± 0.07 b	94.35 ± 0.21 ab
	90.38	99.96 ± 0.01	98.99 ± 0.11	99.00 ± 0.06 b	95.06 ± 0.15 b
	120.51	99.83 ± 0.07	98.56 ± 0.17	99.00 ± 0.02 b	92.90 ±0.99 a
T6	*12.05*	99.79 ± 0.13	99.85 ± 0.09 d	99.83 ± 0.02	99.12 ± 0.06 c
	36.15	99.79 ± 0.12	98.96 ± 0.21 c	99.82 ± 0.01	95.71 ± 0.16 b
	60.26	99.88 ± 0.05	97.65 ± 0.09 b	99.80 ± 0.07	90.45 ± 0.35 a
	90.38	99.90 ± 0.04	97.53 ± 0.01 b	99.81 ± 0.00	91.04 ±0.25 a
	120.51	99.80 ± 0.03	96.84 ± 0.27 a	99.76 ± 0.01	89.19 ±2.12 a

Very high retention > 95% (green), High retention > 50% (yellow), Low retention < 50% (orange), No retention (red). Table S5.2 shows the concentrations of As, Cd, Cu, and Zn in the resulting leachates after the treatment of artificial AMD used to calculate these retention rates together with the concentration of these PHEs in the artificial AMD shown in Table 5.1. Letters represent significant differences between the different added acidities in each treatment (Kruskal-Wallis and Mann-Whitney U tests; $p < 0.05$).

On the other hand, when exposed to increasing volumes of AMD, both polluted and unpolluted soil behave completely differently from Technosols. In leachates from PS, the concentrations of Cu and Zn did not decrease with respect to concentrations in AMD, but on the contrary, they increased (Table S5.2). This degraded soil, instead of retaining these PHEs from the artificial AMD, contributed them by the weathering of the remaining sulphide minerals containing Cu and Zn in this soil (Cu doubled and Zn increased by 10-30%) with a decreasing trend as added acidity increased (Table 5.2). On the contrary, As and Cd in leachates decreased, although far from the retention effectiveness of Technosols. For As, retention rates were close to 99% in the first exposures to AMD, but as the added acidity increased, the retention rate of As decreased to values close to 65%; and for Cd, retention rates were below 50% and with a decreasing tendency. The unpolluted soil did not release PHEs to the leachates as did the polluted soil; however, the retention capacity of the different PHEs when exposed to increasing volumes of AMD was not as high as that in Technosols (except for As, which was also close to 100%) (Table 5.2). At low to medium added acidities (6.03-24.10 mmol H^+ kg^{-1}), the retention of Cd, Cu, and Zn by US was highly effective (between 96% and 99.8%), but sharply decreased with increasing added acidities to end up with retention rates of 69%, 62%, and 14% of Cd, Cu, and Zn, respectively, for the highest added acidity (60.26 mmol H^+ kg^{-1}).

Among these PHEs, Cu and Zn are considered more mobile than As and Cd (Kraus and Wiegand 2006; Simón et al. 2008), being their solubility mainly conditioned by pH directly or indirectly through its influence on the main adsorbing agents in soil (i.e. organic matter, carbonates, Fe, Al, and Mn oxy(hydr)oxides, clay minerals). In general, at acid pH, the mobility of most PHEs increased; therefore, as different soils are exposed to higher acidity, PHEs retention can be expected to decrease, as occurred in US. In this case, the natural buffering capacity of most soils can be observed, which is mainly due to their clay content, SOM, and carbonates. Unpolluted soil was capable of retaining PHEs as acidity increased, until a critical point was reached (36 mmol H^+ kg^{-1}) at which the reserves that provide its buffering capacity were depleted and, thus, its retention was decreased. Being the most drastic drop for Zn due to its high solubility at acid pH (Rutkowska et al. 2015; Romero-Freire et al. 2016), while As retention was practically total for all acidities added due to its low solubility at acid pH and because different soil components (mainly Fe oxy(hydr)oxides and carbonates) adsorb arsenates in non-bioavailable forms (García-Sánchez et al. 2010; Romero-Freire et al. 2014; González et al. 2017) (Figure 5.1 and Table 5.2). When the buffer capacity is exceeded, the soil ceases to protect the ecosystem and can become a source of pollution, as is the case of polluted soil with the release of Cu and Zn into an already PHE-loaded AMD mainly due to its extremely acidity and low SOM content, which in abundance in soil forms very stable compounds with both Cu and Zn (Sun et al. 2019; Padoan et al. 2020).

5.2.3. CRITICAL LOAD OF TECHNOSOLS

In the case of Technosols, it is very important to know their capacity to deal with long-term pollution, since they are established as a sustainable solution for polluted areas. In this sense, it is necessary to assess the amount of PHEs that Technosols can withstand without producing leachates exceeding the regulatory values. To estimate these limits, the soil critical load of the target PHE is usually calculated (de Vries and Groenenberg 2009). This concept refers to the maximum tolerable amount of PHE that can be introduced into a soil ecosystem without affecting the normal structure and functions of the soil ecosystem (Feng et al. 2020). To determine the critical load of Technosols and control soils (PS and US), we calculated the amount of artificial AMD that must be added to these soils (ml AMD per g soil) to exceed the maximum levels allowed in irrigation reuse water (in mg l^{-1}) for As: 0.1, Cd: 0.01, Cu: 0.2, and Zn: 2 (BOE 2007; EPA 2012) in their leachates.

In general, Technosols showed a better critical load than US and especially than PS (Figure 5.2). Indeed, as previously seen, PS was not capable of retaining PHEs since its critical load is exceeded, except As, for which PS had a slight critical load, possibly due to the high presence of iron oxyhydroxides, to which As had a high affinity for adsorption (Romero-Freire et al. 2014); whereas US had moderately low critical load values for the four PHEs considered, so as acidity increased the retention of PHEs was lower (Table 5.2). For As, all Technosols showed a very high critical load up to the acidity levels tested (maximum of 20 ml of AMD used). The critical load for Zn in Technosols was higher than that of US; especially in Technosols composed of CW as carbonate amendment (T1, T2, and T3) with three times more critical load, whereas in the others composed of MS, it was lower (between 1.3 and 2.5 times respect to US). This is probably because CW contains elements other than carbonates, such as silicates, organic matter, and oxy(hydr)oxides of Fe and Al, which have the capacity to immobilise Zn, whereas MS, which is composed almost exclusively of carbonates, does not. In the case of Cd, while in CW-composite Technosols, the critical load for this element was very high, in MS-composite Technosols it was even lower than in US (except in T6, which was about twice higher than in US). The critical load for Cu was the most irregular among Technosols, with the best performance in Technosols composed of VC (very high in T6 and about four times higher in T3 than in US), while in the rest of the Technosols, it was below that of US, except in T1, which was slightly higher.

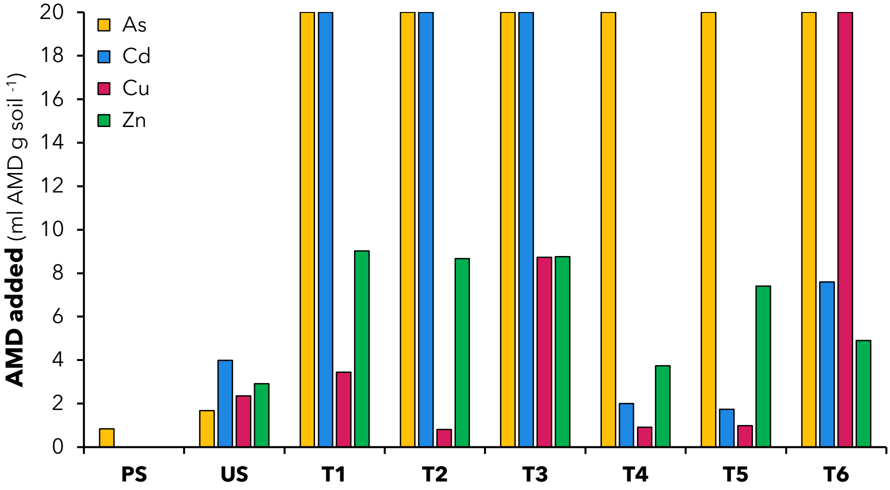

Figure 5.2. *Critical load (ml of artificial AMD received per g of soil to exceed regulatory limits) of polluted soil (PS), unpolluted soil (US), and Technosols (T1-T6) (n = 3).*

In general, these waste-derived Technosols fulfil to a high degree with the ecosystemic function of pollution control normally provided by healthy soils, since they present an appropriate critical load for most PHEs, even superior to that of US when exposed to intense pollution through an artificial AMD with high acidity, salinity, and PHEs concentrations (see section 5.2.1). Indeed, the retention rates were so high (except the slight decrease for Zn at high acidities), that the concentrations of As and Cd in the leachates - AMD treated - for most of the added acidity levels and for all Technosols (Table S5.2) were below the maximum levels for natural surface waters (BOE 2015) and for reclaimed water for irrigation purposes (BOE 2007; EPA 2012). However, the concentrations of Cu and Zn, although markedly reduced (Table S5.2), were not below these levels in most cases. In contrast, in the leachates resulting from PS, most PHEs were found at concentrations well above these levels, as well as in the leachates resulting from US, where although the concentrations of As, Cd, Cu, and Zn remained at values close to or below the regulatory levels at low added acidities, as more acidity was added, the concentrations of Cd, Cu, and Zn far exceeded the previous regulatory levels.

5.3. CONCLUSIONS

Ecotechnology based on Technosols composed of polluted soil and non-hazardous wastes from local industries (mines, urban gardening services, wastewater treatment services, and olive mills) has proven to be a suitable solution for AMD treatment. Indeed, the results of this study can be extrapolated to most acid mine water or AMD treatment situations worldwide; furthermore, the use of Technosols specifically designed for the treatment of real AMD would most likely produce a better quality treated water than that achieved for the artificial AMD used in this study, as its conditions are even more extreme than in some real ones.

All Technosols (T1, T2, T3, T4, T5, and T6) have a significant acid neutralisation capacity, as well as a strong immobilisation capacity for PHEs and critical load, well above US and especially PS. Technosols suffer a sharp drop on first exposure to AMD, but as more acidity is added, they stabilise pH in leachates around slightly acidic values in CW-composite Technosols (T1, T2, and T3) and moderately acidic in MS-composite Technosols (T4, T5, and T6); in contrast to unpolluted soil, which has certain pH buffering capacity at low-medium acidity, but when exposed to higher acidity, this potential is strongly reduced due to the depletion of the main buffering reserves (carbonates and exchangeable bases). Generally, the retention rates of all Technosols were so high (As, Cd, and Cu: > 95% for all acidities tested, Zn: > 85% [> 95% at low–medium acidities]), in line with the considerable critical load measured, that PHEs concentrations in leachates were significantly reduced compared to AMD. Thus, these Technosols constitute a potential ecotechnology that can be applied for the control of AMD. Although, the presence of PHEs with variable chemical behaviour must be controlled, as the presence of a continuous AMD may pose a risk in the medium and long term due to the saturation of their capacity to retain certain PHEs and the loss of effectiveness as a protective barrier over time; therefore, Technosols used in AMD control must be monitored regularly and corrective measures proposed to maintain their functionality over time.


T4 1
T5 2

Lettuce cultivation on polluted soils and Technosols in the growth chamber of the Department of Plant Physiology - UGR

Granada, Spain

November 2022

Antonio Aguilar Garrido

CHAPTER 6

PHYTOTOXICITY REDUCTION IN POLLUTED SOILS BASED ON THE USE OF TECHNOSOLS AND *L. SATIVA* AS BIOINDICATOR

González-Quero, M., **Aguilar-Garrido, A.**, Paniagua-López, M., García-Huertas, C., Sierra-Aragón, M., Blasco, B. (2024). Phytotoxicity reduction in polluted soils based on the use of Technosols and *L. sativa* as a bioindicator. ***Environmental Technology & Innovation*** (Under review)

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ABSTRACT

Residual pollution of soils by potentially harmful elements (PHEs) due to mining activity can lead to serious ecological problems. This study aims to evaluate the effectiveness of three Technosols designed for PHE-polluted soils (PS) remediation. *L. sativa* plants were grown in five treatments: recovered soil (RS) and polluted soil (PS) as controls, and the three Technosols (T4, T5 and T6) consisting of 60% PS mixed with 2% iron sludge, 20% marble sludge, and 18% different organic wastes (T4: composted olive waste, T5: composted sewage sludge, T6: vermicompost of garden waste). The main soil properties and solubility of PHEs, together with several physiological parameters related with phytotoxicity in lettuce plants, such as growth, photosynthetic capacity, oxidative stress, and antioxidant defence, were determined to evaluate the influence of Technosols on PHE-induced soil toxicity. Technosols improved unfavourable conditions of PS (i.e. neutralised acidity, enhanced OC content), leading to a significant decrease in Cd, Cu, and Zn mobility. Nevertheless, T6 was the most effective because the reduction in PHEs mobility was higher. Furthermore, *L. sativa* plants grown on T6 and T4 showed a higher growth rate (+90% and +41%, respectively) than PS, whereas no increase occurred in T5. However, lower oxidative stress and impact on net photosynthetic rate was observed in all Technosols compared to PS (+344% in T6, +157% in T4 and +194% in T5). Thus, this ecotechnology can be a potential solution for the remediation of polluted soils, as a widely used plant such as lettuce can be grown on them without experiencing toxicity; although the effectiveness of Technosols depends largely on their components, as some, such as T6, greatly improve conditions while others, such as T5, may not bring similar benefits.

6.1. BACKGROUND

Most studies on the remediation of polluted soils evaluate the effectiveness of the applied ecotechnology mainly by changes in soil properties and PHEs mobility and bioavailability (Kabata-Pendias 2010; García-Carmona et al. 2017). However, the interaction between PHEs, soil matrix, and organisms, as well as the resulting effects, cannot be assessed solely through a physicochemical study (Leitgib et al. 2007; ISO 17402 2008). Other approaches such as microbial community characterisation (Paniagua-López et al. 2021), ecotoxicity tests (García-Carmona et al. 2017; Pastor-Jáuregui et al. 2022) and phytotoxicity tests (Bhaduri and Fulekar 2012; Hamels et al. 2014) can provide useful insights into remediation techniques from a holistic approach.

Exposure of plants to PHEs can cause various phytotoxic effects, such as growth inhibition, decreased photosynthesis and respiration, oxidative stress, altered enzyme activity and stomatal function, and DNA damage (Romero-Freire et al. 2023). Plants can uptake PHEs through the roots and subsequently be stored in roots or translocated to aerial part, although this tendency depends on the PHE and the plant species (Antoniadis et al. 2017). Furthermore, the toxicity caused by PHEs may differ depending on the interactions between them, through antagonistic or synergistic functions in their absorption and translocation when using, for example, the same transporter (Huang and Gui 2019; Xiang et al. 2021).

These elements can be taken up as free ions and soluble metal complexes, although most of them are captured, transported to the xylem, and translocated to the shoots in the form of chelates. It has sometimes been documented that most PHEs are sequestered in the root, not causing photosynthetic damage, as an adaptative response, due to various processes such as blockage by Casparian strips, chelation and compartmentalisation in rhizodermal vacuoles, and immobilisation in root cell walls (Rascio and Navari-Izzo 2011; Shahid et al. 2015). However, when PHEs accumulate in sensitive parts of plants, they can disrupt photosynthesis, for example, by reducing the presence of photosynthetic pigments such as chlorophyll due to Fe and Mg deficiencies caused by the presence of PHEs (Yildirim et al. 2022) or by decreasing the efficiency of photosystem II through PHEs interference with the electron transport chain (Dias et al. 2013).

The presence of PHEs also triggers the generation of reactive oxygen species (ROS) due to an imbalance in electron flow during photosynthesis or through Haber-Weiss reactions, leading to oxidative stress (Gill and Tuteja 2010). For example, causing membrane destabilisation due to lipid peroxidation and subsequent formation of malondialdehyde (MDA) (Gill and Tuteja 2010; Barrameda-Medina et al. 2014), which can result in increased permeability for some PHEs that would not normally cross the membrane. Other processes related to increased ROS in cells include protein carbonylation, through irreversible oxidation of proteins that inhibits their enzymatic activities or stimulates their degradation, and DNA damage due to increased cross-linking, strand breaks and base exchange or deletion (Huang et al. 2019; Li et al. 2022). To compensate for the effects of ROS, plants develop mechanisms that maintain cellular redox homeostasis, such as the induction of antioxidant systems based on both enzyme and non-enzymatic antioxidants (Israr et al. 2011; Sharma et al. 2012; Kolahi et al. 2020). For example, the ascorbate-glutathione cycle, in which glutathione (GSH) and ascorbate peroxidase (APX) are involved in the removal of H_2O_2 and lipid peroxides and the expression of stress-responsive genes (Gill and Tuteja 2010; Bhaduri and Fulekar 2012; Barrameda-Medina et al. 2014). However, other more basic mechanisms rely on the synthesis of a metabolite such as proline (Pro) (Kolahi et al. 2020).

Therefore, this chapter **aims** to analysis the reduction of phytotoxicity caused by the presence of PHEs in polluted soils (see Figure 1.2) using one of the most widely used bioindicator plants due to its high sensitivity to PHE-pollution, *Lactuca sativa* L. (Bagur-González et al. 2011; Pastor-Jáuregui et al. 2022), in some Technosols composed of polluted soil and inorganic/organic wastes. The assessment of ecotoxicity through physiological parameters related to phytotoxicity in *L. sativa*, such as plant growth, photosynthetic capacity, oxidative stress, and antioxidant defence allows for a more complete evaluation of the effectiveness of this soil remediation ecotechnology based on the use of waste-derived Technosols. For detailed information on the experimental design and analyses implemented in this growth chamber experiment, see section 2.3.2.2.

In this chapter, the evaluation of phytotoxicity reduction is limited to Technosols T4, T5, and T6 (for information of their production, section 2.2.5), as the only difference with T1, T2, and T3 is the carbonated waste used. The sludge from marble cutting and polishing (MS) used in T4, T5, and T6 was preferred to the carbonated waste from peat bog mining (CW) used in the other Technosols because of its greater ubiquity and magnitude, and because in **chapter 4** no major differences were observed between using one or the other.

Note: In this chapter, unlike in the rest of the thesis, letters are not used in the different tables and figures to indicate statistically significant mean differences, but rather Fisher's Least Significant Difference ($LSD_{0.05}$) test values and significance levels expressed as * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ or *NS* (not significant) $p > 0.05$.

6.2. RESULTS AND DISCUSSION

6.2.1. SOIL PROPERTIES AND PHEs CONCENTRATIONS

Polluted soil (PS) was characterised by an extremely acidic pH due to the formation of sulphuric acid by oxidation of sulphides contained in the pyritic sludge dumped in the Aznalcóllar mining accident (Simón et al. 2001), as opposed to the neutral to slightly alkaline pH of recovered soil (RS) (Table 6.1). Hence, one of the first remediation actions undertaken immediately after the accident was the addition of calcium carbonate-rich amendments to neutralise the acidity, and consequent mobilisation of PHEs due to their high pH buffering capacity (Aguilar et al. 2004). This explains the low $CaCO_3$ content in RS (Table 6.1), as partial dissolution of carbonates occurred to buffer pH (Simón et al. 2010). In this regard, because pH exerts a strong influence on PHEs mobility, which generally decreases with increasing pH (Romero-Freire et al. 2016), Technosols are 20% composed of a carbonate-rich waste, the marble sludge (MS), to have buffering capacity. As a result, T4, T5, and T6, despite the high percentage of PS in their composition, had neutral to slightly alkaline pH and considerable $CaCO_3$ content (Table 6.1).

Likewise, PS showed low organic carbon (OC) content and low cation exchange capacity (CEC) (Table 6.1), which limits both chemical and physical fertility. These soils are not only depleted in nutrients but also tend to form crusts on the soil surface, which further limits the possibility of vegetation development (Martín et al. 2004). In contrast to RS, where previous remediation actions allowed the growth and development of plants, significantly increasing the soil organic matter compared to PS (Madejón et al. 2006a). For this reason, an 18% of organic amendment was included in the formulation of the three Technosols, resulting in a much higher OC content than PS. However, there was a large variability in the OC content among Technosols (T4: 4.1%, T5: 5.6%, T6: 2.1%) due to the different OC richness of the organic wastes (Table 3.1). In addition, the nature of the organic waste can lead to variations in the evolution of the organic matter during the incubation period along the production of the Technosols, thereby contributing to differences in the fertility status of each Technosol (Aguilar-Garrido et al. 2023a). In fact, T4 had a higher CEC than T5, despite its lower OC content. Thus, the composted solid olive-mill by-product (OL) used in T4 was much more reactive than the composted sewage sludge (WS) used in T5. In the case of T6, the decrease in its CEC compared to the others was due to the lower OC input with the vermicompost from pruning and gardening waste (VC), which presumably is also more easily mineralised (Tables 3.1 and 6.1).

Table 6.1. *Main properties of recovered soil (RS), polluted soil (PS), and different Technosols (T4, T5, and T6) (mean ± SD; n = 9).*

	pH (H_2O)	**EC** (dS m^{-1})	**OC** (%)	**$CaCO_3$** (%)	**CEC** (cmol+ kg^{-1})	**Na^+** (cmol+ kg^{-1})
RS	7.38 ± 0.12	0.96 ± 0.11	5.89 ± 0.24	0.80 ± 0.09	35.28 ± 3.41	0.60 ± 0.02
PS	4.16 ± 0.09	2.99 ± 0.18	0.87 ± 0.09	0.56 ± 0.06	11.25 ± 1.76	0.53 ± 0.03
T4	7.72 ± 0.15	4.96 ±0.21	4.08 ± 0.14	23.06 ± 1.25	28.50 ± 2.64	2.45 ± 0.13
T5	7.30 ± 0.08	4.34 ± 0.17	5.59 ± 0.17	23.17 ± 1.21	24.34 ± 1.74	2.46 ± 0.16
T6	7.62 ± 0.13	2.96 ± 0.15	2.09 ± 0.11	22.83 ± 1.51	16.51 ± 1.66	0.70 ± 0.02
p-value	***	***	***	***	***	***
$LSD_{0.05}$	0.02	0.08	0.14	0.77	4.15	0.27

EC – Electrical conductivity, OC – Organic carbon, $CaCO_3$ - Calcium carbonate, CEC – Cation exchange capacity, Na^+ - Extractable sodium. Differences between means were compared using Fisher's least-significant difference test ($LSD_{0.05}$). The levels of significance were represented by $p > 0.05$: NS (not significant), $p < 0.05$ (*), $p < 0.01$ (**) and $p < 0.001$ (***).

The high salinity of PS (Table 6.1) was a consequence of the oxidation of sulphides to soluble sulphates remaining in the pyritic sludge upon drying (Simón et al. 2001). The Technosols formulation includes 60% of PS, which justifies the salinity levels (Table 6.1). However, Technosols T4 and T5 showed considerably higher EC than PS and T6 because of the salinity provided by the organic wastes composing them (OL and WS) (Table 3.1). The same occurred with exchangeable sodium, which showed very high values in T4 and T5 (Table 6.1), even reaching levels that can cause stress for plant development and compromise their growth (Parida and Das 2005).

At present, despite the clean-up and remediation measures implemented by the Regional Government of Andalusia and more than 25 years after the accident, some PHEs (As, Cu, Pb, and Zn) are still of concern in the area because of their potential environmental risk due to their solubility and long-term bioavailability (Paniagua-López et al. 2021), especially in the areas without vegetation close to the mine. Indeed, total concentrations of As and Pb in PS (mg kg^{-1}; As: 346, Pb: 546; Table 6.2) exceeded 9.6 and 2 times the Andalusian regulatory levels of 36 and 275 mg kg^{-1} (BOJA 2015), respectively. In any case, these remediation efforts focused on removal of toxic tailings and highly polluted topsoil, application of amendments, and phytostabilisation with native vegetation were effective in almost the entire affected area (Madejón et al. 2018; Pastor-Jáuregui et al. 2021), leading not only to an improvement of soil conditions but also to decreased PHEs concentrations. As shown in our results, the total concentrations of most PHEs in RS were significantly lower than those in PS (Table 6.2). With the exception of Zn, whose total concentration in RS was higher than that in PS because its solubility is strongly controlled by pH. In PS, Zn intensively leached over time due to soil acidity, thus reducing its total concentration compared to RS (Kraus and Wiegand 2006; Martín Peinado et al. 2015).

The potential toxicological risk of these PHEs depends largely on their mobility, rather than their total concentrations (Quevauviller et al. 1998; Giannakis et al. 2021), since the water-soluble fraction is the most readily accessible to plants and living soil organisms and can therefore cause damage to the ecosystem and/or enter the trophic chain (Chang et al. 2014; Son et al. 2019). Therefore, it is essential to assess their mobility, as the risk of pollution could be over- or under-estimated if it is only based on total concentrations. Furthermore, the remediation of residually polluted soils with Technosols is not expected to modify the total PHEs content, but will reduce their mobility and phytoavailability. Indeed, the total PHEs concentrations in Technosols were high, as 60% of them were composed of PS, although in T5, the concentration of Zn_T was even higher because of the Zn concentration in the sewage sludge (Table 3.2).

The solubility of PHEs in Technosols strongly changed compared to that of PS. The high water-soluble concentrations of Cd, Cu, and Zn in PS decreased significantly in most Technosols to values roughly close to those in RS (Table 6.2). Because the solubility of Cd, Cu, and Zn is strongly conditioned by soil properties, it decreases as pH rises (García-Carmona et al. 2019a). The pH neutralisation in Technosols was mediated by carbonates (Cravotta and Trahan 1999) provided by the marble sludge used in their formulations. Furthermore, the increased OC content in Technosols may also contribute to the decrease in the solubility of these elements (Bur et al. 2010; García-Carmona et al. 2019a). However, in T4, Cu_W concentration did not decrease with respect to PS, and in contrast, it was even higher (Table 6.2); this may be due to the fact that Cu is not as pH-dependent as Cd and Zn (McBride et al. 1997) and that this element has a strong affinity for organic matter (García-Carmona et al. 2019b; Paniagua-López et al. 2021). This was in agreement with our data, as the organic waste used in T4 (OL) was considered more reactive than the other two organic wastes (WS and VC). The composted solid olive-mill by-product (OL) showed the highest CEC (Table 3.1) (Aguilar-Garrido et al. 2023a), which could allow the adsorption of a greater amount of Cu in exchangeable form that can be more available to the plant. Furthermore, soluble Cd and Zn presented significantly lower concentrations in T6 than in T4 and T5 (Table 6.2), with the only difference between Technosols being the organic waste used in their formulation. On the other hand, although the same proportion of iron oxyhydroxide-rich sludge was added to all Technosols, Fe_W concentration was significantly higher in T5 (Table 6.2) due to the contribution of more forms in this Technosol from the added organic waste, sewage sludge (WS) (Table 3.1). Hence, the nature, composition and interaction of the components of Technosols have a major influence on the solubility of PHEs.

Water-soluble As in Technosols significantly increased compared to PS, varying among Technosols (Table 6.2). Arsenic solubility is strongly conditioned by pH; however, As solubility in soils decreases at slightly acidic pH, while it increases under strongly acidic and close to neutral conditions (Romero-Freire et al. 2014). In

Table 6.2. *Total and water-soluble PHEs concentrations in recovered soil (RS), polluted soil (PS), and different Technosols (T4, T5, and T6) in mg kg^{-1} (mean ± SD; n = 9).*

	Total fraction (T) *(mg kg^{-1})*						***Water-soluble fraction (W)*** *(mg kg^{-1})*					
	$\mathrm{As_T}$	**$\mathrm{Cd_T}$**	**$\mathrm{Cu_T}$**	**$\mathrm{Fe_T}$ ***	**$\mathrm{Pb_T}$**	**$\mathrm{Zn_T}$**	**$\mathrm{As_W}$**	**$\mathrm{Cd_W}$**	**$\mathrm{Cu_W}$**	**$\mathrm{Fe_W}$**	**$\mathrm{Pb_W}$**	**$\mathrm{Zn_W}$**
RS	51.44 ± 0.63	1.98 ± 0.05	112.03 ± 5.12	29.88 ± 0.25	117.36 ± 1.01	420.35 ± 4.37	0.269 ± 0.015	0.010 ± 0.001	0.405 ± 0.015	9.943 ± 0.706	0.078 ± 0.010	0.606 ± 0.034
PS	345.79 ± 5.64	7.85 ± 0.13	241.34 ± 5.60	52.98 ± 0.79	545.86 ± 9.05	189.14 ± 2.40	0.135 ± 0.009	0.101 ± 0.010	2.617 ± 0.038	3.209 ± 0.125	bdl	7.061 ± 0.098
T4	207.84 ± 1.85	4.21 ± 0.27	99.65 ± 1.19	38.25 ± 0.64	329.30 ± 5.85	163.07 ± 1.73	0.377 ± 0.013	0.015 ± 0.001	3.301 ± 0.053	7.718 ± 0.409	0.089 ± 0.007	1.585 ± 0.043
T5	178.71 ± 3.75	5.86 ± 0.13	137.63 ± 3.06	38.29 ± 0.39	281.54 ± 4.90	315.76 ± 5.71	0.457 ± 0.014	0.014 ± 0.001	0.696 ± 0.015	19.971 ± 1.010	0.046 ± 0.006	0.913 ± 0.019
T6	212.20 ± 1.76	5.88 ± 0.09	93.70 ± 1.95	39.91 ± 0.54	333.45 ± 3.44	181.46 ± 2.41	0.199 ± 0.012	0.006 ± 0.001	0.130 ± 0.007	3.251 ± 0.194	bdl	0.321 ± 0.018
p-value	***	***	***	***	***	***	***	***	***	***	**	***
$\mathrm{LSD_{0.05}}$	9.29	0.44	10.86	1.58	15.82	10.41	0.04	0.01	0.09	1.69	0.02	0.15

** in g kg^{-1}, bdl – below detection limit. Differences between means were compared using Fisher's least-significant difference test ($\mathrm{LSD_{0.05}}$). The levels of significance were represented by $p > 0.05$: NS (not significant), $p < 0.05$ (*), $p < 0.01$ (**) and $p < 0.001$ (***).*

this sense, Simón et al. (2010) pointed out that liming of soils polluted by PHEs should be cautious, because above pH 6.5, this element can be solubilised. To counteract this, 2% of iron oxyhydroxide-rich sludge was added to Technosols for the immobilisation of As, since in soils, it usually appears in anionic forms ($HAsO_4^{2-}$, $H_2AsO_4^{-}$), so it can be fixed to the positive charges that dominate in iron oxyhydroxides (Aguilar et al. 2007; Romero-Freire et al. 2014). The significant increase in As_W concentration in T4 and T5 compared to PS (Table 6.2) may be due to the potential competition of arsenates with SOM for Fe oxyhydroxides binding sites (Wang and Mulligan 2006; Sierra Aragón et al. 2019; Pastor-Jáuregui et al. 2021). Arsenic solubility in T6, although higher than that in PS, was significantly lower compared to T5 and T4 because the lower OC content in the former decreased the competitive effect of organic matter for As fixation sites.

A similar behaviour was observed with Pb solubility, where the higher organic matter content in RS, T4, and T5 could increase Pb_w. In contrast, T6 did not show an increase in Pb solubility compared to PS, with the OC content being the lowest among the three Technosols (Tables 6.1 and 6.2). The relationship between Pb solubility and organic matter content is in agreement with previous studies (García-Carmona et al. 2019a; Pastor-Jáuregui et al. 2021), which observed a slight increase in Pb solubility in reclaimed soils of the GGC compared to soils that remain polluted and have very low organic matter content. However, several authors have also highlighted the affinity of Pb for organic matter (Coppola et al. 2010; Romero-Freire et al. 2015; Sierra Aragón et al. 2019) by the formation of nearly stable forms, which can condition its solubility. Therefore, this controversy highlights the need for further research on the interaction between organic matter and Pb in the remediation of polluted soils.

6.2.2. GROWTH OF L. SATIVA

The highest relative growth rates (RGR) were recorded for *L. sativa* plants grown on RS and T6, whereas the lowest rates were on PS and T5 (Figure 6.1). According to these results, the lower growth of *L. sativa* plants may be due to the increased toxicity resulting from the presence of PHEs, as observed in several studies such as Moreira et al. (2020), who point out that the toxicity caused by several PHEs (i.e. Cd, Cr, Cu, Mn, Ni, Pb, Zn) can result in visible adverse symptoms in seedlings (inhibition of growth, browning of roots, reduction of leaf size, chlorosis and necrosis of roots), reduction of shoot and root biomass, root length, seedling vigour, and tolerance index. Similarly, Li et al. (2020a) reported that the accumulation of Pb and Cu in the root system of lettuce plants resulted in lettuce seed death. Nevertheless, the toxic effect of PHEs is not limited to lettuce, Navarro-León et al. (2020) reported that exposure to Cd toxicity caused a 60% decrease in the leaf biomass of *Brassica rapa* L. subsp. *trilocularis* (Roxb.), and Zaier et al. (2010) showed that the presence of Pb strongly inhibited the growth of *Brassica juncea* (L.).

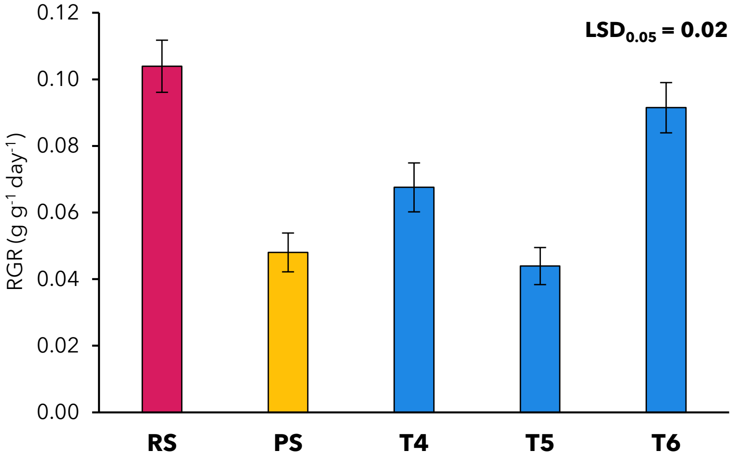

Figure 6.1. *Relative growth rate (RGR) of the aerial part of L. sativa grown in recovered soil (RS), polluted soil (PS), and different Technosols (T4, T5, and T6) (n = 9).*

6.2.3. PHEs ACCUMULATION IN L. SATIVA

High concentrations of PHEs present in polluted areas can exert a detrimental effect on plants, which respond at the molecular, cellular, physiological, anatomical, and morphological levels (Dola et al. 2022). Thus, plants have evolved over time complex mechanisms of hyperaccumulation, tolerance, exclusion, and chelation with organic molecules to overcome this abiotic stress (Angulo-Bejarano et al. 2021). In this experiment, translocation processes did not appear to be prominent because PHEs accumulation in the aerial part of *L. sativa* plants was much lower than the accumulation in the roots in all treatments and for all PHEs considered (Table 6.3). This could be caused by the Casparian strip, which acts as a selective barrier by blocking the entry of a large part of PHEs into the xylem to ensure selective ion

Table 6.3. *Concentrations of PHEs in the aerial part and roots of L. sativa plants grown in recovered soil (RS), polluted soil (PS), and different Technosols (T4, T5, and T6) in mg kg^{-1} (mean ± SD; n = 9).*

	Aerial part *(mg kg^{-1})*						***Roots*** *(mg kg^{-1})*					
	As	**Cd**	**Cu**	**Fe**	**Pb**	**Zn**	**As**	**Cd**	**Cu**	**Fe**	**Pb**	**Zn**
RS	2.21 ± 0.22	0.88 ± 0.03	57.14 ± 0.79	85.80 ± 7.14	bdl	55.37 ± 0.46	5.10 ± 0.86	0.97 ± 0.04	101.40 ± 2.81	5587 ± 132	6.51 ± 0.48	81.89 ± 6.08
PS	0.65 ± 0.35	0.55 ± 0.02	152.59 ± 1.08	70.32 ± 13.79	0.78 ± 0.26	129.31 ± 3.21	0.78 ± 0.68	4.51 ± 0.72	225.53 ± 6.00	6074 ± 139	2.40 ± 0.30	893.85 ± 23.45
T4	1.79 ± 0.14	0.27 ± 0.01	185.56 ± 3.12	73.98 ± 7.04	0.38 ± 0.14	49.53 ± 0.28	18.23 ± 0.72	0.95 ± 0.81	87.58 ± 9.39	7322 ± 313	11.28 ± 0.42	65.27 ± 3.36
T5	1.55 ± 0.18	0.23 ± 0.04	363.08 ± 7.08	164.51 ± 12.10	1.32 ± 0.41	44.06 ± 1.03	17.15 ± 0.68	0.83 ± 0.68	101.62 ± 7.07	8003 ± 628	13.12 ± 0.49	63.53 ± 5.03
T6	2.56 ± 0.17	1.57 ± 0.02	68.86 ± 1.53	67.01 ± 6.71	bdl	92.97 ± 1.76	14.90 ± 0.81	1.40 ± 0.81	112.82 ± 2.07	7741 ± 113	19.88 ± 0.34	83.87 ± 1.12
p-value	*	***	***	***	**	***	***	***	***	***	***	***
LSD$_{0.05}$	1.12	0.08	10.22	28.10	0.62	4.92	2.18	2.24	17.10	883.71	1.11	31.84

bdl – below detection limit. Differences between means were compared using Fisher's least-significant difference test (LSD$_{0.05}$). The levels of significance were represented by $p > 0.05$: NS (not significant), $p < 0.05$ (*), $p < 0.01$ (**) and $p < 0.001$ (***).

transport while retaining ions (Alassimone et al. 2012). In treatments RS and T6, the highest concentrations of Cd and As were found in the aerial part. However, no accumulation was observed for Pb and Cu in the same treatments (Table 6.3). This is probably due to the low solubility of both elements in T6 and Pb in RS (Table 6.2), as well as the competition between certain PHEs for transporters and possible antagonistic effects (Adamczyk-Szabela et al. 2020). The latter could be the case of Cd and Zn, which although both were at high concentrations in T6, their combined effect could result in less stress than either of them separately (Gao et al. 2022). On the other hand, at high concentrations, a potent synergy between the two elements has also been demonstrated (Adamczyk-Szabela et al. 2020). In this study, T5 showed the highest concentrations of PHEs such as Pb, Fe, and Cu in shoots of *L. sativa* plants, even higher than those grown in PS (Table 6.3). However, the higher solubility of pH-dependent elements such as Cd, Cu, and Zn in PS resulted in higher root concentrations than in the other treatments. In contrast, As and Pb, generally less mobile elements, showed the highest root concentrations in soils with higher content in OC at neutral to slightly alkaline pH (RS, T4, T5 and T6), especially in Technosols with 2-3 times higher concentrations of these PHEs than in RS (Table 6.3). Therefore, toxicity may not be directly related to higher PHEs concentrations due to their compartmentalisation and chelation by binding to different ligands for accumulation in shoots and roots in a non-toxic way (Shahid et al. 2015), as seems to be occurring in T6 where, despite lower solubility for Cd, Cu and Zn, high concentrations of these PHEs were accumulated in *L. sativa* roots.

6.2.4. PHOTOSYNTHESIS AND GAS EXCHANGE IN L. SATIVA

A reduction in the process of photosynthesis and gas exchange due to alterations in the photosynthetic apparatus caused by the presence of PHEs impairs plant development, resulting in various damages, such as reduced growth and nutrient uptake (Guala et al. 2010; Dias et al. 2013; Shahid et al. 2015; Yildirim et al. 2022). In this sense, *L. sativa* plants grown on PS and Technosol T5 showed a drastic depletion in photosynthetic and transpiration rates, as well as stomatal conductance compared to RS (Table 6.4), possibly due to the damage caused by the presence of PHEs, together with other stress conditions such as the high soil acidity in the case of PS (Table 6.1) (Clemente et al. 2005; Dias et al. 2013; Bazihizina et al. 2015).

Table 6.4. *Photosynthetic and gas exchange parameters of L. sativa plants grown in recovered soil (RS), polluted soil (PS), and different Technosols (T4, T5, and T6) (mean ± SD; n = 9).*

	ACO_2 (μmol m^{-2} s^{-1})	**E** (mol m^{-2} s^{-1})	**g_s** (mmol m^{-2} s^{-1})	**C_i** (μmol mol^{-1} air)	**WUE** (μmol mol^{-1})
RS	4.34 ± 0.15	1.21 ± 0.05	93.40 ± 3.62	311.63 ± 0.77	3589.78 ± 30.19
PS	0.98 ± 0.09	0.16 ± 0.01	11.50 ± 0.63	257.85 ± 7.80	5827.05 ± 338.38
T4	2.52 ± 0.19	0.80 ± 0.07	59.40 ± 5.22	313.34 ± 5.82	3436.03 ± 256.61
T5	2.89 ± 0.09	0.37 ± 0.01	26.80 ± 0.89	215.43 ± 4.18	7836.31 ± 196.14
T6	4.38 ± 0.14	1.26 ± 0.03	95.00 ± 3.36	311.94 ± 0.08	3491.64 ± 42.17
p-value	***	***	***	***	***
$LSD_{0.05}$	0.36	0.11	8.60	12.82	563.33

ACO_2 - net photosynthetic rate, E - transpiration rate, g_s - stomatal conductance, C_i - intracellular CO_2 concentration, WUE - water use efficiency. Differences between means were compared using Fisher's least-significant difference test ($LSD_{0.05}$). The levels of significance were represented by $p > 0.05$: NS (not significant), $p < 0.05$ (*), $p < 0.01$ (**) and $p < 0.001$ (***).

Moreover, the low values of intracellular CO_2 concentration in plants grown in PS and T5 (Table 6.4) may indicate that not only was the photosynthetic apparatus damaged but also that the stomatal conductance limitations could have caused a decrease in rubisco activity and, thus, the collapse of photosynthesis (Adamczyk-Szabela et al. 2023). The same tendency was observed in plants grown on T4 for all parameters except the intracellular CO_2 concentration, which was similar to RS (Table 6.4). Therefore, these low photosynthetic rates in T4 and T5, and especially in PS, in addition to the RGR values (Figure 6.1) could be due to the high PHEs toxicity problem in the soil of these treatments (Dias et al. 2013). Furthermore, the severe reduction in transpiration derived from stomatal closure increased the WUE of T5 and PS, probably due to the increase in soil solute potential caused by soluble PHEs in soil solution, which could be more severe in T5 because of the higher salt stress (Parida and Das 2005). On the other hand, transpiration drives the transport and translocation of ions to the leaves; therefore, treatments in which plants showed a higher transpiration rate together with a better physiological state may accumulate more PHEs in the aerial part (Liu et al. 2010), as shown in RS and T6 (Tables 6.3 and 6.4). Finally, the values for all these growth and photosynthetic parameters for T6 were similar to those for RS (Figure 6.1, Table 6.4), showing the high effectiveness of this Technosol in the remediation of polluted soils, allowing the establishment of healthy vegetation. This is the same Technosols that showed in a previous study (Aguilar-Garrido et al. 2023c) the highest biomass production in a bioassay with *Trifolium campestre* Schreb.

6.2.5. OXIDATIVE STRESS AND ANTIOXIDANT ACTIVITY IN L. SATIVA

In *L. sativa* plants grown on PS, the concentration of malondialdehyde (MDA) generated was 12-fold higher than in plants grown on RS, whereas this increase was only 5-fold greater in the other treatments compared to RS (Figure 6.2A). This higher concentration of MDA produced in lipid peroxidation responds to oxidative stress caused by PHEs toxicity and other negative conditions such as salinity (Gill and Tuteja 2010; Barrameda-Medina et al. 2014). These results are consistent with those of Barrameda-Medina et al. (2014) and Buturi et al. (2022), where an increase in MDA levels of 126% and 30% was reported in *L. sativa* plants under toxic concentrations of Zn and Fe, respectively. Likewise, proline (Pro) levels also increased in PS and T5 compared to RS, although the highest values were recorded in T5 (Figure 6.2B). Similar results were observed by Yang et al. (2011) when assessing Pb toxicity in *Tritium aestivum* L. with a 207% increase in Pro concentration under these unfavourable conditions. Thus, the increased synthesis of this amino acid can be interpreted as an adaptive mechanism under the stress conditions of PS and T5, as proline is involved in reactive oxygen species (ROS) extinction processes by functioning as an antioxidant metabolite and having the capacity to chelate PHEs (Gill and Tuteja 2010; Kolahi et al. 2020). This stress was more pronounced in PS than in T5, as reflected by the lower MDA concentration recorded for this treatment (Figure 6.2A). This could be due to the higher proline synthesis reported in T5, as it also has an osmoprotective behaviour and is a target of the hydroxyl radical, which could mitigate the effects of salinity and oxidative stress given to the higher Fe and Cu concentrations in the aerial part of plants grown in T5 (Table 6.3) could be responsible for the formation of the hydroxyl radical through Haber-Weiss and Fenton reactions (Parida and Das 2005; Kolahi et al. 2020). This phenomenon probably prevents ROS concentrations in T5 from ultimately leading to lipid peroxidation and MDA formation (Figure 6.2A). In fact, Siripornadulsil et al.

(2002) correlated a higher Pro content with a mitigation of oxidative damage (lower MDA) in the presence of PHEs, in accordance with our results.

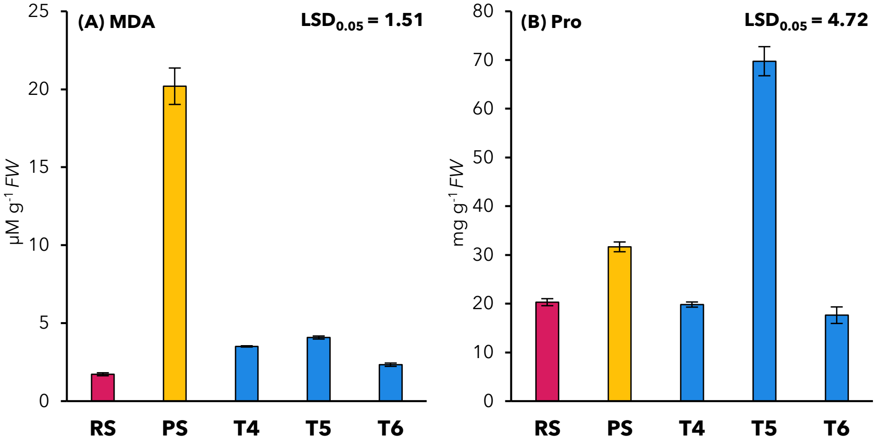

Figure 6.2. *Malondialdehyde [MDA] (A) and proline [Pro] (B) concentrations in L. sativa plants grown in recovered soil (RS), polluted soil (PS), and different Technosols (T4, T5, and T6) (n = 9).*

Regarding ROS production, significant differences of O_2^- concentration were observed in PS and T5 with respect to RS (Figure 6.3A), as well as H_2O_2 production, which was also higher in T5 (Figure 6.3B). Increased ROS production is a very common effect in plants subjected to abiotic stresses such as PHEs toxicity, known as oxidative burst (Gill and Tuteja 2010; Kolahi et al. 2020). According to our results, Barrameda-Medina et al. (2014) and Buturi et al. (2022) showed that plant exposure to toxic concentrations of Fe and Zn lead to increased ROS production in *Brassica oleracea* L. and *L. sativa*. In addition, antioxidant enzymatic activity was measured, and data showed that superoxide dismutase (SOD) and catalase (CAT) activities were increased in PS, T4 and T5 respect to RS. Similar to the rest of assays, the highest values were observed in PS and T5 (Figure 6.3C, D). In this study, the greater ROS production in T4 and T5, and especially in PS, was combined with an increase in antioxidant enzymatic activity that was not observed in either RS or T6 (Figure 6.3C, D). This higher antioxidant enzymatic activity mitigates plant oxidative stress, such as SOD activity, which transforms O_2^- into less reactive H_2O_2, and CAT activity, which removes H_2O_2 (Gill and Tuteja 2010; Yang et al. 2011). However, in T5 and PS, O_2^- and H_2O_2 levels remained high, despite high SOD and CAT activities (Figure

6.3), thus reflecting a higher degree of stress. Furthermore, H_2O_2 concentration at T5 was decreased not only by CAT activity but also by the high Fe and Cu contents in the aerial part (Table 6.3), which led to Haber-Weiss and Fenton reactions to form the much more reactive hydroxyl radical (Gill and Tuteja 2010). On the other hand, the high H_2O_2 concentration in T4 could be related to lower CAT activity but high SOD activity, which transformed much of the O_2^- to a concentration similar to that of RS (Figure 6.3). Finally, it is worth noting again the similarity between T6 and RS, revealing a low stress due to the strong efficacy of this Technosol.

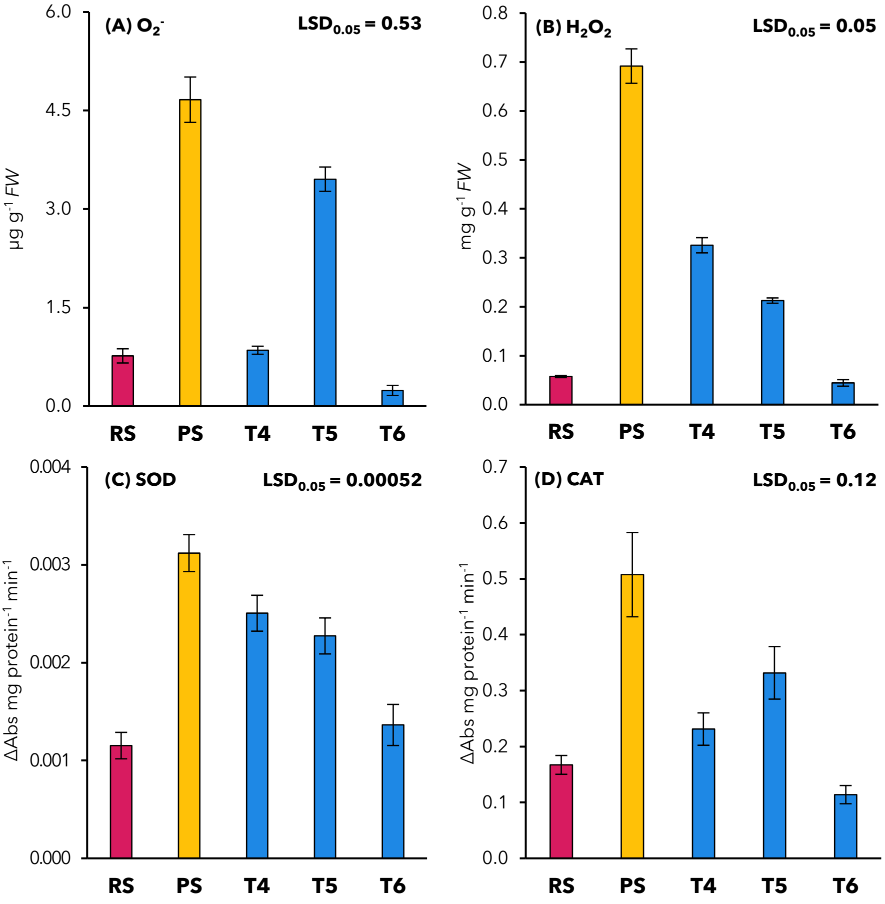

Figure 6.3. *Reactive oxygen species (ROS) [A: superoxide ion (O_2^-), B: hydrogen peroxide (H_2O_2)] and associated enzymatic activities [C: superoxide dismutase (SOD), D: catalase (CAT)] in L. sativa plants grown in recovered soil (RS), polluted soil (PS), and different Technosols (T4, T5, and T6) (n = 9).*

6.2.6. ASCORBATE-GLUTATHIONE CYCLE IN L. SATIVA

The ascorbate-glutathione cycle is another important mechanism for plants to limit oxidative stress, maintain cell redox status, and decrease ROS levels. This network involves important antioxidant compounds and enzymes with great antioxidant capacity and a special protection function against oxidative damage caused by exposure to PHEs (Gill and Tuteja 2010). In this study, the highest total glutathione (GSH Total) concentrations were recorded in PS, T5, and T6 compared to RS, with the highest values in PS and T5. As for oxidised glutathione (GSH Oxid), an increase in this compound was observed in PS compared to RS (Figure 6.4A). Likewise, a large decrease in GSH Oxid concentration in relation to GSH Total was observed only in T5 (Figure 6.4A), revealing that more reduced glutathione (GSH Red) could be available in T5. On the other hand, compared to RS plants grown in PS registered the highest concentration of total ascorbate (AsA Total), followed by those grown in T5, and lastly, those grown in the other treatments that presented similar values (Figure 6.4B). However, the reduced ascorbate form (AsA Red) increased significatily in PS (Figure 6.4B). Glutathione reductase (GR) activity was increased in PS, T5, and T6 compared to RS (Figure 6.4C). Finally, the ascorbate peroxidase (APX) activity was higher for PS and all Technosols used, and the highest values were found in PS and T5, followed by T4 and T6 with intermediate values, and RS with the lowest values (Figure 6.4D). Our results were similar to those of other studies reporting an increase in GSH Total concentration, and especially in GSH Red, to mitigate the oxidative stress caused by Pb and Zn toxicity in *T. aestivum* and *B. oleracea* plants (Barrameda-Medina et al. 2014; Kaya et al. 2022). Moreover, the lower glutathione reductase (GR) activity in PS with respect to T5 (Figure 6.4C) revealed a higher stress in PS, as Barrameda-Medina et al. (2014) described for *L. sativa* exposed to high Zn contents. This fact would explain why our results showed higher GSH Oxid accumulation and less glutathione recycling in PS than in T5.

Furthermore, the availability of GSH not only contributes to the reduction of ROS but also contributes to the chelation of PHEs, either directly through its reduced form or by the formation of phytochelatins (Yadav 2010). Ascorbate is another antioxidant non-enzymatic metabolite with a function similar to that of glutathione in ROS detoxification. In this sense, the existence of a large difference

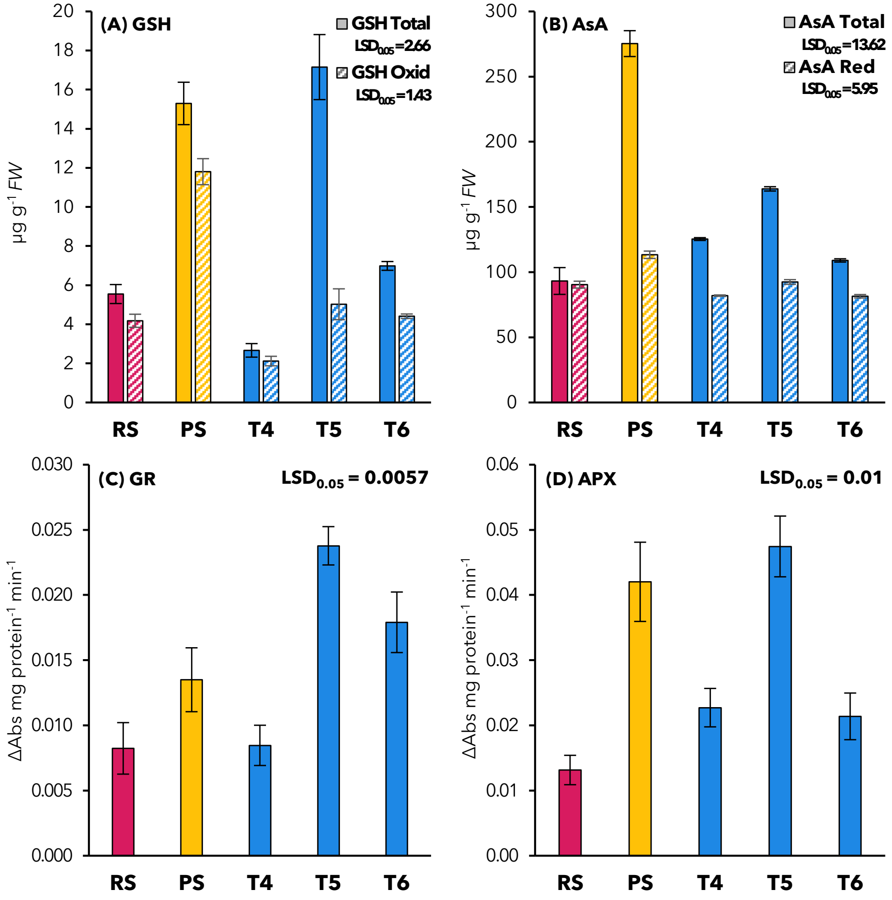

Figure 6.4. *Antioxidant metabolites [A: glutathione (GSH), B: ascorbate (AsA)] and associated enzymatic activities [C: glutathione reductase (GR), D: ascorbate peroxidase (APX)] in L. sativa plants grown in recovered soil (RS), polluted soil (PS), and different Technosols (T4, T5, and T6) (n = 9).*

between AsA Total concentration and AsA Red in T5 and especially in PS (Figure 6.4B) showed that this metabolite was being oxidised to mitigate the abiotic stress produced by PHEs (Barrameda-Medina et al. 2014; Buturi et al. 2022), which did not occur in RS and T6, possibly due to the lower stress conditions and lower APX activity with respect to PS and T5 (Figure 6.4D), agreeing with Buturi et al. (2022). In addition, the higher APX activity achieved at T5 also accounted for the lower H_2O_2 concentration compared T4. Finally, the degree of stress generally leads to an increase in the activity of antioxidant enzymes such as SOD, CAT, APX, and GR

(Figure 6.3C, D and 6.4C, D), which are aimed at reducing oxidative stress caused by exposure to PHEs, as occurred in PS (Gill and Tuteja 2010; Kaya et al. 2022).

6.3. CONCLUSIONS

The three designed Technosols (T4, T5 and T6), composed of polluted soil (PS) with a combination of organic and inorganic wastes from local industries (mines, urban gardening services, wastewater treatment services, and olive mills), improved the unfavourable conditions of PS (neutralised acidity and increased OC content) and, consequently, modified the mobility of most PHEs. However, the application of Technosols induced a differential response in each case, which was mainly influenced by the composition of the organic waste used in each Technosol. Thus, Cd, Cu, and Zn solubility decreased in all Technosols (except Cu in T4, which increased), whereas As was re-solubilised in all Technosols and Pb solely in T4 and T5. Moreover, the added wastes not only affect to PHEs solubility, but can also represent an input of PHEs. This is the case of T5 with respect to Fe_W and Zn_T, or T4 and T5 where Na^+ ions contribute to the exchange complex causing salt stress.

The presence of PHEs, especially in their soluble form, caused oxidative stress in *L. sativa* grown in T5 and particularly in PS, triggering a strong antioxidant response as a tolerance mechanism. Detoxification of ROS occurred through the production of antioxidant metabolites such as glutathione and ascorbate, as well as increased enzymatic activity (i.e. catalase, superoxide dismutase, ascorbate peroxidase, glutathione reductase). Another effect of PHEs exposure was the limitation of photosynthesis and gas exchange processes; in both T5 and PS, the photosynthetic rate was reduced, leading to a decrease in *L. sativa* plant growth due to the stomatal limitations and damage to the photosynthetic machinery caused by PHEs.

Thus, the ecotechnology of Technosols can constitute a potential solution for the remediation of persistent polluted soils, although, in particular, the Technosol composed of vermicompost from pruning and gardening waste (T6) showed an overall performance far superior to that of the other Technosols. In addition to improving soil properties and reducing the mobility of most PHEs, phytotoxicity evaluated in *L. sativa* plants grown on T6 was minimal, with great similarity to the recovered soil in most of the parameters analysed.

Monitoring of the established vegetation on Technosols in the experimental plot "El Vicario", Guadiamar Green Corridor

Seville, Spain

April 2022

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CHAPTER 7

ENHANCING MICROBIAL BIODIVERSITY AND VEGETATION GROWTH IN POLLUTED SOILS BY TECHNOSOLS INTEGRATION

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CHAPTER 7: ENHANCING MICROBIAL BIODIVERSITY AND VEGETATION GROWTH IN POLLUTED SOILS BY TECHNOSOLS INTEGRATION

ABSTRACT

The objective of this study was to evaluate the effectiveness of two waste-derived Technosols in the *in situ* remediation of soils affected by potentially harmful elements (PHEs). Both Technosols (T4 and T6) consisted of polluted soil mixed with two inorganic mining wastes (iron oxyhydroxide-rich sludge and marble cutting and polishing sludge). In addition to an organic waste, in the case of T4, it was of agro-industrial origin (composted solid olive-mill by-product), while T6 incorporates one of urban origin (vermicompost from pruning and gardening). Technosols were applied superficially on polluted soils under field conditions in an area affected by residual pollution, 25 years after the Aznalcóllar mining spill. After one year, both the Technosols and the underlying polluted soils were analysed for main soil properties, solubility of PHEs, soil enzymatic activities, soil microbial composition and diversity, and the recovery of indigenous vegetation. The two selected Technosols remediated the adverse state of polluted soils, both by their integration into the Technosols and by the effect of their surface application, neutralising acidity, boosting organic carbon levels, minimising the mobility of PHEs, and stimulating soil enzymatic functions. This changes promoted the enhancement of the microbial community, mainly plant growth-promoting microorganisms, which positively influenced the establishment of a biodiverse and functional vegetation cover with autochthonous plants.

7.1. BACKGROUND

After evaluating in **chapters 4**, **5**, and **6** the effectiveness of the Technosols composed of polluted soil with a mixture of organic/inorganic wastes from local industries (mines, urban gardening services, wastewater treatment services, and olive mills) in remediating soils and water affected by PHEs at laboratory and microcosm level; the next step is the application under real conditions of the two best performing Technosols, **T4** and **T6**, selected in previous experiments. This chapter presents the results of the field application of these Technosols on unvegetated patches of polluted soils in the GGC (see Figure 2.7). In particular, the aim of this chapter is to comprehensively evaluate the effectiveness of *in situ* remediation of polluted soils through the application of these two Technosols by analysing the changes that occurred in: i) soil physicochemical properties, ii) PHEs solubility, iii) soil enzymatic activity, iv) soil microbial composition and diversity, and v) vegetation recovery.

Since, although the mobility and toxicity of PHEs in soils are strongly influenced by key properties (i.e. pH, OC, $CaCO_3$) (García-Carmona et al. 2017), to understand the complex interactions between PHEs, soil matrix, and organisms, as well as the resulting effects, we must extend beyond the scope of a mere physicochemical study (Leitgib et al. 2007; ISO 17402 2008). For example, as indicated in section 1.3.6, the characterisation of soil microbial communities provides valuable information on soil health status because microorganisms are sensitive to environmental stresses, such as PHEs pollution, serving as an indicator of ecosystem recovery (Schloter et al. 2018). Alterations in microbial composition (abundance, diversity, and metabolism) after remediation actions may signify a recovery in soil ecosystem functions (Muñoz-Rojas 2018). In this chapter, both the determination of soil enzymatic activities and the examination of the V3/V4 region of 16S rDNA extracted from soil have been used to determine soil microbial characteristics. Similarly, as stated in section 1.3.5, vegetation also plays an important role in the remediation processes of polluted areas, with its presence or absence serving as a significant indicator of soil pollution severity (Paniagua-López et al. 2024). For this reason, this chapter also analyses the vegetation developed on Technosols after one year of their field application to evaluate the potential assisted natural remediation by native vegetation of these polluted areas. The establishment of vegetation cover is highly relevant as it favours the enhancement of physicochemical and biological conditions of these degraded soils, for instance, by increasing SOM and nutrient availability, preventing the dispersion of pollutants in the ecosystem, helping to reestablish the water cycle, and avoiding soil erosion (Madejón et al. 2006b; Pérez-de-Mora et al. 2006; Bolan et al. 2011).

For a description of the experimental design and the analyses conducted in this field experience, see section 2.3.3. In this chapter, the changes over time of most parameters are tested both in the Technosols (T4 and T6; ~ 25 cm thickness) and in the underlying polluted soil (T4-PS and T6-PS; 25-30 cm depth) at 0, 6, and 12 months after application.

7.2. RESULTS AND DISCUSSION

7.2.1. SOIL PROPERTIES AND PHEs CONCENTRATIONS

Polluted soils sampled in the experimental plot "El Vicario" (T4-PS and T6-PS, Figure 2.7) showed a strongly acidic pH (Table 7.1) due to the reactions of hydration, oxidation, and hydrolysis of sulphides remaining in the soil after the remediation actions, which generated the conditions of acidity and solubilisation of PHEs (Simón et al. 2005). However, Technosols T4 and T6, despite the high percentage of polluted soil in their composition, showed a neutral pH from the beginning of the assay (Table 7.1) because during the incubation period, the calcium carbonate added as marble sludge exerted a pH buffering effect (Simón et al. 2010). Likewise, after the application of Technosols, a significant increase in pH was observed over time in both the Technosols and especially in the underlying polluted soils, from strong acidity to neutrality due to the dissolution of carbonates (Simón et al. 2005). The pH increases in polluted soils (T4-PS and T6-PS) may be due to leaching of carbonates from Technosols by rainfall and sporadic irrigation applied manually with 28 l m^{-2} at three different times (0, 3, and 6 months).

The high EC in polluted soils (Table 7.1) also resulted from the sludge deposited in the accident, which, upon drying, generated oxidising conditions that transformed sulphides into soluble sulphates (Simón et al. 2001). In Technosols, EC was even higher than in polluted soils at baseline conditions, especially in T4, due to the high EC of the composted solid olive-mill by-product (3.7 dS m^{-1}, Table 3.1) used as organic amendment in this Technosol (Aguilar-Garrido et al. 2023a). Nevertheless, in Technosols, EC decreased over time, whereas in underlying polluted soils, it decreased after six months (sampling in October) but increased again after a year (sampling in April). These changes could be related to the improvement of physical properties with Technosol treatment (e.g. soil structure and permeability), mainly related to the increase in organic matter, that may favour the mobility of soluble salts such as sulphates, conditioning salinity in depth, together with the seasonal influence.

Table 7.1. *Changes over time in the main physicochemical properties of Technosols (T4 and T6) and underlying polluted soils (T4-PS and T6-PS) (mean ± SD; n = 3).*

	pH			**$CaCO_3$** (%)			**EC** (dS m^{-1})			**OC** (%)		
Months	0	6	12	0	6	12	0	6	12	0	6	12
T4	7.03 ± 0.07 a	7.78 ± 0.14 b	7.56 ± 0.02 b	21.60 ± 1.27	19.47 ± 4.79	22.24 ± 2.09	3.34 ± 0.19 b	3.12 ± 0.11 b	2.79 ± 0.08 a	2.76 ± 0.36	2.16 ± 0.28	2.66 ± 0.12
T4-PS	3.61 ± 0.13 a	7.33 ± 0.26 b	7.04 ± 0.16 b	0.68 ± 0.04 a	0.41 ± 0.05 a	2.05 ± 0.83 b	2.88 ± 0.05 ab	2.72 ± 0.05 a	2.96 ± 0.04 b	0.46 ± 0.23 a	0.87 ± 0.08 b	0.87 ± 0.15 b
T6	7.12 ± 0.08 a	7.79 ± 0.07 c	7.48 ± 0.04 b	20.39 ± 0.99	19.10 ± 3.24	22.68 ± 0.72	3.05 ± 0.14 b	2.73 ± 0.02 a	2.77 ± 0.04 a	2.29 ± 0.09	2.40 ± 0.52	2.25 ± 0.22
T6-PS	3.60 ± 0.24 a	7.38 ± 0.19 b	7.27 ± 0.04 b	0.62 ± 0.14 a	0.38 ± 0.10 a	2.02 ± 0.66 b	2.81 ± 0.15	2.69 ± 0.11	2.88 ± 0.16	0.59 ± 0.19 a	0.92 ± 0.10 ab	1.12 ± 0.34 b

$CaCO_3$ - Calcium carbonate, EC - Electrical conductivity, OC - Organic carbon. Letters indicate significant differences in each soil between the three times (Dunn test, $p < 0.05$).

Furthermore, the underlying polluted soils showed a low OC content (Table 7.1), which limited both chemical and physical fertility. Such soils are not only depleted in nutrients but also tend to form surface crusts, which further limits the possibility of vegetation development (Martín et al. 2004). Therefore, in addition to buffering the acidic pH, it is imperative to increase the organic matter content to enhance its chemical and physical fertility. Organic matter is an important reservoir of nutrients and improves physical properties (e.g. porosity, structure), being one of the main structure-forming agents (Oades and Waters 1991; Aerts 1997). For that reason, both Technosols (T4 and T6) are composed in a considerable proportion (18%) of organic amendment, so that technosols had an OC content about 4-6 times higher than that of underlying polluted soils (T4-PS and T6-PS). In T6, the OC content was lower because the amendment used, pruning and gardening vermicompost, was poorer in OC than solid olive-mill by-product used in T4 (10.5% in VC vs. 29% in OL, Table 3.1) (Aguilar-Garrido et al. 2023a). The OC content did not show statistically significant differences over time in Technosols, remaining above 2.5% in T4 and 2% in T6. Moreover, OC content increased significantly over time in T4-PS and T6-PS, which may be due to physical stabilisation of the soil profile after wetting and drying processes, leading to expansion and contraction phenomena that mobilise soil particles, blurring the boundary between Technosol and underlying polluted soil.

After the sludge spill, the PHEs of greatest concern due to their concentration and potential toxicity were As, Cd, Cu, Sb, Pb, Tl, and Zn (Simón et al. 1999). However, with the clean-up and remediation measures carried out by the Regional Government of Andalusia and more than two decades after the accident, the main PHEs remaining in the area are As, Cu, Pb, and Zn (Paniagua-López et al. 2021); as these may represent an environmental risk due to their solubility and long-term bioavailability. In this sense, both in underlying polluted soils (T4-PS and T6-PS) and Technosols (T4 and T6), the total concentrations of As and Pb exceeded the guideline values proposed for soil protection in Andalusia (As: 36 mg kg^{-1}, Pb: 275 mg kg^{-1}), but not for Cu (595 mg kg^{-1}) and Zn (10,000 mg kg^{-1}) (BOJA 2015). In any case, the total PHEs concentrations measured exceeded the geochemical background of the area (in mg kg^{-1}; As: 17.8, Cu: 38.4, Pb: 39.1, Zn: 108.2) (Simón et al. 1999), which is in concordance with those reported by García-Carmona et al. (2019a) in bare soils located near the old tailing dump in the GGC.

However, the toxicity of PHE in soil is predominantly governed by its mobility and availability rather than its total concentration; therefore, PHE fractionation in soils is crucial for a comprehensive assessment of soil pollution risk (Giannakis et al. 2021). For example, PHEs that are encapsulated, insoluble, or tightly bound to solids may not be prone to biological absorption or cause a biological response, whereas those that are dissolved may be readily available (Kuppusamy et al. 2017). In this regard, the determination of water-soluble forms of PHEs is widely accepted in pollution studies because of its simplicity and cost-effectiveness. Water-soluble forms constitute the portion of PHEs that can be readily absorbed, and released to surface water and groundwater, potentially posing a threat to living organisms upon reaching critical endpoints (Bagherifam et al. 2019). Additionally, these forms may accumulate in plants, introducing a risk through their incorporation into food chain.

Among these PHEs, Cu and Zn are considered more mobile than As and Pb (Kraus and Wiegand 2006; Simón et al. 2008). The mobility of Cu and Zn is strongly conditioned by soil properties, mainly by pH, with solubility increasing at acidic pH (García-Carmona et al. 2019b). In this sense, the water-soluble (W) concentrations of Cu and Zn were negatively related to pH and $CaCO_3$ content (Figure S7.1). The initial acidity of the polluted soils (T4-PS and T6-PS) resulted in higher water-soluble concentrations of Cu and Zn than As and Pb (Table 7.2). Indeed, the acidity of polluted soils together with high Cu_W and Zn_W concentrations are the main soil stressors, inhibiting the development of vegetation in these persistently polluted soils in the GGC (García-Carmona et al. 2019a). Moreover, water-soluble concentrations of Cu and Zn were not only negatively related to pH and $CaCO_3$ content but also to OC content (Figure S7.1). All of these edaphic properties condition their solubility in soil (García-Carmona et al. 2019b; Sierra Aragón et al. 2019; Pastor-Jáuregui et al. 2021). Thus, the increase in pH and OC content in underlying polluted soils over time (Table 7.1) leads to a significant decrease in Cu_W and Zn_W concentrations (Table 7.2). This shows that Technosols applied on top of polluted soils gradually improve their properties and their effect is not limited to the uppermost 25 cm of the applied Technosol.

Table 7.2. *Changes over time in the water-soluble (W) concentrations of PHEs in Technosols (T4 and T6) and underlying polluted soils (T4-PS and T6-PS) in mg kg^{-1} (mean ± SD; n = 3).*

	As$_W$ (mg kg^{-1})			**Cu**$_W$ (mg kg^{-1})			**Pb**$_W$ (mg kg^{-1})			**Zn**$_W$ (mg kg^{-1})		
Months	0	6	12	0	6	12	0	6	12	0	6	12
T4	0.26 ± 0.03 a	0.51 ± 0.05 c	0.39 ± 0.01 b	0.42 ± 0.04 b	0.20 ± 0.05 a	0.14 ± 0.06 a	0.0017 ± 0.0029	bdl	bdl	0.30 ± 0.06 b	0.10 ± 0.05 a	0.07 ± 0.01 a
T4-PS	0.27 ± 0.04 b	0.17 ± 0.01 a	0.21 ± 0.03 ab	3.90 ± 1.07 b	0.08 ± 0.03 a	0.31 ± 0.04 a	0.0177 ± 0.0099	bdl	bdl	40.73 ± 6.49 b	0.04 ± 0.02 a	0.12 ± 0.12 a
T6	0.34 ± 0.03 a	0.56 ± 0.03 c	0.46 ± 0.03 b	0.25 ± 0.01 b	0.07 ± 0.04 a	0.10 ± 0.03 a	0.0013 ± 0.0023	bdl	bdl	0.21 ± 0.04 b	0.05 ± 0.01 a	0.05 ± 0.02 a
T6-PS	0.28 ± 0.03 b	0.16 ± 0.05 a	0.18 ± 0.04 a	3.62 ± 0.98 b	0.49 ± 0.22 a	0.24 ± 0.09 a	0.0250 ± 0.0333	bdl	bdl	32.36 ± 8.21 b	1.73 ± 0.48 a	0.10 ± 0.05 a

bdl – below detection limit. Letters indicate significant differences in each soil between the three times (Dunn test, $p < 0.05$).

In the case of Pb, the very low water-soluble concentration at initial conditions in both Technosols and underlying polluted soils decreased over time (Table 7.2), in line with the low mobility described for this element in soil (García-Carmona et al. 2019a; Alghamdi and Alasmary 2023). This reduction of Pb_W was related to the liming amendment with marble sludge, as it contributed to the immobilisation of Pb through precipitation in poorly soluble carbonates and/or chemisorption on carbonates (Simón et al. 2005; Mourid 2014).

Arsenic in soils, under oxidising conditions, is mainly present as As(V) in anionic form (Al-Abed et al. 2007). Therefore, the anionic behaviour of arsenate ($HAsO_4^{2-}$, $H_2AsO_4^-$) implies that this element has a special affinity for iron oxyhydroxides, thus being one of the main mechanisms of As immobilisation in soils (Romero-Freire et al. 2014). For this reason, the Technosols are composed of 2% iron oxyhydroxide-rich sludge (IO); however, it appears that this added amount was insufficient, as the As_W concentration increased significantly in Technosols after field application (Table 7.2). This may be caused by the increase in pH in Technosols, as arsenate in soils has a maximum adsorption between pH 3 and 5 (Arco-Lázaro et al. 2016). Moreover, its solubility increases with increasing pH, which favours As desorption from iron oxides (Sigfusson et al. 2008). On the other hand, organic matter added to Technosols can also increase As solubility by competition between organic matter and As forms for adsorption sites or by forming As soluble complexes (Wang and Mulligan 2009).

7.2.2. SOIL ENZYME ACTIVITY

Soil microorganisms are key in the stabilisation and degradation of organic matter, soil nutrient cycling, and maintenance of soil structure (Agegnehu et al. 2016); therefore, they can be used as bioindicators of soil quality. Among the enzymes commonly used to assess the effectiveness in the remediation of degraded environments (e.g. polluted soils, mining waste) in the short-medium term are dehydrogenase, cellulase, protease, and acid phosphatase (de Varennes et al. 2010; Kumar et al. 2013; Santos et al. 2016a). Enzyme diversity in soil is extremely important because chemical transformations will be complemented when a full set of enzymes is present (Walmsley and Sklenička 2017), as each enzyme is related to a specific function and chemical reaction to be catalysed.

Overall, under initial conditions, polluted soils (T4-PS and T6-PS) showed low enzymatic activities, whereas Technosols (T4 and T6) exhibited higher enzymatic activities. In this regard, the principal component analysis (PCA) performed considering soil properties, total and water-soluble forms of PHEs studied, and soil enzymatic activities, in addition to grouping these variables into two components that explain 65.2% of the variance, distinguished two main groups: Technosols (T4 and T6) and polluted soils (T4-PS and T6-PS) (Figure S7.1). This distinction is based, with a greater weight of the initial assay conditions, in the case of polluted soils mainly on the total and water-soluble concentrations of PHEs, whereas in the case of Technosols, it is based on soil physicochemical properties and soil enzymatic activity. Anyway, over time, most of the enzymes increased not only in Technosols but also in underlying polluted soils due to the influence of Technosols, although to a lesser extent (Figure 7.1). The increased enzymatic activities found in Technosols suggest a well functioning of microbial communities involved in organic matter degradation, mineralisation processes, and nutrient cycling (Arán et al. 2022), highlighting the improved quality of Technosols (T4 and T6) compared to the polluted soil composing them.

Dehydrogenase activity has been extensively employed as a key metric to assess overall microbial activity in both natural and PHEs-affected soils (Kumar et al. 2013; Dotaniya et al. 2019). This enzymatic activity serves as an indicative measure of the microbiological redox system and microbial oxidative activities in soils (Casida et al. 1964). Dehydrogenase is significantly influenced by several environmental factors, including soil moisture, oxygen availability, pH, SOM content, soil depth, temperature, season, and PHE pollution (Wolińska and Stępniewsk 2012). Thus, the unfavourable conditions (i.e. acidity, nutrient depletion, high PHEs concentrations) in polluted soils would explain the low dehydrogenase activity measured (< 2 µg TPF g^{-1} 16 h^{-1}). Likewise, after one year of Technosol application, because of the improvement in quality and the decrease in the solubility of PHEs studied, the dehydrogenase activity slightly increased in underlying polluted soils (Figure 7.1), although it was only statistically significant at T4-PS. This is consistent with other studies, such as Santos et al. (2016a), which reported an increase in dehydrogenase activity when acidity is reduced in soils affected by high PHEs concentrations. In Technosols, dehydrogenase activity also increased over time, reaching the maximum values recorded in this assay after one year (85 and 60 μg TPF g^{-1} 16 h^{-1} in T4 and T6, respectively), which correspond to similar or even higher values observed in healthy grassland soils, agricultural soils, and Mediterranean forest soils (Kumar et al. 2013).

Cellulases, which consist of a system of three enzymes (endo-1,4-glucanase, exo-1,4-glucanase, and β-d-glucosidase), are responsible for degrading cellulose into glucose (Deng and Tabatabai 1994) and are therefore strongly linked to the C-cycle (Bai et al. 2013). Cellulase activity in soils may be inhibited by the presence of PHEs (Makoi and Ndakidemi 2008; Martinez-Salgado et al. 2010), as occurred in the initial conditions of polluted soils (Figure 7.1), where cellulase activity was relatively low. In contrast, the initial cellulase activity in Technosols was clearly higher than that measured in polluted soils because a considerable amount of organic matter was added to Technosols. After one year, cellulase activity increased significantly in both Technosols and underlying polluted soils, exhibiting a faster response to improved soil properties (i.e. pH rise, OC increase, and PHEs solubility decrease) compared to the other enzymes.

Phosphatase activity related to the P cycle (Krämer and Green 2000) has also been studied as an indicator of organic phosphorus availability for plants and organisms (Piotrowska-Długosz and Charzyński 2015). However, although it has been reported that the presence of PHEs can diminish phosphatase activity (Kandeler et al. 1996), other studies suggest that the impact of PHEs on phosphatase activity is not very pronounced, demonstrating a certain degree of resilience to these perturbations (Aponte et al. 2020; Aguilar-Garrido et al. 2023b). This coincides with our results, as polluted soils (T4-PS and T6-PS) showed a certain phosphatase activity at the initial stage (0.25 μmol ρ-nitrophenol g^{-1} h^{-1}) (Figure 7.1). The increased phosphatase activity over time in Technosols is in accordance with the findings of Qu and de Varennes (2010), de Varennes et al. (2010), and Santos et al. (2014b), who reported a positive relationship between acid phosphatase activity and a significant increase in SOM content through the application of organic amendments to polluted soils or the production of tailor-made Technosols.

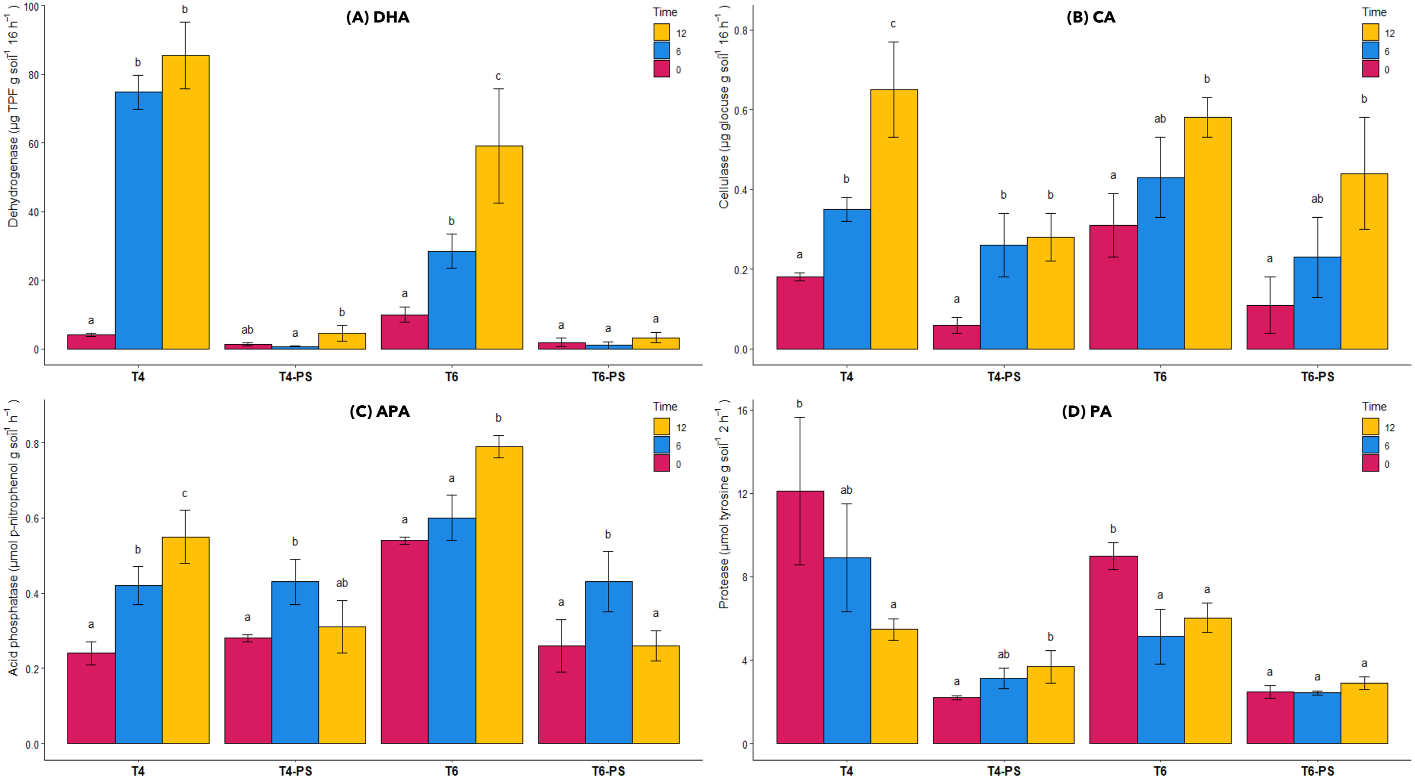

Figure 7.1. *Changes over time (0, 6, and 12 months) in enzymatic activities [DHA: dehydrogenase (A), CA: cellulase (B), APA: acid phosphatase (C), PA: protease (D)] in Technosols (T4 and T6) and underlying polluted soils (T4-PS and T6-PS) (n = 3).* Letters *indicate significant differences in each soil between the three times (Dunn test,* $p < 0.05$*).*

Proteases, which are responsible for breaking down proteins into amino acids through proteolysis, play a key role in N mineralisation (Jan et al. 2009). Therefore, they are essential N-cycle enzymes (Liang et al. 2003), as approximately 40% of the organic N in soil is in the form of proteins (Schulten and Schnitzer 1997). In this sense, the organic waste added to Technosols could explain the higher protease activity that occurred in Technosols at the initial time and that decreased over time depending on the evolution of SOM over the year of the assay (Figure 7.1). High N-cycle enzyme activity, particularly protease, has been documented in polluted soils amended with different organic wastes such as sewage sludge (Achberger and Ohlinger 1988), effluents from cotton ginning mills (Narasimha et al. 2012), and pig slurry (Plaza et al. 2002). In this regard, protease activity tended to show a slight increase in these polluted soils (T4-PS and T6-PS), although only statistically significant in T4-PS, potentially attributed to improved soil properties, especially linked to increased organic matter content, which exhibited a positive correlation with protease activity (Figure S7.1).

7.2.3. VEGETATION GROWTH

One year after the application of the Technosols (T4 and T6), total vegetation cover was established on both Technosols (Table 7.3), with the identification of 15 species (Table S7.1). All these species are characteristic of the Guadiamar river basin (Pérez Latorre et al. 2002) and are prevalent in the remediated area affected by the Aznalcóllar spill (García-Carmona et al. 2019a), as these soils were used as the seed bank (SB) to accelerate the establishment of natural vegetation.

Table 7.3. *Comparison of vegetation cover and diversity between Technosols T4 and T6 after one year.*

	Technosol T4	**Technosol T6**
Cover (%)	100	100
Specific richness (no species)	13	9
H’	2.93	2.51

However, although the vegetation cover was 100% in both Technosols, there was a higher specific richness and biodiversity index in T4 than in T6 (Table 7.3).

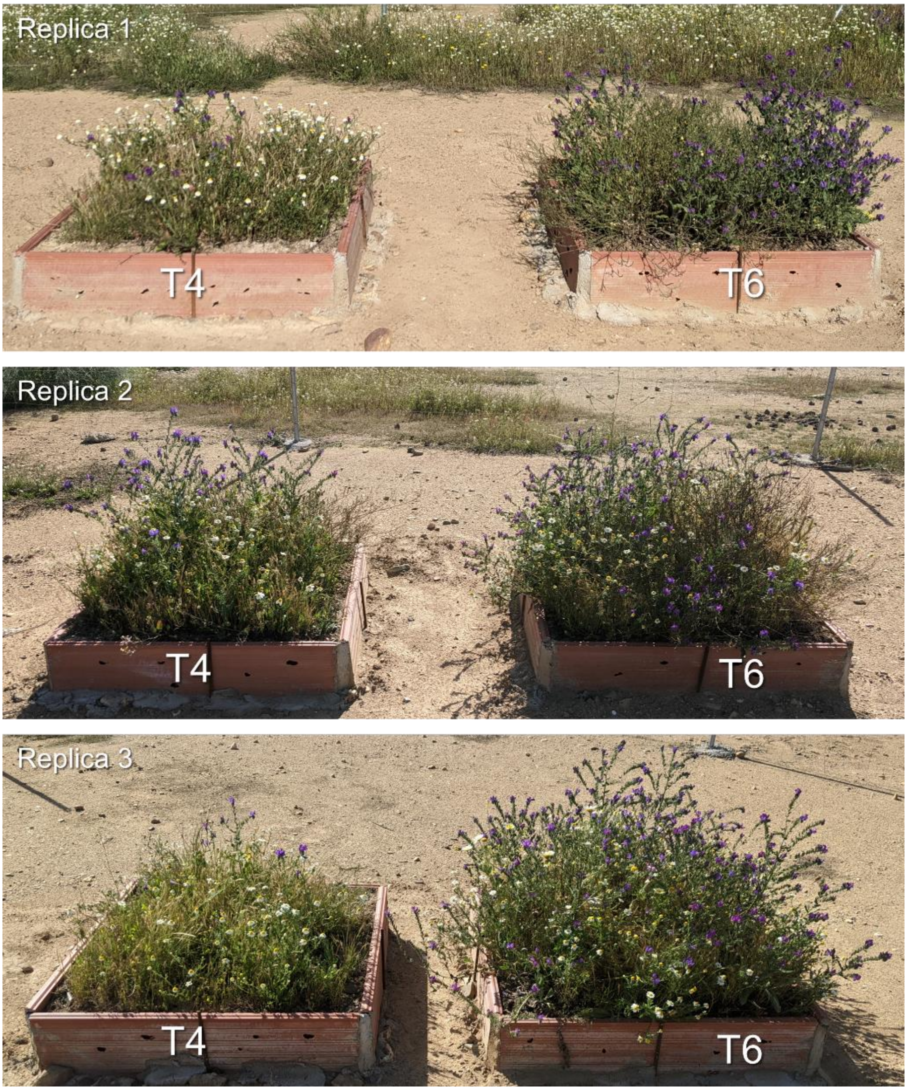

Figure 7.2. *General aspect of the natural vegetation developed after one year in Technosols T4 and T6 (n = 3).*

In T6, the most predominant species were *Echium plantagineum* L., *Brassica oxyrrhina* Willk. (Coss.), *Anthemis arvensis* L., and *Vulpia membranacea*, (L.) Dumort., whereas in T4 *Spergularia rubra* (L.) J. Presl & C. Presl. and *Lamarckia aurea* (L.) Moench also predominated, but *E. plantagineum* was not abundant (Table S7.1). The organic matter of the vermicompost of pruning and gardening waste used in T6 promoted more favourable conditions for the development of fast-growing and vigorous vegetation, which prevented the growth of later species. In T6, *E. plantagineum* was the most abundant species and the largest in size (Figure 7.2), which may lead to a competitive effect with other species, limiting their growth. While, in T4, species such as *S. rubra* and *L. aurea,* identified as bioindicator (pioneer plants) in this region of partially remediated soils (García-Carmona et al. 2019a), exhibited a significant presence in this soil. Both species are highly tolerant in persistently PHE-polluted soils, suggesting still potentially unfavourable conditions for vegetation growth in T4. In this sense, soil conditions in T4 could limit the vigorous growth of species such as *E. plantagineum*, allowing the development of other species and increasing biodiversity.

7.2.4. MICROBIAL DIVERSITY

The microbial diversity of Technosols and polluted soils was highly linked to the soil enzyme activity and vegetation development (see sections 7.2.2 and 7.2.3) previously described. Initially, Technosol T6 contained higher diversity indices than Technosol T4, probably because of the nature of the organic carbon in the vermicompost of pruning and gardening waste (VC), which was more readily usable by soil microorganisms compared to that of the composted solid olive-mill by-product (OL) (Aguilar-Garrido et al. 2023a). Moreover, OL commonly contains high concentrations of phenolic acids, which have toxic effects on most living organisms, from plants to microorganisms (Pinho et al. 2017; Panigrahy et al. 2022), hence the lower diversity indices in initial T4 samples.

The low diversity indices (Shannon, Simpson, and Chao1) observed in the initial polluted soil samples (T4-PS and T6-PS) gradually increased to levels observed in the seed bank and Technosols samples (Table 7.4). The seed bank (SB) corresponds to the uppermost 5 cm of vegetated topsoil from recovered areas, of which a small layer was applied over Technosols to provide indigenous seeds (see section 2.3.3). Similar results were observed in the cluster analysis of the microbial relative abundance data (Figure 7.3), where at time 0, the microbial profile of polluted samples shifted towards that of recovered soil, similar to the profiles of the seed bank and Technosols. Over time, rainfall and irrigation may have increased microbial mobility from the seed bank and Technosols to the deeper layers of polluted soils. However, the survival of the microbial profile throughout the year reflects the recovery of the soil in terms of microbial diversity. Nevertheless, the presence of a similar microbial community profile does not necessarily mean full functional recovery of polluted soil, as observed in the microbial enzymatic activities (Figure 7.1), although the microbial activities showed an improvement in cellulase activities.

Table 7.4. *Diversity indices of the samples of seed bank (SB), Technosols (T4 and T6), and polluted soils (T4-PS and T6-PS) at the ASV level.*

		Mean diversity indices		
Sample	**Months**	**Shannon**	**Simpson**	**Chao1**
SB	0	6.718	0.997	1715
T4	0	5.691	0.991	1039
	6	6.211	0.995	1200
	12	6.682	0.997	1673
T4-PS	0	3.777	0.927	360
	6	5.598	0.985	988
	12	6.002	0.987	1193
T6	0	7.038	0.999	1812
	6	6.625	0.997	1439
	12	6.914	0.998	1705
T6-PS	0	4.548	0.960	607
	6	6.465	0.996	1354
	12	6.567	0.996	1464

The network analysis performed on the relative abundance data (Figure S7.2) shows the strength of pairwise interactions among the microbial operational taxonomic units (OTUs) at the species level. The clustering coefficients in Technosols were much higher (T4: 0.48, T6: 0.31) than those of polluted soils (T4-PS: 0.13, T6-PS: 0.12), which is a measure of the degree to which nodes cluster together. The latter indicates a lower connectivity between the microbial species in polluted soils than that established in Technosols; hence, Technosol accelerates ecological interactions.

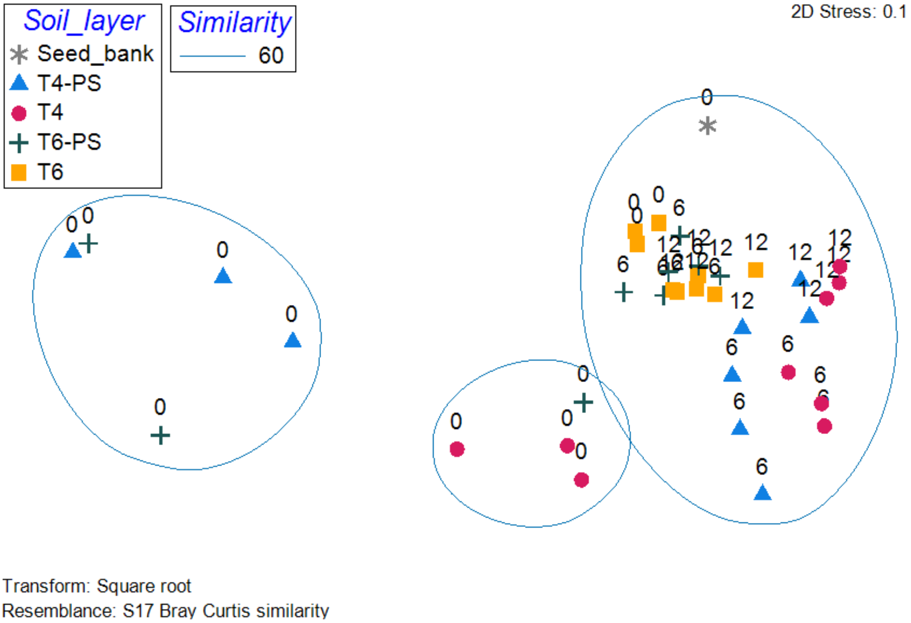

Figure 7.3. *Multi-dimensional scaling (MDS) of bacterial communities at the species level based on Bray-Curtis dissimilarity index. Each data point label represents sampling time (0, 6, and 12 months). Clustering of samples with similarity ≥ 60%, with replicates represented for each condition.*

The meta-taxonomic analysis of the most abundant species is shown in Figure 7.4, and they are clustered into 10 groups according to their abundance profiles across the samples. One of the most abundant OTUs observed mainly in polluted samples (T4-PS and T6-PS) was *g_Actinocorallia* (cluster ii. spore-forming, Gram-positive bacteria. Phylum: Actinomycetota). In the literature, *Actinocorallia populi* is described as containing functional genes encoding substances against toxic compounds such as Co, Zn, Cd, As, and Cr (Li et al. 2020b). Other studies have described high isolation numbers (Remenár et al. 2014) or high relative abundances (Paniagua-López et al. 2021) of Actinomycetota (previously Actinobacteria) from PHE-polluted soil sites.

Other OTUs were found at constant relative abundances throughout all samples (cluster i and v); therefore, they were impervious to soil physicochemical changes. Moreover, in clusters iv and viii, we observed a high relative abundance of these OTUs in both Technosols and underlying polluted soils, which decreased with time.

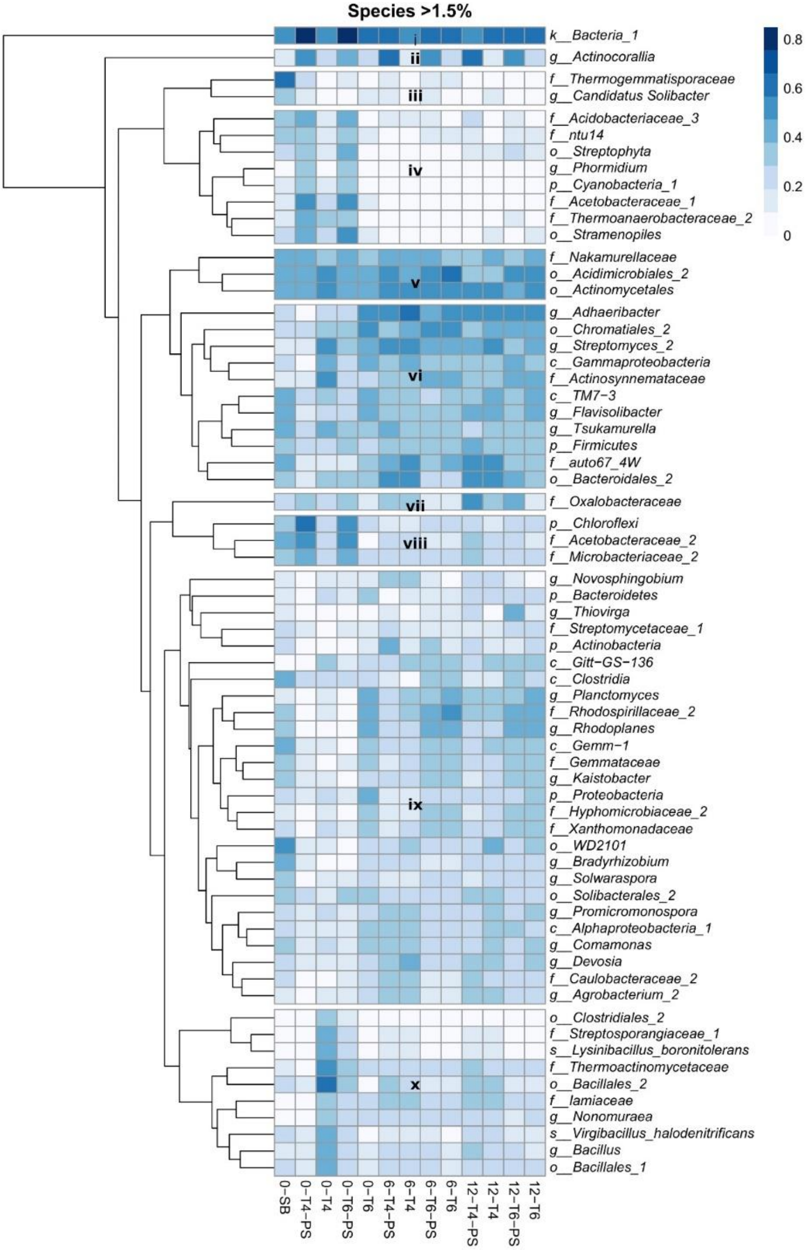

Figure 7.4. *Comparative bacterial cluster analysis across of seed bank (SB), Technosols (T4 and T6), and polluted soils (T4-PS and T6-PS). The colour scale represents the $\log_{10}$ of the square root of the mean relative abundance. Rows are clustered using Euclidean distance. Taxa are grouped at the species level (97% similarity), with only those exceeding 1.5% relative abundance in at least one sample displayed. Unclassified taxa are labelled according to their last known taxon.*

The most significant shift in relative abundance was observed in cluster vi, with an increase in these OTUs over time. The latter cluster includes species from the genus Adhaeribacter, Flavisolibacter, and Tsukamurella, which have been described in PLABA database (https://plabase.cs.uni-tuebingen.de/pb/plabase.php) as containing attributes of plant growth-promoting microorganisms (PGPMs). Therefore, the increase in the relative abundance of PGPMs in Technosols and underlying polluted soils, accompanied by full vegetation growth, describes the early stage of recovery of soil ecosystem functions.

7.3. CONCLUSIONS

The PHE-polluted soils exhibited strong acidity, high salinity, and low organic carbon (OC) levels, as well as high PHEs concentrations in total and soluble forms. Technosols supplemented with liming, iron-rich, and organic amendments significantly improved soil properties (pH, EC, $CaCO_3$) and notably increased OC content, thus enhancing fertility. This improvement in soil properties reduced the mobility and potential risk of the most potentially harmful elements (PHEs). However, the progressive increase in soil enzyme activity over time during the remediation process implied an improvement in the microbial community. In addition, the increase in microbial diversity favoured greater connectivity among microbial species, indicating an acceleration of ecological interactions. This was further evidenced by the increasing presence of plant growth-promoting microorganisms in the studied soils over time, in correlation with vegetation growth, which revealed the recovery of soil ecosystem functions. Consequently, the integration of Technosols resulted in a substantial enhancement of soil quality, creating a favourable environment for vegetation growth while mitigating the risks associated with PHEs in polluted soils. However, differences related to the type of organic matter and the richness and diversity of plant and microorganism species should be studied in the long term to evaluate the recovery of biological properties in both Technosols and underlying polluted soils.


50 (1)
75 (1)

Hyacinth bean growing on gossan waste-derived Technosols in an open greenhouse of the Instituto Superior de Agronomia (ISA) - Universidade de Lisboa

Lisbon, Portugal

May 2019

Patrícia Vidigal

CHAPTER 8

A GREEN SOLUTION FOR THE REHABILITATION OF MARGINAL LANDS: THE CASE OF *LABLAB PURPUREUS* (L.) SWEET GROWN IN TECHNOSOLS

Aguilar-Garrido, A., Reyes-Martín, M. P., Vidigal, P., Abreu, M. M. (2023). A green solution for the rehabilitation of marginal lands: the case of *Lablab purpureus* (L.) Sweet grown in Technosols. ***Plants***, 12(14), 2682. DOI: 10.3390/plants12142682

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ABSTRACT

Reclamation of abandoned mining areas can be a potentially viable solution to tackle three major problems: waste mismanagement, environmental pollution, and growing food demand. This study aims to evaluate the conversion of mining areas into agricultural production areas using integrated biotechnology based on the combination of Technosols with a multipurpose (forage, food, ornamental and medicinal) drought-resistant legume, the *Lablab purpureus* (L.) Sweet. Two Technosols were prepared by mixing gossan waste (GW) from an abandoned mining area with low-cost organic and inorganic materials. Before and after plant growth, several parameters were analysed, such as soil physicochemical characteristics, nutritional status, bioavailable concentrations of potentially harmful elements (PHEs), soil enzymatic activities, and development and accumulation of PHEs in *L. purpureus*, among others. Both Technosols improved physicochemical conditions, nutritional status, and microbiological activity, and reduced the bioavailability of most PHE (except As) of GW. *L. purpureus* thrived in both Technosols and showed PHEs accumulation mainly in roots, with PHEs concentrations in shoots that are safe for cattle and sheep consumption. Thus, this is a potential plant that, in conjunction with Technosols, constitutes a potential integrated biotechnology approach for the conversion of marginal lands, such as abandoned mining areas, into food production areas under the condition of exhaustive quality and food safety controls.

8.1. BACKGROUND

In this chapter, we focus on the remediation of mining tailings such as gossan waste, which is one of the main sources of pollution in sulphide mining. Because the exposure to oxidising and rainfall conditions of the sulphides (mainly pyrite [FeS_2]) contained in these wastes leads to the generation of AMD that can result in soil pollution; consequently, the reclamation of these mining wastes is vital to prevent ecosystem degradation in the surroundings of mining areas.

Alongside this, there is a major global problem of increasing food demand due to population growth. The current population of eight billion people is expected to reach 8.6 billion in 2030, 9.8 billion in 2050, and 11.2 billion in 2100 (United Nations 2022), putting severe pressure on land and water resources. Thus, it is imperative to safeguard our natural resources and remediate degraded areas. In fact, both society and the scientific community demand a change in food production that allows for lower land use and reduces water consumption and greenhouse gas emissions (Willett et al. 2019; James-Martin et al. 2022). In this study, the potential of using a green technology for the remediation of marginal lands, such as abandoned mining areas, is explored. To this end, the combination of waste management allied to the production of a forgotten crop, *Lablab purpureus* (L.) Sweet (common name: hyacinth bean or Lablab bean) was studied, evaluating its viability towards the reclamation of marginal lands. In this sense, the combination of Technosols and phytostabilisation techniques has emerged recently as one of the most effective ecotechnologies for the remediation of degraded areas (Macías 2004; Macías et al. 2007; Macías et al. 2011; Santos et al. 2014a; Santos et al. 2016a; Santos et al. 2018), again enabling different potential human uses in them, from recreational to agricultural and livestock activities.

L. purpureus is a stress-tolerant, versatile, and multipurpose crop native to tropical and subtropical Africa, and is recognised as a crop of the future (Vidigal et al. 2018; Vidigal et al. 2019). It is one of the oldest domesticated and cultivated legumes because of its versatility and multifunctionality (legume, vegetable [leaf, pod, and flower], forage/green manure, medicinal, phytopharmaceutical, and ornamental) (Maass et al. 2010). Furthermore, it is very tolerant to abiotic stress and adapts well to different soil conditions. In particular, it is highly resistant to drought via physiological adaptations (e.g. insolation-dependent leaf movement) in low rainfall areas (Younis 2010) and moderately tolerant to salinity (D'Souza and Devaraj 2009). This legume has also been shown to be a potential phytoremediator of the herbicide trifloxysulfuron sodium (Procópio et al. 2004) and a phytostabiliser of Cd (Souza et al. 2017). This suggests that this legume is a potential alternative for the reclamation of abandoned mining areas, especially in harsh climates that are prone to worsen with climate change, as in the south of the Iberian Peninsula.

In particular, the objective of this chapter is to evaluate the potential of a green biotechnology in mining reclamation that combines the widely tolerant multifunctional legume, *L. purpureus*, with two designed Technosols derived from mixing gossan waste with a combination of organic/inorganic wastes from local industries (urban gardening services, quarries, cafes, and breweries) (see section 2.2.5 for details of Technosols). Specifically, the purpose is to promote favourable conditions in the gossan waste from the São Domingos mine (see section 2.2.3) for optimal growth of *L. purpureus*, as well as to evaluate the safety of this plant for animal consumption. For complete information on the experimental design and analyses implemented in this greenhouse experiment, see section 2.3.2.3.

Note: In this chapter, unlike in other chapters, the letters appearing in different tables and figures indicate statistically significant differences in the means by comparison between one treatment/substrate and another, not integrating all treatments/substrates.

8.2. RESULTS AND DISCUSSION

8.2.1. PHYSICOCHEMICAL CHARACTERISATION OF GOSSAN WASTE

Physicochemical characteristics of the base gossan waste (GW_b) from the São Domingos mining area show that these materials were highly acidic (pH ~ 3.4) and poorly fertile, manifested by a very low cation exchange capacity (CEC < 2.5 $cmol_{(+)}$ kg^{-1}), very low OC content (5.1 g kg^{-1}), as well as total N (0.19 g kg^{-1}), extractable P (0.003 mg kg^{-1}), and extractable K (7.3 mg kg^{-1}) (Table 8.1) (Veloso et al. 2022).

After incubation (GW_i), gossan wastes showed, overall, no significant differences in physicochemical properties from GW_b (Table 8.1), except for EC, which showed an increase of approximately 36%. Slight increases were also observed in some properties, such as pH and total N, whereas others, such as OC and extractable P and K, decreased slightly during the incubation period. The development of *L. purpureus* (GW_f) had no great influence on GW_i improvement, as no statistically significant changes were found between these two. The high ratio of Ca/Mg both in GW_i and GW_f (> 8.0) indicates that these substrates were very unfavourable for plant nutrition (Veloso et al. 2022). However, most macro- and micronutrient concentrations in gossan waste suffered an overall increase from GW_i to GW_f (Table 8.2). Although there were no statistically significant differences, except for Mn, there was an increase of 35% in Fe and 39% in Ca, an effect observed with other legume crops (Marcos-Pérez et al. 2023; Mthimunye et al. 2023).

Table 8.1. *Physicochemical characteristics of gossan waste (GW) and Technosols (TC50 and TC75) before (i) and after (f) L. purpureus growth (mean ± SD; n = 6).*

	GW_b	**GW_i**	**$TC50_i$**	**$TC75_i$**	**GW_f**	**$TC50_f$**	**$TC75_f$**
pH (H_2O)	3.41 ± 0.01 bc	3.58 ± 0.09 de	6.79 ± 0.03 bdfj	7.04 ± 0.02 cefk	3.73 ± 0.04 gh	6.26 ± 0.02 gj	6.36 ± 0.02 hk
EC (dS m^{-1})	0.14 ± 0.02 abc	0.43 ± 0.03 de	0.75 ± 0.06 dfj	1.12 ± 0.05 ef	0.29 ± 0.06 gh	0.54 ± 0.06 gij	0.97 ± 0.03 hi
OC (g kg^{-1})	5.14 ± 1.18 bc	3.46 ± 0.46 de	15.07 ± 2.01 bd	20.32 ± 1.30 ce	4.22 ± 0.17 gh	14.36 ± 0.86 g	19.71 ± 1.90 h
N_T (g kg^{-1})	0.19 ± 0.01 bc	0.30 ± 0.03 de	1.12 ± 0.06 bdf	1.82 ± 0.14 cef	0.19 ± 0.04 gh	1.06 ± 0.04 gi	1.94 ± 0.07 hi
P_A (mg kg^{-1})	0.003 ± 0.001 bc	bdl de	161.66 ± 16.10 bdfj	358.33 ± 15.31 ef	0.75 ± 0.41 gh	180.59 ± 9.50 gij	421.38 ± 30.99 hi
K_A (mg kg^{-1})	7.31 ± 3.37 bc	6.50 ± 2.86 de	156.45 ± 7.47 bdf	276.15 ± 16.88 efk	6.70 ± 2.41 gh	161.52 ± 6.93 gi	292.90 ± 11.20 hik
CEC ($cmol_{(+)}$ kg^{-1})	2.44 ± 0.49	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.

GW_b – Base gossan waste (prior to incubation); EC – electrical conductivity; OC – organic carbon; N_T – total N; P_A, K_A – extractable P and K; CEC – cation exchange capacity; bdl - below detection limit; n.a. – not analysed. Letters represent significant differences between substrates (ANOVA and Tukey's post hoc test, $p < 0.05$): (a) GW_b vs. GW_i, (b) GW_b vs. $TC50_i$, (c) GW_b vs. $TC75_i$, (d) GW_i vs. $TC50_i$, (e) GW_i vs. $TC75_i$, (f) $TC50_i$ vs. $TC75_i$, (g) GW_f vs. $TC50_f$, (h) GW_f vs. $TC75_f$, (i) $TC50_f$ vs. $TC75_f$, (j) $TC50_i$ vs. $TC50_f$, (k) $TC75_i$ vs. $TC75_f$.

The gossan wastes had high total concentrations of several PHEs (in g kg^{-1}; As: 9.13, Cu: 0.22, Hg: 0.03, Pb: 29.63; Table 8.3). Thus, these gossan wastes can be considered polluted with As, Cu, Hg, and Pb for agricultural, residential/parkland, commercial, and industrial uses in most cases, as the concentrations exceeded 830, 3.5, 169, and 658 times, respectively, the most restrictive regulatory level (usually agricultural use) (CCME 2007; APA 2019). On the other hand, PHEs concentrations in the bioavailable fraction of GW$_i$ and GW$_f$ were low, ranging between 0.0034% for As and 9.09% for Cd of the total concentrations in GW$_b$. Furthermore, PHEs bioavailability did not change significantly between GW$_i$ and GW$_f$, although there were slight changes, such as a decrease of about 40% of bioavailable Pb in GW$_f$ (Table 8.3), probably due to Pb uptake by *L. purpureus* roots.

Table 8.2. *Concentration of extractable macro- (Ca, Mg, Na, K) and micronutrients (Fe, Mn, Zn, and Cu) in gossan waste (GW) and Technosols (TC50 and TC75) before (i) and after (f) L. purpureus growth in mg kg^{-1} (mean ± SD; n = 6).*

Nutrients (mg kg^{-1})	**GW$_i$**	**TC50$_i$**	**TC75$_i$**	**GW$_f$**	**TC50$_f$**	**TC75$_f$**
Ca	80.63 ± 11.47 ab	1075.65 ± 96.90 ah	1501.75 ± 71.08 bi	205.28 ± 25.53 de	3017.38 ± 212.05 dfh	4132.25 ± 337.64 efi
Mg	6.13 ± 0.43 ab	52.25 ± 2.95 ach	85.20 ± 4.20 bci	8.40 ± 1.02 de	66.50 ± 1.21 dfh	109.96 ± 5.14 efi
Na	44.25 ± 3.72 ab	131.90 ± 34.95 a	162.40 ± 6.75 b	41.05 ± 2.63 de	104.38 ± 4.41 df	178.17 ± 10.53 ef
K	12.13 ± 1.30 ab	192.85 ± 11.02 ac	285.35 ± 12.05 bc	17.15 ± 2.33 de	177.75 ± 7.73 df	301.83 ± 13.38 ef
Fe	24.44 ± 0.70 ab	1031.35 ± 8.69 ach	1797.95 ± 102.65 bci	69.85 ± 2.14 de	850.71 ± 19.99 dfh	2024.83 ± 169.79 efi
Mn	<0.01 abg	3.72 ± 0.11 ach	7.03 ± 0.30 bci	1.95 ± 0.34 deg	9.83 ± 0.87 dfh	14.63 ± 0.82 efi
Zn	1.31 ± 0.12 ab	5.22 ± 0.26 ach	7.61 ± 0.77 bc	1.31 ± 0.21 de	3.88 ± 0.07 dfh	7.59 ± 0.51 ef
Cu	6.03 ± 2.05 g	7.10 ± 0.72	5.58 ± 0.32	1.24 ± 0.03 g	3.04 ± 0.08	4.78 ± 0.31

Letters represent significant differences between substrates (ANOVA and Tukey's post hoc test, $p < 0.05$): (a) GW$_i$ vs. TC50$_i$, (b) GW$_i$ vs. TC75$_i$, (c) TC50$_i$ vs. TC75$_i$, (d) GW$_f$ vs. TC50$_f$, (e) GW$_f$ vs. TC75$_f$, (f) TC50$_f$ vs. TC75$_f$, (g) GW$_i$ vs. GW$_f$; (h) TC50$_i$ vs. TC50$_f$; (i) TC75$_i$ vs. TC75$_f$.

The gossan wastes from the São Domingos mine area constitute degraded environments characterised by very acidic pH, low CEC, low nutrient availability, poor OC, and high total concentrations of several PHEs, similar to most gossan waste from sulphide mining areas. Therefore, it is necessary to neutralise the acid pH, increase nutrient availability and OC content, and improve structure and water-holding capacity, which are essential characteristics in the remediation of Mediterranean mining areas (Madejón et al. 2021). For this purpose, the addition of amendments has been widely used, asthey can also provide the ability to immobilise PHEs by various chemical processes (Adriano et al. 2004; Kumpiene et al. 2008), although some of these effects may not last over time (Macías 2004; Macías et al. 2011). Therefore, it might be more efficient to produce specific Technosols adjusted to the conditions of each mining waste by mixing amendments for better promotion and maintenance of biogeochemical processes and decrease of bioavailability of PHEs for plants (Yao et al. 2009; Macías et al. 2011). In this case, a mixture of organic wastes with different OC sources and stability (biomass pruning, coffee grounds, sludge, and waste kieselguhr) was used to improve fertility in Technosols, as they have high contents of OC, total N, extractable P and K, and N-NH_4^+, as well as to promote soil aggregate formation to improve soil structure, together with limestone rock wastes to provide acidity buffering capacity (Cortinhas et al. 2020, 2021). Although this was not the only criterion for their selection in the construction of Technosols, the fact that they all come from common activities around the world in urban, mining, and agro-industrial environments was also considered; therefore, it is assumed that they are readily available in other regions affected by the mining industry.

8.2.2. PHYSICOCHEMICAL CHARACTERISATION OF TECHNOSOLS

The technology behind Technosols offered a significant improvement to the physicochemical properties and nutritional status (Tables 8.1 and 8.2), demonstrating the beneficial influence that the Technosols approach has on the improvement of gossan waste. Regardless of the ratio of the GW–organic and inorganic waste mixture ($TC50_i$ and $TC75_i$), both Technosols exhibited significant increases in all parameters studied. pH increased from acidic values in GW_i to a near neutral pH in Technosols, and EC increased 1.7 and 2.6 times in $TC50_i$ and $TC75_i$, respectively (Table 8.1). The contents of OC and total N increased by 77% in $TC50_i$ and in $TC75_i$ increased by 83% but, extractable P and K contents showed the highest significant increase, over 96% (Table 8.1). Similarly, the concentration of extractable macro- and micronutrients significantly increased by more than 66% in both $TC50_i$ and $TC75_i$ (Table 8.2) because of the added organic and inorganic waste mixture, as observed in Aguilar-Garrido et al. (2022b), but $TC75_i$ was more favourable than in $TC50_i$.

The concentrations of PHEs in the bioavailable fraction of Technosols, in comparison with the total concentrations in GW_b (Table 8.3), showed a decrease of

Table 8.3. *Concentrations of PHEs in pseudototal fraction of base gossan waste (GW$_b$) and in bioavailable fraction of gossan waste (GW) and Technosols (TC50 and TC75) before (i) and after (f) L. purpureus growth in mg kg^{-1} (mean ± SD; n = 6).*

	Pseudototal fraction (mg kg^{-1})	***Bioavailable fraction*** (mg kg^{-1})					
PHEs	**GW$_b$**	**GW$_i$**	**TC50$_i$**	**TC75$_i$**	**GW$_f$**	**TC50$_f$**	**TC75$_f$**
As	9126.67 ± 238.77	0.31 ± 0.06 ab	2.14 ± 0.47 a	2.57 ± 0.38 b	0.32 ± 0.03 c	3.23 ± 0.62 ce	1.59 ± 0.22 e
Cd	0.11 ± 0.08	0.01 ± 0.00	<0.01	<0.01	0.01 ± 0.00	<0.01	<0.01
Cr	21.00 ± 1.15	0.02 ± 0.00 b	0.07 ± 0.01	0.12 ± 0.01 b	0.01 ± 0.00 cd	0.10 ± 0.02 c	0.09 ± 0.01 d
Cu	218.67 ± 5.81	0.92 ± 0.11 a	0.48 ± 0.09 a	0.56 ± 0.08	0.89 ± 0.05	0.58 ± 0.10	0.73 ± 0.03
Hg	26.67 ± 6.67	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Mn	27.67 ± 1.76	0.36 ± 0.04 ab	3.40 ± 0.50 a	5.28 ± 0.35 b	0.85 ± 0.15 cd	4.17 ± 0.78 c	5.73 ± 0.58 d
Ni	2.77 ± 0.43	0.09 ± 0.01	0.17 ± 0.05	0.22 ± 0.02	0.10 ± 0.01	0.19 ± 0.04	0.18 ± 0.03
Pb	29,633.33 ± 554.78	2.64 ± 0.35	0.52 ± 0.36	0.15 ± 0.10	1.61 ± 0.24	0.27 ± 0.12	0.10 ± 0.03
Zn	83.33 ± 6.62	3.57 ± 0.54	2.49 ± 0.69	3.59 ± 0.98	5.07 ± 0.35	2.71 ± 0.71	2.53 ± 0.42

Letters represent significant differences between substrates (ANOVA and Tukey's post hoc test, $p < 0.05$): (a) GW$_i$ vs. TC50$_i$, (b) GW$_i$ vs. TC75$_i$, (c) GW$_f$ vs. TC50$_f$, (d) GW$_f$ vs. TC75$_f$, (e) TC50$_f$ vs. TC75$_f$,

more than 81% (Mn in TC75$_i$), with some of PHEs below the detection limit (<0.01 mg kg^{-1}), such as Hg and Cd. The bioavailability of PHEs in Technosols compared to GW$_i$ has undergone element-dependent variations, mainly driven by the increase in OC and/or pH (Kabata-Pendias 2010; Alloway 2012), although these variations between Technosols were negligible. In both Technosols, As increased in the bioavailable fraction while Pb decreased, which is consistent with previous studies (Beesley et al. 2010; Paradelo et al. 2011; Santos et al. 2019), where an increase in OC can result in reduced mobility/bioavailability of Pb and increased bioavailability of other PHEs (e.g. As). Moreover, when pH was higher than 6.5 in non- or low-carbonated soils such as Technosols, As bioavailability increased, as it could be desorbed from iron oxides and/or organic matter (Wang and Mulligan 2006; Aguilar-Garrido et al. 2020; Paniagua-López et al. 2021). The increase of As bioavailability can also be attributed to the increase of extractable P concentration

(Table 8.1) in Technosols (Santos et al. 2019), as phosphate anions can compete with As anions for binding sites in soil components, increasing As bioavailability (Bolan et al. 2014).

Throughout the vegetative growth of *L. purpureus* ($TC50_f$ and $TC75_f$), the physicochemical characteristics, nutrient content, and PHEs concentration in their bioavailable fractions varied in both Technosols (Tables 8.1, 8.2 and 8.3). A decrease of 8% in pH from $TC50_i$ to $TC50_f$ was observed and of 10% from $TC75_i$ to $TC75_f$. In addition, EC decreased with plant development in both Technosols, with a decrease of 29% in $TC50_f$ and 13% in $TC75_f$ (Table 8.1). Contrary to what is expected, since *L. purpureus* is a legume crop (Adesoji et al. 2014), the contents of OC and total N decreased by less than 6% with the development of the crop in both Technosols, except for $TC75_f$, which showed an increase of 7% in total N, probably related to the absence of root nodulation inhibited by the presence of PHEs (Haddad et al. 2015). However, there was a significant increase in extractable P content (12% for $TC75_f$ and 18% for $TC50_f$) and a slight non-significant increase in K content (6% for $TC75_f$ and 3% for $TC50_f$), which was probably due to the release of these elements by organic matter mineralisation and mineral weathering (Table 8.1). Changes in other macro-and micronutrient contents during plant growth were much more variable between Technosols. In $TC50_f$, a significant increase in Ca, Mg, and Mn concentrations occurred, whereas Na, K, Fe, Zn, and Cu concentrations decreased (Table 8.2). In contrast, there was an increase in the concentration of all nutrients in $TC75_f$, except for Cu, which decreased, and Zn, which remained unchanged. These results contrast with those obtained by Santos et al. (2014b), who reported a decrease in nutrient concentration with *C. ladanifer* growth in similar gossan wastes amended by a mixture of inorganic and organic wastes. The development of *L.* purpureus in both Technosols resulted in no significant differences in PHEs bioavailability; however, in $TC50_f$, there was an overall increase in most PHEs measured, except for Hg and Pb. Conversely, *L. purpureus* in $TC75_f$ resulted in an overall decrease for most of PHEs, except for Cd, Cu, and Mn (Table 8.3). These differences between TC50*f* and TC75*f* in PHEs bioavailability could be the result of differences in physicochemical characteristics due to the increased dosage of waste mixture between the two Technosols (Xu et al. 2022).

8.2.3. BIOLOGICAL CHARACTERISATION OF GOSSAN WASTE AND TECHNOSOLS

The remediation effectiveness of Technosols containing gossan waste was also assessed by measuring soil enzymatic activities as biological indicators, which reflect soil functional diversity, changes in microbial community composition, and microbial status (Martinez-Salgado et al. 2010; Kumar et al. 2013). Soil-enzyme activity is influenced by soil characteristics related to nutrient availability, soil microbial activity, and land use management processes that modify potential soil-enzyme-mediated substrate catalysis (Kandeler et al. 1996). The most important soil enzymes involved in the C, N, and P cycles in soil are dehydrogenase, β-glucosidase, cellulase, protease, urease, and phosphatase (Sardans and Peñuelas 2005; Sardans et al. 2008; Aragón et al. 2014). Both cellulase and β-glucosidase break down labile cellulose and related carbohydrates, facilitating the activities of other enzymes such as protease and phosphatase (Debosz et al. 1999; Sardans et al. 2008; Stege et al. 2010). Proteases are involved in the first phase of N mineralisation, whereas urease regulates the release of N-NH_4^+ by hydrolysis of urea. Phosphatase regulates the hydrolysis of O-P bonds, releasing phosphate from organic matter (Sardans and Peñuelas 2005).

Overall, GW_i and GW_f showed low enzymatic activities, whereas $TC75_i$ and $TC75_f$ exhibited higher enzymatic activities (Figure 8.1). However, at the end of the trial, most of the enzymatic activities decreased. The higher enzymatic activities found in Technosols indicate the good performance of the overall microbial communities involved in organic matter degradation, mineralisation processes, and nutrient cycling (Arán et al. 2022). The enzyme activities determined are involved in nutrient cycling, such as C (β-glucosidase and cellulase), N (protease, urease), and P (acid phosphatase).

Dehydrogenase activity often serves as an indicator of the microbiological redox system and microbial oxidative activities in soils (Casida et al. 1964), but its activity can be significantly affected by several environmental factors such as soil moisture, oxygen availability, oxidation-reduction potential, pH, SOM content, soil profile depth, temperature, season, presence of PHEs or pesticides, and fertilisation (Wolińska and Stępniewsk 2012). Thus, explaining the low dehydrogenase activity

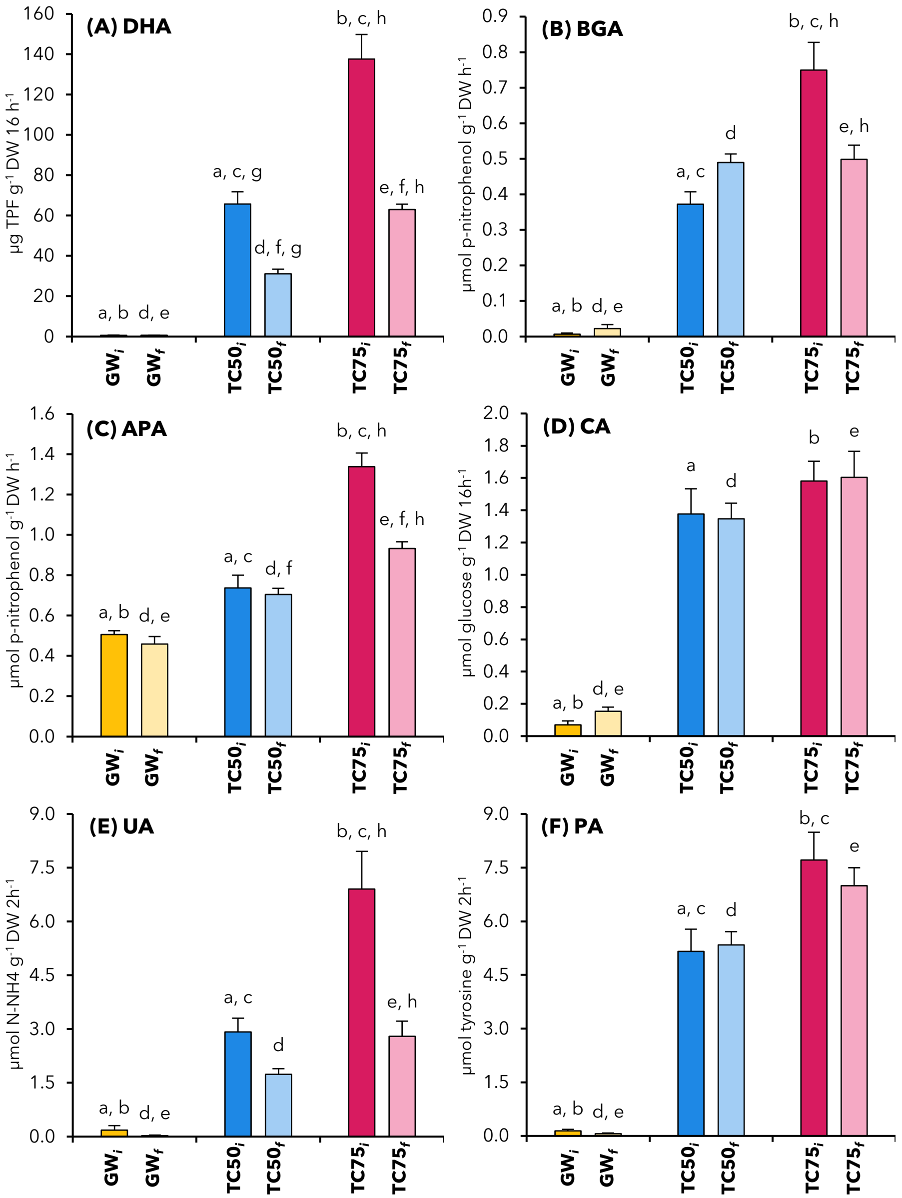

Figure 8.1. *Enzymatic activities [DHA: dehydrogenase (A), BGA: β-glucosidase (B), APA: acid phosphatase (C), CA: cellulase (D), UA: urease (E), PA: protease (F)] in gossan waste (GW) and Technosols (TC50 and TC75) before (i) and after (f) L. purpureus growth (n = 6).*

DW – dry weight. Letters represent significant differences between substrates (ANOVA and Tukey's post hoc test, $p < 0.05$): (a) GWi vs. TC50i, (b) GWi vs. TC75i, (c) TC50i vs. TC75i, (d) GWf vs. TC50f, (e) GWf vs. TC75f, (f) TC50f vs. TC75f, (g) TC50i vs. TC50f; (h) TC75i vs. TC75f.

observed in both GW_i and GW_f, (<1 μg TPF g^{-1} 16 h^{-1}). In comparison, both Technosols showed higher dehydrogenase activity, at $TC50_i$ about 70 μg TPF g^{-1} 16 h^{-1} and at $TC75_i$ approximately 140 μg TPF g^{-1} 16 h^{-1}, almost twice as high as $TC50_i$ (Figure 8.1). These results are consistent with those reported previously in different studies (Alvarenga et al. 2008; Burgos et al. 2010; Santos et al. 2014b), indicating that soil microorganism activity, evaluated by dehydrogenase activity, is stimulated by the addition of both organic and inorganic amendments to inhospitable materials (e.g. polluted soil or gossan waste), regardless of the presence or absence of plants. However, in contrast with other species such as *C. ladanifer* (Santos et al. 2014b) or *Triticum aestivum* L. (Piotrowska and Długosz 2012), which increased dehydrogenase activity, *L. purpureus* negatively affected dehydrogenase activity in both Technosols, especially in $TC75_f$. In a study with post-agricultural forest soils (Wiatrowska et al. 2021), dehydrogenase activity was related to the SOM content, possibly explaining the decrease observed in both final Technosols, as a slight decrease in OC was observed in $TC50_f$ and $TC75_f$.

Regarding enzymatic activities related to C-cycle, the same trend of enzymatic activity stimulation was observed in Technosols (Figure 8.1). Cellulase showed similar values in both Technosols (TC50: 1.38 μmol glucose g^{-1} 16 h^{-1}, TC75: 1.58 μmol glucose g^{-1} 16 h^{-1}), without temporal changes, and was higher compared to GW (0.07 μmol glucose g^{-1} 16 h^{-1}). β-glucosidase activity after *L. purpureus* growth was similar in both Technosols ($TC50_f$ and $TC75_f$: ~ 0.5 μmol ρ-nitrophenol g^{-1} h^{-1}) but, in $TC50_i$, it was half the activity recorded in $TC75_i$ (0.37 μmol ρ-nitrophenol g^{-1} h^{-1} vs. 0.75 μmol ρ-nitrophenol g^{-1} h^{-1}). Thus, in $TC50_f$, it did not significantly increase and, on the contrary, in $TC75_f$, there was a significant decrease.

Unlike the other enzyme activities studied here, GW showed considerable acid phosphatase activity (0.57 μmol ρ-nitrophenol g^{-1} h^{-1}), although lower than in TC50 and much lower than in TC75 (0.74 μmol ρ-nitrophenol g^{-1} h^{-1} and 1.34 μmol ρ-nitrophenol g^{-1} h^{-1}, respectively) (Figure 8.1). At the end of *L. purpureus* plant development, phosphatase activity was only modified at TC75, where it decreased significantly. Thus, the results obtained are in line with many studies that reported a significant increase in OC content by the application of amendments in gossan wastes or the construction of tailor-made Technosols, which have a positive effect not only on dehydrogenase activity but also on the enzymatic activities related to the C-cycle (β-glucosidase and cellulase) and the P-cycle (acid phosphatase) (Qu and de Varennes 2010; de Varennes et al. 2010; Santos et al. 2014b).

The behaviour of enzymes related to N-cycle (protease and urease) was similar. The lowest values of protease and urease were found in GW (0.14 μmol tyrosine g^{-1} 16 h^{-1} and 0.18 μmol $N\text{-}NH_4$ g^{-1} 2 h^{-1}), while intermediate values were found in $TC50_i$ (5.16 μmol tyrosine g^{-1} 16 h^{-1} and 2.92 μmol $N\text{-}NH_4$ g^{-1} 2 h^{-1}); and in $TC75_i$, the maximum activity occurred (7.72 μmol tyrosine g^{-1} 16h^{-1} and 6.91 μmol $N\text{-}NH_4$ g^{-1} 2h^{-1}) (Figure 8.1). This is related to the higher concentration of total N in Technosols than in GW (0.30 g kg^{-1}) and TC75 (1.80 g kg^{-1}) compared to TC50 (1.10 g kg^{-1}); as high activity, for example of urease, is related to a higher fraction of plant available N (He et al. 2010). Similar results of N-cycle enzymatic activity (mainly protease) have been detailed for metal- and semimetal-polluted soils amended with different wastes, e.g., sewage sludge (Achberger and Ohlinger 1988), effluents from cotton ginning mills (Narasimha et al. 2012), and pig slurry (Plaza et al. 2002). In this study, the urease activity was reduced in both Technosols at the end of the experiment, while the protease enzyme was not. The activity of urease is closely related to the biological cycle transformation and the bioavailability of N, whilst proteolysis is a process of the N cycle and is considered as a stage limiting the rate of N mineralisation in soils. Thus, the increase in total N occurred in $TC75_f$ could be linked to protease activity that did not suffer great variation, rather than to urease.

8.2.4. ECOTOXICOLOGICAL CHARACTERISATION OF GOSSAN WASTE AND TECHNOSOLS

To assess the ecotoxicological risk of the reclaimed materials (gossan waste) by Technosols, a bioassay with *L. purpureus* was performed. In this way, the growing conditions on these materials can also be examined to diagnose the suitability of the combined use of Technosols and this wide-tolerant multifunctional legume to reconvert these degraded areas into productive ones.

The germination percentage of *L. purpureus* was greater in Technosols (TC50: 50%, TC75: 75%) than in GW (38%). This increase in germination can be explained, as Santos et al. (2019) reported for other species (*Lavandula pedunculata* (Mill.) Cav.

and *C. ladanifer*), by an improvement in water-holding capacity in the studied Technosols, which is related to an enhancement in structure due to wastes used in their preparation. Likewise, the development of *L. purpureus*, measured in terms of length and dry biomass, was superior to that of GW, especially in dry biomass (Figures 8.2 and 8.3). The shoot length of *L. purpureus* developed on both Technosols was approximately 20 times greater than in GW. As for root length, greater variability was observed than in shoot length, but also much more evident growth in Technosols than in GW. Although *L. purpureus* seeds were inoculated with *Bradyrhizobium* sp. (strain CB1024), nodulation failed to occur, as previously reported in Abd-Alla et al. (2014) and Dhakal et al. (2016). The greatest differences between the substrates were observed in plant biomass. Growth stimulation of these plants is evident in both Technosols, with high biomass production in both shoot and root, while in GW, it is practically nil. Similar results have been reported for *E. globulus*, *C. ladanifer*, *D. glomerata*, *E. australis*, and pasture species grown in Technosols composed of mining waste/polluted soils, at medium–long term, either in field or greenhouse assays (de Varennes et al. 2010; Macías et al. 2011; Santos et al. 2014b). Plant growth can be explained by the improvement of some substrates properties, but mainly by the increase in nutrient and OC contents, as well as biological activity (Santos et al. 2019). No statistically significant variations were observed in the analysed growth parameters between TC50 and TC75.

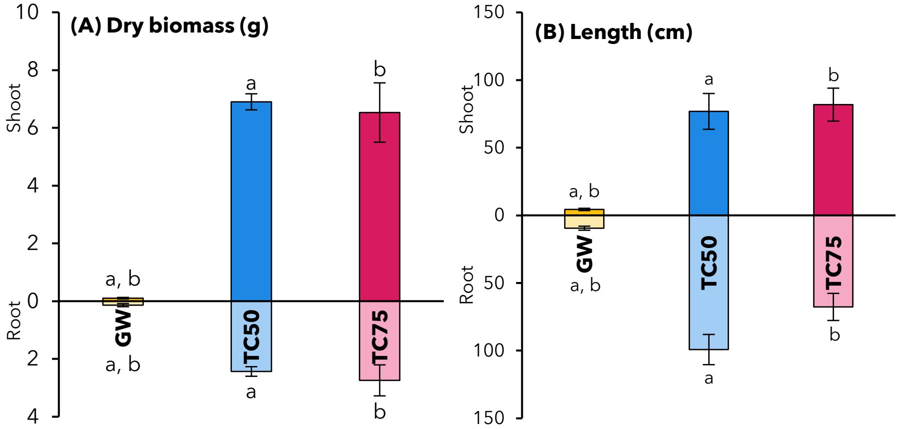

Figure 8.2. *Dry biomass (A) and length (B) of L. purpureus after six months of growth in gossan waste (GW) and Technosols (TC50 and TC75) (n = 6).* Letters represent significant differences between substrates (ANOVA and Tukey's post hoc test, $p < 0.05$): *(a) GW vs. TC50; (b) GW vs. TC75.*

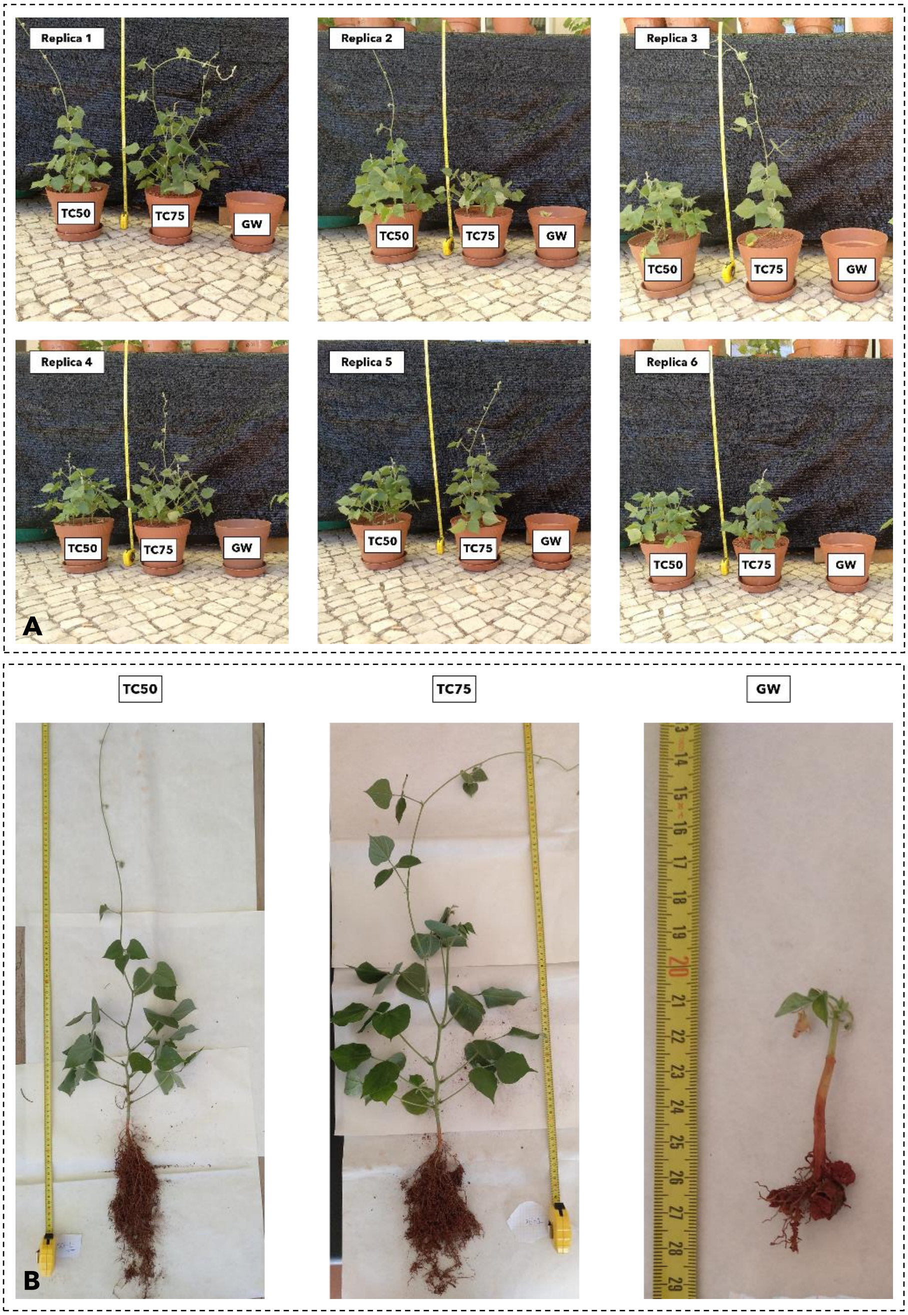

Figure 8.3. *Aspect of L. purpureus after six months of growth in gossan waste (GW) and Technosols (TC50 and TC75): General view (A) and details of the roots and aerial part (B).*

The concentrations of PHEs and nutrients in plant tissues of *L. purpureus* (shoots and roots) were determined to test whether this legume can uptake them from soil to roots and then translocate them to shoots; thus, determining the potential use of *L. purpureus* in phytostabilisation of gossan wastes and as a crop for feed production. In general, PHEs concentrations in shoots and roots were higher in plants grown on GW than in those grown on Technosols (Table 8.4), which is reflected in the higher biological absorption and soil–plant transfer coefficients (Table 8.5). While plant uptake and accumulation of nutrients in shoots and roots was more variable depending on the nutrient, which may be due to the physiology of this species, some nutrients were at higher concentrations (Ca, K, and Mn) in plants from Technosols and others in lower (Cu, Zn, Fe, and Na) or similar (Mg). However, independently of the studied Technosol, no significant differences were obtained among concentrations, both in shoots and roots, of the nutrients studied in *L. purpureus* plants. The concentration of Fe in plant shoots grown in GW exceeded by far the normal values in plants of 50–250 mg kg^{-1} (de Varennes 2003), in TC75 slightly, and in TC50 it was within these. In both GW and Technosols plants, Ca and Mg were found in concentrations within the normal range (Ca: 2–40 g kg^{-1}, Mg: 1–8 g kg^{-1}); however, K was in deficit concentrations in GW plants (<20–25 g kg^{-1}) (de Varennes 2003). Even some elements (Cu and Zn), which have a micronutrient–PHE duality depending on the concentration, were in the optimal range for good plant development (Cu: 5–20 mg kg^{-1}, Zn: 25–150 mg kg^{-1}) (de Varennes 2003) in both GW and Technosols plants.

Concentrations of most PHEs studied in shoots of *L. purpureus* growing in Technosols (Table 8.4) were considered as normal/sufficient and/or below the phytotoxicity level, except for As, which accumulated in shoots in concentrations that may be toxic to plants (5–20 mg kg^{-1}) (Kabata-Pendias 2010), although they showed no signs of toxicity (Figure 8.3). However, in shoots of *L. purpureus* grown in GW, the concentrations of As (168 mg kg^{-1}) and Pb (498 mg kg^{-1}) were well above the maximum phytotoxic limit considered for plants in general (Pb: 30–300 mg kg^{-1}) and Cr and Ni were close to the limits (5 and 10 mg kg^{-1}) (Kabata-Pendias 2010), respectively. The high concentrations of these elements in shoots and roots can be responsible for the growth impairment of the plants on GW (Figures 8.2 and 8.3).

Table 8.4. *Total concentrations of PHEs and nutrients accumulated in the shoots and roots of L. purpureus grown in gossan waste (GW) and Technosols (TC50 and TC75) in mg kg^{-1} (mean ± SD; n = 6).*

	Shoots			***Roots***		
mg kg^{-1}	**GW**	**TC50$_i$**	**TC75**	**GW**	**TC50**	**TC75**
As	167.62 ± 66.59 d	7.50 ± 1.87	19.52 ± 11.77 f	3228.30 ± 1116.92 d	1,303.01 ± 92.06	2559.03 ± 440.64 f
Cd	0.13 ± 0.03	0.04 ± 0.00	0.04 ± 0.00	2.47 ± 2.26	0.12 ± 0.01	0.08 ± 0.01
Cr	2.76 ± 0.31	1.51 ± 0.07	1.65 ± 0.13	33.90 ± 13.44	6.69 ± 0.86	64.33 ± 41.15
Cu	22.63 ± 1.90 d	7.63 ± 0.32 e	7.73 ± 1.06 f	120.35 ± 17.51 bcd	54.96 ± 3.14 be	66.76 ± 0.78 cf
Hg	0.64 ± 0.15 d	0.05 ± 0.00	0.05 ± 0.01	10.36 ± 4.80 d	2.09 ± 0.07	2.49 ± 0.16
Mn	63.32 ± 25.18	80.22 ± 7.04	82.62 ± 9.48	30.70 ± 3.29	43.48 ± 3.79	50.17 ± 2.08
Ni	7.99 ± 1.65	1.88 ± 0.10	1.46 ± 0.14	19.09 ± 2.50	4.02 ± 0.23	18.17 ± 9.37
Pb	497.79 ± 204.05 d	19.61 ± 4.42	60.14 ± 27.34 f	8268.16 ± 2728.47 d	3510.74 ± 240.93	6841.61 ± 1228.20 f
Zn	77.93 ± 27.08 d	33.85 ± 2.88	28.71 ± 2.81	252.21 ± 44.02 bcd	115.95 ± 18.28 b	92.94 ± 5.82 c
Fe	3455.75 ± 1763.80 d	186.60 ± 37.68	443.60 ± 191.42	72,248.52 ± 27,067.29 d	26,385.28 ± 2722.85	43,524.84 ± 4186.81
Ca	14,283.28 ± 6080.72	14,497.45 ± 324.90	20,635.08 ± 1584.08	5131.99 ± 1367.83	9684.34 ± 718.13	14,012.42 ± 1983.44
Mg	2235.75 ± 330.09 d	1828.00 ± 55.37	2241.47 ± 167.64	615.73 ± 138.83 bcd	1907.58 ± 118.10 b	1581.21 ± 51.54 c
Na	2729.43 ± 82.55 d	1612.31 ± 41.47	1177.15 ± 274.94	6499.61 ± 1863.28 d	3439.55 ± 366.84	3787.01 ± 437.27
K	16,144.79 ± 1886.70 ad	20,481.38 ± 505.07	24,214.06 ± 1769.29 af	7053.64 ± 2300.41 bd	18,057.22 ± 2174.36 b	11,592.39 ± 361.87 f

Letters represent significant differences between substrates (ANOVA and Tukey's post hoc test, p < 0.05): (a) GW shoot vs. TC75 shoot, (b) GW root vs. TC50 root, (c) GW root vs. TC75 root, (d) GW shoot vs. GW root, (e) TC50 shoot vs. TC50 root, (f) TC75 shoot vs. TC75 root.

Most PHEs and nutrient concentrations in roots, except Mn, Ca, Mg, and K, were much higher than in shoots, both in GW and Technosols (Table 8.4). Moreover, except for Mn, the translocation coefficient was less than a unit in all cases (Table 8.5), indicating a low transfer of the elements between the root and aerial part. The calculated soil-plant coefficient transfer (Table 8.5) had values for plants from GW that were much higher than those of plants growing in Technosols. Although these values were also higher than a unit, indicating a high level of tolerance of this legume to PHEs, they showed no signs of toxicity, in agreement with several studies (D'Souza and Devaraj 2013; Ruthrof et al. 2018; Pandey et al. 2022). Thus, *L. purpureus* could have mechanisms of tolerance to high concentrations of PHEs in soil, as has been observed in many other species of *L. pedunculata* and *C. ladanifer* (Santos et al. 2019). Therefore, it could be used in phytostabilisation programmes for gossan waste. Since it is a plant capable of absorbing PHEs in their bioavailable form and concentrating them in roots (biological absorption coefficient > 1, translocation coefficient < 1, respectively; Table 8.5), the combined use of this species with Technosols is a good option as the organic and inorganic wastes composing Technosols decreased the bioavailability of most PHEs in soil and, therefore, the potential uptake by the plant (Table 8.4).

On the other hand, to verify whether this strategy of converting degraded areas into production areas by combining Technosols and *L. purpureus* growth is valid, the safety of the consumption of this plant by animals must be evaluated. For this purpose, the concentrations of PHEs in shoots were compared to the maximum tolerable levels of PHEs in feed for different species (rodents, poultry, pigs, horses, cattle, and sheep) (National Research Council 2005). In this sense, although the concentrations of As in *L. purpureus* shoots grown in Technosols exceed the phytotoxicity limit, this would not represent a tolerable maximum level of this element (30 mg kg^{-1}) in the diet of the aforementioned animals (National Research Council 2005). However, the maximum tolerable level of Pb in *L. purpureus* grown in Technosols was exceeded for rodents, poultry, pigs, and horses (10 mg kg^{-1}), but not for cattle or sheep feed (100 mg kg^{-1}) (National Research Council 2005). For plants grown on gossan waste, there is a risk of As and Pb affection if used in the production of feed for all animal species considered, of Hg in the case of feed for

Table 8.5. *Calculated biological absorption, translocation, and soil-plant transfer coefficients of PHEs in L. purpureus grown in gossan waste (GW) and Technosols (TC50 and TC75) (mean ± SD; n = 6).*

	Biological Absorption Coefficient (1)			***Translocation Coefficient*** (2)			***Soil-Plant Transfer Coefficient*** (3)		
PHEs	**GW**	**TC50**	**TC75**	**GW**	**TC50**	**TC75**	**GW**	**TC50**	**TC75**
As	10,197.2 ± 3635.5 ab	465.8 ± 118.0 a	1,795.6 ± 544.6 b	0.09 ± 0.05	0.01 ± 0.00	0.01 ± 0.01	590.3 ± 284.0	2.3 ± 0.3	13.9 ± 8.8
Cd	605.7 ± 576.7	97.5 ± 65.8	29.0 ± 4.1	0.48 ± 0.16	0.30 ± 0.02	0.47 ± 0.02	20.1 ± 4.0	24.8 ± 14.9	13.7 ± 2.2
Cr	2273.9 ± 767.7 a	92.3 ± 36.2 a	648.8 ± 390.0	0.1± 0.03 a	0.22 ± 0.02 ac	0.06 ± 0.02 c	193.7 ± 34.4 ab	18.9 ± 5.3 a	19.0 ± 4.3 b
Cu	139.7 ± 26.6	106.0 ± 24.3	92.0 ± 5.1	0.20 ± 0.03 b	0.14 ± 0.01	0.12 ± 0.02 b	25.7 ± 2.6 ab	14.4 ± 2.4 a	10.6 ± 1.4 b
Hg	3171.6 ± 1444.4	647.6 ± 25.2	773.3 ± 45.3	0.09 ± 0.04	0.02 ± 0.00	0.02 ± 0.00	196.8 ± 47.6 ab	14.1 ± 1.0 a	16.2 ± 4.1 b
Mn	39.6 ± 7.6 ab	12.6 ± 3.9 a	9.1 ± 1.1 b	2.03 ± 0.69	1.85 ± 0.08	1.64 ± 0.16	82.2 ± 36.8	22.7 ± 6.3	15.1 ± 2.6
Ni	207.9 ± 54.1 a	24.8 ± 7.3 a	95.8 ± 43.5	0.45 ± 0.14	0.47 ± 0.04	0.16 ± 0.06	82.3 ± 17.6 ab	11.5 ± 3.0 a	9.2 ± 2.7 b
Pb	4935.7 ± 1291.6	23,498.4 ± 10,331.7	45,658.8 ± 1378.0	0.11 ± 0.06	0.01 ± 0.00	0.01 ± 0.01	391.0 ± 210.7	115.5 ± 38.2	1095.0 ± 526.2
Zn	52.0 ± 12.6	59.7 ± 24.0	41.7 ± 10.7	0.29 ± 0.05	0.30 ± 0.03	0.31 ± 0.04	16.4 ± 6.8	16.7 ± 5.5	12.4 ± 2.4

(1) Biological absorption coefficient = [Root (x)]$_{(y)}$/[PHE in bioavailable fraction (x)]$_{(y)}$; (2) Translocation coefficient = [shoots (x)]$_{(y)}$/[roots (x)]$_{(y)}$; (3) Soil-plant transfer coefficient = [shoots (x)]$_{(y)}$/[PHE in bioavailable fraction (x)]$_{(y)}$. x – PHE concentration in y, y – GW$_f$, TC50$_f$, TC75$_f$. Letters represent significant differences between substrates (ANOVA and Tukey's post hoc test, $p < 0.05$: (a) GW vs. TC50; (b) GW vs. TC75; (c) TC50 vs. TC75.

rodents, poultry, pigs and horses, and of Cu for sheep feed; as their concentrations in *L. purpureus* shoots exceeded 30, 10, 0.2, and 15 mg kg^{-1} the most restrictive maximum tolerable level in feed (National Research Council 2005), respectively. All other PHEs considered were present in *L. purpureus* shoots at concentrations below the maximum tolerable level in feed for the considered species.

8.3. CONCLUSIONS

The gossan waste present in the abandoned mine area of São Domingos constitute a degraded environment characterised by very low pH values, low CEC, low nutrient availability, poor organic matter content, high total concentrations of several PHEs, and low microbiological activity, similar to most gossan waste from sulphide mining areas. Therefore, this case could be a reference model for the study of remediation solutions for sulphide mining areas, particularly in harsh climates that are prone to worsen with climate change, such as the Mediterranean region or the south of the Iberian Peninsula.

The integrated green biotechnology approach combining the remediation of sulphide mine areas by the use of designed Technosols constructed from mixing gossan waste with a combination of organic/inorganic wastes from local industries (urban gardening services, quarries, cafes, and breweries), followed by the development of *L. purpureus*, is efficient over the time span tested (174 days) under greenhouse conditions. Both Technosols improved the physicochemical conditions, nutrient status, and microbiological activity of the gossan waste and reduced the bioavailability of most PHEs (with the exception of As); although, in general, conditions in TC75 were more favourable than in TC50. This, in turn, allowed *L. purpureus* growth with no visible signs of toxicity and low translocation of PHEs to the shoots, with PHEs concentrations suitable for cattle or sheep but not for poultry, pig, and horse due to the Pb concentration. Whereas in pure gossan wastes, not only was the growth of *L. purpureus* limited (very low biomass and signs of toxicity), but As, Cu, Hg and Pb concentrations in plants exceeded the limits for most animal feed. Therefore, the combination of Technosols with the *L. purpureus* crop is a potentially valuable green biotechnology approach for the remediation of degraded/polluted environments, but also for converting them into food production areas under the condition of exhaustive quality and food safety controls.

Furthermore, in the case of Technosols implementation in mining areas, monitoring should be carried out to assess over time whether conditions are maintained or, for example, whether the addition (or not) of certain amendments to Technosols needs to be readjusted.

Mixture of constituents of Technosol T4 on site in the experimental plot "El Vicario", Guadiamar Green Corridor

Seville, Spain

February 2021

Antonio Aguilar Garrido

CHAPTER 9

GENERAL DISCUSSION

9.1. PHEs-POLLUTION IN A WORLD IN TRANSITION

The mining industry is expanding to meet the demand for strategic elements for energy and digital transition, which raises serious health and environmental concerns as a major source of PHEs into the environment (Watari et al. 2021). In this context, sulphide mining can have serious impacts on different environmental compartments that need to be addressed (Mahar et al. 2016). For example, in the IPB, this problem may occur because extensive past, present, and future mining activity has generated and will continue to produce large quantities of waste (i.e. gossan waste, acid mine water) that, either accidentally through spillage or mismanagement, can lead to soil and water pollution. This concern should help to seek strategies to make the production of mining resources compatible with the protection of ecosystems, in order to avoid cases such as the Aznalcóllar mining accident, which has led to residual soil pollution and ecosystem degradation in the GGC that persists more than 25 years later. On the other hand, the increase in waste production that has occurred in industrialised countries leads to the proposal of circular economy and zero waste strategies as essential for the preservation of the environment.

According to these two statements, this PhD thesis comprehensively evaluates the effectiveness of different waste-derived Technosols in the remediation of the following environments degraded by sulphide mining, with high levels of PHEs: (i) laboratory-created acid mine drainage (**chapter 3** and **5**), (ii) polluted soils from the GGC (**chapter 4**, **6** and **7**), and (iii) gossan waste from the São Domingos mine (**chapter 8**).

The **artificial acid mine drainage (AMD)** prepared in the laboratory by oxidation of pyritic tailings from the Aznalcóllar spillage, studied in **chapter 3** and **5**, represents AMD generated worldwide (Akcil and Koldas 2006), as they show the same extreme conditions involving extremely acidic pH (2.2-2.9), high electrical conductivity (3.7-7.2 dS m^{-1}) and very high concentrations of several PHEs (i.e. As, Cd, Co, Cr, Cu, Mn, Pb, Sb, V, Zn).

The **residually polluted soils (PS)** in the GGC, targeted for remediation in **chapter 4**, **6**, and **7**, are characterised by high concentrations of several PHEs, acidic pH (3.5), low fertility (OC: 0.4%, N_T: 0.1%), high presence of soluble salts (EC > 2.5 dS m^{-1}) and formation of surface crust during the dry season. The total concentrations of some PHEs (As, Cu, Pb, Zn) exceeded the background values reported by Simón et al. (1999) for unaffected soils in the area (from 3 to 20 times, depending on PHE); especially As and Pb, which in many of these unvegetated areas, even surpassed by far (9.6 and 2.3 times, respectively) the levels to declare a soil as potentially polluted in Andalusia (Spain) (BOJA 2015). Nevertheless, considering the solubility and bioavailability of PHEs, As and Pb were not of critical concern, as the water-soluble and EDTA-bioavailable concentrations were similar to those in unaffected soils. The risk of leaching of these two PHEs is very limited because of their low solubility (García-Carmona et al. 2019a) and the low precipitation in the area (mean annual of 490 mm). In contrast, Cd, Cu, and Zn, with total concentrations not exceeding the regulatory values, showed concentrations high enough to potentially cause toxicity (Guo et al. 2011; Noulas et al. 2018; Dutta et al. 2021), especially in the water-soluble fraction, which is the most readily accessible to organisms. Indeed, these soils show a certain toxicity manifested by low microbiological activity (i.e. low diversity and abundance of microbial communities, practically no soil enzymatic activity) and a high impact on vegetation development. In the field, these areas are devoid of vegetation; and in growth chamber tests, very limited growth of *T. campestre* and *L. sativa* was observed (**chapter 4**), as well as strong phytotoxicity in *L. sativa* plants (**chapter 6**).

The **gossan waste (GW)** from the abandoned mine area of São Domingos, analysed in **chapter 8**, are representative of sulphide mining wastes as they are characterised by a very acidic pH (< 3.5), low CEC (< 2.5 $cmol_{(+)}$ kg^{-1}), low nutrient availability (N_T: 0.19 g kg^{-1}, P_A: 0.003 mg kg^{-1}, K_A: 7.3 mg kg^{-1}), poor OC content (0.5%), and high total concentrations of several PHEs (in g kg^{-1}; As: 9.13, Cu: 0.22, Hg: 0.03, Pb: 29.63). Conditions that lead to GW exhibiting some toxicity signified by low microbiological activity (i.e. practically no enzymatic activity) and low potential for plant growth (i.e. limited biomass production and high accumulation of PHEs in tissues of *L. purpureus*). Therefore, their treatment is necessary to prevent the formation of AMD/acid and PHEs-laden water if exposed to oxidising conditions that could constitute a source of pollution in the IPB.

9.2. WASTE-DERIVED TECHNOSOLS FOR ECOLOGICAL RESTORATION

In this PhD thesis, different Technosols have been designed and produced to specifically provide them with the necessary conditions to remediate these degraded environments, since by mixing amendments, a better promotion and maintenance of biogeochemical processes and a better decrease of bioavailability of PHEs are achieved (Yao et al. 2009; Macías et al. 2011). A wide variety of materials have been reported in the scientific literature for the production of Technosols, both as main components and as amendments (Table 9.1).

Table 9.1. *Materials used for the design and production of Technosols.*

Main component	Amendments
☐ Mining waste ▪ Gossan and sulphide waste (Santos et al. 2019; Barba-Brioso et al. 2023) ▪ Carbonatite and sepertine-magnesite waste (Slukovskaya et al. 2019) ▪ Coal waste (Walmsley et al. 2022; Zocche et al. 2023) ▪ Limestone/dolomite spoil (Jordán et al. 2017; Ruiz et al. 2020) ▪ Bauxite residue (Santini and Fey 2016) ☐ Mine soils (Asensio et al. 2013; Martínez-Sánchez et al. 2021) ☐ Agricultural soils (Martínez-Sánchez et al. 2021) ☐ Urban soils (Barredo et al. 2020) ☐ Construction and demolition debris (Barredo et al. 2020; Ramos et al. 2021) ☐ Thermally-treated industrial soils (Séré et al. 2010; Colombini et al. 2022) ☐ Dredged marine sediments (Macía et al. 2014)	☐ Composted agricultural waste (Santos et al. 2019; Ramos et al. 2021; Zocche et al. 2023) ☐ Composted/digested sewage sludge (Yao et al. 2009; Jordán et al. 2017; Hbaieb et al. 2018) ☐ Paper mill sludge (Séré et al. 2010; Asemaninejad et al. 2018) ☐ Biochar (Moreno-Barriga et al. 2017; Nandillon et al. 2019) ☐ Coffee grounds (Cortinhas et al. 2020) ☐ Compost from urban biowaste/green waste (food and garden) (Rodríguez-Vila et al. 2016; Cannavo et al. 2018; Coull et al. 2021) ☐ Bio-stabilised municipal solid waste (Herrán Fernández et al. 2016; Barredo et al. 2020) ☐ Industrial waste materials (Camps Arbestain et al. 2008; Yao et al. 2009; Walmsley et al. 2022) ▪ Fly ash ▪ Steel slag ▪ Foundry sand ▪ Blasting machinery scrap ☐ Marble sludge (Moreno-Barriga et al. 2017) ☐ Bentonite (Asensio et al. 2019; Barredo et al. 2020; Ramos et al. 2021) ☐ Sand from treatment plants (Rodríguez-Vila et al. 2016)

The Technosols designed in this thesis are mainly composed of the degraded material to be remediated, (i) polluted soil from GGC (T1, T2, T3, T4, T5, and T6) (**chapter 4**, **5**, **6**, and **7**), and (ii) gossan waste from São Domingos mine (TC50 and

TC75) (**chapter 8**), combined with various organic and inorganic wastes as amendments from mining (iron oxyhydroxide-rich sludge, carbonated waste from peat bog, marble cutting and polishing sludge, limestone rock waste), agro-industry (solid olive mill by-product, sludge and waste kieselguhr from breweries, coffee grounds), and urban activity (composted sewage sludge, vermicompost from pruning and gardening waste).

In general, the main recommendations on the use of wastes of different nature for the construction of Technosols should focus on the use of waste materials coming from areas close to the intervention site (to reduce transport costs), produced in large quantities, and based on a detailed characterisation of their physicochemical and biological properties, to verify that they are compatible as useful materials in the regeneration of degraded areas. For example, it is particularly important to consider the presence of substances with potential toxic properties (e.g. PHEs, Na, phenolic compounds) and, in the case of organic wastes, that are sanitised and pathogen-free due to the composting process and/or the raw material used (EPA 2003; Wichuk and McCartney 2015).

9.2.1. POTENTIAL OF WASTES AND TECHNOSOLS TO CONTROL AMD

Before the design and production of Technosols from PS, in **chapter 3**, a laboratory test of treatment of an artificial AMD, with extreme acidity and loaded with PHEs (see section 3.2.1), was conducted with 10 wastes to select those with the best performance to be used as amendments in Technosols. These are: i) iron oxyhydroxide-rich sludge (IO), ii) marble sludge (MS), iii) carbonated waste (CW), iv) gypsum mining spoil (GS), v) composted sewage sludge (WS), vi) bio-stabilised material (BM), vii) vermicompost from gardening waste (VC), viii and ix) two composted solid olive-mill by-products (OW: irrigated with drinking water, OL: irrigated with liquid olive waste), and x) compost from agricultural waste (AW).

Some wastes have a strong carbonate character (MS and CW), others are highly organic (VC, AW, OL, WS, OW and BM), and other have a high iron oxyhydroxide content (IO); all these properties play an important role in the immobilisation of PHEs and acid neutralisation (Merdy et al. 2009; Bolan et al. 2011). Many wastes show considerable acid neutralisation and PHEs immobilisation capacity. The pH in leachates increases from pH < 3 in AMD to values above 6 and close to neutrality in all cases. Likewise, the concentrations of several PHEs in AMD after treatment decreased significantly. The main PHEs (i.e. As, Cd, Cr, Cu, Pb, and Zn) were successfully removed (close to 100%) from artificial AMD. Inorganic wastes and VC have the highest capacity, with concentrations in the treated water below regulatory levels for irrigation and surface water in most cases (BOE 2007; EPA 2012; BOE 2015). In contrast, with organic wastes, PHEs concentrations were significantly reduced but remained above regulatory levels in most cases. Furthermore, the retention of other less studied PHEs (i.e. In, Sc, Sn, Th, Tl, V and Y) was also remarkable, as retention rates were higher than those obtained for materials with similar characteristics in other studies. For example, close to 100% removal of V with inorganic wastes and VC compared to 85% with commercial iron products and a ferric residue from groundwater treatment (Zhang et al. 2022b); Tl with removal rates above 75% for all wastes (and 90% with IO, CW, WS and VC) versus rates below 50% with lime (Liu et al. 2019); and Sb removed over 95% by inorganic wastes and VC, while a commercial ferric chloride coagulant retained 80% (Inam et al. 2019).

Similarly, the removal efficiencies of the main PHEs with these wastes were also much higher than those achieved in other studies (Zhang 2011; Kefeni et al. 2017; Dhir 2018). Inorganic wastes were much more effective in retaining PHEs than organic wastes. The decreasing order of effectiveness was: **IO** > **CW** ≥ **MS** ≥ **VC** > GS > OW ≥ **OL** > **WS** > AW > BM; where wastes rich in iron oxyhydroxides and carbonates are more effective than OM-rich wastes. The removal rates for wastes dominated by carbonates (CW and MS) or iron oxyhydroxides (IO) were above 95% for most PHEs, whereas they were below 95% for organic wastes. On the other hand, the PHEs content in some wastes should be considered as a potential risk. For example, IO and GS have slightly elevated concentrations of As, Pb, and Sb, but without major concern as they do not exceed the regulatory maximum levels to be used as fertiliser products (BOE 2013). Moreover, AW, WS, and BM also have high concentrations of certain PHEs not exceeding these values (Cr, Cu, V and Zn) and very high salinity, being also the wastes with the lowest retention capacity.

Thus, to produce Technosols, the wastes selected are those with the highest PHEs removal rates and which have the conditions needed to cope with pollution by favouring PHEs immobilisation and acidity neutralisation, such as carbonate character (CW and MS), richness in OM (VC, OL, WS) and iron oxyhydroxides (IO). Carbonated wastes provide pH buffering capacity, organic wastes favour the recovery of biological activity and contribute to soil aggregation, and iron-rich wastes promote the retention capacity of anionic PHEs (i.e. As, Sb).

The combination of these wastes with polluted soil to form efficient Technosols for AMD control may be a viable and effective ecotechnology, as discussed in **chapter 5**. However, the responses of the different Technosols studied indicate that the results may differ depending on the doses, type, and combination of waste used; therefore, the final application must be adjusted to solve this problem in each specific situation.

The studied Technosols were made with a high capacity to cope with acidity, being this capacity directly related to the $CaCO_3$ content; however, the presence of other components and properties (organic matter, cation exchange capacity, exchangeable bases, Fe and Al oxy(hydr)oxides, silicates) must be considered. In our case, T1, T2, and T3 made with CW (a soil horizon containing, in addition to carbonates, silicates, organic matter, and Fe and Al oxy(hydr)oxides), have a higher pH buffering capacity than T4, T5, and T6 made with MS (marble sludge constituted by ~ 99.9% $CaCO_3$). The capacity of Technosols to reduce the leaching of PHEs was very high in all cases, with retention rates above 85% in relation to AMD. The neutralising power of PHEs was estimated from the critical load of PHEs and compared to unpolluted soil (US). The critical load is expressed as the amount (ml) of AMD that a soil can support without producing leachate that exceeds the regulatory values. In the case of As, US presents values of 1.7 ml AMD g soil^{-1}, while in all Technosols the values are very high (> 20 ml g^{-1}). For the other PHEs of interest, the results are highly variable. For Cd, Technosols made with CW (T1, T2, and T3) showed very high values (> 20 ml g^{-1}) and Technosols made with MS (T4, T5, and T6) presented lower values (2.0, 1.7, and 7.6 ml g^{-1}, respectively), highlighting T4 and T5 with values even lower than those in US (4 mg l^{-1}). For Cu, T6 and T3 presented the highest values (> 20 and 8.7 ml g^{-1}, respectively), followed by T1 (3.5

ml g^{-1}); the other Technosols showed moderately to low values for this PHE, even lower than in US (2.4 ml g^{-1}). Finally, for Zn, Technosols made with CW showed high values (~ 9 ml g^{-1}), whereas MS-composed Technosols showd lower values (ranging between 3.8 and 7.4 ml g^{-1}), somewhat higher than those in US (2.9 ml g^{-1}).

Technosols have a very high acidity neutralisation capacity and variable critical load of PHEs. In this sense, the presence of PHEs with variable chemical behaviour must be controlled, since the presence of a continuous AMD can pose a risk in the medium-long term due to the saturation of its capacity to retain certain elements and the loss of effectiveness as a protective barrier over time; so Technosols used in the control of AMD must be monitored regularly and corrective measures proposed to maintain their functionality over time.

9.2.2. POTENTIAL OF TECHNOSOLS TO REMEDIATE POLLUTED SOILS AND GOSSAN WASTES

This study advances the knowledge of Technosols as a useful tool in the remediation of degraded mining areas. The use of different waste materials makes this technology advance in the implementation of circular economy policies while being highly recommended for the improvement of the previous conditions of polluted areas. In this study, we investigated a wide variety and combinations of wastes for the design and production of Technosols, with the aim of evaluating the best possible combinations and waste materials to address the problems arising from these degraded environments (e.g. residual pollution, regeneration of vegetation, low microbiological activity). In this PhD thesis, we have deepened our knowledge of Technosols applied to real situations, and we have seen that they produce a significant **improvement** in severely degraded areas affected by pollution, while we have found some **drawbacks** or potential risks that must be carefully managed for each specific situation.

The characteristics of Technosols T1–T6 were significantly better than those of PS due to the addition of carbonate, organic-rich, and iron oxyhydroxide-rich wastes, as well as those of Technosols TC50 and TC75 with respect to GW also because of the different organic and inorganic wastes added; demonstrating the beneficial influence of Technosols in improving these degraded environments.

According to the main physicochemical properties and constituents in relation to the polluted soil (PS), Technosols produced a **significant increase in pH**, with values between slightly (T2, T5, and T6) and moderately alkaline (T1, T3, and T4), due to the moderately high CaCO3 content (> 25%). The EC strongly decreased in Technosols in relation to PS, although the **EC remained considerably high** (> 1 dS m^{-1}) in all cases, especially in Technosols made of sewage sludge (T2 and T5) with values above 2 dS m^{-1}. **Organic carbon and total N contents increased** considerably in relation to PS; T2 and T5 had the highest values content (around 6.4% in OC and 0.85% in N), followed by those amended with composted solid olive mill by-product (T1 and T4) with 5.8% in OC and 0.5% in N, and those composed of vermicompost from pruning and gardening waste (T3 and T6) had the lowest (2.4% in OC and 0.22% in N). Similar results in relation to the physicochemical properties were obtained for the GW-composed Technosols.

The solubility and bioavailability of PHEs in Technosols also underwent major changes. The **decrease in water-soluble concentrations of Cd, Cu, and Zn** in all Technosols to values approximately close to those in US is highly significant, possibly due to the increase in pH and $CaCO_3$ provided by carbonated waste (CW and MS). These elements in acidic soils are usually adsorbed in non-specific forms and thus in reversible processes, whereas at neutral or alkaline pH, they can be specifically adsorbed or even occluded by iron oxy(hydr)oxides (Reed and Martens 1996; Bradl 2004) or co-precipitated as iron-sulphate or hydrosulphate complexes (Simón et al. 2010), which are considered less reversible reactions. Furthermore, the higher OC content in Technosols may also contribute to the lower solubility of these PHEs (Bur et al. 2010; García-Carmona et al. 2019a). In contrast, an **increase in the soluble and bioavailable concentrations of As, Pb, and Sb** in Technosols in relation to PS was observed. This may be related to the formation of more available organic–metallic complexes when pH rises (Wang and Mulligan 2009; Nakamaru and Martín Peinado 2017). In particular, the lower As adsorption may be due to a higher solubility at near-neutral pH (> 6.5) (Simón et al. 2010), and arsenate species are more negatively charged at this pH, repelling anion exchange sites (Simón et al. 2005). The increased As mobilisation may also be caused by the reductive dissolution of As-containing iron oxides (e.g. ferrihydrite) (Burton et al.

2011), as well as possible competition of arsenates with SOM for Fe oxyhydroxides binding sites (Redman et al. 2002; Wang and Mulligan 2006; Fleming et al. 2013; Sierra Aragón et al. 2019). On the other hand, increased OC may be responsible for more soluble Pb, as reported in previous studies (García-Carmona et al. 2019a; Pastor-Jáuregui et al. 2021). However, several authors also highlighted the affinity of Pb for SOM (Coppola et al. 2010; Romero-Freire et al. 2015; Sierra Aragón et al. 2019), which can interact with it in a nearly stable manner, conditioning its solubility.

For Technosols composed of GW, PHEs concentrations in the bioavailable fraction of most elements decrease by more than 81% in relation to total concentrations in GW. The bioavailability of PHEs in Technosols compared to GW has undergone element-dependent variations, mainly driven by increases in OC and/or pH (Kabata-Pendias 2010; Alloway 2012). Notably, As increased in the bioavailable fraction while Pb decreased, in line with previous studies (Beesley et al. 2010; Paradelo et al. 2011; Santos et al. 2019), which reported that an increase in OC can result in both reduced mobility/bioavailability of Pb and increased bioavailability of other PHEs (e.g. As). The As increase in this fraction may be related to the fact that, at pH close to neutral, As can be desorbed from iron oxy(hydr)oxides and/or SOM (Wang and Mulligan 2006; Aguilar-Garrido et al. 2020; Paniagua-López et al. 2021) or by the competing effect of phosphate anions with As anions for binding sites in soil components (Santos et al. 2019).

The microbial activity in Technosols was measured by enzyme activity as an indicator of the recovery of soil functional diversity, changes in microbial community composition, and microbial status (Kumar et al. 2013). **Improvements in enzymatic activities** were also observed in Technosols with respect to PS, but with a large variability among them. Dehydrogenase, widely used as a global microbial activity index (Nannipieri et al. 1990), strongly increased, especially in Technosols composed of WS (T2 and T5) and OL (T1 and T4), with values above 100 µg TPF g^{-1} in 16 h^{-1}, while in VC-derived Technosols (T3 and T6), values were of 27 and 11 µg TPF g^{-1} in 16 h^{-1}, respectively. On the other hand, the microbial operational taxonomic units (OTUs) at the species level (**chapter 7**) showed a **relative abundance of plant growth-promoting microorganisms** in some Technosols, such as species of the genus *Adhaeribacter* sp., *Flavisolibacter* sp., and

Tsukamurella sp. However, different responses of the different enzyme activities tested in the studied Technosols indicate that **the full recovery of biological activity is limited in some cases**, at least in the time periods used in this study; therefore, longer-term studies are required. For example, T3 and T6 were the Technosols with the lowest dehydrogenase activities, but they had the highest β-glucosidase activity of all tested Technosols; indicating that the enzymatic activity related to the C-cycle (e.g. β-glucosidase) may be related to a higher proportion of more easily decomposable organic compounds (Hadas et al. 2004) dominant in the pruning and gardening vermicompost (T3 and T6), in relation to the more resistant ones presented in the other Technosols. In any case, the values of 0.8–0.9 p-nitrophenol g^{-1} h^{-1} in T3 and T6 are less than half that obtained in the US, indicating a partial recovery of this biological cycle.

Similarly, in GW-composed Technosols, the addition of organic and inorganic amendments stimulated microorganism activity. Both tested Technosols showed a much higher dehydrogenase activity than GW (<1 μg TPF g^{-1} 16 h^{-1}), with approximately 70 and 140 μg TPF g^{-1} 16 h^{-1} at TC50 and TC75, respectively. The same stimulation trend occurred for the enzymes related to the cycles of C (cellulase and β-glucosidase), N (protease and urease), and P (acid phosphatase). In any case, it would be necessary to compare with a reference soil in the area to be able to evaluate the degree of recovery of the biological activity in the studied Technosols.

On the other hand, a novelty of this thesis focused on the effect of Technosols on the underlying polluted soil (**chapters 4** and **7**). **The overlying Technosols improved the physicochemical properties of the underlying polluted soils** by increasing the pH to values near neutrality and raising the OC and $CaCO_3$ contents. These changes in physicochemical properties influenced the **reduction of solubility and bioavailability of PHEs (As, Cd, Cu, and Zn)**, although, in the time span of our experiment, the **soluble concentration of most PHEs in the underlying polluted soils was higher than in Technosols**, with special attention to Sb solubility probably due to the increase in pH and SOM in this intermediate soil layer, which may be related to the formation of bioavailable Fe-SOM-Sb complexes (Nakamaru and Martín Peinado 2017). On the other hand, although surface application of Technosols improved the health status of the underlying polluted soils, **the improvement in microbiological activity was not as marked**. Little or no significant changes occurred in enzymes (dehydrogenase, β-glucosidase, acid phosphatase and cellulase) in underlying soils compared to the previous conditions of PS under laboratory conditions (**chapter 4**), indicating that improvements in some physicochemical properties and the slight decrease in solubility of many PHEs in the short time elapsed during the experiment were not sufficient to promote microbial activity in treated soils. While with the field application of Technosols T4 and T6 (**chapter 7**), after one year, **an improvement of the microbiological activity in the underlying polluted soils** was observed, although not as pronounced as in Technosols.

9.2.4. POTENTIAL OF TECHNOSOLS IN REDUCING ECOTOXICITY

The characterisation of soil physicochemical properties and the mobility and availability of PHEs content gives an insufficient idea of the degree of remediation; therefore, it is essential to assess the effects on living organisms (Ojuederie and Babalola 2017). Thereby, in **chapter 4**, we implemented two toxicity bioassays with fairly representative plant species used globally as bioindicators of toxic effects (*T. campestre* and *L. sativa*) (Bagur-González et al. 2011; Graziano et al. 2022) to properly assess environmental risks and ensure the efficacy of this soil remediation technique. Since the effects of PHEs on organisms vary widely due to the different exposure routes, the microcosm assay with *T. campestre* was conducted directly in soil and the toxicity bioassay with *L. sativa* in a soil extract.

The treatment of PHE-polluted soil with **Technosols decreases the ecotoxicological risk**, as it favours an optimal development of *T. campestre* and *L. sativa*, unlike the null and low growth of these plants, respectively, when sown in PS. This stimulation in plant growth is related to the improvement of some soil properties (i.e. neutralised pH, increased OC, $CaCO_3$, and N_T), microbiological activity, and decreased solubility/bioavailability of most PHEs in both Technosols and underlying polluted soil (Santos et al. 2019). *T. campestre* plants cultivated in Technosols have higher survival rate, biomass, and SPAD index than those grown in PS, in line with results observed for other plant species (e.g. *E. globulus*, *C. ladanifer*, *D. glomerata*, *E. australis*) on different Technosols also composed of mining waste/polluted soils, either in field or greenhouse assays (de Varennes et al.

2010; Macías et al. 2011; Santos et al. 2014b). Likewise, most treatments had a similar response for *L. sativa*, with seed germination and root elongation similar to those of US. However, **some problems of potential phytotoxicity were detected in some cases**, like in Technosols T2 and T5, which presented low germination; in this case, this problem may be related to the type of organic waste used in these Technosols (sewage sludge), which often contains a complex mixture of pollutants (e.g. heavy metals and emerging organic pollutants like pharmaceuticals) potentially harmful to organisms (da Silva Souza et al. 2020).

In order to gain a detailed understanding of how PHE-polluted soil causes phytotoxicity, as well as the role of Technosols in mitigating these effects, in **chapter 6** we focused on the comprehensive assessment of ecotoxicity through physiological parameters related to phytotoxicity in *L. sativa*, such as plant growth, photosynthetic capacity, oxidative stress, and antioxidant defence. In this regard, *L. sativa* plants showed the highest growth when organic material composed of pruning and gardening vermicompost was used (T6), with values similar to that in recovered soil (RS). The use of other organic waste like composted solid olive mill by-product or sewage sludge produced a lower growth rate, in some cases (T5) even slightly lower than in PS. This lower growth of *L. sativa* in PS and T5 may be due to the higher toxicity resulting from the presence of PHEs (T4 and T5 presented a critical load for Cd and Cu lower than US). Moreover, when exposed to PHEs, ***L. sativa* plants tend to accumulate PHEs in their tissues**, potentially affecting their physiological mechanisms. In general, PHEs accumulate mainly in the roots, not in the aerial part of *L. sativa*, as translocation processes are limited by the Casparian stripe, acting as a selective barrier blocking the entry of many PHEs into the xylem to ensure selective ion transport, while retaining them (Alassimone et al. 2012); although in some cases (T5) the concentrations of Pb, Fe, and Cu in shoots were very high, even greater than in PS. Therefore, toxicity may not be directly related to higher PHEs concentrations due to their compartmentalisation and chelation by binding to different ligands for accumulation in shoots and roots in a non-toxic way (Shahid et al. 2015), as seems to occur in T6 where, despite lower solubility for Cd, Cu, and Zn, high concentrations of these PHEs accumulated in *L. sativa* roots.

In the GW-composed Technosols, ***L. purpureus* plants presented a higher survival rate and development** without visible signs of toxicity, unlike those grown in GW. Moreover, the **low translocation factor of PHEs concentrations to the shoots** grown in these Technosols caused the values to be within the normal and/or below phytotoxicity concentrations. Considering the consumption assessment of this plant by animals, PHEs concentrations in shoots were compared to their maximum tolerable levels in feed for different species (rodents, poultry, pigs, horses, cattle, and sheep) (National Research Council 2005). For plants grown in GW, there is a risk of As and Pb affection if used in feed production for all animal species considered, of Hg for rodents, poultry, pigs and horses feed, and of Cu for sheep feed; while in Technosols, *L. purpureus* plants presented **PHEs concentrations** that could be **suitable for cattle or sheep feed**, but **not for poultry, pigs and horses were because of Pb levels**. Therefore, the combination of Technosols with *L. purpureus* plants could be a potentially valuable ecotechnology for the remediation of gossan waste that should be carefully considered before its conversion into production areas under the condition of thorough quality and safety controls.

9.3. LESSONS LEARNED AND FUTURE RESEARCH DIRECTIONS

The ecotechnology based on Technosols constructed from the degraded elements to be remediated (polluted soil and gossan waste) together with a mixture of local organic and inorganic wastes has proven to be effective in the remediation of degraded environments by sulphide mining (i.e. soils, gossan wastes and AMD) with high levels of PHEs, although some concerns have been detected that should continue to be investigated.

The nature and characteristics of the wastes used in Technosols are key factors to be considered, especially the content, quality, and stability of the organic matter of organic wastes, as it greatly influences the improvement in soil conditions, PHEs mobility, microbiological activity and, thus, its capacity to provide ecosystem functions such as vegetation support. For example, Technosols composed of pruning and gardening vermicompost (T3 and T6) showed the best overall improvement of properties and reduction of toxicity, whereas those composed of sewage sludge (T2 and T5) performed the worst. In addition, some wastes may supply certain substances with toxic properties for most living organisms and should therefore be added with caution. For example, sewage sludge contains high concentrations of Fe and Zn, as well as high levels of Na ions that contribute to the exchange complex causing salt stress. Similarly, solid olive-mill by-product also contain potentially harmful concentrations of Na, and often high levels of phenolic acids. Thus, the full characterisation of potential organic contaminants (e.g. pesticides, antibiotics) should be considered in future studies.

Technosols, by improving the properties of these degraded environments, contributed to reduce the contents of those PHEs with high concentration in the most readily accessible fractions (i.e. Cd, Cu, Zn), and consequently their toxicity to living organisms; however, there are other PHEs (i.e. As, Sb and Pb) that in some cases did not reduce their already low mobility, but rather the opposite, although they are not observed to exert toxicity. As and Sb in soils, under oxidising conditions, are mainly present in anionic form like arsenate ($HAsO_4^{2-}$, $H_2AsO_4^{-}$) and antimonate ($Sb(OH)_6^{-1}$) (Al-Abed et al. 2007), implying that these PHEs may have a special affinity for iron oxyhydroxides, thus being one of the main mechanisms of As and Sb immobilisation in soils (Romero-Freire et al. 2014). Therefore, when these PHEs are present, Technosols must contain iron oxyhydroxide-rich material in sufficient quantities to reduce their mobility and availability. Another element of concern is Pb, that in the microcosm experiments (**chapter 4** and **6**) an increase in Pb mobility was observed; whereas in the field experiment (**chapter 7**), the very low water-soluble concentration at initial conditions decreased over time. This reduction of Pb was related to the liming amendment, as it contributed to the immobilisation of Pb through precipitation in poorly soluble carbonates and/or chemisorption on carbonates (Simón et al. 2005; Mourid 2014); while the increase in Pb mobility could be caused by the increase in SOM (Pastor-Jáuregui et al. 2021). However, other authors also highlighted the affinity of Pb for SOM (Coppola et al. 2010; Sierra Aragón et al. 2019), which can interact with organic matter in a nearly stable manner, reducing its solubility. Indeed, in GW-composed Technosols, the bioavailable Pb decreased due to OC increase (**chapter 8**), as in other studies (Beesley et al. 2010; Paradelo et al. 2011) Therefore, this controversy highlights the need for further research on the interaction between OM and Pb in Technosols.

In this thesis, in order to assess the environmental risks and ensure the efficacy of this remediation ecotechnology, different toxicity bioassays have been applied covering different exposure pathways, but limited to plants (i.e. *T. campestre*, *L. sativa*, and *L. purpureus*) and microorganisms (i.e. soil enzymatic activities). Therefore, it is convenient to complement this battery of tests with species representative of different taxonomic groups, mainly soil invertebrates. For this reason, to complement the evaluation of the effectiveness of Technosols, the behaviour of earthworms (*Eisenia andrei* (Bouché, 1972)) in the presence of PHEs toxicity and their capacity to develop their complete biological cycle in Technosols is being studied.

On the other hand, the effect of Technosols has been evaluated mainly by studying changes in the mobility, bioavailability, and toxicity of PHEs, physicochemical properties with a marked influence on PHEs toxicity, and the life-sustaining capacity from microorganism to plants. These have been the main methods used to assess the success of remediation of polluted areas with Technosols integration, but the physical properties of soils should be evaluated in more detail. The use of Technosols plays an important role in the improvement of the physical quality of polluted soils, which usually have severe physical limitations (e.g. soil crusting, low water-holding capacity, low aggregate stability and porosity, high bulk density), affecting the potential productivity and limiting or conditioning the remediation efforts (Fageria and Nascente 2014). Therefore, the research of this PhD thesis will be complemented by an ongoing study focusing on the changes in bulk density and the improvement of aggregate stability and porosity through the micromorphological study of inclusions prepared from these Technosols alongside the underlying polluted soil.

Monitoring the long-term evolution of the application of these Technosols is another point of interest for future studies, since, as we can see in Figure 9.1 after almost three years, at least a biodiverse and functioning vegetation cover is being maintained. In particular, it is of interest to assess whether the concentrations of PHEs readily accessible to organisms are still maintained at non-hazardous levels, and especially what variations are occurring in the mobility and potential toxicity of some PHEs like As and Sb that were increasing their bioavailability. In general, monitoring of these remediated areas after the application of Technosols is necessary to reinforce knowledge on remediation dynamics of PHE-polluted soils, mainly in the soil-plant system.

Figure 9.1. *General aspect of the natural vegetation developed after almost three years in Technosols T4 and T6 (n = 3).*

Another line of research opened is the phytoremediation of polluted soils through a strategy that combines these Technosols with the cultivation of species with potential to hyperaccumulate or phytostabilise PHEs, namely *Helianthus annuus* L., *Hordeum vulgare* L., *Lamarckia aurea* L., and *Spergularia rubra* L. (January et al. 2008; García-Carmona et al. 2019a; Haddad et al. 2023), identified as plants of interest in this region with high potential to cope with pollution and to grow under unfavourable conditions, promoting the first stages of the recovery of soil biological properties and favouring the re-establishment of their essential functions.

On the other hand, the combination of plant-based techniques with the application Technosols has recently been developed as a viable option to accelerate the recovery of salinity-affected areas (Navarro-Torre et al. 2023). Previous studies reported that Technosols constructed with aquaculture sediments from coastal ponds or saline Fluvisols from salt marshes together with organic and inorganic wastes improved soil physicochemical characteristics and fertility, allowing the cultivation of halophyte species (Cortinhas et al. 2020, 2021; Aguilar-Garrido et al. 2022c). In this respect, future studies are underway in which Technosols have been constructed from both gossan waste and saline soils, and the potential cultivation of halophytic plants (i.e. the critically endangered endemic *Limonium daveaui* Erben) and glycophytic plants such as a biodiverse pasture composed of legumes (*Trifolium* sp. and *Medicago* sp.) and grasses (*Lolium* sp.) is being evaluated. In the latter case, to assess whether these degraded environments can be recovered as grazing areas, additional studies should be conducted to ensure that there is no risk of transfer of PHEs to the food chain due to the translocation of PHEs to the aerial part of the plants growing on Technosols.

Native vegetation growing on Technosols T4 and T6 installed in the experimental plot "El Vicario", Guadiamar Green Corridor

Seville, Spain

April 2022

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CHAPTER 10

CONCLUSIONS

CHAPTER 10: CONCLUSIONS

The research conducted in this PhD thesis aims to advance the scientific knowledge of ecotechnology based on the use of waste-derived Technosols for the remediation of degraded environments by sulphide mining (i.e. soils, tailings and water) with high levels of potentially harmful elements (PHEs). As the global mining industry is expanding to meet the demands of today's energy and digital transition, it is vital to develop such sustainable solutions to address its key threats.

In view of this general objective, the following specific conclusions can be drawn from the methodology and results obtained and discussed in this thesis:

Chapter 3: Remediation potential of mining, agro-industrial, and urban wastes against acid mine drainage

- ☐ Inorganic wastes, together with vermicompost from pruning and gardening waste, decrease most of the concentrations of PHEs in a highly polluted artificial AMD over 95%, while organic wastes retain between 50% and 95%. The potential efficacy was in the following order: **IO** > **CW** ≥ **MS** ≥ **VC** > GS > OW ≥ **OL** > **WS** > AW > BM.
- ☐ Waste treatment has successfully removed the main PHEs (i.e. As, Cd, Cr, Cu, Pb and Zn) from artificial AMD, but the retention of other less studied PHEs (i.e. In, Sc, Sn, Th, Tl, V and Y) is also remarkable.

Chapter 4: Waste-derived Technosols for the remediation of PHE-polluted soil: a microcosm assay

- ☐ The conditions of Technosols are more favourable than those of polluted soils (i.e. neutral pH, increased OC content, decreased solubility and bioavailability of most PHEs), which stimulates soil enzymatic activity and growth of *T. campestre* and *L. sativa*, thus reducing their potential toxicity. Technosols also improve the physicochemical properties and reduce the mobility of most PHEs in the underlying polluted soils, although the biological activity evaluated through enzyme activity is not significantly altered over the experiment time.
- ☐ Technosols composed of pruning and gardening vermicompost (T3 and T6) are the most effective, whereas those of sewage sludge (T2 and T5) show the worst performance, especially reducing toxicity on *T. campestre* and *L. sativa*.

This highlights the relevance of the organic matter nature in the waste used, as it greatly influences the improvement in soil conditions, mobility of PHEs, microbiological activity and, thus, its capacity to favour vegetation growth.

Chapter 5: Assessment of the critical load of waste-derived Technosols for acid mine drainage control

- ☐ All Technosols (T1, T2, T3, T4, T5, and T6) show a significant acid neutralisation capacity, as well as a strong immobilisation capacity for PHEs and critical load, well above unpolluted soil and, especially, polluted soil. The retention rates of all Technosols are so high (As, Cd, and Cu: > 95% for all acidities tested, Zn: > 85% [> 95% at low–medium acidities]), in line with the considerable critical load measured, that the concentrations of PHEs in leachates are significantly decreased compared to AMD.

- ☐ Technosols constitute a potential ecotechnology that can be applied to control AMD. Although, the presence of PHEs with variable chemical behaviour must be controlled, as the presence of a continuous AMD may pose a risk in the medium and long term due to the saturation of their capacity to retain certain PHEs and the loss of effectiveness as a protective barrier over time; therefore, Technosols used in AMD control must be monitored regularly and corrective measures proposed to maintain their functionality over time.

Chapter 6: Phytotoxicity reduction in polluted soils based on the use of Technosols and L. sativa as bioindicator

- ☐ Technosols T4, T5, and T6 improve the unfavourable conditions of PHE-polluted soils (neutralised acidity, increased OC) and consequently modify the mobility of most PHEs. However, with a differential response in each Technosol, depending mainly on the nature of the organic waste used. The solubility of Cd, Cu, and Zn decreases in all Technosols (except Cu in T4, which increases), while As resolubilises in all Technosols and Pb in T4 and T5.

- ☐ The presence of PHEs causes oxidative stress in *L. sativa* grown in T5 and particularly in polluted soil, triggering a strong antioxidant response as a tolerance mechanism. Likewise, exposure to PHEs limits photosynthesis and gas exchange processes in *L. sativa*, causing together with oxidative stress a decline in growth due to stomatal limitations and damage to the photosynthetic machinery.

- ☐ Technosol T6, composed of vermicompost from pruning and gardening waste, shows a much higher overall performance in improving soil properties and reducing the mobility of most PHEs than T4 and T5. Phytotoxicity evaluated in *L. sativa* plants grown on T6 is minimal, with great similarity to the recovered soil in most of the parameters analysed.

Chapter 7: Enhancing microbial biodiversity and vegetation growth in polluted soils by Technosols integration

- ☐ The progressive increase in soil enzyme activity over time during the remediation process implies an improvement of the microbial community. In addition, increased microbial diversity favours greater connectivity between microbial species, indicating an acceleration of ecological interactions as evidenced by the increasing presence of plant growth-promoting microorganisms in soils over time, correlated with vegetation growth.
- ☐ The integration of Technosols results in a substantial improvement of soil quality along with the recovery of its ecosystem functions, creating a favourable environment for vegetation growth while mitigating the risks associated with PHEs in polluted soils.

Chapter 8: A green solution for the rehabilitation of marginal lands: the case of Lablab purpureus (L.) Sweet grown in Technosols

- ☐ The ecotechnology combining the remediation of sulphide mine areas using designed Technosols made from mixing gossan waste with a combination of organic/inorganic wastes from local industries improves the physicochemical conditions, nutrient status, and microbiological activity of the gossan waste and reduces the bioavailability of most PHEs (with the exception of As); although, in general, conditions in TC75 are more favourable than in TC50.
- ☐ *L. purpureus* grows on Technosols with no visible signs of toxicity and low translocation of PHEs to shoots, with PHEs concentrations suitable for cattle or

sheep but not for poultry, pigs and horses due to Pb concentration. While in gossan waste, *L. purpureus* growth is limited (low biomass and signs of toxicity) and with levels of As, Cu, Hg and Pb above the limits for most animal feeds.

- ☐ The combination of Technosols with *L. purpureus* crop could be a potentially valuable ecotechnology for the remediation of degraded/polluted environments, but also for converting them into food production areas under the condition of exhaustive quality and food safety controls.

CHAPTER 10

CONCLUSIONES

La investigación realizada en esta tesis doctoral pretende avanzar en el conocimiento científico de la ecotecnología basada en el uso de Tecnosoles derivados de residuos para la remediación de entornos degradados por la minería de sulfuros (es decir, suelos contaminados, residuos gossan y aguas acidas mineras) con altos niveles de elementos potencialmente contaminantes (EPCs). Como la industria minera mundial se está expandiendo para satisfacer las demandas de la actual transición energética y digital, es vital desarrollar este tipo de soluciones sostenibles para hacer frente a sus principales amenazas.

Atendiendo a este objetivo general, cabe extraer las siguientes conclusiones específicas a partir de la metodología y los resultados obtenidos en esta tesis:

Capítulo 3: Potencial de residuos mineros, agroindustriales y urbanos para el control del drenaje ácido de minas

- ☐ Los residuos inorgánicos, junto con el vermicompost procedente de restos de poda y jardinería, disminuyen las concentraciones de la mayoría de EPCs en un AMD artificial altamente contaminado en más de un 95%, mientras que los orgánicos retuvieron entre un 50% y un 95%. La eficacia potencial sigue el siguiente este orden: **IO** > **CW** ≥ **MS** ≥ **VC** > GS > OW ≥ **OL** > **WS** > AW > BM.
- ☐ El tratamiento de residuos ha eliminado con éxito los principales EPCs (As, Cd, Cr, Cu, Pb y Zn) del AMD artificial, pero también es destacable la retención de otros EPCs menos estudiados (In, Sc, Sn, Th, Tl, V e Y).

Capítulo 4: Tecnosoles derivados de residuos para la remediación de suelos contaminados con EPCs: un ensayo en microcosmos

- ☐ Las condiciones de los Tecnosoles son más favorables que las del suelo contaminado (es decir, pH neutro, mayor contenido de OC, menor solubilidad y biodisponibilidad de la mayoría de los EPCs), lo que estimula la actividad enzimática del suelo y el crecimiento de *T. campestre* y *L. sativa*, reduciendo así su toxicidad potencial. Los Tecnosoles también mejoran las propiedades fisicoquímicas y redujeron la movilidad de la mayoría de los EPCs en los suelos contaminados subyacentes, aunque la actividad biológica evaluada por la actividad enzimática no se altera significativamente en el tiempo del experimento.

- ❑ Los Tecnosoles compuestos por vermicompost de restos de poda y jardinería (T3 y T6) son los más eficaces, mientras que los compuestos por lodos de depuradora (T2 y T5) muestran el peor rendimiento, especialmente reduciendo la toxicidad sobre *T. campestre* y *L. sativa*. Esto pone de manifiesto la relevancia de la naturaleza de la materia orgánica de los residuos, ya que influye en gran medida en la mejora de las condiciones del suelo, la movilidad de los EPCs, la actividad microbiológica y, por tanto, su capacidad para favorecer el crecimiento de la vegetación.

Capítulo 5: Evaluación de la carga crítica de los Tecnosoles derivados de residuos para el control del drenaje ácido de minas

- ❑ Todos los Tecnosoles (T1, T2, T3, T4, T5 y T6) muestran una importante capacidad de neutralización de ácidos, así como una gran capacidad de inmovilización de EPCs y carga crítica, muy por encima de la del suelo no contaminado y especialmente del suelo contaminado. Las tasas de retención de todos los Tecnosoles son tan elevadas (As, Cd y Cu: > 95% para todas las acideces, Zn: > 85% [> 95% a acididades bajas-medias]), en consonancia con la considerable carga crítica medida, que las concentraciones de EPCs en los lixiviados disminuyen significativamente en comparación con los AMD.

- ❑ Los Tecnosoles constituyen una ecotecnología potencial que puede aplicarse para el control de AMD. Si bien, la presencia de EPCs con comportamiento químico variable debe ser controlada, ya que la presencia de un AMD continuo puede suponer un riesgo a medio y largo plazo debido a la saturación de su capacidad de retención de determinados EPCs y a la pérdida de eficacia como barrera protectora con el paso del tiempo; por lo que los Tecnosoles utilizados en el control del AMD deben ser monitorizados periódicamente y proponer medidas correctoras para mantener su funcionalidad en el tiempo.

Capítulo 6: Reducción de la fitotoxicidad en suelos contaminados mediante el uso de Tecnosoles y L. sativa como bioindicador

- ❑ Los Tecnosoles T4, T5 y T6 mejoran las condiciones desfavorables del suelo contaminado por EPCs (neutralización de la acidez, aumento en OC) y, en consecuencia, modifican la movilidad de la mayoría de los EPCs. Sin embargo, con una respuesta diferencial en cada Tecnosol, dependiendo principalmente de la naturaleza de los residuos orgánicos utilizados. La solubilidad del Cd, Cu y Zn disminuye en todos los Tecnosoles (excepto Cu en T4, que aumenta), mientras que As se resolubiliza en todos los Tecnosoles y Pb en T4 y T5.

- ☐ La presencia de EPCs provoca estrés oxidativo en las plantas de *L. sativa* cultivadas en T5 y particularmente en suelo contaminado, desencadenando una fuerte respuesta antioxidante como mecanismo de tolerancia. Asimismo, la exposición a EPCs limita los procesos de fotosíntesis e intercambio gaseoso, provocando junto con el estrés oxidativo una disminución del crecimiento debido a limitaciones estomáticas y daños en la maquinaria fotosintética.

- ☐ El Tecnosol T6, compuesto por vermicompost procedente de restos de poda y jardinería, muestra un rendimiento global muy superior en la mejora de las propiedades del suelo y reduciendo la movilidad de la mayoría de los EPCs que los Tecnosoles T4 y T5. La fitotoxicidad evaluada en plantas de *L. sativa* en T6 fue mínima, con gran similitud a las cultivadas en el suelo recuperado en la mayoría de los parámetros analizados.

Capítulo 7: Mejora de la biodiversidad microbiana y del crecimiento de la vegetación en suelos contaminados mediante la integración de Tecnosoles

- ☐ El aumento progresivo de la actividad enzimática del suelo a lo largo del tiempo durante el proceso de remediación implica una mejora de la comunidad microbiana. Además, el aumento de la diversidad microbiana favorece una mayor conectividad entre las especies microbianas, lo que indica una aceleración de las interacciones ecológicas, como demuestra la presencia creciente de microorganismos promotores del crecimiento vegetal en los suelos a lo largo del tiempo, correlacionada con el crecimiento de la vegetación.

- ☐ La integración de Tecnosoles se traduce en una mejora sustancial de la calidad del suelo junto con la recuperación de sus funciones ecosistémicas, creando un entorno favorable para el crecimiento de la vegetación y mitigando al mismo tiempo los riesgos asociados a los EPCs en suelos contaminados.

Capítulo 8: Una solución verde para la rehabilitación de tierras marginales: el caso de Lablab purpureus (L.) Sweet cultivada en Tecnosoles

- ☐ La ecotecnología que combina la remediación de zonas de minas de sulfuros utilizando Tecnosoles diseñados a partir de la mezcla de residuos de gossan con una combinación de residuos orgánicos/inorgánicos procedentes de industrias locales mejora las condiciones fisicoquímicas, el estado de los nutrientes y la actividad microbiológica de los residuos de gossan y reduce la biodisponibilidad de la mayoría de los EPCs (a excepción del As); no obstante, en general, las condiciones en TC75 son más favorables que en TC50.

- ☐ Las plantas de *L. purpureus* crecen en los Tecnosoles sin signos visibles de toxicidad y con una baja translocación de EPCs a los brotes, con concentraciones de EPCs adecuadas para destinarlas al uso en alimentación de ganado vacuno u ovino, pero no para las aves de corral, los cerdos y los caballos debido a la concentración de Pb. Mientras que en los residuos de gossan, el crecimiento de *L. purpureus* es limitado (baja biomasa y mostrando signos de toxicidad) y con niveles de As, Cu, Hg y Pb por encima de los límites para la mayoría de los piensos animales.

- ☐ La combinación de Tecnosoles con el cultivo de *L. purpureus* podría ser una ecotecnología potencialmente valiosa para la remediación de entornos degradados/contaminados, pero también para convertirlos en zonas de producción de alimentos bajo la condición de exhaustivos controles de calidad y seguridad alimentaria.


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REFERENCES

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Abd-Alla MH, Issa AA, Ohyama T. 2014. Impact of Harsh Environmental Conditions on Nodule Formation and Dinitrogen Fixation of Legumes. In: Ohyama T, editor. Advances in Biology and Ecology of Nitrogen Fixation. London, United Kingdom: IntechOpen Limited. p. 131–193.

Abreu MM, Batista MJ, Magalhães MCF, Matos JX. 2010. Acid Mine Drainage in the Portuguese Iberian Pyrite Belt. In: Robinson BC, editor. Mine Drainage and Related Problems. New York, USA: Nova Science Publisher, Inc. p. 77–118.

Abreu MM, Tavares MT, Batista MJ. 2008. Potential use of Erica andevalensis and Erica australis in phytoremediation of sulphide mine environments: São Domingos, Portugal. J Geochem Explor. 96:210–222. doi:10.1016/J.GEXPLO.2007.04.007.

Achberger K, Ohlinger R. 1988. Effect of sewage sludge and waste compost on some enzymatic activities tested in a field experiments. In: Proceedings of the European Water Pollution Control Association symposium (19–23 September 1988). Amsterdam, The Netherlands.

Activation Laboratories Ltd. 2005a. Code 1 - Aqua Regia "Partial" Digestion by ICP-MS. Ancaster, Ontario, Canada. [accessed 2021 Nov 12]. https://actlabs.com/wp-content/uploads/2021/07/Actlabs-Schedule-of-Services-Euro-2021.pdf.

Activation Laboratories Ltd. 2005b. Code 6 - Overrange elements in Code 6 MB reanalyzed by ICP-MS if required. Ancaster, Ontario, Canada. [accessed 2021 Nov 12]. https://actlabs.com/wp-content/uploads/2021/07/Actlabs-Schedule-of-Services-Euro-2021.pdf.

Activation Laboratories Ltd. 2005c. Code 6 - Total Recoverable Natural Waters with low TDS (<0.05%), analysed by ICP-MS. Ancaster, Ontario, Canada. [accessed 2021 Nov 12]. https://actlabs.com/wp-content/uploads/2021/07/Actlabs-Schedule-of-Services-Euro-2021.pdf.

Adamczyk-Szabela D, Chrześcijańska E, Zielenkiewicz P, Wolf WM. 2023. Antioxidant Activity and Photosynthesis Efficiency in Melissa officinalis Subjected to Heavy Metals Stress. Molecules. 28:2642. doi:10.3390/MOLECULES28062642.

Adamczyk-Szabela D, Lisowska K, Romanowska-Duda Z, Wolf WM. 2020. Combined cadmium-zinc interactions alter manganese, lead, copper uptake by Melissa officinalis. Scientific Reports . 10:1675. doi:10.1038/s41598-020-58491-9.

Adesoji AG, Abubakar IU, Labe DA. 2014. Soil Chemical Properties as Affected by Incorporated Legumes and Nitrogen in Soil with Maize (Zea mays L.) in a Semi-Arid Environment. Int J Agric Innov Res. 3:888–894.

Adhikari K, Hartemink AE. 2016. Linking soils to ecosystem services – A global review. Geoderma. 262:101–111. doi:10.1016/J.GEODERMA.2015.08.009.

Adriano DC. 2001. Trace elements in terrestrial environments. Biogeochemistry, bioavailability and risks of metals, 2nd ed. New York, United States: Springer Science & Business Media.

Adriano DC, Wenzel WW, Vangronsveld J, Bolan NS. 2004. Role of assisted natural remediation in environmental cleanup. Geoderma. 122:121–142. doi:10.1016/J.GEODERMA.2004.01.003.

REFERENCES

Aerts R. 1997. Climate, leaf litter chemistry and leaf litter decomposition in terrestrial ecosystems: a triangular relationship. Oikos. 79(3):439–449. doi:10.2307/3546886.

AGAPA (Agencia de Gestión Agraria y Pesquera de la Junta de Andalucía). 2015. Evaluación de la producción y usos de los subproductos de las agroindustrias del olivar en Andalucía. Seville, Spain: Junta de Andalucía. Consejería de Agricultura, Pesca y Desarrollo.

Agegnehu G, Bass AM, Nelson PN, Bird MI. 2016. Benefits of biochar, compost and biochar-compost for soil quality, maize yield and greenhouse gas emissions in a tropical agricultural soil. Science of the Total Environment. 543:295–306. doi:10.1016/J.SCITOTENV.2015.11.054.

Aguilar J, Bouza P, Dorronsoro C, Fernández E, Fernández J, García I, Martín F, Ortiz I, Simón M. 2003. Contaminación de los suelos afectados por el vertido de Aznalcóllar y su evolución en el tiempo (1998 - 2001). Edafología. 10(1):65–73.

Aguilar J, Dorronsoro C, Fernández E, Fernández J, García I, Martín F, Sierra M, Simón M. 2007. Remediation of As-contaminated soils in the Guadiamar river basin (SW, Spain). Water Air Soil Pollut. 180:109–118. doi:10.1007/s11270-006-9254-3.

Aguilar J, Dorronsoro C, Fernández E, Fernández J, García I, Martín F, Simón M. 2004. Soil pollution by a pyrite mine spill in Spain: evolution in time. Environmental Pollution. 132:395–401. doi:10.1016/j.envpol.2004.05.028.

Aguilar-Garrido A, García-Carmona M, Sierra-Aragón M, Martín-Peinado FJ, Martínez Garzón FJ. 2022a. Carbonated waste valorisation from a peat bog exploitation in the treatment of arsenic-polluted waters. International Journal of Environmental Science and Technology. 19:3457–3468. doi:10.1007/s13762-021-03445-5.

Aguilar-Garrido A, Paniagua-López M, Romero-Freire A, Martínez Garzón FJ, Fernández Ondoño E, Martín Peinado FJ. 2022b. Short-term evolution of physico-chemical properties of Technosols made from contaminated soils by pyritic sludge. Revista de Ciências Agrárias. 45(4):627–631. doi:10.19084/rca.28744.

Aguilar-Garrido A, Paniagua-López M, Sierra-Aragón M, Martínez Garzón FJ, Martín-Peinado FJ. 2023a. Remediation potential of mining, agro-industrial, and urban wastes against acid mine drainage. Sci Rep. 13:12120. doi:10.1038/s41598-023-39266-4.

Aguilar-Garrido A, Reyes-Martín MP, Vidigal P, Abreu MM. 2023b. A Green Solution for the Rehabilitation of Marginal Lands: The Case of Lablab purpureus (L.) Sweet Grown in Technosols. Plants. 12:2682. doi:10.3390/plants12142682.

Aguilar-Garrido A, Romero-Freire A, García-Carmona M, Martín Peinado FJ, Sierra Aragón M, Martínez Garzón FJ. 2020. Arsenic Fixation in Polluted Soils by Peat Applications. Minerals. 10:968. doi:10.3390/min10110968.

Aguilar-Garrido A, Romero-Freire A, Paniagua-López M, Martínez-Garzón FJ, Martín-Peinado FJ, Sierra-Aragón M. 2023c. Technosols Derived from Mining, Urban, and Agro-Industrial Waste for the Remediation of Metal(loid)-Polluted Soils: A Microcosm Assay. Toxics. 11(10):854. doi:10.3390/toxics11100854.

REFERENCES

Aguilar-Garrido A, Vidigal P, Caperta AD, Abreu MM. 2022c. Development capacity of a biodiverse pasture on Technosols for the rehabilitation of marginal lands (saline soils and mining waste). In: European Geosciences Union General Assembly 2022. Vienna, Austria. p. EGU22-121. [accessed 2024 Apr 29]. https://doi.org/10.5194/egusphere-egu22-121.

Akcil A, Koldas S. 2006. Acid Mine Drainage (AMD): causes, treatment and case studies. J Clean Prod. 14:1139–1145. doi:10.1016/J.JCLEPRO.2004.09.006.

Al-Abed SR, Jegadeesan G, Purandare J, Allen D. 2007. Arsenic release from iron rich mineral processing waste: Influence of pH and redox potential. Chemosphere. 66:775–782. doi:10.1016/J.CHEMOSPHERE.2006.07.045.

Alassimone J, Roppolo D, Geldner N, Vermeer JEM. 2012. The endodermis-development and differentiation of the plant's inner skin. Protoplasma. 249(3):433–443. doi:10.1007/S00709-011-0302-5.

Alfaia RG de SM, Costa AM, Campos JC. 2017. Municipal solid waste in Brazil: A review. Waste Management & Research. 35(12):1195–1209. doi:10.1177/0734242X17735375.

Alghamdi AG, Alasmary Z. 2023. Fate and Transport of Lead and Copper in Calcareous Soil. Sustainability. 15:775. doi:10.3390/SU15010775.

Alloway BJ. 2012. Heavy metals in soils. Trace metals and metalloids in soils and their bioavailability. 3rd ed. Dordrecht, Netherlands: Springer.

Almodóvar GR, Yesares L, Sáez R, Toscano M, González F, Pons JM. 2019. Massive Sulfide Ores in the Iberian Pyrite Belt: Mineralogical and Textural Evolution. Minerals. 9(11):653. doi:10.3390/min9110653.

Alvarenga P, Gonçalves AP, Fernandes RM, de Varennes A, Vallini G, Duarte E, Cunha-Queda AC. 2008. Evaluation of composts and liming materials in the phytostabilization of a mine soil using perennial ryegrass. Science of The Total Environment. 406:43–56. doi:10.1016/J.SCITOTENV.2008.07.061.

Álvarez-Rogel J, Peñalver-Alcalá A, Jiménez-Cárceles FJ, Carmen Tercero M, Nazaret González-Alcaraz M. 2021. Evidence supporting the value of spontaneous vegetation for phytomanagement of soil ecosystem functions in abandoned metal(loid) mine tailings. Catena (Amst). 201:105191. doi:10.1016/J.CATENA.2021.105191.

Álvarez-Valero AM, Pérez-López R, Matos J, Capitán MA, Nieto JM, Sáez R, Delgado J, Caraballo M. 2008. Potential environmental impact at São Domingos mining district (Iberian Pyrite Belt, SW Iberian Peninsula): evidence from a chemical and mineralogical characterization. Environmental Geology. 55:1797–1809. doi:10.1007/S00254-007-1131-X.

Anawar HM, Freitas MC, Canha N, Santa Regina I. 2011. Arsenic, antimony, and other trace element contamination in a mine tailings affected area and uptake by tolerant plant species. Environ Geochem Health. 33:353–362. doi:10.1007/S10653-011-9378-2.

REFERENCES

Angai JU, Ptacek CJ, Pakostova E, Bain JG, Verbuyst BR, Blowes DW. 2022. Removal of arsenic and metals from groundwater impacted by mine waste using zero-valent iron and organic carbon: Laboratory column experiments. J Hazard Mater. 424:127295. doi:10.1016/J.JHAZMAT.2021.127295.

Angulo-Bejarano PI, Puente-Rivera J, Cruz-Ortega R. 2021. Metal and Metalloid Toxicity in Plants: An Overview on Molecular Aspects. Plants. 10:635. doi:10.3390/PLANTS10040635.

Antoniadis V, Levizou E, Shaheen SM, Ok YS, Sebastian A, Baum C, Prasad MNV, Wenzel WW, Rinklebe J. 2017. Trace elements in the soil-plant interface: Phytoavailability, translocation, and phytoremediation–A review. Earth Sci Rev. 171:621–645. doi:10.1016/J.EARSCIREV.2017.06.005.

APA (Agência Portuguesa do Ambiente). 2019. Solos Contaminados - Guia Técnico - Valores de referência para solo. Revisão 3 - Setembro de 2022. Lisbon, Portugal: Agência Portuguesa do Ambiente. [accessed 2022 Nov 23]. https://sniambgeoviewer.apambiente.pt/GeoDocs/geoportaldocs/AtQualSolos/Guia_Tecnico_Valores%20de%20Referencia_2019_01.pdf.

Aparicio JD, Raimondo EE, Saez JM, Costa-Gutierrez SB, Álvarez A, Benimeli CS, Polti MA. 2022. The current approach to soil remediation: A review of physicochemical and biological technologies, and the potential of their strategic combination. J Environ Chem Eng. 10:107141. doi:10.1016/J.JECE.2022.107141.

Aponte H, Meli P, Butler B, Paolini J, Matus F, Merino C, Cornejo P, Kuzyakov Y. 2020. Meta-analysis of heavy metal effects on soil enzyme activities. Science of The Total Environment. 737:139744. doi:10.1016/J.SCITOTENV.2020.139744.

Aragón R, Sardans J, Peñuelas J. 2014. Soil enzymes associated with carbon and nitrogen cycling in invaded and native secondary forests of northwestern Argentina. Plant Soil. 384:169–183. doi:10.1007/s11104-014-2192-8.

Arán D, Santos ES, Abreu MM, Antelo J, Macías F. 2022. Use of combined tools for effectiveness evaluation of tailings rehabilitated with designed Technosol. Environ Geochem Health. 44:1857–1873. doi:10.1007/s10653-021-01118-3.

Arco-Lázaro E, Agudo I, Clemente R, Bernal MP. 2016. Arsenic(V) adsorption-desorption in agricultural and mine soils: Effects of organic matter addition and phosphate competition. Environmental Pollution. 216:71–79. doi:10.1016/j.envpol.2016.05.054.

Asemaninejad A, Arteaga J, Spiers G, Beckett P, McGarry S, Mykytczuk N, Basiliko N. 2018. Blended pulp mill, forest humus and mine residual material Technosols for mine reclamation: A growth-chamber study to explore the role of physiochemical properties of substrates and microbial inoculation on plant growth. J Environ Manage. 228:93–102. doi:10.1016/j.jenvman.2018.08.114.

Asensio V, Flórido FG, Ruiz F, Perlatti F, Otero XL, Oliveira DP, Ferreira TO. 2019. The potential of a Technosol and tropical native trees for reclamation of copper-polluted soils. Chemosphere. 220:892–899. doi:10.1016/j.chemosphere.2018.12.190.

REFERENCES

Asensio V, Vega FA, Andrade ML, Covelo EF. 2013. Technosols Made of Wastes to Improve Physico-Chemical Characteristics of a Copper Mine Soil. Pedosphere. 23(1):1–9. doi:10.1016/S1002-0160(12)60074-5.

Ayala J, Fernández B. 2020. Industrial waste materials as adsorbents for the removal of As and other toxic elements from an abandoned mine spoil heap leachate: a case study in Asturias. J Hazard Mater. 384:121446. doi:10.1016/j.jhazmat.2019.121446.

Ayala-Carcedo FJ. 2004. The Aznalcollar (Spain) tailings pond failure of 1998 and the ecological disaster of Guadiamar river: causes, effects and lessons. Boletin Geologico y Minero. 115(4):711–714.

Azhar U, Ahmad H, Shafqat H, Babar M, Shahzad Munir HM, Sagir M, Arif M, Hassan A, Rachmadona N, Rajendran S, et al. 2022. Remediation techniques for elimination of heavy metal pollutants from soil: A review. Environ Res. 214:113918. doi:10.1016/J.ENVRES.2022.113918.

Azubuike CC, Chikere CB, Okpokwasili GC. 2016. Bioremediation techniques–classification based on site of application: principles, advantages, limitations and prospects. World J Microbiol Biotechnol. 32:180. doi:10.1007/S11274-016-2137-X.

Bagherifam S, Brown TC, Fellows CM, Naidu R. 2019. Bioavailability of Arsenic and Antimony in Terrestrial Ecosystems: A Review. Pedosphere. 29(6):681–720. doi:10.1016/S1002-0160(19)60843-X.

Bagur-González MG, Estepa-Molina C, Martín-Peinado F, Morales-Ruano S. 2011. Toxicity assessment using Lactuca sativa L. bioassay of the metal(loid)s As, Cu, Mn, Pb and Zn in soluble-in-water saturated soil extracts from an abandoned mining site. J Soils Sediments. 11(2):281–289. doi:10.1007/s11368-010-0285-4.

Bai H, Wang H, Sun J, Irfan M, Han M, Huang Y, Han X, Yang Q. 2013. Production, purification and characterization of novel beta glucosidase from newly isolated penicillium simplicissimum h-11 in submerged fermentation. EXCLI J. 12:528–540.

Barahona E. 1984. Determinaciones analíticas en suelos: carbonatos totales y caliza activa. In: I Congreso Nacional de la Ciencia del Suelo. Madrid, Spain: Sociedad Española de la Ciencia del Suelo.

Barba-Brioso C, Hidalgo PJ, Fernández-Landero S, Giráldez I, Fernández-Caliani JC. 2023. Phytoaccumulation of trace elements (As, Cd, Co, Cu, Pb, Zn) by Nicotiana glauca and Euphorbia segetalis growing in a Technosol developed on legacy mine wastes (Domingo Rubio wetland, SW Spain). Environ Geochem Health. 45:9541–9557. doi:10.1007/S10653-023-01523-W.

Barrameda-Medina Y, Montesinos-Pereira D, Romero L, Blasco B, Ruiz JM. 2014. Role of GSH homeostasis under Zn toxicity in plants with different Zn tolerance. Plant Science. 227:110–121. doi:10.1016/J.PLANTSCI.2014.07.010.

Barredo O, Vilela J, Garbisu C, Besga G, Alkorta I, Epelde L. 2020. Technosols made from urban and industrial wastes are a good option for the reclamation of abandoned city plots. Geoderma. 377:114563. doi:10.1016/J.GEODERMA.2020.114563.

Bazihizina N, Colzi I, Giorni E, Mancuso S, Gonnelli C. 2015. Photosynthesizing on metal excess: Copper differently induced changes in various photosynthetic parameters in copper tolerant and sensitive Silene paradoxa L. populations. Plant Science. 232:67–76. doi:10.1016/J.PLANTSCI.2014.12.015.

Beesley L, Moreno-Jiménez E, Gomez-Eyles JL. 2010. Effects of biochar and greenwaste compost amendments on mobility, bioavailability and toxicity of inorganic and organic contaminants in a multi-element polluted soil. Environmental Pollution. 158(6):2282–2287. doi:10.1016/J.ENVPOL.2010.02.003.

Bennardi DO, Díaz Gorostegui A, Juan L, Millan G, Pellegrini A, Vázquez ME. 2018. Evaluación de la capacidad buffer de suelos ácidos de la región Pampeana. Ciencias del Suelo (Asociación Argentina Ciencia del Suelo). 36(1):30–38.

Beyer WF, Fridovich I. 1987. Assaying for superoxide dismutase activity: Some large consequences of minor changes in conditions. Anal Biochem. 161(2):559–566. doi:10.1016/0003-2697(87)90489-1.

Bhaduri AM, Fulekar MH. 2012. Antioxidant enzyme responses of plants to heavy metal stress. Rev Environ Sci Biotechnol. 11:55–69. doi:10.1007/S11157-011-9251-X.

Bhaduri D, Sihi D, Bhowmik A, Verma BC, Munda S, Dari B. 2022. A review on effective soil health bio-indicators for ecosystem restoration and sustainability. Front Microbiol. 13:938481. doi:10.3389/FMICB.2022.938481.

Bieleski RL, Turner NA. 1966. Separation and estimation of amino acids in crude plant extracts by thin-layer electrophoresis and chromatography. Anal Biochem. 17:278–293. doi:10.1016/0003-2697(66)90206-5.

Bini C, Bech J. 2014. PHEs, Environment and Human Health: Potentially harmful elements in the environment and the impact on human health. Berlin, Germany: Springer Science & Business Media.

Bissen M, Frimmel FH. 2003. Arsenic - a review. Part I: Occurrence, toxicity, speciation, mobility. Acta hydrochimical and hydrobiological. 31:9–18. doi:10.1002/aheh.200390025.

BOE. 1995. Resolución 11463, por el que se aprueba el Plan Nacional de Recuperación de Suelos Contaminados. BOE-A-1995-11463. Madrid, Spain: Ministerio de Obras Públicas, Transportes y Medio Ambiente.

BOE. 2007. Real Decreto 1620/2007, de 7 de diciembre, por el que se establece el régimen jurídico de la reutilización de aguas depuradas. BOE-A-2007-21092. Madrid, Spain: Ministerio de la Presidencia.

BOE. 2013. Real Decreto 506/2013, de 28 de junio, sobre productos fertilizantes. BOE-A-2013-7540. Madrid, Spain: Ministerio de la Presidencia.

BOE. 2015. Real Decreto 817/2015, de 11 de septiembre, por el que se establecen los criterios de seguimiento y evaluación del estado de las aguas superficiales y las normas de calidad ambiental. BOE-A-2015-9806. Madrid, Spain: Ministerio de Agricultura, Alimentación y Medio Ambiente.

BOJA. 2015. Decreto 18/2015, por el que se aprueba el reglamento que regula el régimen aplicable a los suelos contaminados. Seville, Spain: Consejería de Medio Ambiente y Ordenación del Territorio.

BOJA. 2018. Orden de 27 de abril de 2018, por la que se aprueba el Programa Andaluz de Suelos Contaminados 2018-2023. Seville, Spain: Consejería de Medio Ambiente y Ordenación del Territorio.

Bolan N, Kunhikrishnan A, Thangarajan R, Kumpiene J, Park J, Makino T, Kirkham MB, Scheckel K. 2014. Remediation of heavy metal(loid)s contaminated soils – To mobilize or to immobilize? J Hazard Mater. 266:141–166. doi:10.1016/J.JHAZMAT.2013.12.018.

Bolan NS, Park JH, Robinson B, Naidu R, Huh KY. 2011. Phytostabilization: A Green Approach to Contaminant Containment. In: Sparks DL, editor. Advances in Agronomy. Vol. 112. Cambridge, MA, USA: Academic Press. p. 145–204.

Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, Alexander H, Alm EJ, Arumugam M, Asnicar F, et al. 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat Biotechnol. 37:852–857. doi:10.1038/s41587-019-0209-9.

Bradford MM. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 72:248–254. doi:10.1016/0003-2697(76)90527-3.

Bradl HB. 2004. Adsorption of heavy metal ions on soils and soils constituents. J Colloid Interface Sci. 277:1–18. doi:10.1016/J.JCIS.2004.04.005.

De Bruijn J, Hansen B, Johansson S, Luotamo M, Munn S, Musset C, Olsen S, Olsson H, Paya-Perez A, Pedersen F, et al. 2002. Technical Guidance Document on Risk Assessment. Part 1. Part 2. EUR 20418 EN. JRC23785. Ispra, Italy: European Commission Joint Research Centre.

Bünemann EK, Bongiorno G, Bai Z, Creamer RE, De Deyn G, de Goede R, Fleskens L, Geissen V, Kuyper TW, Mäder P, et al. 2018. Soil quality – A critical review. Soil Biol Biochem. 120:105–125. doi:10.1016/J.SOILBIO.2018.01.030.

Bu-Olayan AH, Thomas B V. 2009. Translocation and bioaccumulation of trace metals in desert plants of Kuwait governorates. Res J Environ Sci. 3(5):581–587. doi:10.3923/rjes.2009.581.587.

Bur T, Probst A, Bianco A, Gandois L, Crouau Y. 2010. Determining cadmium critical concentrations in natural soils by assessing Collembola mortality, reproduction and growth. Ecotoxicol Environ Saf. 73:415–422. doi:10.1016/J.ECOENV.2009.10.010.

Burgos P, Madejón E, Pérez-de-Mora A, Cabrera F. 2008a. Horizontal and vertical variability of soil properties in a trace element contaminated area. International Journal of Applied Earth Observation and Geoinformation. 10(1):11–25. doi:10.1016/J.JAG.2007.04.001.

Burgos P, Madejón P, Cabrera F, Madejón E. 2010. By-products as amendment to improve biochemical properties of trace element contaminated soils: Effects in time. Int Biodeterior Biodegradation. 64:481–488. doi:10.1016/J.IBIOD.2010.05.009.

Burgos P, Pérez-de-Mora A, Madejón P, Cabrera F, Madejón E. 2008b. Trace elements in wild grasses: a phytoavailability study on a remediated field. Environ Geochem Health. 30:109–114. doi:10.1007/S10653-008-9135-3.

Burns RG, DeForest JL, Marxsen J, Sinsabaugh RL, Stromberger ME, Wallenstein MD, Weintraub MN, Zoppini A. 2013. Soil enzymes in a changing environment: Current knowledge and future directions. Soil Biol Biochem. 58:216–234. doi:10.1016/J.SOILBIO.2012.11.009.

Burton ED, Johnston SG, Bush RT. 2011. Microbial sulfidogenesis in ferrihydrite-rich environments: Effects on iron mineralogy and arsenic mobility. Geochim Cosmochim Acta. 75(11):3072–3087. doi:10.1016/J.GCA.2011.03.001.

Buturi CV, Sabatino L, Mauro RP, Navarro-León E, Blasco B, Leonardi C, Giuffrida F. 2022. Iron Biofortification of Greenhouse Soilless Lettuce: An Effective Agronomic Tool to Improve the Dietary Mineral Intake. Agronomy. 12:1793. doi:10.3390/AGRONOMY12081793.

Cabrera F, Clemente L, Díaz Barrientos E, López R, Murillo JM. 1999. Heavy metal pollution of soils affected by the Guadiamar toxic flood. Science of The Total Environment. 242:117–129. doi:10.1016/S0048-9697(99)00379-4.

Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. 2016. DADA2: High-resolution sample inference from Illumina amplicon data. Nature Methods . 13(7):581–583. doi:10.1038/nmeth.3869.

Campillo-Cora C, Conde-Cid M, Arias-Estévez M, Fernández-Calviño D, Alonso-Vega F. 2020. Specific Adsorption of Heavy Metals in Soils: Individual and Competitive Experiments. Agronomy. 10:1113. doi:10.3390/AGRONOMY10081113.

Camps Arbestain M, Madinabeitia Z, Anza Hortalà M, Macías-García F, Virgel S, Macías F. 2008. Extractability and leachability of heavy metals in Technosols prepared from mixtures of unconsolidated wastes. Waste Management. 28(12):2653–2666. doi:10.1016/j.wasman.2008.01.008.

Cannavo P, Guénon R, Galopin G, Vidal-Beaudet L. 2018. Technosols made with various urban wastes showed contrasted performance for tree development during a 3-year experiment. Environ Earth Sci. 77:1–13. doi:10.1007/s12665-018-7848-x.

Carrillo-González R, Gatica García BG, Del Carmen A. M, González-Chávez CA, Solís Domínguez FA. 2022. Trace elements adsorption from solutions and acid mine drainage using agricultural by-products. Soil and Sediment Contamination: An International Journal. 31(3):348–366. doi:10.1080/15320383.2021.1942430.

Casentini B, Lazzazzara M, Amalfitano S, Salvatori R, Guglietta D, Passeri D, Belardi G, Trapasso F. 2019. Mining rock wastes for water treatment: Potential reuse of Fe- and Mn-rich materials for arsenic removal. Water (Basel). 11:1897. doi:10.3390/W11091897.

Casida L, Klein D, Santoro T. 1964. Soil dehydrogenase activity. Soil Sci. 98(6):371–376. doi:10.1097/00010694-196412000-00004.

CCME (Canada Council of Ministers of the Environment). 2007. Canadian Soil Quality Guidelines for the Protection Of Environmental and Human Health: Summary Tables (Updated September, 2007). Winnipeg, Canada: Canada Council of Ministers of the Environment.

Chang Y-T, Hseu Z-Y, Zehetner F. 2014. Evaluation of Phytoavailability of Heavy Metals to Chinese Cabbage (Brassica chinensis L.) in Rural Soils. The Scientific World Journal. 2014:309396. doi:10.1155/2014/309396.

Chopin EIB, Alloway BJ. 2007. Distribution and mobility of trace elements in soils and vegetation around the mining and smelting areas of Tharsis, Ríotinto and Huelva, Iberian Pyrite Belt, SW Spain. Water Air Soil Pollut. 182:245–261. doi:10.1007/S11270-007-9336-X.

Clemente R, Pardo T, Madejón P, Madejón E, Bernal MP. 2015. Food byproducts as amendments in trace elements contaminated soils. Food Research International. 73:176–189. doi:10.1016/J.FOODRES.2015.03.040.

Clemente R, Walker DJ, Bernal MP. 2005. Uptake of heavy metals and As by Brassica juncea grown in a contaminated soil in Aznalcóllar (Spain): The effect of soil amendments. Environmental Pollution. 138:46–58. doi:10.1016/J.ENVPOL.2005.02.019.

Colombini G, Watteau F, Auclerc A. 2022. Technosol rehabilitation strategies drive soil physico-chemical properties and fauna diversity on a former coking plant area. Applied Soil Ecology. 177:104542. doi:10.1016/J.APSOIL.2022.104542.

Consejería de Medio Ambiente. 2003. Ciencia y restauración del Río Guadiamar. PICOVER 1998-2002. Seville, Spain: Junta de Andalucía.

Coppola E, Capra GF, Odierna P, Vacca S, Buondonno A. 2010. Lead distribution as related to pedological features of soils in the Volturno River low Basin (Campania, Italy). Geoderma. 159:342–349. doi:10.1016/J.GEODERMA.2010.08.010.

Cortinhas A, Caperta AD, Teixeira G, Carvalho L, Abreu MM. 2020. Harnessing sediments of coastal aquaculture ponds through technosols construction for halophyte cultivation using saline water irrigation. J Environ Manage. 261:109907. doi:10.1016/J.JENVMAN.2019.109907.

Cortinhas A, Ferreira TC, Abreu MM, Caperta AD. 2021. Conservation of a critically endangered endemic halophyte of West Portugal: A microcosm assay to assess the potential of soil technology for species reintroduction. Front Ecol Evol. 9:604509. doi:10.3389/fevo.2021.604509.

Costantini EAC, Branquinho C, Nunes A, Schwilch G, Stavi I, Valdecantos A, Zucca C. 2016. Soil indicators to assess the effectiveness of restoration strategies in dryland ecosystems. Solid Earth. 7:397–414. doi:10.5194/SE-7-397-2016.

Coull M, Butler B, Hough R, Beesley L. 2021. A Geochemical and Agronomic Evaluation of Technosols Made from Construction and Demolition Fines Mixed with Green Waste Compost. Agronomy. 11:649. doi:10.3390/AGRONOMY11040649.

Cravotta CA, Trahan MK. 1999. Limestone drains to increase pH and remove dissolved metals from acidic mine drainage. Applied Geochemistry. 14:581–606. doi:10.1016/S0883-2927(98)00066-3.

Dağhan H, Öztürk M. 2015. Soil Pollution in Turkey and Remediation Methods. In: Hakeem KR, Sabir M, Öztürk M, Mermut AR, editors. Soil Remediation and Plants: Prospects and Challenges. Cambridge, MA, USA: Academic Press. p. 287–312.

Davis JA. 1984. Complexation of trace metals by adsorbed natural organic matter. Geochim Cosmochim Acta. 48(4):679–691. doi:10.1016/0016-7037(84)90095-4.

Debosz K, Rasmussen PH, Pedersen AR. 1999. Temporal variations in microbial biomass C and cellulolytic enzyme activity in arable soils: effects of organic matter input. Applied Soil Ecology. 13:209–218. doi:10.1016/S0929-1393(99)00034-7.

Deng SP, Tabatabai MA. 1994. Cellulase activity of soils. Soil Biol Biochem. 26(10):1347–1354. doi:10.1016/0038-0717(94)90216-X.

Dhakal Y, Meena RS, Kumar S. 2016. Effect of INM on nodulation, yield, quality and available nutrient status in soil after harvest of greengram. Legume Research. 39(4):590–594. doi:10.18805/lr.v0iOF.9435.

Dhir B. 2018. Biotechnological tools for remediation of acid mine drainage (removal of metals from wastewater and leachate). In: Prasad MNV, Favas PJ de C, Maiti SK, editors. Bio-Geotechnologies for Mine Site Rehabilitation. Amsterdam, Netherlands: Elsevier Inc. p. 67–82.

Dias MC, Monteiro C, Moutinho-Pereira J, Correia C, Gonçalves B, Santos C. 2013. Cadmium toxicity affects photosynthesis and plant growth at different levels. Acta Physiol Plant. 35:1281–1289. doi:10.1007/S11738-012-1167-8.

Dick RP. 1997. Soil enzyme activities as integrative indicators of soil health. In: Pankhurst C, Doube BM, Gupta VVSR, editors. Biological indicators of soil health. Wallingford, United Kingdom: Centre for Agricultural Bioscience International. p. 121–156.

Díez M, Simón M, García I, Martín F. 2009. Assessment of the Critical Load of Trace Elements in Soils Polluted by Pyrite tailings. A Laboratory Experiment. Water Air Soil Pollut. 199:381–387. doi:10.1007/s11270-008-9886-6.

DOG (Diario Oficial de Galicia). 2009. Decreto 60/2009, do 26 de febreiro, sobre solos potencialmente contaminados e procedemento para a declaración de solos contaminados. Santiago de Compostela, Spain: Conselleria de Medio Ambiente y Cambio Climático.

Dola DB, Mannan MA, Sarker U, Mamun MA Al, Islam T, Ercisli S, Saleem MH, Ali B, Pop OL, Marc RA. 2022. Nano-iron oxide accelerates growth, yield, and quality of Glycine max seed in water deficits. Front Plant Sci. 13:992535. doi:10.3389/FPLS.2022.992535.

Doran JW, Zeiss MR. 2000. Soil health and sustainability: managing the biotic component of soil quality. Applied Soil Ecology. 15(1):3–11. doi:10.1016/S0929-1393(00)00067-6.

Dotaniya ML, Aparna K, Dotaniya CK, Singh M, Regar KL. 2019. Role of soil enzymes in sustainable crop production. In: Kuddus M, editor. Enzymes in Food Biotechnology: Production, Applications, and Future Prospects. Amsterdam, The Netherlands: Elsevier. p. 569–589.

Draszawka-Bołzan B. 2017. Effect of pH and soil environment. World News of Natural Sciences. 8:50–60.

D'Souza MR, Devaraj VR. 2009. Biochemical responses of Hyacinth bean (Lablab purpureus) to salinity stress. Acta Physiol Plant. 32:341–353. doi:10.1007/s11738-009-0412-2.

D'Souza MR, Devaraj VR. 2013. Oxidative stress biomarkers and metabolic changes associated with cadmium stress in hyacinth bean (Lablab purpureus). Afr J Biotechnol. 12(29):4670–4682. doi:10.5897/ajb2013.12385.

Dutta A, Patra A, Singh Jatav H, Singh Jatav S, Kumar Singh S, Sathyanarayana E, Verma S, Singh P. 2021. Toxicity of Cadmium in Soil-Plant-Human Continuum and Its Bioremediation Techniques. In: Larramendy ML, Soloneski S, editors. Soil Contamination - Threats and Sustainable Solutions. London, United Kingdom: IntechOpen. p. 59–80.

EEA. 2019. EEA Signals 2019 - Land and soil in Europe. Copenhagen, Denmark: European Environmental Agency.

Egnér H, Riehm H, Domingo WR. 1960. Chemical extraction methods for phosphorus and potassium. Chemical analyses of soil as a basis for determining soil fertility Lantbr Högsk-Annlr. 26:199–215.

Eivazi F, Tabatabai MA. 1977. Phosphatases in soils. Soil Biol Biochem. 9:167–172. doi:10.1016/0038-0717(77)90070-0.

Eivazi F, Tabatabai MA. 1988. Glucosidases and galactosidases in soils. Soil Biol Biochem. 20(5):601–606. doi:10.1016/0038-0717(88)90141-1.

EPA. 1996. Ecological effects test guidelines. Seed germination/root elongation toxicity test. OPPTS 850.4200. Washington DC, USA: United States Environmental Protection Agency.

EPA. 2003. Environmental Regulations and Technology. Control of Pathogens and Vector Attraction in Sewage Sludge. EPA/625/R-92/013. Washington, DC, USA: United States Environmental Protection Agency.

EPA. 2007. Framework for Metals Risk Assessment. EPA 120/R-07/00. Washington DC, USA: United States Environmental Protection Agency.

EPA. 2012. Guidelines for Water Reuse. EPA/600/R-12/618. Washington, D.C., USA: United States Environmental Protection Agency.

EPA. 2022. National Overview: Facts and Figures on Materials, Wastes and Recycling. [accessed 2022 Apr 19]. https://www.epa.gov/facts-and-figuresabout-materials-waste-and-recycling/national-overview-facts-andfigures-materials.

Eurostat. 2020. Municipal waste statistics. [accessed 2022 Apr 19]. https://ec.europa.eu/eurostat/statistics-explained/index.php?title=Municipal_waste_statistics.

Eurostat. 2023. Waste statistics. Generation of waste by waste category, hazardousness and NACE Rev. 2 activity. [accessed 2024 Apr 23]. https://ec.europa.eu/eurostat/statistics-explained/index.php?title=Waste_statistics#Total_waste_generation.

Evans DM, Zipper CE, Hester ET, Schoenholtz SH. 2015. Hydrologic effects of surface coal mining in Appalachia (U.S.). J Am Water Resour Assoc. 51(5):1436–1452. doi:10.1111/1752-1688.12322.

Fageria NK, Nascente AS. 2014. Management of Soil Acidity of South American Soils for Sustainable Crop Production. Advances in Agronomy. 128:221–275. doi:10.1016/B978-0-12-802139-2.00006-8.

FAO, ITPS. 2015. Status of the world's soil resources (SWSR) – Main report. Rome, Italy: Food and Agriculture Organization of the United Nations and Intergovernmental Technical Panel on Soils.

FAO, ITPS, GSBI, SCBD, EC. 2020. State of knowledge of soil biodiversity - Status, challenges and potentialities. Rome, Italy: Food and Agriculture Organization of the United Nations.

Feng MH, Shan XQ, Zhang S, Wen B. 2005. A comparison of the rhizosphere-based method with DTPA, EDTA, CaCl2, and NaNO3 extraction methods for prediction of bioavailability of metals in soil to barley. Environmental Pollution. 137(2):231–240. doi:10.1016/j.envpol.2005.02.003.

Feng W, Guo Z, Xiao X, Peng C, Shi L, Ran H, Xu W. 2020. A dynamic model to evaluate the critical loads of heavy metals in agricultural soil. Ecotoxicol Environ Saf. 197:110607. doi:10.1016/J.ECOENV.2020.110607.

Fernández-González R, Martín-Lara MA, Iáñez-Rodríguez I, Calero M. 2018. Removal of heavy metals from acid mining effluents by hydrolyzed olive cake. Bioresour Technol. 268:169–175. doi:10.1016/J.BIORTECH.2018.07.124.

Finger SE, Church SE, Von Guerard P. 2007. Potential for Successful Ecological Remediation, Restoration, and Monitoring. In: Church SE, Von Guerard P, Finger SE, editors. Integrated Investigations of Environmental Effects of Historical Mining in the Animas River Watershed, San Juan County, Colorado. Vol. 2. San Juan County, CO, USA: U.S. Geological Survey. p. 1067–1078.

Fleming M, Tai Y, Zhuang P, Mcbride MB. 2013. Extractability and bioavailability of Pb and As in historically contaminated orchard soil: Effects of compost amendments. Environmental Pollution. 177:90–97. doi:10.1016/j.envpol.2013.02.013.

Freitas H, Prasad MNV, Pratas J. 2004. Plant community tolerant to trace elements growing on the degraded soils of São Domingos mine in the south east of Portugal: Environmental implications. Environ Int. 30(1):65–72. doi:10.1016/S0160-4120(03)00149-1.

Fu J, Huang B. 2001. Involvement of antioxidants and lipid peroxidation in the adaptation of two cool-season grasses to localized drought stress. Environ Exp Bot. 45(2):105–114. doi:10.1016/S0098-8472(00)00084-8.

Gao F, Zhang X, Zhang J, Li J, Niu T, Tang C, Wang C, Xie J. 2022. Zinc oxide nanoparticles improve lettuce (Lactuca sativa L.) plant tolerance to cadmium by stimulating antioxidant defense, enhancing lignin content and reducing the metal accumulation and translocation. Front Plant Sci. 13:1015745. doi:10.3389/FPLS.2022.1015745.

García I, Diez M, Martín F, Simón M, Dorronsoro C. 2009. Mobility of arsenic and heavy metals in a sandy-loam textured and carbonated soil. Pedosphere. 19(2):166–175. doi:10.1016/S1002-0160(09)60106-5.

García Robles H. 2020. The role of gypsum in bioremediation; recovery of ecosystem functions and services of polluted soils. University of Granada. https://digibug.ugr.es/bitstream/handle/10481/63067/Tesis_HGR.pdf?sequence=4&isAllowed=y.

García-Carmona M, García-Robles H, Turpín Torrano C, Fernández Ondoño E, Lorite Moreno J, Sierra Aragón M, Martín Peinado FJ. 2019a. Residual pollution and vegetation distribution in amended soils 20 years after a pyrite mine tailings spill (Aznalcóllar, Spain). Science of the Total Environment. 650:933–940. doi:10.1016/j.scitotenv.2018.09.092. https://doi.org/10.1016/j.scitotenv.2018.09.092.

García-Carmona M, Romero-Freire A, Sierra Aragón M, Martín Peinado FJ. 2019b. Effectiveness of ecotoxicological tests in relation to physicochemical properties of Zn and Cu polluted Mediterranean soils. Geoderma. 338:259–268. doi:10.1016/j.geoderma.2018.12.016.

García-Carmona M, Romero-Freire A, Sierra Aragón M, Martínez Garzón FJ, Martín Peinado FJ. 2017. Evaluation of remediation techniques in soils affected by residual contamination with heavy metals and arsenic. J Environ Manage. 191:228–236. doi:10.1016/j.jenvman.2016.12.041.

García-Sánchez A, Alonso-Rojo P, Santos-Francés F. 2010. Distribution and mobility of arsenic in soils of a mining area (Western Spain). Science of The Total Environment. 408(19):4194–4201. doi:10.1016/J.SCITOTENV.2010.05.032.

Gaspar OC. 1998. História da mineração dos depósitos de sulfuretos maciços vulcanogénicos da Faixa Piritosa Portuguesa. Boletim de Minas. 35(4):401–414.

Gebremeskel G, Gebremicael TG, Girmay A. 2018. Economic and environmental rehabilitation through soil and water conservation, the case of Tigray in northern Ethiopia. J Arid Environ. 151:113–124. doi:10.1016/J.JARIDENV.2017.12.002.

Gerwing TG, Hawkes VC, Gann GD, Murphy SD. 2022. Restoration, reclamation, and rehabilitation: on the need for, and positing a definition of, ecological reclamation. Restor Ecol. 30(7):e13461. doi:10.1111/REC.13461.

van Gestel CAM. 2012. Soil ecotoxicology: state of the art and future directions. Zookeys. 176:275–296. doi:10.3897/ZOOKEYS.176.2275.

Giannakis I, Emmanouil C, Mitrakas M, Manakou V, Kungolos A. 2021. Chemical and ecotoxicological assessment of sludge-based biosolids used for corn field fertilization. Environmental Science and Pollution Research. 28(4):3797–3809. doi:10.1007/S11356-020-09165-6.

Giannopolitis CN, Ries SK. 1977. Superoxide Dismutases: I. Occurrence in Higher Plants. Plant Physiol. 59(2):309–314. doi:10.1104/PP.59.2.309.

Gill SS, Tuteja N. 2010. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiology and Biochemistry. 48:909–930. doi:10.1016/J.PLAPHY.2010.08.016.

Giller KE, Witter E, Mcgrath SP. 1998. Toxicity of heavy metals to microorganisms and microbial processes in agricultural soils: a review. Soil Biol Biochem. 30(10–11):1389–1414. doi:10.1016/S0038-0717(97)00270-8.

Goldberg S, Glaubig RA. 1988. Anion sorption on a calcareous, montmorillonitic soil-arsenic. Soil Science Society of America Journal. 52:1297–1300. doi:10.2136/sssaj1988.03615995005200050015x.

González V, Salinas J, García I, del Moral F, Simón M. 2017. Using marble sludge and phytoextraction to remediate metal(loid) polluted soils. J Geochem Explor. 174:29–34. doi:10.1016/j.gexplo.2016.03.008.

Grande JA, Santisteban M, de la Torre ML, Fortes JC, de Miguel E, Curiel J, Dávila JM, Biosca B. 2018. The paradigm of Circular Mining in the world: the Iberian Pyrite Belt as a potential scenario of interaction. Environ Earth Sci. 77(10):1–6. doi:10.1007/s12665-018-7577-1.

Graziano M, Martín-Peinado FJ, Delgado-Moreno L. 2022. Application of Biochar for the Restoration of Metal(loid)s Contaminated Soils. Applied Sciences. 12(4):1918. doi:10.3390/APP12041918.

Greyson J. 2007. An economic instrument for zero waste, economic growth and sustainability. J Clean Prod. 15(13–14):1382–1390. doi:10.1016/J.JCLEPRO.2006.07.019.

Guala SD, Vega FA, Covelo EF. 2010. The dynamics of heavy metals in plant-soil interactions. Ecol Modell. 221:1148–1152. doi:10.1016/J.ECOLMODEL.2010.01.003.

Guo G, Yuan T, Wang W, Li D, Cheng J, Gao Y, Zhou P. 2011. Bioavailability, mobility, and toxicity of Cu in soils around the Dexing Cu mine in China. Environ Geochem Health. 33(2):217–224. doi:10.1007/S10653-010-9334-6.

Hadas A, Kautsky L, Goek M, Kara EE. 2004. Rates of decomposition of plant residues and available nitrogen in soil, related to residue composition through simulation of carbon and nitrogen turnover. Soil Biol Biochem. 36(2):255–266. doi:10.1016/J.SOILBIO.2003.09.012.

Haddad M, Nassar D, Shtaya M. 2023. Heavy metals accumulation in soil and uptake by barley (Hordeum vulgare) irrigated with contaminated water. Sci Rep. 13:4121. doi:10.1038/s41598-022-18014-0.

Haddad SA, Tabatabai MA, Abdel-Moneim AMA, Loynachan TE. 2015. Inhibition of nodulation and nitrogen nutrition of leguminous crops by selected heavy metals. Air, Soil and Water Research. 8:1–7. doi:10.4137/ASWR.S21098.

Hamels F, Malevé J, Sonnet P, Kleja DB, Smolders E. 2014. Phytotoxicity of trace metals in spiked and field-contaminated soils: Linking soil-extractable metals with toxicity. Environ Toxicol Chem. 33(11):2479–2487. doi:10.1002/ETC.2693.

Harter RD, Naidu R. 1995. Role of metal-organic complexation in metal sorption by soils. Advances in Agronomy. 55:219–263. doi:10.1016/S0065-2113(08)60541-6.

Hbaieb R, Soubrand M, Joussein E, Medhioub M, Casellas M, Gady C, Saladin G. 2018. Assisted phytostabilisation of As, Pb and Sb-contaminated Technosols with mineral and organic amendments using Douglas fir (Pseudotsuga menziesii (Mirb.) Franco). Environmental Science and Pollution Research. 25(32):32292–32302. doi:10.1007/s11356-018-3213-6.

He Z, Honeycutt CW, Griffin TS, Larkin RP, Olanya M, Halloran JM. 2010. Increases of soil phosphatase and urease activities in potato fields by cropping rotation practices. J Food Agric Environ. 8(2):1112–1117.

He Z, Shentu J, Yang X, Baligar VC, Zhang T, Stoffella PJ. 2015. Heavy metal contamination of soils: sources, indicators, and assessment. Journal of Environmental Indicators. 9:17–18.

Herrán Fernández Á, Lacalle RG, Iturritxa Vélez del Burgo MJ, Martínez Azkuenaga M, Vilela Lozano J. 2016. Firsts results of Technosols constructed from municipal waste in Vitoria-Gasteiz (Spain). Spanish Journal of Soil Science. 6(1):64–81. doi:10.3232/SJSS.2016.V6.N1.06.

Hettelingh JP, Downing RJ, de Smet PAM. 1991. Mapping critical loads for Europe. CCE Technical Report No. 1. Bilthoven, The Netherlands: National Institute of Public Health and Environmental Protection.

Heviánková S, Bestová I, Kyncl M, Šimková L, Zechner M. 2013. Calcium carbonate as an agent in acid mine water neutralization. Inzynieria Mineralna. 14(2):159–166.

Hla SS, Roberts D. 2015. Characterisation of chemical composition and energy content of green waste and municipal solid waste from Greater Brisbane, Australia. Waste Management. 41:12–19. doi:10.1016/j.wasman.2015.03.039.

Hogsden KL, Harding JS. 2012. Consequences of acid mine drainage for the structure and function of benthic stream communities: A review. Freshwater Science. 31(1):108–120. doi:10.1899/11-091.1.

Hope CFA, Burns RG. 1987. Activity, origins and location of cellulases in a silt loam soil. Biol Fertil Soils. 5:164–170. doi:10.1007/BF00257653.

Hou D, O'Connor D, Igalavithana AD, Alessi DS, Luo J, Tsang DCW, Sparks DL, Yamauchi Y, Rinklebe J, Ok YS. 2020. Metal contamination and bioremediation of agricultural soils for food safety and sustainability. Nature Reviews Earth & Environment . 1:366–381. doi:10.1038/s43017-020-0061-y.

Huang D, Gui H. 2019. Distribution features and internal relations of heavy metals in soil–maize system of mining area, Anhui Province, Eastern China. Human and Ecological Risk Assessment: An International Journal. 25(4):863–881. doi:10.1080/10807039.2018.1453298.

Huang H, Ullah F, Zhou DX, Yi M, Zhao Y. 2019. Mechanisms of ROS regulation of plant development and stress responses. Front Plant Sci. 10:440478. doi:10.3389/FPLS.2019.00800.

Huang M, Zhu Y, Li Z, Huang B, Luo N, Liu C, Zeng G. 2016. Compost as a Soil Amendment to Remediate Heavy Metal-Contaminated Agricultural Soil: Mechanisms, Efficacy, Problems, and Strategies. Water Air Soil Pollut. 227(10). doi:10.1007/S11270-016-3068-8.

Inam MA, Khan R, Akram M, Khan S, Yeom IT. 2019. Effect of water chemistry on antimony removal by chemical coagulation: Implications of ζ-potential and size of precipitates. Int J Mol Sci. 20:2945. doi:10.3390/ijms20122945.

IPBES (Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services). 2018. The IPBES assessment report on land degradation and restoration. Montanarella L, Scholes R, Brainich A, editors. Bonn, Germany: Secretariat of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services.

ISO 17155. 2002. Soil quality. Determination abundance activity soil microflora using respiration curves. International Standard ISO No.17155. Geneva, Switzerland: International Organization for Standardization.

ISO 17402. 2008. Soil quality. Requirements and guidance for the selection and application of methods for the assessment of bioavailability of contaminants in soil and soil materials. International Standard ISO No.17402. Geneva, Switzerland: International Organization for Standardization.

ISO/IEC 17025. 2017. General requirements for the competence of testing and calibration laboratories. Geneva, Switzerland: ISO (International Organization for Standardization) and IEC (International Electrotechnical Commission).

Israr M, Jewell A, Kumar D, Sahi S V. 2011. Interactive effects of lead, copper, nickel and zinc on growth, metal uptake and antioxidative metabolism of Sesbania drummondii. J Hazard Mater. 186:1520–1526. doi:10.1016/J.JHAZMAT.2010.12.021.

IUSS Working Group WRB. 2022. World Reference Base for Soil Resources. International soil classification system for naming soils and creating legends for soil maps. 4th edition. Vienna, Austria: International Union of Soil Sciences (IUSS).

Ivezić V, Almås ÅR, Singh BR. 2012. Predicting the solubility of Cd, Cu, Pb and Zn in uncontaminated Croatian soils under different land uses by applying established regression models. Geoderma. 170:89–95. doi:10.1016/J.GEODERMA.2011.11.024.

James-Martin G, Baird DL, Hendrie GA, Bogard J, Anastasiou K, Brooker PG, Wiggins B, Williams G, Herrero M, Lawrence M, et al. 2022. Environmental sustainability in national food-based dietary guidelines: a global review. Lancet Planet Health. 6(12):e977–e986. doi:10.1016/S2542-5196(22)00246-7.

Jan MT, Roberts P, Tonheim SK, Jones DL. 2009. Protein breakdown represents a major bottleneck in nitrogen cycling in grassland soils. Soil Biol Biochem. 41:2272–2282. doi:10.1016/J.SOILBIO.2009.08.013.

REFERENCES

January MC, Cutright TJ, Keulen H Van, Wei R. 2008. Hydroponic phytoremediation of Cd, Cr, Ni, As, and Fe: Can Helianthus annuus hyperaccumulate multiple heavy metals? Chemosphere. 70(3):531–537. doi:10.1016/J.CHEMOSPHERE.2007.06.066.

Jensen J, Mesman M. 2006. Ecological Risk Assessment of Contaminated Land: Decision support for site specific investigations. Bilthoven, The Netherlands: National Institute for Public Health and the Environment (RIVM).

Jordán MM, García-Sánchez E, Almendro-Candel MB, Pardo F, Vicente AB, Sanfeliu T, Bech J. 2017. Technosols designed for rehabilitation of mining activities using mine spoils and biosolids. Ion mobility and correlations using percolation columns. Catena (Amst). 148:74–80. doi:10.1016/j.catena.2016.02.027.

Juhasz AL, Naidu R, Zhu YG, Wang LS, Jiang JY, Cao ZH. 2003. Toxicity issues associated with geogenic arsenic in the groundwater – soil – plant – human continuum. Bull Environ Contam Toxicol. 71:1100–1107. doi:10.1007/s00128-003-0231-x.

Junglee S, Urban L, Sallanon H, Lopez-Lauri F. 2014. Optimized Assay for Hydrogen Peroxide Determination in Plant Tissue Using Potassium Iodide. Am J Analyt Chem. 5:730–736. doi:10.4236/AJAC.2014.511081.

Kabata-Pendias A. 2010. Trace elements in soils and plants: Fourth edition. Florida, USA: CRC Press (Taylor & Francis Group).

Kandeler E, Gerber H. 1988. Short-term assay of soil urease activity using colorimetric determination of ammonium. Biol Fertil Soils. 6:68–72. doi:10.1007/BF00257924.

Kandeler E, Kampichler C, Horak O. 1996. Influence of heavy metals on the functional diversity of soil microbial communities. Biol Fertil Soils. 23:299–306. doi:10.1007/BF00335958.

Kaur G, Couperthwaite SJ, Hatton-Jones BW, Millar GJ. 2018. Alternative neutralisation materials for acid mine drainage treatment. Journal of Water Process Engineering. 22:46–58. doi:10.1016/j.jwpe.2018.01.004.

Kaya C, Ugurlar F, Farooq S, Ashraf M, Alyemeni MN, Ahmad P. 2022. Combined application of asparagine and thiourea improves tolerance to lead stress in wheat by modulating AsA-GSH cycle, lead detoxification and nitrogen metabolism. Plant Physiology and Biochemistry. 190:119–132. doi:10.1016/J.PLAPHY.2022.08.014.

Kefeni KK, Msagati TAM, Mamba BB. 2017. Acid mine drainage: Prevention, treatment options, and resource recovery: A review. J Clean Prod. 151:475–493. doi:10.1016/j.jclepro.2017.03.082.

Khalid S, Shahid M, Niazi NK, Murtaza B, Bibi I, Dumat C. 2017. A comparison of technologies for remediation of heavy metal contaminated soils. J Geochem Explor. 182:247–268. doi:10.1016/J.GEXPLO.2016.11.021.

Khan MA, Ding X, Khan S, Brusseau ML, Khan A, Nawab J. 2018. The influence of various organic amendments on the bioavailability and plant uptake of cadmium present in mine-degraded soil. Science of The Total Environment. 636:810–817. doi:10.1016/J.SCITOTENV.2018.04.299.

Khan S, Naushad M, Lima EC, Zhang S, Shaheen SM, Rinklebe J. 2021. Global soil pollution by toxic elements: Current status and future perspectives on the risk assessment and remediation strategies – A review. J Hazard Mater. 417:126039. doi:10.1016/J.JHAZMAT.2021.126039.

Kizilkaya R. 2008. Dehydrogenase activity in Lumbricus terrestris casts and surrounding soil affected by addition of different organic wastes and Zn. Bioresour Technol. 99:946–953. doi:10.1016/J.BIORTECH.2007.03.004.

Kjeldahl C. 1883. A new method for the determination of nitrogen in organic matter. Z Anal Chem. 22(1):366. doi:10.1007/BF01338151.

Kolahi M, Mohajel Kazemi E, Yazdi M, Goldson-Barnaby A. 2020. Oxidative stress induced by cadmium in lettuce (Lactuca sativa Linn.): Oxidative stress indicators and prediction of their genes. Plant Physiology and Biochemistry. 146:71–89. doi:10.1016/J.PLAPHY.2019.10.032.

Kolde R. 2015. Pheatmap: Pretty Heatmaps. R package version 1.0.8. [accessed 2023 Oct 16]. https://CRAN.R-project.org/package=pheatmap.

Kopittke PM, Menzies NW, Wang P, McKenna BA, Lombi E. 2019. Soil and the intensification of agriculture for global food security. Environ Int. 132:105078. doi:10.1016/J.ENVINT.2019.105078.

Krämer S, Green DM. 2000. Acid and alkaline phosphatase dynamics and their relationship to soil microclimate in a semiarid woodland. Soil Biol Biochem. 32:179–188. doi:10.1016/S0038-0717(99)00140-6.

Kraus U, Wiegand J. 2006. Long-term effects of the Aznalcóllar mine spill—heavy metal content and mobility in soils and sediments of the Guadiamar river valley (SW Spain). Science of The Total Environment. 367(2–3):855–871. doi:10.1016/J.SCITOTENV.2005.12.027.

Kubiś J. 2008. Exogenous spermidine differentially alters activities of some scavenging system enzymes, H2O2 and superoxide radical levels in water-stressed cucumber leaves. J Plant Physiol. 165:397–406. doi:10.1016/J.JPLPH.2007.02.005.

Kumar S, Chaudhuri S, Maiti SK. 2013. Soil Dehydrogenase Enzyme Activity in Natural and Mine Soil - A Review. Middle-East Journal of Scientific Research. 13(7):898–906. doi:10.5829/idosi.mejsr.2013.13.7.2801.

Kumar V, Rout C, Singh J, Saharan Y, Goyat R, Umar A, Akbar S, Baskoutas S. 2023. A review on the clean-up technologies for heavy metal ions contaminated soil samples. Heliyon. 9(5):e15472. doi:10.1016/J.HELIYON.2023.E15472.

Kumpiene J, Antelo J, Brännvall E, Carabante I, Ek K, Komárek M, Söderberg C, Wårell L. 2019. In situ chemical stabilization of trace element-contaminated soil – Field demonstrations and barriers to transition from laboratory to the field – A review. Applied Geochemistry. 100:335–351. doi:10.1016/J.APGEOCHEM.2018.12.003.

Kumpiene J, Lagerkvist A, Maurice C. 2008. Stabilization of As, Cr, Cu, Pb and Zn in soil using amendments - A review. Waste Management. 28(1):215–225. doi:10.1016/J.WASMAN.2006.12.012.

Kuppusamy S, Venkateswarlu K, Megharaj M, Mayilswami S, Lee YB. 2017. Risk-based remediation of polluted sites: A critical perspective. Chemosphere. 186:607–615. doi:10.1016/J.CHEMOSPHERE.2017.08.043.

Labanowski J, Monna F, Bermond A, Cambier P, Fernandez C, Lamy I, van Oort F. 2008. Kinetic extractions to assess mobilization of Zn, Pb, Cu, and Cd in a metal-contaminated soil: EDTA vs. citrate. Environmental Pollution. 152:693–701. doi:10.1016/J.ENVPOL.2007.06.054.

Ladd JN, Butler JHA. 1972. Short-term assays of soil proteolytic enzyme activities using proteins and dipeptide derivatives as substrates. Soil Biol Biochem. 4(1):19–30. doi:10.1016/0038-0717(72)90038-7.

Lakanen E, Erviö R. 1971. A comparison of eight extractants for the determination of plant available micronutrients in soils. Acta Agriculturae Fenniae. 123:223–232.

Larney FJ, Angers DA. 2012. The role of organic amendments in soil reclamation: A review. Can J Soil Sci. 92:19–38. doi:10.4141/CJSS2010-064.

Law MY, Charles SA, Halliwell B. 1983. Glutathione and ascorbic acid in spinach (Spinacia oleracea) chloroplasts. The effect of hydrogen peroxide and of Paraquat. Biochemical Journal. 210(3):899–903. doi:10.1042/BJ2100899.

Lèbre É, Corder G, Golev A. 2017. The role of the mining industry in a circular economy. A framework for resource management at the mine site level. J Ind Ecol. 21(3):662–672. doi:10.1111/jiec.12596.

Leistel JM, Marcoux E, Thiéblemont D, Quesada C, Sánchez A, Almodóvar GR, Pascual E, Sáez R. 1997. The volcanic-hosted massive sulphide deposits of the Iberian Pyrite Belt: Review and preface to the Thematic Issue. Miner Depos. 33:2–30. doi:10.1007/s001260050130.

Leitgib L, Kálmán J, Gruiz K. 2007. Comparison of bioassays by testing whole soil and their water extract from contaminated sites. Chemosphere. 66:428–434. doi:10.1016/J.CHEMOSPHERE.2006.06.024.

Li BB, Zhang SB, Lv YY, Wei S, Hu Y Sen. 2022. Reactive oxygen species-induced protein carbonylation promotes deterioration of physiological activity of wheat seeds. PLoS One. 17(3):e0263553. doi:10.1371/JOURNAL.PONE.0263553.

Li J, Qiu Y, Zhao Q, Chen D, Wu Z, Peng A an, Niazi NK, Trakal L, Sakrabani R, Gao B, et al. 2020a. Lead and copper-induced hormetic effect and toxicity mechanisms in lettuce (Lactuca sativa L.) grown in a contaminated soil. Science of The Total Environment. 741:140440. doi:10.1016/J.SCITOTENV.2020.140440.

Li K, Yao H. 2023. Revitalizing our earth: unleashing the power of green energy in soil remediation for a sustainable future. Environmental Science and Pollution Research. 30:109007–109020. doi:10.1007/S11356-023-29672-6.

Li X, Wang Z, Gan L, Tian Y. 2020b. Draft genome sequence of Actinocorallia populi A251T, an actinomycetes producing polyketides and nonribosomal polypeptides. 3 Biotech. 10(2):79. doi:10.1007/S13205-020-2070-4.

Liang Y, Yang Y, Yang C, Shen Q, Zhou J, Yang L. 2003. Soil enzymatic activity and growth of rice and barley as influenced by organic manure in an anthropogenic soil. Geoderma. 115:149–160. doi:10.1016/S0016-7061(03)00084-3.

Liao Z, Fan J, Lai Z, Bai Z, Wang H, Cheng M, Zhang F, Li Z. 2023. Response network and regulatory measures of plant-soil-rhizosphere environment to drought stress. In: Sparks DL, editor. Advances in Agronomy. Vol. 180. Cambridge, MA, USA: Academic Press. p. 93–196.

Lima AT, Mitchell K, O'Connell DW, Verhoeven J, Van Cappellen P. 2016. The legacy of surface mining: Remediation, restoration, reclamation and rehabilitation. Environ Sci Policy. 66:227–233. doi:10.1016/J.ENVSCI.2016.07.011.

Liu J, Luo X, Sun Y, Tsang DCW, Qi J, Zhang W, Li N, Yin M, Wang J, Lippold H, et al. 2019. Thallium pollution in China and removal technologies for waters: A review. Environ Int. 126:771–790. doi:10.1016/J.ENVINT.2019.01.076.

Liu L, Li W, Song W, Guo M. 2018. Remediation techniques for heavy metal-contaminated soils: Principles and applicability. Science of the Total Environment. 633:206–219. doi:10.1016/j.scitotenv.2018.03.161.

Liu X, Peng K, Wang A, Lian C, Shen Z. 2010. Cadmium accumulation and distribution in populations of Phytolacca americana L. and the role of transpiration. Chemosphere. 78:1136–1141. doi:10.1016/J.CHEMOSPHERE.2009.12.030.

Lombi E, Zhao FJ, Zhang G, Sun B, Fitz W, Zhang H, McGrath SP. 2002. In situ fixation of metals in soils using bauxite residue: chemical assessment. Environmental Pollution. 118(3):435–443. doi:10.1016/S0269-7491(01)00294-9.

López-Pamo E, Barettino D, Antón-Pacheco C, Ortiz G, Arránz JC, Gumiel JC, Martínez-Pledel B, Aparicio M, Montouto O. 1999. The extent of the Aznalcollar pyritic sludge spill and its effects on soils. Science of the Total Environment. 242(1–3):57–88. doi:10.1016/S0048-9697(99)00376-9.

Lottermoser BG. 2010. Mine Wastes. Characterization, treatment and environmental impacts. Third Edition. Berlin, Germany: Springer.

Maass BL, Knox MR, Venkatesha SC, Tolera Angessa T, Ramme S, Pengelly BC. 2010. Lablab purpureus- A crop lost for Africa? Trop Plant Biol. 3:123–135. doi:10.1007/s12042-010-9046-1.

Macía P, Fernández-Costas C, Rodríguez E, Sieiro P, Pazos M, Sanromán MA. 2014. Technosols as a novel valorization strategy for an ecological management of dredged marine sediments. Ecol Eng. 67:182–189. doi:10.1016/j.ecoleng.2014.03.020.

Macías F. 2004. Recuperación de suelos degradados, reutilización de residuos y secuestro de carbono. Una alternativa integral de mejora de la calidad ambiental. Recursos Rurais. 1:49–56.

Macías F, Bao M, Macías-García F, Camps Arbestain M. 2007. Valorización biogeoquímica de residuos por medio de la elaboración de Tecnosoles con diferentes aplicaciones ambientales. Agua Residuos. 5:12–25.

Macías F, Macías-García F, Nieto C, Verde JR, Pérez C, Bao M, Camps-Arbestain M. 2011. Gestión de residuos y cambio climático. In: Mosquera MEL, Osés MJS, editors. Gestión de residuos orgánicos de uso agrícola. Santiago de Compostela, Spain: Servizo de Publicacións e Intercambio Científico de la Universidade de Santiago de Compostela. p. 11–24.

Macías F, Rodríguez Lado L, Camps Arbestain M, Barreal E. 2005. Mecanismos de amortiguación de impacto acidificantes. Influencia de la litología, propiedades superficiales y grado de desarrollo edáfico. Edafología. 12(2):115–126.

Madejón E, de Mora AP, Felipe E, Burgos P, Cabrera F. 2006a. Soil amendments reduce trace element solubility in a contaminated soil and allow regrowth of natural vegetation. Environmental Pollution. 139:40–52. doi:10.1016/J.ENVPOL.2005.04.034.

Madejón P, Caro-Moreno D, Navarro-Fernández CM, Rossini-Oliva S, Marañón T. 2021. Rehabilitation of waste rock piles: Impact of acid drainage on potential toxicity by trace elements in plants and soil. J Environ Manage. 280:111848. doi:10.1016/j.jenvman.2020.111848.

Madejón P, Domínguez MT, Madejón E, Cabrera F, Marañón T, Murillo JM. 2018. Soil-plant relationships and contamination by trace elements: A review of twenty years of experimentation and monitoring after the Aznalcóllar (SW Spain) mine accident. Science of The Total Environment. 625:50–63. doi:10.1016/J.SCITOTENV.2017.12.277.

Madejón P, Murillo JM, Marañón T, Cabrera F. 2006b. Bioaccumulation of trace elements in a wild grass three years after the Aznalcóllar mine spill (South Spain). Environ Monit Assess. 114:169–189. doi:10.1007/s10661-006-2523-1.

Mahar A, Wang P, Ali A, Awasthi MK, Lahori AH, Wang Q, Li R, Zhang Z. 2016. Challenges and opportunities in the phytoremediation of heavy metals contaminated soils: A review. Ecotoxicol Environ Saf. 126:111–121. doi:10.1016/J.ECOENV.2015.12.023.

Makoi JHJR, Ndakidemi PA. 2008. Selected soil enzymes: Examples of their potential roles in the ecosystem. Afr J Biotechnol. 7(3):181–191.

Manzano M, Custodio E, Ayora C, Navarrete P. 2000. Contamination of the Guadiamar river aquifer after the Aznalcóllar mine accident, SW Spain. Boletín Geológico y Minero. 111(6):93–106.

Marcos-Pérez M, Sánchez-Navarro V, Zornoza R. 2023. Intercropping systems between broccoli and fava bean can enhance overall crop production and improve soil fertility. Sci Hortic. 312:111834. doi:10.1016/J.SCIENTA.2023.111834.

Maree JP, du Plessis P. 1994. Neutralization of acid mine water with calcium carbonate. Water Science and Technology. 29(9):285–296. doi:10.2166/wst.1994.0496.

MARM (Ministerio de Medio Ambiente y Medio Rural y Marino). 2007. Memoria del Plan Nacional Integrado de Residuos (PNIR) 2007-2015. Madrid, Spain: Ministerio de Medio Ambiente y Medio Rural y Marino. Gobierno de España.

Márquez-García B, Pérez-López R, Ruíz-Chancho MJ, López-Sánchez JF, Rubio R, Abreu MM, Nieto JM, Córdoba F. 2012. Arsenic speciation in soils and Erica andevalensis Cabezudo & Rivera and Erica australis L. from São Domingos Mine area, Portugal. J Geochem Explor. 119–120:51–59. doi:10.1016/j.gexplo.2012.06.012.

Martín F, García I, Díez M, Sierra M, Simon M, Dorronsoro C. 2008. Soil alteration by continued oxidation of pyrite tailings. Applied Geochemistry. 23(5):1152–1165. doi:10.1016/j.apgeochem.2007.11.012.

Martín F, García I, Dorronsoro C, Simón M, Aguilar J, Ortíz I, Fernández E, Fernández J. 2004. Thallium Behavior in Soils Polluted by Pyrite Tailings (Aznalcóllar, Spain). Soil Sediment Contam. 13(1):25–36. doi:10.1080/10588330490269769.

Martin M. 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet J. 17(1):10–12. doi:10.14806/EJ.17.1.200.

Martín Peinado FJ. 2001. Contaminación de suelos por el vertido de una mina de pirita (Aznalcóllar, España). Universidad de Granada. [accessed 2024 Apr 30]. http://hdl.handle.net/10481/28793.

Martín Peinado FJ, Romero-Freire A, García Fernández I, Sierra Aragón M, Ortiz-Bernad I, Simón Torres M. 2015. Long-term contamination in a recovered area affected by a mining spill. Science of the Total Environment. 514:219–223. doi:10.1016/j.scitotenv.2015.01.102.

Martínez CE, Motto HL. 2000. Solubility of lead, zinc and copper added to mineral soils. Environmental Pollution. 107:153–158. doi:10.1016/S0269-7491(99)00111-6.

Martinez-Salgado MM, Gutiérrez-Romero V, Jannsens M, Ortega-Blu R. 2010. Biological soil quality indicators: a review. In: Méndez-Vilas A, editor. Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology. Badajoz, Spain: Formatex Research Center. p. 319–328.

Martínez-Sánchez MJ, Pérez-Sirvent C, Martínez-López S, García-Lorenzo ML, Agudo I, Martínez-Martínez LB, Hernández-Pérez C, Bech J. 2021. Uptake of potentially toxic elements by edible plants in experimental mining Technosols: preliminary assessment. Environ Geochem Health. 44:1649–1665. doi:10.1007/s10653-021-01091-x.

Maximillian J, Brusseau ML, Glenn EP, Matthias AD. 2019. Pollution and Environmental Perturbations in the Global System. In: Brusseau ML, Pepper IL, Gerba CP, editors. Environmental and Pollution Science. Cambridge, MA, USA: Academic Press. p. 457–476.

McBride M, Sauvé S, Hendershot W. 1997. Solubility control of Cu, Zn, Cd and Pb in contaminated soils. Eur J Soil Sci. 48:337–346. doi:10.1111/J.1365-2389.1997.TB00554.X.

REFERENCES

McGrath SP, Zhao FJ. 2003. Phytoextraction of metals and metalloids from contaminated soils. Curr Opin Biotechnol. 14:277–282. doi:10.1016/S0958-1669(03)00060-0.

MEEPRC (Ministry of Ecology and Environment of the People's Republic of China). 2018. Soil Environmental Quality Risk. Control Standard for Soil Contamination of Agricultural Land (GB 15618–2018). Beijing, China: Ministry of Ecology and Environment of the People's Republic of China.

Merdy P, Gharbi LT, Lucas Y. 2009. Pb, Cu and Cr interactions with soil: Sorption experiments and modelling. Colloids Surf A Physicochem Eng Asp. 347:192–199. doi:10.1016/J.COLSURFA.2009.04.004.

Montanarella L. 2007. Trends in land degradation in Europe. In: Sivakumar MannvaVK, Ndiang'ui Ndegwa, editors. Climate and Land Degradation. Environmental Science and Engineering. Berlin, Germany: Springer. p. 83–104.

Moreira IN, Martins LL, Mourato MP. 2020. Effect of Cd, Cr, Cu, Mn, Ni, Pb and Zn on seed germination and seedling growth of two lettuce cultivars (Lactuca sativa L.). Plant Physiology Reports. 25(2):347–358. doi:10.1007/S40502-020-00509-5.

Moreno JL, Bastida F, Ros M, Hernández T, García C. 2009. Soil organic carbon buffers heavy metal contamination on semiarid soils: Effects of different metal threshold levels on soil microbial activity. Eur J Soil Biol. 45(3):220–228. doi:10.1016/J.EJSOBI.2009.02.004.

Moreno-Barriga F, Faz Á, Acosta JA, Soriano-Disla M, Martínez-Martínez S, Zornoza R. 2017. Use of Piptatherum miliaceum for the phytomanagement of biochar amended Technosols derived from pyritic tailings to enhance soil aggregation and reduce metal(loid) mobility. Geoderma. 307:159–171. doi:10.1016/J.GEODERMA.2017.07.040.

Mourid SS. 2014. Effect of Calcium Carbonate Content on Potential Toxic Heavy Metals Adsorption In Calcareous Soils. Curr Sci Int. 3(2):141–149.

Mourinha C, Palma P, Alexandre C, Cruz N, Rodrigues SM, Alvarenga P. 2022. Potentially Toxic Elements' Contamination of Soils Affected by Mining Activities in the Portuguese Sector of the Iberian Pyrite Belt and Optional Remediation Actions: A Review. Environments . 9(1):11. doi:10.3390/ENVIRONMENTS9010011.

Mthimunye LM, Managa GM, Nemadodzi LE. 2023. The influence of Lablab purpureus growth on nitrogen availability and mineral composition concentration in nutrient poor savanna soils. Agronomy. 13:622. doi:10.3390/agronomy13030622.

Muñoz-Rojas M. 2018. Soil quality indicators: critical tools in ecosystem restoration. Curr Opin Environ Sci Health. 5:47–52. doi:10.1016/J.COESH.2018.04.007.

Muñoz-Rojas M, Erickson TE, Dixon KW, Merritt DJ. 2016a. Soil quality indicators to assess functionality of restored soils in degraded semiarid ecosystems. Restor Ecol. 24(S2):S43–S52. doi:10.1111/REC.12368.

Muñoz-Rojas M, Erickson TE, Martini D, Dixon KW, Merritt DJ. 2016b. Soil physicochemical and microbiological indicators of short, medium and long term post-fire recovery in semi-arid ecosystems. Ecol Indic. 63:14–22. doi:10.1016/J.ECOLIND.2015.11.038.

Murphy AM, Colucci PE. 1999. A tropical forage solution to poor quality ruminant diets: A review of Lablab purpureus. Livestock Research for Rural Development. 11:96–113.

Naidu G, Ryu S, Thiruvenkatachari R, Choi Y, Jeong S, Vigneswaran S. 2019. A critical review on remediation, reuse, and resource recovery from acid mine drainage. Environmental Pollution. 247:1110–1124. doi:10.1016/J.ENVPOL.2019.01.085.

Nakamaru YM, Martín Peinado FJ. 2017. Effect of soil organic matter on antimony bioavailability after the remediation process. Environmental Pollution. 228:425–432. doi:10.1016/j.envpol.2017.05.042.

Nakano Y, Asada K. 1981. Hydrogen Peroxide is Scavenged by Ascorbate-specific Peroxidase in Spinach Chloroplasts. Plant Cell Physiol. 22(5):867–880. doi:10.1093/OXFORDJOURNALS.PCP.A076232.

Nandillon R, Miard F, Lebrun M, Gaillard M, Sabatier S, Bourgerie S, Battaglia-Brunet F, Morabito D. 2019. Effect of biochar and amendments on Pb and As phytotoxicity and phytoavailability in a Technosol. Clean (Weinh). 47:1–11. doi:10.1002/clen.201800220.

Nannipieri P, Greco S, Ceccanti B. 1990. Ecological significance of the biological activity in soil. In: Soil Biochemistry. New York, USA: Routledge. p. 293–356.

Narasimha G, Sridevi A, Venkata G, Reddy S, Banu T, Reddy BR. 2012. Effect of cotton ginning mill industrial effluents on soil dehydrogenase, phosphatase, amylase and invertase enzyme activities. International Journal of Agricultural and Food Science. 2(1):1–6.

National Research Council. 1992. Restoration of Aquatic Ecosystems: Science, Technology, and Public Policy. Washington DC, USA: National Academies Press.

National Research Council. 2005. Mineral tolerance of animals: Second revised edition, 2005. Washington DC, USA: National Academies Press.

Navarro-Cano JA, Verdú M, Goberna M. 2018. Trait-based selection of nurse plants to restore ecosystem functions in mine tailings. Journal of Applied Ecology. 55:1195–1206. doi:10.1111/1365-2664.13094.

Navarro-León E, Ruiz JM, Albacete A, Blasco B. 2020. Tolerance to cadmium toxicity and phytoremediation potential of three Brassica rapa CAX1a TILLING mutants. Ecotoxicol Environ Saf. 189:109961. doi:10.1016/J.ECOENV.2019.109961.

Navarro-Torre S, Garcia-Caparrós P, Nogales A, Abreu MM, Santos E, Cortinhas AL, Caperta AD. 2023. Sustainable agricultural management of saline soils in arid and semi-arid Mediterranean regions through halophytes, microbial and soil-based technologies. Environ Exp Bot. 212:105397. doi:10.1016/J.ENVEXPBOT.2023.105397.

Nawaz R, Anurakpongsatorn P, Chandio AA, Ponza S. 2010. Sodium and Calcium Depletion by Acid Rain from Clayey Soils of Thailand. In: GMSTEC 2010:

International Conference for a Sustainable Greater Mekong Subregion. Bangkok, Thailand: Greater Mekong Subregion Tertiary Education Consortium. p. 233–236.

Nenova L, Zgorelec Z, Benkova M, Semeomova C, Velichkova N, Atanassova I. 2018. Solubility and availability of copper, zinc, lead and iron in technosols under the effect of increasing copper levels. International Journal of Hydrology. 2(3):379–386. doi:10.15406/ijh.2018.02.00100.

Nguyen TV, Nguyen TVT, Pham TL, Vigneswaran S, Ngo HH, Kandasamy J, Nguyen HK, Nguyen DT. 2009. Adsorption and removal of arsenic from water by iron ore mining waste. Water Science and Technology. 60(9):2301–2308. doi:10.2166/wst.2009.667.

Nielsen MN, Winding A. 2002. Microorganisms as indicators of soil health. Technical Report No. 388. Himmelev, Denmark: National Environmental Research Institute.

Nighojkar AK, Vijay A, Kumavat A, Gupta S, Satankar RK, Plappally A. 2019. Use of marble and iron waste additives for enhancing arsenic and E. Coli contaminant removal capacity and strength of porous clay ceramic materials for point of use drinking water treatment. Desalination Water Treat. 157:290–302. doi:10.5004/dwt.2019.23553.

Nikolic N, Kostic L, Djordjevic A, Nikolic M. 2011. Phosphorus deficiency is the major limiting factor for wheat on alluvium polluted by the copper mine pyrite tailings: A black box approach. Plant Soil. 339(1):485–498. doi:10.1007/s11104-010-0605-x.

Nocete F, Sáez R, Bayona MR, Nieto JM, Peramo A, López P, Gil-Ibarguchi JI, Inácio N, García S, Rodríguez J. 2014. Gold in the Southwest of the Iberian Peninsula during the 3rd Millennium BC. J Archaeol Sci. 41:691–704. doi:10.1016/j.jas.2013.10.006.

Noctor G, Foyer CH. 1998. Simultaneous Measurement of Foliar Glutathione, γ-Glutamylcysteine, and Amino Acids by High-Performance Liquid Chromatography: Comparison with Two Other Assay Methods for Glutathione. Anal Biochem. 264:98–110. doi:10.1006/ABIO.1998.2794.

Noulas C, Tziouvalekas M, Karyotis T. 2018. Zinc in soils, water and food crops. Journal of Trace Elements in Medicine and Biology. 49:252–260. doi:10.1016/J.JTEMB.2018.02.009.

Nriagu JO, Bhattacharya P, Mukherjee AB, Bundschuh J, Zevenhoven R, Loeppert RH. 2007. Arsenic in soil and groundwater: an overview. In: Bhattacharya P, Mukherjee AB, Bundschuh J, Zevenhoven R, Loeppert RH, editors. Trace Metals and other Contaminants in the Environment. Vol. 9. Amsterdam, Netherlands: Elsevier. p. 3–60.

Oades JM, Waters AC. 1991. Aggregate Hierarchy in Soils. Australian Journal of Soil Research. 29:5–28. doi:10.1071/SR9910815.

Ojuederie OB, Babalola OO. 2017. Microbial and Plant-Assisted Bioremediation of Heavy Metal Polluted Environments: A Review. Int J Environ Res Public Health. 14:1504. doi:10.3390/ijerph14121504.

Ok YS, Rinklebe J, Hou D, Tsang DCW, Tack FMG. 2020. Soil and Groundwater Remediation Technologies. A Practical Guide. Boca Raton, Florida, USA : CRC Press.

Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin PR, O'Hara RB, Simpson GL, Solymos P, et al. 2017. Vegan: Community Ecology Package. R package version 2.4-3. [accessed 2023 Oct 16]. https://CRAN.R-project.org/package=vegan.

Oldfield TL, Sikirica N, Mondini C, López G, Kuikman PJ, Holden NM. 2018. Biochar, compost and biochar-compost blend as options to recover nutrients and sequester carbon. J Environ Manage. 218:465–476. doi:10.1016/J.JENVMAN.2018.04.061.

Olsen SR, Sommers LE. 1982. Determination of available phosphorus. In: Page AL, Miller RH, Keeney DR, editors. Method of Soil Analysis. Part 2, second ed. ASA and ASSA. Vol. 2. Madison, WI, USA: Agronomy. p. 403–430.

Ordóñez A, Guerra J, Cano MJ. 2003. The bryophyte diaspore bank in soils affected by the Aznalcóllar (Seville, SW Spain) mine disaster. An Biol. 25:37–48.

Padoan E, Hernandez Kath A, Vahl LC, Ajmone-Marsan F. 2020. Potential Release of Zinc and Cadmium From Mine-Affected Soils Under Flooding, a Mesocosm Study. Arch Environ Contam Toxicol. 79(4):421–434. doi:10.1007/S00244-020-00777-0.

Palansooriya KN, Shaheen SM, Chen SS, Tsang DCW, Hashimoto Y, Hou D, Bolan NS, Rinklebe J, Ok YS. 2020. Soil amendments for immobilization of potentially toxic elements in contaminated soils: A critical review. Environ Int. 134:105046. doi:10.1016/j.envint.2019.105046.

Pandey AK, Zorić L, Sun T, Karanović D, Fang P, Borišev M, Wu X, Luković J, Xu P. 2022. The anatomical basis of heavy metal responses in legumes and their impact on plant–rhizosphere interactions. Plants. 11(19):2554. doi:10.3390/plants11192554.

Paniagua-López M, Aguilar-Garrido A, Contero-Hurtado J, García-Romera I, Sierra-Aragón M, Romero-Freire A. 2023. Ecotoxicological Assessment of Polluted Soils One Year after the Application of Different Soil Remediation Techniques. Toxics. 11(4):298. doi:10.3390/toxics11040298.

Paniagua-López M, García-Robles H, Aguilar-Garrido A, Romero-Freire A, Lorite J, Sierra-Aragón M. 2024. Vegetation establishment in soils polluted by heavy metal(loid)s after assisted natural remediation. Plant Soil. doi:10.1007/s11104-024-06521-0.

Paniagua-López M, Vela-Cano M, Correa-Galeote D, Martín-Peinado F, Marínez Garzón FJ, Pozo C, González-López J, Sierra Aragón M. 2021. Soil remediation approach and bacterial community structure in a long-term contaminated soil by a mining spill (Aznalcóllar, Spain). Science of The Total Environment. 777:145128. doi:10.1016/J.SCITOTENV.2021.145128.

Panigrahy N, Priyadarshini A, Sahoo MM, Verma AK, Daverey A, Sahoo NK. 2022. A comprehensive review on eco-toxicity and biodegradation of phenolics: Recent progress and future outlook. Environ Technol Innov. 27:102423. doi:10.1016/J.ETI.2022.102423.

Paradelo R, Villada A, Barral MT. 2011. Reduction of the short-term availability of copper, lead and zinc in a contaminated soil amended with municipal solid waste compost. J Hazard Mater. 188:98–104. doi:10.1016/J.JHAZMAT.2011.01.074.

Parida AK, Das AB. 2005. Salt tolerance and salinity effects on plants: a review. Ecotoxicol Environ Saf. 60:324–349. doi:10.1016/J.ECOENV.2004.06.010.

Pascual JA, Hernandez T, Garcia C, Ayuso M. 1998. Enzymatic activities in an arid soil amended with urban organic wastes: Laboratory experiment. Bioresour Technol. 64:131–138. doi:10.1016/S0960-8524(97)00171-5.

Pastor-Jáuregui R, Paniagua-López M, Aguilar-Garrido A, Martínez-Garzón FJ, Romero-Freire A, Sierra-Aragón M. 2022. Ecotoxicological risk assessment in soils contaminated by Pb and As 20 years after a mining spill. J Contam Hydrol. 251:104100. doi:10.1016/j.jconhyd.2022.104100.

Pastor-Jáuregui R, Paniagua-López M, Aguilar-Garrido A, Martín-Peinado FJ, Sierra-Aragón M. 2021. Long-term assessment of remediation treatments applied to an area affected by a mining spill in Spain. Land Degrad Dev. 32(8):2481–2492. doi:10.1002/ldr.3911.

Pérez Latorre A V., Navas P, Navas D, Gil Y, Cabezudo B. 2002. Datos sobre la flora y vegetación de la cuenca del río Guadiamar (Sevilla-Huelva, España). Acta Botanica Malacitana. 27:189–228. doi:10.24310/abm.v27i0.7326.

Pérez-de-Mora A, Madejón E, Burgos P, Cabrera F. 2006. Trace element availability and plant growth in a mine-spill contaminated soil under assisted natural remediation I. Soils. Science of The Total Environment. 363:28–37. doi:10.1016/J.SCITOTENV.2005.10.015.

Pérez-De-Mora A, Madejón P, Burgos P, Cabrera F, Lepp NW, Madejón E. 2011. Phytostabilization of semiarid soils residually contaminated with trace elements using by-products: Sustainability and risks. Environmental Pollution. 159(10):3018–3027. doi:10.1016/J.ENVPOL.2011.04.015.

Pérez-López R, Álvarez-Valero AM, Nieto JM, Sáez R, Matos JX. 2008. Use of sequential extraction procedure for assessing the environmental impact at regional scale of the São Domingos Mine (Iberian Pyrite Belt). Applied Geochemistry. 23:3452–3463. doi:10.1016/J.APGEOCHEM.2008.08.005.

Pesciaroli C, Purswani J, Mestelan S, Lett L, Portela G, Medici S, Morillo JA, Pozo C, González-López J, Rivadeneyra MA. 2021. Bacterial Diversity in Calcium Carbonate Paleo Accretions (Tosca) in the Southern Pampas, Argentina. Geomicrobiol J. 38(10):869–878. doi:10.1080/01490451.2021.1968980.

Pinho IA, Lopes D V., Martins RC, Quina MJ. 2017. Phytotoxicity assessment of olive mill solid wastes and the influence of phenolic compounds. Chemosphere. 185:258–267. doi:10.1016/J.CHEMOSPHERE.2017.07.002.

Piotrowska A, Długosz J. 2012. Spatio–temporal variability of microbial biomass content and activities related to some physicochemical properties of Luvisols. Geoderma. 173–174:199–208. doi:10.1016/J.GEODERMA.2011.12.014.

Piotrowska-Długosz A, Charzyński P. 2015. The impact of the soil sealing degree on microbial biomass, enzymatic activity, and physicochemical properties in the Ekranic Technosols of Toruń (Poland). J Soils Sediments. 15(1):47–59. doi:10.1007/S11368-014-0963-8.

Plaza C, Garcia-Gil JC, Soler-Revira P, Polo A. 2002. Effect of agricultural application of pig slurry on soil enzyme activities. In: Centro de Ciencias Medioambientales (CSIC). Madrid, Spain: Consejo Superior de Investigaciones Científica.

Póvoas I, Barral MF. 1992. Métodos de análise de solos. Comunicações do Instituto de Investigação Científica Tropical. In: Série de Ciências Agrárias N.º 10. Lisbon, Portugal: Instituto de Investigação Científica Tropical.

Procópio SO, Santos JB, Silva AA, Pires FR, Ribeiro Júnior JI, Santos EA, Ferreira LR. 2004. Seleção de plantas com potencial para fitorremediação de solos contaminados com o herbicida trifloxysulfuron sodium. Planta Daninha. 22(2):315–322.

Qu G, de Varennes A. 2010. Use of hydrophilic polymers from diapers to aid the establishment of Spergularia purpurea in a mine soil. J Hazard Mater. 178:956–962. doi:10.1016/j.jhazmat.2010.02.031.

Queiroz HM, Ferreira AD, Ruiz F, Bovi RC, Deng Y, de Souza Júnior VS, Otero XL, Bernardino AF, Cooper M, Ferreira TO. 2022. Early pedogenesis of anthropogenic soils produced by the world's largest mining disaster, the "Fundão" dam collapse, in southeast Brazil. Catena (Amst). 219:106625. doi:10.1016/J.CATENA.2022.106625.

Quevauviller Ph, Lachica M, Barahona E, Gomez A, Rauret G, Ure A, Muntau H. 1998. Certified reference material for the quality control of EDTA- and DTPA-extractable trace metal contents in calcareous soil (CRM 600). Fresenius' Journal of Analytical Chemical. 360:505–511. doi:10.1007/s002160050750.

Raffa CM, Chiampo F, Shanthakumar S. 2021. Remediation of Metal/Metalloid-Polluted Soils: A Short Review. Applied Sciences. 11(9):4134. doi:10.3390/APP11094134.

Ramos LL, Solé Benet A, Lázaro Suau R, Arzadun Larrucea A, Hens Del Campo L, Matilla AU. 2021. Field-testing and characterization of Technosols made from industrial and agricultural residues for restoring degraded slopes in semiarid SE Spain. J Soils Sediments. 21:1989–1997. doi:10.1007/s11368-021-02918-x/Published.

Rao M V., Paliyath G, Ormrod DP. 1996. Ultraviolet-B- and Ozone-Induced Biochemical Changes in Antioxidant Enzymes of Arabidopsis thaliana. Plant Physiol. 110:125–136. doi:10.1104/PP.110.1.125.

Rao M V., Paliyath G, Ormrod DP, Murr DP, Watkins CB. 1997. Influence of Salicylic Acid on H2O2 Production, Oxidative Stress, and H2O2-Metabolizing Enzymes (Salicylic Acid-Mediated Oxidative Damage Requires H2O2). Plant Physiol. 115:137–149. doi:10.1104/PP.115.1.137.

Rao MA, Scelza R, Acevedo F, Diez MC, Gianfreda L. 2014. Enzymes as useful tools for environmental purposes. Chemosphere. 107:145–162. doi:10.1016/J.CHEMOSPHERE.2013.12.059.

Rascio N, Navari-Izzo F. 2011. Heavy metal hyperaccumulating plants: How and why do they do it? And what makes them so interesting? Plant Science. 180:169–181. doi:10.1016/J.PLANTSCI.2010.08.016.

Redman AD, Macalady DL, Ahmann D. 2002. Natural organic matter affects arsenic speciation and sorption onto hematite. Environ Sci Technol. 36(13):2889–2896. doi:10.1021/es0112801.

Reed TS, Martens DC. 1996. Copper and Zinc. In: Sparks DL, Page AL, Helmke PA, Loeppert RH, Soltanpour PN, Tabatabai MA, Johnston CT, Sumner ME, editors. Methods of soil analysis. Part 3 - Chemical methods. Madison, WI, USA: Soil Science Society of America, American Society of Agronomy. (SSSA Book Series). p. 703–722.

Remenár M, Karelová E, Harichová J, Zámocký M, Krčová K, Ferianc P. 2014. Actinobacteria occurrence and their metabolic characteristics in the nickel-contaminated soil sample. Biologia (Bratisl). 69(11):1453–1463. doi:10.2478/S11756-014-0451-Z.

Roberts D, Nachtegaal M, Sparks DL. 2005. Speciation of metals in soils. In: Tabatabai MA, Sparks DL, editors. Chemical processes in soils. Washington DC, USA: Soil Science Society of America. p. 619–654.

Rodríguez Martín JA, De Arana C, Ramos-Miras JJ, Gil C, Boluda R. 2015. Impact of 70 years urban growth associated with heavy metal pollution. Environmental Pollution. 196:156–163. doi:10.1016/J.ENVPOL.2014.10.014.

Rodríguez-Eugenio N, McLaughlin MJ, Pennock D. 2018. Soil pollution: a hidden reality. Rome, Italy: Food and Agriculture Organization of the United Nations (FAO).

Rodríguez-Jordá MP, Garrido F, García-González MT. 2012. Effect of the addition of industrial by-products on Cu, Zn, Pb and As leachability in a mine sediment. J Hazard Mater. 213–214:46–54. doi:10.1016/J.JHAZMAT.2012.01.049.

Rodriguez-Ruiz A, Asensio V, Zaldibar B, Soto M, Marigómez I. 2014. Toxicity assessment through multiple endpoint bioassays in soils posing environmental risk according to regulatory screening values. Environmental Science and Pollution Research. 21(16):9689–9708. doi:10.1007/S11356-014-2915-7.

Rodríguez-Vila A, Asensio V, Forján R, Covelo EF. 2016. Assessing the influence of technosol and biochar amendments combined with Brassica juncea L. on the fractionation of Cu, Ni, Pb and Zn in a polluted mine soil. J Soils Sediments. 16(2):339–348. doi:10.1007/s11368-015-1222-3.

Rodríguez-Vila A, Covelo EF, Forján R, Asensio V. 2014. Phytoremediating a copper mine soil with Brassica juncea L., compost and biochar. Environmental Science and Pollution Research. 21(19):11293–11304. doi:10.1007/S11356-014-2993-6.

Rognes T, Flouri T, Nichols B, Quince C, Mahé F. 2016. VSEARCH: A versatile open source tool for metagenomics. PeerJ. 2016(10):e2584. doi:10.7717/PEERJ.2584.

Romero-Freire A. 2015. Influence of soil properties on the toxicity of metal-polluted soils: Comparison of different bioassay methods. [accessed 2024 Apr 30]. http://hdl.handle.net/10481/40798.

Romero-Freire A, García Fernández I, Simón Torres M, Martínez Garzón FJ, Martín Peinado FJ. 2016. Long-term toxicity assessment of soils in a recovered area affected by a mining spill. Environmental Pollution. 208:553–561. doi:10.1016/J.ENVPOL.2015.10.029.

Romero-Freire A, Martin Peinado FJ, van Gestel CAM. 2015. Effect of soil properties on the toxicity of Pb: Assessment of the appropriateness of guideline values. J Hazard Mater. 289:46–53. doi:10.1016/j.jhazmat.2015.02.034.

Romero-Freire A, Minguez L, Pelletier M, Cayer A, Caillet C, Devin S, Gross EM, Guérold F, Pain-Devin S, Vignati DAL, et al. 2018. Assessment of baseline ecotoxicity of sediments from a prospective mining area enriched in light rare earth elements. Science of The Total Environment. 612:831–839. doi:10.1016/J.SCITOTENV.2017.08.128.

Romero-Freire A, Sierra-Aragón M, Ortiz-Bernad I, Martín-Peinado FJ. 2014. Toxicity of arsenic in relation to soil properties: implications to regulatory purposes. J Soils Sediments. 14(5):968–979. doi:10.1007/s11368-014-0845-0.

Romero-Freire A, Sierra-Aragón M, Qiu H, He E. 2023. Editorial for the Special Issue "Phytotoxicity of Heavy Metals in Contaminated Soils." Toxics. 11:536. doi:10.3390/TOXICS11060536.

Ruiz F, Perlatti F, Oliveira DP, Ferreira TO. 2020. Revealing tropical Technosols as an alternative for mine reclamation and waste management. Minerals. 10(2):110. doi:10.3390/min10020110.

Russell EW, Wild A. 1988. Russell's soil conditions and plant growth. London, United Kingdom: Longman Scientific & Technical.

Ruthrof KX, Fontaine JB, Hopkins AJM, McHenry MP, O'Hara G, McComb J, Hardy GESJ, Howieson J. 2018. Potassium amendment increases biomass and reduces heavy metal concentrations in Lablab purpureus after phosphate mining. Land Degrad Dev. 29(3):398–407. doi:10.1002/ldr.2866.

Rutkowska B, Szulc W, Bomze K, Gozdowski D, Spychaj-Fabisiak E. 2015. Soil factors affecting solubility and mobility of zinc in contaminated soils. International Journal of Environmental Science and Technology. 12(5):1687–1694. doi:10.1007/S13762-014-0546-7.

Salvany JM, Mediavilla C, Mantecón C, Manzano M. 2001. Geología del Valle del Guadiamar y áreas colindantes. Boletín Geológico y Minero. Vol. Especial:57–68.

Sánchez España J, López Pamo E, Santofimia E, Aduvire O, Reyes J, Barettino D. 2005. Acid mine drainage in the Iberian Pyrite Belt (Odiel river watershed, Huelva, SW Spain): Geochemistry, mineralogy and environmental implications. Applied Geochemistry. 20:1320–1356. doi:10.1016/j.apgeochem.2005.01.011.

Sánchez-Castro I, Molina L, Prieto-Fernández MÁ, Segura A. 2023. Past, present and future trends in the remediation of heavy-metal contaminated soil - Remediation techniques applied in real soil-contamination events. Heliyon. 9(6):e16692. doi:10.1016/J.HELIYON.2023.E16692.

Santini TC, Fey M V. 2016. Assessment of Technosol formation and in situ remediation in capped alkaline tailings. Catena (Amst). 136:17–29. doi:10.1016/j.catena.2015.08.006.

Santos ES, Abreu MM, Batista MJ, Magalhães MCF, Fernandes E. 2014a. Inter-population variation on the accumulation and translocation of potentially harmful chemical elements in Cistus ladanifer L. from Brancanes, Caveira, Chança, Lousal, Neves Corvo and São Domingos mines in the Portuguese Iberian Pyrite Belt. J Soils Sediments. 14:758–772. doi:10.1007/s11368-014-0852-1.

Santos ES, Abreu MM, Macías F. 2019. Rehabilitation of mining areas through integrated biotechnological approach: Technosols derived from organic/inorganic wastes and autochthonous plant development. Chemosphere. 224:765–775. doi:10.1016/j.chemosphere.2019.02.172.

Santos ES, Abreu MM, Macías F, de Varennes A. 2014b. Improvement of chemical and biological properties of gossan mine wastes following application of amendments and growth of Cistus ladanifer L. J Geochem Explor. 147:173–181. doi:10.1016/j.gexplo.2014.07.007.

Santos ES, Abreu MM, Macías F, de Varennes A. 2016a. Chemical quality of leachates and enzymatic activities in Technosols with gossan and sulfide wastes from the São Domingos mine. J Soils Sediments. 16(4):1366–1382. doi:10.1007/s11368-015-1068-8.

Santos ES, Abreu MM, Saraiva JA. 2016b. Mutielemental concentration and physiological responses of Lavandula pedunculata growing in soils developed on different mine wastes. Environmental Pollution. 213:43–52. doi:10.1016/j.envpol.2016.02.001.

Santos ES, Arán D, Abreu MM, de Varennes A. 2018. Engineered soils using amendments for in situ rehabilitation of mine lands. In: Prasad MNV, Favas PJ de C, Maiti SK, editors. Bio-Geotechnologies for Mine Site Rehabilitation. Amsterdam, Netherlands: Elsevier Inc. p. 131–146.

Sardans J, Peñuelas J. 2005. Drought decreases soil enzyme activity in a Mediterranean Quercus ilex L. forest. Soil Biol Biochem. 37:455–461. doi:10.1016/j.soilbio.2004.08.004.

Sardans J, Peñuelas J, Ogaya R. 2008. Experimental drought reduced acid and alkaline phosphatase activity and increased organic extractable P in soil in a Quercus ilex Mediterranean forest. Eur J Soil Biol. 44:509–520. doi:10.1016/j.ejsobi.2008.09.011.

Schloter M, Nannipieri P, Sørensen SJ, van Elsas JD. 2018. Microbial indicators for soil quality. Biol Fertil Soils. 54:1–10. doi:10.1007/S00374-017-1248-3.

Schulten HR, Schnitzer M. 1997. The chemistry of soil organic nitrogen: A review. Biol Fertil Soils. 26(1):1–15. doi:10.1007/S003740050335.

SCS - USDA. 1972. Soil Survey Manual. Methods and procedures for collecting soil samples. Washington, DC, USA: Soil Conservation Service, United States Department of Agriculture.

Seraj F, Rahman T. 2018. Heavy Metals, Metalloids, Their Toxic Effect and Living Systems. Am J Plant Sci. 9:2626–2643. doi:10.4236/AJPS.2018.913191.

Séré G, Schwartz C, Ouvrard S, Renat JC, Watteau F, Villemin G, Morel JL. 2010. Early pedogenic evolution of constructed Technosols. J Soils Sediments. 10(7):1246–1254. doi:10.1007/s11368-010-0206-6.

Shaheen SM, Shams MS, Khalifa MR, El-Dali MA, Rinklebe J. 2017. Various soil amendments and environmental wastes affect the (im)mobilization and phytoavailability of potentially toxic elements in a sewage effluent irrigated sandy soil. Ecotoxicol Environ Saf. 142:375–387. doi:10.1016/j.ecoenv.2017.04.026.

Shahid M, Khalid S, Abbas G, Shahid N, Nadeem M, Sabir M, Aslam M, Dumat C. 2015. Heavy metal stress and crop productivity. In: Hakeem KR, editor. Crop Production and Global Environmental Issues. Basel, Switzerland: Springer International Publishing. p. 1–25.

Shannon CE. 1948. A Mathematical Theory of Communication. Bell System Technical Journal. 27(3):379–423. doi:10.1002/J.1538-7305.1948.TB01338.X.

Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, Amin N, Schwikowski B, Ideker T. 2003. Cytoscape: A Software Environment for Integrated Models of Biomolecular Interaction Networks. Genome Res. 13:2498–2504. doi:10.1101/GR.1239303.

Sharholy M, Ahmad K, Mahmood G, Trivedi RC. 2008. Municipal solid waste management in Indian cities - A review. Waste Management. 28(2):459–467. doi:10.1016/j.wasman.2007.02.008.

Sharma P, Jha AB, Dubey RS, Pessarakli M. 2012. Reactive Oxygen Species, Oxidative Damage, and Antioxidative Defense Mechanism in Plants under Stressful Conditions. J Bot. 2012:217037. doi:10.1155/2012/217037.

Shaw GTW, Pao YY, Wang D. 2016. MetaMIS: A metagenomic microbial interaction simulator based on microbial community profiles. BMC Bioinformatics. 17:488. doi:10.1186/S12859-016-1359-0.

Sheppard SC, Evenden WG. 1988. The assumption of linearity in soil and plant concentration ratios: An experimental evaluation. J Environ Radioact. 7(3):221–247. doi:10.1016/0265-931X(88)90030-6.

Shukurov N, Kodirov O, Peitzsch M, Kersten M, Pen-Mouratov S, Steinberger Y. 2014. Coupling geochemical, mineralogical and microbiological approaches to assess the health of contaminated soil around the Almalyk mining and smelter complex, Uzbekistan. Science of The Total Environment. 476–477:447–459. doi:10.1016/J.SCITOTENV.2014.01.031.

Sierra Aragón M, Nakamaru YM, García-Carmona M, Martínez Garzón FJ, Martín Peinado FJ. 2019. The role of organic amendment in soils affected by residual pollution of potentially harmful elements. Chemosphere. 237:124549. doi:10.1016/j.chemosphere.2019.124549.

Sigfusson B, Meharg AA, Gislason SR. 2008. Regulation of Arsenic Mobility on Basaltic Glass Surfaces by Speciation and pH. Environ Sci Technol. 42:8816–8821. doi:10.1021/ES8001458.

da Silva Souza T, Lacerda D, Aguiar LL, Martins MNC, Augusto de Oliveira David J. 2020. Toxic potential of sewage sludge: Histopathological effects on soil and aquatic bioindicators. Ecol Indic. 111:105980. doi:10.1016/J.ECOLIND.2019.105980.

Simate GS, Ndlovu S. 2014. Acid mine drainage: Challenges and opportunities. J Environ Chem Eng. 2:1785–1803. doi:10.1016/j.jece.2014.07.021.

Simón M, Diez M, González V, García I, Martín F, de Haro S. 2010. Use of liming in the remediation of soils polluted by sulphide oxidation: A leaching-column study. J Hazard Mater. 180(1–3):241–246. doi:10.1016/J.JHAZMAT.2010.04.020.

Simón M, García I, Martín F, Díez M, del Moral F, Sánchez JA. 2008. Remediation measures and displacement of pollutants in soils affected by the spill of a pyrite mine. Science of The Total Environment. 407(1):23–39. doi:10.1016/J.SCITOTENV.2008.07.040.

Simón M, Martín F, García I, Bouza P, Dorronsoro C, Aguilar J. 2005. Interaction of limestone grains and acidic solutions from the oxidation of pyrite tailings. Environmental Pollution. 135(1):65–72. doi:10.1016/j.envpol.2004.10.013.

Simón M, Martín F, Ortiz I, García I, Fernández J, Fernández E, Dorronsoro C, Aguilar J. 2001. Soil pollution by oxidation of tailings from toxic spill of a pyrite mine. Science of The Total Environment. 279:63–74. doi:10.1016/S0048-9697(01)00726-4.

Simón M, Ortiz I, García I, Fernández E, Fernández J, Dorronsoro C, Aguilar J. 1998. El desastre ecológico de doñana. Edafología. 5:153–161.

Simón M, Ortiz I, García I, Fernández E, Fernández J, Dorronsoro C, Aguilar J. 1999. Pollution of soils by the toxic spill of a pyrite mine (Aznalcollar, Spain). Science of the Total Environment. 242(1–3):105–115. doi:10.1016/S0048-9697(99)00378-2.

Siripornadulsil S, Traina S, Verma DPS, Sayre RT. 2002. Molecular Mechanisms of Proline-Mediated Tolerance to Toxic Heavy Metals in Transgenic Microalgae. Plant Cell. 14(11):2837–2847. doi:10.1105/TPC.004853.

Skousen JG, Ziemkiewicz PF, McDonald LM. 2019. Acid mine drainage formation, control and treatment: Approaches and strategies. Extractive Industries and Society. 6(1):241–249. doi:10.1016/j.exis.2018.09.008.

Slukovskaya M V., Vasenev VI, Ivashchenko K V., Morev D V., Drogobuzhskaya S V., Ivanova LA, Kremenetskaya IP. 2019. Technosols on mining wastes in the subarctic: Efficiency of remediation under Cu-Ni atmospheric pollution. International Soil and Water Conservation Research. 7:297–307. doi:10.1016/j.iswcr.2019.04.002.

Smith P, Keesstra SD, Silver WL, Adhya TK. 2021. The role of soils in delivering Nature's Contributions to People. Philosophical Transactions of the Royal Society B. 376:20200169. doi:10.1098/RSTB.2020.0169.

Soil Survey Staff. 2014. Keys to soil taxonomy (12th edition). Washington, USA: USDA-Natural Resources Conservation Service.

Solomon M, Tornos F, Large RR, Badham JNP, Both RA, Zaw K. 2004. Zn–Pb–Cu volcanic-hosted massive sulphide deposits: criteria for distinguishing brine pool-type from black smoker-type sulphide deposition. Ore Geol Rev. 25(3–4):259–283. doi:10.1016/J.OREGEOREV.2004.01.003.

Son J, Kim JG, Hyun S, Cho K. 2019. Screening level ecological risk assessment of abandoned metal mines using chemical and ecotoxicological lines of evidence. Environmental Pollution. 249:1081–1090. doi:10.1016/J.ENVPOL.2019.03.019.

Song J, Guo Z hui, Xiao X yuan, Miao X feng, Wang F yong. 2009. Environmental availability and profile characteristics of arsenic, cadmium, lead and zinc in metal-contaminated vegetable soils. Transactions of Nonferrous Metals Society of China. 19(3):765–772. doi:10.1016/S1003-6326(08)60347-7.

Song J, Zhao F-JJ, McGrath SP, Luo Y-MM. 2006. Influence of soil properties and aging on arsenic phytotoxicity. Environ Toxicol Chem. 25(6):1663–1670. doi:10.1897/05-480R2.1.

Soria R, Ortega R, Bastida F, Miralles I. 2021. Role of organic amendment application on soil quality, functionality and greenhouse emission in a limestone quarry from semiarid ecosystems. Applied Soil Ecology. 164:103925. doi:10.1016/J.APSOIL.2021.103925.

Souza LA, Piotto FA, Dourado MN, Schmidt D, Franco MR, Boaretto LF, Tezotto T, Ferreira RR, Azevedo RA. 2017. Physiological and biochemical responses of Dolichos lablab L. to cadmium support its potential as a cadmium phytoremediator. J Soils Sediments. 17:1413–1426. doi:10.1007/s11368-015-1322-0.

Sposito G, Lund LJ, Chang AC. 1982. Trace Metal Chemistry in Arid-zone Field Soils Amended with Sewage Sludge: I. Fractionation of Ni, Cu, Zn, Cd, and Pb in Solid Phases. Soil Science Society of America Journal. 46:260–264. doi:10.2136/sssaj1982.03615995004600020009x.

Stefanowicz AM, Niklińska M, Laskowski R. 2008. Metals affect soil bacterial and fungal functional diversity differently. Environ Toxicol Chem. 27(3). doi:10.1897/07-288.1.

Stege PW, Messina GA, Bianchi G, Olsina RA, Raba J. 2010. Determination of β-glucosidase activity in soils with a bioanalytical sensor modified with multiwalled carbon nanotubes. Anal Bioanal Chem. 397:1347–1353. doi:10.1007/s00216-010-3634-7.

Stigliani WM. 1991. Chemical time bombs: definition, concepts, and examples. Laxenburg, Austria: International Institute for Applied Systems Analysis.

Sun Q, Li T, Alva AK, Li YC. 2019. Mobility and fractionation of copper in sandy soils. Environmental Pollutants and Bioavailability. 31(1):18–23. doi:10.1080/09542299.2018.1558114.

Tabatabai MA. 1994. Soil enzymes. In: Mickelson SH, Bigham JM, editors. Methods of Soil Analysis, Part 2. Microbiological and Biochemical Properties. Madison, WI, USA: Soil Science Society of America. (SSSA Book Series). p. 775–833.

Takahashi S, Tomita J, Nishioka K, Hisada T, Nishijima M. 2014. Development of a Prokaryotic Universal Primer for Simultaneous Analysis of Bacteria and Archaea Using Next-Generation Sequencing. Bourtzis K, editor. PLoS One. 9(8):e105592. doi:10.1371/journal.pone.0105592.

Takamatsu T, Kusakabe R, Yoshida T. 1983. Analysis of metal-humic acid interaction by paper chromatography using humic acid-impregnated filter paper. Soil Sci. 136(6):371–381.

Tao Y, Shen L, Feng C, Yang R, Qu J, Ju H, Zhang Y. 2022. Distribution of rare earth elements (REEs) and their roles in plant growth: A review. Environmental Pollution. 298:118540. doi:10.1016/J.ENVPOL.2021.118540.

Tapia A, Cornejo-La Torre M, Santos ES, Arán D, Gallardo A. 2019. Improvement of chemical quality of percolated leachates by in situ application of aqueous organic wastes on sulfide mine tailings. J Environ Manage. 244:154–160. doi:10.1016/j.jenvman.2019.05.040.

Tayebi-Khorami M, Edraki M, Corder G, Golev A. 2019. Re-thinking mining waste through an integrative approach led by circular economy aspirations. Minerals. 9(5):286–299. doi:10.3390/min9050286.

Theodorsson-Norheim E. 1986. Kruskal-Wallis test: BASIC computer program to perform nonparametric one-way analysis of variance and multiple comparisons on ranks of several independent samples. Comput Methods Programs Biomed. 23:57–62. doi:10.1016/0169-2607(86)90081-7.

Tornos F, López Pamo E, Sánchez España FJ. 2008. The Iberian Pyrite Belt. In: García-Cortes Á, editor. Contextos geológicos españoles: una aproximación al patrimonio geológico de relevancia internacional. Madrid, Spain: Instituto Geológico y Minero de España. p. 56–64.

Tripathi N, Hills CD, Singh RS, Atkinson CJ. 2019. Biomass waste utilisation in low-carbon products: harnessing a major potential resource. NPJ Clim Atmos Sci. 2:35. doi:10.1038/S41612-019-0093-5.

Turdi M, Yang L. 2016. Trace Elements Contamination and Human Health Risk Assessment in Drinking Water from the Agricultural and Pastoral Areas of Bay County, Xinjiang, China. International Journal of Environmental Research and Public Health . 13(10):938. doi:10.3390/IJERPH13100938.

Tyurin I V. 1951. Analytical procedure for a comparative study of soil humus. Trudy poch Inst Dokuchaev. 33:5–21.

UNEP (United Nations Environment Programme). 2015. Converting waste agricultural biomass into a resource. Osaka/Shiga, Japan: United Nations

Environment Programme Division of Technology, Industry and Economics International Environmental Technology Centre.

United Nations. 2022. World Population Prospects 2022: Summary of Results. Paris, France: United Nations Department of Economic and Social Affairs, Population Division.

de Varennes A. 2003. Produtividade dos Solos e Ambiente. Lisbon, Portugal: Escolar editora.

de Varennes A, Abreu MM, Qu G, Cunha-Queda C. 2010. Enzymatic Activity of a Mine Soil Varies According to Vegetation Cover and Level of Compost Applied. Int J Phytoremediation. 12(4):371–383. doi:10.1080/15226510903051757.

Vaughan DJ. 2005. MINERALS | Sulphides. In: Selley RC, Cocks LRobinM, Plimer IR, editors. Encyclopedia of Geology. Amsterdam, The Netherlands: Elsevier Ltd. Academic Press. p. 574–586.

Vavrek M. 2011. fossil: Palaeoecological and palaeogeographical analysis tools. Palaeontologia Electronica. 14(1):1–16.

Veloso A, Sempiterno C, Calouro F, Rebelo F, Pedra F, Castro I V, da Conceição Gonçalves M, da Encarnação Marcelo M, Pereira P, Fareleira P, et al. 2022. Manual de fertilização das culturas. 3rd edition. Calouro F, editor. Lisbon, Portugal: INIAV (Instituto Nacional de Investigação Agrária e Veterinária, I.P.).

Vidigal P, Duarte B, Cavaco AR, Caçador I, Figueiredo A, Matos AR, Viegas W, Monteiro F. 2018. Preliminary diversity assessment of an undervalued tropical bean (Lablab purpureus (L.) Sweet) through fatty acid profiling. Plant Physiology and Biochemistry. 132:508–514. doi:10.1016/J.PLAPHY.2018.10.001.

Vidigal P, Romeiras MM, Monteiro F. 2019. Crops diversification and the role of orphan legumes to improve the Sub-Saharan Africa farming systems. In: Hasanuzzaman M, Teixeira Filho MCM, Fujita M, Rodrigues Nogueira TA, editors. Sustainable Crop Production. London, United Kingdom: IntechOpen.

de Vries W, Groenenberg JE. 2009. Evaluation of approaches to calculate critical metal loads for forest ecosystems. Environmental Pollution. 157(12):3422–3432. doi:10.1016/J.ENVPOL.2009.06.021.

Walkley A, Black IA. 1934. An examination of the degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. Soil Sci. 37:29–38. doi:10.1097/00010694-193401000-00003.

Walmsley A, Mundodi L, Sederkenny A, Anderson N, Missen J, Yellishetty M. 2022. From spoil to soil: utilising waste materials to create soils for mine rehabilitation. In: Tibbett M, Fourie AB, Boggs G, editors. Proceedings of the International Conference on Mine Closure. Vol. 1. Brisbane, Australia. p. 1237–1248.

Walmsley A, Sklenička P. 2017. Various effects of land tenure on soil biochemical parameters under organic and conventional farming – Implications for soil quality restoration. Ecol Eng. 107:137–143. doi:10.1016/J.ECOLENG.2017.07.006.

Wang S, Mulligan CN. 2006. Occurrence of arsenic contamination in Canada: sources, behavior and distribution. Science of the Total Environment. 366:701–721. doi:10.1016/j.scitotenv.2005.09.005.

Wang S, Mulligan CN. 2009. Enhanced mobilization of arsenic and heavy metals from mine tailings by humic acid. Chemosphere. 74(2):274–279. doi:10.1016/J.CHEMOSPHERE.2008.09.040.

Watari T, Nansai K, Nakajima K. 2021. Major metals demand, supply, and environmental impacts to 2100: A critical review. Resour Conserv Recycl. 164:105107. doi:10.1016/J.RESCONREC.2020.105107.

Wiatrowska K, Komisarek J, Olejnik J. 2021. Variations in Organic Carbon Content and Dehydrogenases Activity in Post-Agriculture Forest Soils: A Case Study in South-Western Pomerania. Forests. 12(4):459. doi:10.3390/f12040459.

Wichuk KM, McCartney D. 2015. A review of the effectiveness of current time–temperature regulations on pathogen inactivation during composting. Journal of Environmental Engineering and Science. 6(5):573–586. doi:10.1139/S07-011.

Willett W, Rockström J, Loken B, Springmann M, Lang T, Vermeulen S, Garnett T, Tilman D, DeClerck F, Wood A, et al. 2019. Food in the Anthropocene: the EAT–Lancet Commission on healthy diets from sustainable food systems. The Lancet. 393(10170):447–492. doi:10.1016/S0140-6736(18)31788-4.

Wolińska A, Stępniewsk Z. 2012. Dehydrogenase Activity in the Soil Environment. In: Canuto RA, editor. Dehydrogenases. London, United Kingdom: IntechOpen Limited. p. 183–210.

World Bank Group. 2022. Solid Waste Management. [accessed 2024 Apr 30]. https://www.worldbank.org/en/topic/urbandevelopment/brief/solid-waste-management.

Xiang M, Li Y, Yang J, Lei K, Li Y, Li F, Zheng D, Fang X, Cao Y. 2021. Heavy metal contamination risk assessment and correlation analysis of heavy metal contents in soil and crops. Environmental Pollution. 278:116911. doi:10.1016/J.ENVPOL.2021.116911.

Xiong J, Madejon P, Madejon E, Cabrera F. 2015. Assisted Natural Remediation of a Trace Element-Contaminated Acid Soil: An Eight-Year Field Study. Pedosphere. 25(2):250–262. doi:10.1016/S1002-0160(15)60010-8.

Xu D, Shen Z, Dou C, Dou Z, Li Y, Gao Y, Sun Q. 2022. Effects of soil properties on heavy metal bioavailability and accumulation in crop grains under different farmland use patterns. Sci Rep. 12:9211. doi:10.1038/s41598-022-13140-1.

Xu D-M, Fu R-B, Liu H-Q, Guo X-P. 2021. Current knowledge from heavy metal pollution in Chinese smelter contaminated soils, health risk implications and associated remediation progress in recent decades: A critical review. J Clean Prod. 286:124989. doi:10.1016/J.JCLEPRO.2020.124989.

Xu M, Mckay G. 2017. Removal of heavy metals, lead, cadmium, and zinc, using adsorption processes by cost-effective adsorbents. In: Bonilla-Petriciolet A, Mendoza-Castillo DI, Reynel-Ávila HE, editors. Adsorption Processes for Water

Treatment and Purification. New York, USA: Springer International Publishing AG. p. 1–256.

Yadav SK. 2010. Heavy metals toxicity in plants: An overview on the role of glutathione and phytochelatins in heavy metal stress tolerance of plants. South African Journal of Botany. 76:167–179. doi:10.1016/J.SAJB.2009.10.007.

Yang B, Cao Y, Ren J, Wang M, Luo H, Li F. 2019. Water incubation-induced fluctuating release of heavy metals in two smelter-contaminated soils. Journal of Environmental Sciences . 82:14–23. doi:10.1016/j.jes.2019.02.026.

Yang Y, Zhang Y, Wei X, You J, Wang W, Lu J, Shi R. 2011. Comparative antioxidative responses and proline metabolism in two wheat cultivars under short term lead stress. Ecotoxicol Environ Saf. 74:733–740. doi:10.1016/J.ECOENV.2010.10.035.

Yao FX, Macías F, Santesteban A, Virgel S, Blanco F, Jiang X, Camps Arbestain M. 2009. Influence of the acid buffering capacity of different types of Technosols on the chemistry of their leachates. Chemosphere. 74(2):250–258. doi:10.1016/j.chemosphere.2008.09.031.

Yildirim E, Ekinci M, Turan M. 2022. Mitigation of heavy metal toxicity by plant growth-promoting rhizobacteria. In: Seymen M, Kurtar ES, Erdinc C, Kumar A, editors. Sustainable Horticulture: Microbial Inoculants and Stress Interaction. Cambridge, MA, USA: Academic Press. p. 97–123.

Younis M. 2010. Responses of Lablab purpureus (L.) sweet / Rhizobium symbiosis and growth to potassium supply under different water regimes. J Plant Nutr. 33(9):1400–1409. doi:10.1080/01904167.2010.484287.

Yu Q, Osborne L, Rengel Z. 1998. Micronutrient deficiency changes activities of superoxide dismutase and ascorbate peroxidase in tobacco plants. J Plant Nutr. 21(7):1427–1437. doi:10.1080/01904169809365493.

Zaier H, Ghnaya T, Lakhdar A, Baioui R, Ghabriche R, Mnasri M, Sghair S, Lutts S, Abdelly C. 2010. Comparative study of Pb-phytoextraction potential in Sesuvium portulacastrum and Brassica juncea: Tolerance and accumulation. J Hazard Mater. 183:609–615. doi:10.1016/J.JHAZMAT.2010.07.068.

Zhang D, Chen H, Xia J, Nie Z, Fan X, Liu H, Zheng L, Zhang L, Yang H. 2020. Humic acid promotes arsenopyrite bio-oxidation and arsenic immobilization. J Hazard Mater. 384(July 2019):121359. doi:10.1016/j.jhazmat.2019.121359.

Zhang M. 2011. Adsorption study of Pb(II), Cu(II) and Zn(II) from simulated acid mine drainage using dairy manure compost. Chemical Engineering Journal. 172(1):361–368. doi:10.1016/J.CEJ.2011.06.017.

Zhang M, Dong LG, Fei SX, Zhang JW, Jiang XM, Wang Y, Yu X. 2021. Responses of Soil Organic Carbon Mineralization and Microbial Communities to Leaf Litter Addition under Different Soil Layers. Forests. 12:170. doi:10.3390/F12020170.

Zhang M, Sun Q, Chen P, Wei X, Wang B. 2022a. How microorganisms tell the truth of potentially toxic elements pollution in environment. J Hazard Mater. 431:128456. doi:10.1016/j.jhazmat.2022.128456.

Zhang R, Lu J, Dopson M, Leiviskä T. 2022b. Vanadium removal from mining ditch water using commercial iron products and ferric groundwater treatment residual-based materials. Chemosphere. 286:131817. doi:10.1016/J.CHEMOSPHERE.2021.131817.

Zhao Z, Xiong Y, Cheng X, Hou X, Yang Y, Tian Y, You J, Xu L. 2020. Adsorptive removal of trace thallium(I) from wastewater: A review and new perspectives. J Hazard Mater. 393:122378. doi:10.1016/J.JHAZMAT.2020.122378.

Zhu Y, Zhang Y, Luo D, Chong Z, Li E, Kong X. 2021. A review of municipal solid waste in China: characteristics, compositions, influential factors and treatment technologies. Environ Dev Sustain. 23:6603–6622. doi:10.1007/s10668-020-00959-9.

Zocche JJ, Sehn LM, Pillon JG, Schneider CH, Olivo EF, Raupp-Pereira F. 2023. Technosols in coal mining areas: Viability of combined use of agro-industry waste and synthetic gypsum in the restoration of areas degraded. Clean Eng Technol. 13:100618. doi:10.1016/J.CLET.2023.100618.

Samples of soils and Technosols collected after the microcosm assay with big hop clover

Granada, Spain

December 2020

Antonio Aguilar Garrido

SUPPLEMENTARY MATERIALS

- CHAPTER 2 -

Table S2.1. *PHEs content of the toxic tailing used to obtain the artificial AMD in mg kg^{-1} (mean ± SD, n = 3).*

PHEs	Maximum	Minimum	Median
Ag	41.0	25.0	34.0 ± 4.8 *
As	4122.6	1473.0	3749.0
Ba	804.1	353.7	650.8
Be	1.7	0.4	0.7
Bi	86.6	37.9	76.4
Cd	36.8	22.9	28.6
Ce	50.0	13.0	28.0 ± 9.1 *
Co	54.0	32.6	49.8
Cr	78.6	34.5	63.4
Cu	2175.8	1100.7	2009.1
Hg	4.9	2.4	3.2
In	3.0	1.5	2.7
La	40.0	12.0	21.0 ± 7.2 *
Mn	1097.5	667.3	726.3
Mo	8.6	3.2	7.8
Ni	31.4	15.8	18.7
Pb	9635.9	4352.9	9507.3
Sb	957.9	394.1	786.2
Sc	7.1	0.0	3.0
Se	5.3	0.0	4.4
Sn	24.0	6.0	7.3
Tl	66.8	33.1	57.1
Th	6.9	3.3	4.6
U	2.4	1.5	2.2
V	78.0	34.8	35.9
Y	14.4	5.2	6.5
Zn	8063.4	6246.6	7183.5

Data belonging to Simón et al. (1999), *except those with asterisks that belong to* López-Pamo et al. (1999)

Table S2.2. *Results of loamy clay soil CRM 052-050 (RT-Corporation Limited, Salisbury, UK) analysed by X-ray fluorescence (XRF) with a portable NITON XL3t-980 GOLDD+ analyser (Thermo Fisher Scientific, Waltham, MA, USA).*

Element (mg kg^{-1})	**Certified value**	**Prediction interval**		**Measured value**	**LOD**
		Low	*High*		
As	14.60 (1.71)	10.90	18.30	15.53 (3.96)	7
Cd	35.60 (1.89)	31.60	39.60	41.15 (4.96)	12
Cu	44.20 (3.11)	37.60	50.80	48.51 (8.01)	13
Fe	12,400 (1,170)	9,870	14,900	11,187 (96)	N/A
Pb	82.60 (5.10)	71.80	93.40	92.62 (4.84)	8
Sb	20.10 (6.23)	6.13	34.20	30.25 (5.42)	20
Zn	89.00 (8.56)	70.90	107.00	94.41 (6.23)	10

Measured values are the mean of six replicates and SD in parentheses. The limits of detection (LOD) are also shown. All values are given in mg kg^{-1}. N/A: Not applicable.

Table S2.3. *Results of Lichen BCR® - 482 (EC-JRC-IRMM, Geel, Belgium) analysed by ICP-OES in PerkinElmer Avio 500 (PerkinElmer, Inc., Waltham, MA, USA) after acid digestion in a Mars XP1500 Plus microwave (CEM Corporation, Matthews, CN, USA).*

Element (mg kg^{-1})	**Certified value**	**Measured value**	**Variance +/- (%)**
As	0.85 (0.07)	0.65 (0.17)	- 24
Cd	0.56 (0.02)	0.40 (0.03)	- 29
Cr	4.12 (0.15)	1.89 (0.34)	- 54
Cu	7.03 (0.19)	6.37 (0.28)	- 9
Ni	2.47 (0.07)	2.12 (1.19)	- 14
Pb	40.90 (1.40)	29.66 (1.11)	- 27
Zn	100.60 (2.20)	72.00 (2.76)	- 30

Measured values are the mean of three replicates and SD in parentheses. All values are given in mg kg^{-1}.

Table S2.4. *Results of loamy clay soil CRM 052-050 (RT-Corporation Limited, Salisbury, UK) analysed by ICP-OES in PerkinElmer Avio 500 (Perkin Elmer, Inc., Waltham, MA, USA) after acid digestion in a Mars XP1500 Plus microwave (CEM Corporation, Matthews, CN, USA).*

Element (mg kg^{-1})	**Certified value**	**Prediction interval**		**Measured value**	**Variance +/- (%)**
		Low	*High*		
As	14.60	10.90	18.30	18.30	+ 25
Cu	44.20	37.60	50.80	32.70	- 26
Pb	82.60	71.80	93.40	74.80	- 9
Zn	89.00	70.90	107.00	77.00	- 13

Measured values are the mean of six replicates. All values are given in mg kg^{-1}.

Table S2.5. *Results of Lichen BCR® - 482 (EC-JRC-IRMM, Geel, Belgium) analysed by ICP-MS in PerkinElmer NexION 300D (PerkinElmer, Inc., Waltham, MA, USA) after acid digestion in a Mars XP1500 Plus microwave (CEM Corporation, Matthews, CN, USA).*

Element (mg kg^{-1})	**Certified value**	**Measured value**	**Variance +/- (%)**
As	0.85	0.73	- 16
Cd	0.56	0.43	- 23
Cu	7.03	6.57	- 7
Pb	40.9	33.75	- 17
Zn	100.6	76.75	- 24

Measured values are the mean of three replicates. All values are given in mg kg^{-1}.

Table S2.6. *Results of loamy clay soil CRM 052-050 (RT-Corporation Limited, Salisbury, UK) analysed by ICP-MS in PerkinElmer NexION 300D (Perkin Elmer, Inc., Waltham, MA, USA) after acid digestion in a Mars XP1500 Plus microwave (CEM Corporation, Matthews, CN, USA).*

Element (mg kg^{-1})	**Certified value**	**Prediction interval**		**Measured value**
		Low	*High*	
As	14.60	10.90	18.30	12.80 - 13.00
Cd	35.60	31.60	39.60	31.60 - 33.49
Cu	44.20	37.60	50.80	32.70 - 38.20
Fe	12400	9870	14900	11190
Pb	82.60	71.80	93.40	74.80 - 75.30
Sb	20.10	16.60	23.60	23.73
Zn	89.00	70.90	107.00	77.0 - 77.85

Measured values are the mean of three replicates. All values are given in mg kg^{-1}.

- CHAPTER 3 -

Table S3.1. *PHEs concentration in the leachates resulting from the treatment of the artificial AMD by the different waste materials in μg l^{-1} (mean ± SD, n = 3).*

PHEs	AMD	IO	MS	CW	GS	WS	BM	VC	OW	OL	AW
As	2859.70 ± 270.16 e	1.16 ± 0.13 a	13.51 ± 7.85 a	7.48 ± 1.99 a	3.84 ± 0.96 a	870.45 ± 199.50 c	1517.10 ± 99.78 d	9.57 ± 0.47 a	339.45 ± 105.17 ba	522.84 ± 86.88 b	652.12 ± 83.41 bc
Ba	34.27 ± 9.83 ba	74.91 ± 1.67 bc	20.47 ± 1.43 a	22.69 ± 12.51 a	34.24 ± 3.18 ba	103.20 ± 37.49 cd	128.26 ± 15.69 de	162.25 ± 8.46 e	652.16 ± 20.47 g	794.77 ± 25.55 h	401.61 ± 14.63 f
Be	3.19 ± 0.36 e	bdl	bdl	bdl	0.02 ± 0.01 a	0.29 ± 0.07 ab	0.94 ± 0.07 cd	0.01 ± 0.02 a	0.62 ± 0.15 bc	1.19 ± 0.15 d	0.62 ± 0.06 bc
Bi	180.20 ± 10.98 e	0.82 ± 0.43 a	5.72 ± 2.56 ab	3.74 ± 1.18 ab	2.37 ± 0.33 a	34.48 ± 14.60 c	53.09 ± 3.96 d	0.44 ± 0.08 a	20.21 ± 4.08 bc	14.12 ± 0.25 ab	4.81 ± 0.87 ab
Cd	452.02 ± 5.82 f	4.94 ± 0.15 ab	3.27 ± 0.10 a	1.47 ± 0.06 a	18.87 ± 2.29 c	11.56 ± 1.32 b	112.47 ± 3.30 e	6.59 ± 0.31 ab	7.86 ± 1.32 ab	23.27 ± 1.32 c	46.80 ± 3.27 d
Co	434.61 ± 8.20 g	5.23 ± 0.09 a	155.37 ± 3.01 e	19.59 ± 0.95 a	268.59 ± 28.43 f	71.38 ± 3.63 cd	263.02 ± 10.66 f	20.97 ± 2.56 a	28.88 ± 1.41 ba	56.66 ± 2.03 bc	87.17 ± 10.31 d
Cr	351.89 ± 5.66 bc	bdl	bdl	bdl	bdl	58.01 ± 9.33 a	293.58 ± 15.32 bc	bdl	43.20 ± 12.69 a	49.69 ± 5.72 a	374.82 ± 86.08 c
Cu	6238.22 ± 67.11 f	4.47 ± 0.43 a	33.56 ± 2.12 a	96.91 ± 0.29 a	27.72 ± 2.93 a	468.53 ± 23.56 c	2051.00 ± 56.47 e	11.38 ± 0.35 a	266.31 ± 29.56 b	621.56 ± 37.37 c	1323.50 ± 150.79 d
In	31.56 ± 0.38 d	bdl	0.02 ± 0.02 a	bdl	bdl	2.57 ± 0.52 c	2.72 ± 0.10 c	bdl	1.78 ± 0.51 bc	1.96 ± 0.19 bc	2.29 ± 0.27 bc
Mn	12,937.64 ± 216.04 d	256.76 ± 21.24 a	2791.30 ± 38.72 bca	740.01 ± 39.57 ba	28,157.19 ± 3067.08 e	3476.60 ± 1517.82 bc	15,777.99 ± 582.29 d	3668.79 ± 323.63 bc	3209.11 ± 87.35 bca	3825.76 ± 133.26 bc	3940.75 ± 366.19 c
Mo	6.33 ± 0.30 a	6.11 ± 5.14 a	1.70 ± 0.18 a	424.28 ± 18.58 b	8.05 ± 1.21 a	138.45 ± 49.01 a	174.72 ± 7.15 a	12.31 ± 0.72 a	14.35 ± 1.50 a	14.00 ± 0.45 a	1568.89 ± 222.68 c

Continued on next page

SUPPLEMENTARY MATERIALS

PHEs	AMD	IO	MS	CW	GS	WS	BM	VC	OW	OL	AW
Ni	197.82 ± 1.89 de	29.08 ± 0.32 ab	73.24 ± 1.50 bc	49.96 ± 1.06 abc	220.00 ± 22.01 e	158.30 ± 34.39 d	848.79 ± 27.45 f	24.90 ± 2.45 a	59.58 ± 1.85 abc	103.29 ± 3.26 c	103.34 ± 12.87 c
Pb	597.69 ± 81.54 c	bdl	0.18 ± 0.32 a	bdl	bdl	40.70 ± 27.76 ab	106.51 ± 7.74 b	0.42 ± 0.05 a	32.85 ± 10.93 ab	54.38 ± 8.73 ab	51.36 ± 8.27 ab
Sb	817.85 ± 32.29 e	5.50 ± 0.27 a	11.83 ± 0.47 a	15.34 ± 0.81 a	14.12 ± 1.13 a	310.24 ± 51.76 c	483.36 ± 28.70 d	14.19 ± 0.93 a	120.71 ± 21.16 b	128.32 ± 2.43 b	120.21 ± 15.61 b
Sc	29.93 ± 0.19 e	2.45 ± 0.03 a	0.80 ± 0.13 a	1.34 ± 0.04 a	1.30 ± 0.06 a	6.72 ± 1.44 b	14.92 ± 0.64 d	2.89 ± 0.23 a	9.67 ± 1.76 c	8.46 ± 0.30 bc	6.39 ± 0.74 b
Sn	443.91 ± 32.71 c	bdl	2.75 ± 2.41 a	bdl	bdl	251.51 ± 100.17 b	1224.84 ± 55.40 d	1.34 ± 1.86 a	74.47 ± 20.92 a	57.20 ± 4.02 a	43.94 ± 11.04 a
Th	8.25 ± 1.43 c	0.09 ± 0.04 a	0.34 ± 0.08 a	0.37 ± 0.09 a	0.22 ± 0.03 a	3.15 ± 0.74 b	6.89 ± 0.17 c	0.08 ± 0.02 a	3.60 ± 0.61 b	3.99 ± 0.15 b	4.63 ± 0.43 b
Tl	25.00 ± 2.19 f	1.04 ± 0.01 a	9.98 ± 0.19 e	2.14 ± 0.04 ab	3.57 ± 0.37 bc	2.26 ± 0.73 ab	6.22 ± 0.17 d	2.08 ± 0.01 ab	3.40 ± 0.07 bc	4.19 ± 0.18 bcd	5.49 ± 0.99 cd
U	14.55 ± 0.68 c	2.89 ± 0.15 ba	0.83 ± 0.02 a	39.31 ± 3.82 d	1.17 ± 0.15 ba	4.90 ± 1.04 b	3.00 ± 0.14 ba	0.83 ± 0.20 a	3.72 ± 0.23 ba	4.12 ± 0.11 ba	16.31 ± 1.95 c
V	54.92 ± 9.45 bc	bdl	bdl	bdl	bdl	85.29 ± 15.20 c	139.09 ± 12.21 d	bdl	42.78 ± 11.30 b	72.57 ± 9.86 bc	147.81 ± 28.19 d
Y	39.86 ± 0.29 f	0.03 ± 0.04 a	0.04 ± 0.02 a	0.05 ± 0.09 a	0.07 ± 0.04 a	8.27 ± 4.21 bc	26.01 ± 0.93 e	0.14 ± 0.02 a	7.24 ± 1.55 bc	16.11 ± 0.85 d	11.36 ± 0.40 c
Zn	32,208.45 ± 495.51 e	259.29 ± 14.41 a	2050.18 ± 43.75 b	45.44 ± 2.20 a	2297.60 ± 207.18 bc	1558.11 ± 267.60 b	11,592.10 ± 504.43 d	669.57 ± 60.86 a	655.22 ± 126.60 a	1581.21 ± 114.97 b	2869.79 ± 240.30 c

IO – Dry sludge rich in iron oxyhydroxide, MS – Dry marble sludge, CW – Carbonated waste of a peat exploitation, GS – Gypsum mining spoil, WS – Composted sewage sludge, BM – Bio-stabilised material of municipal solid waste, VC – Vermicompost from pruning and gardening, OW – Composted solid olive-mill by-product irrigated with drinking water, OL – Composted solid olive-mill by-product irrigated with leachates of the olive-mill, AW – Composted greenhouse plant waste, bdl – below detection limit. Letters represent significant differences among different waste materials for a same element (Kruskal-Wallis and Dunn tests, $p < 0.05$).

Table S3.2. *Spearman correlation coefficients relating PHEs concentrations in leachates (L) with waste properties (EC: electrical conductivity; OC: organic carbon content; $CaCO_3$: calcium carbonate content; CEC: cation exchange capacity; N_T/C_T: total concentrations of N and C; BR: basal respiration rate). See Table 3.1 for waste properties and Table S3.1 for PHEs concentration in the leachates. Correlations above ± 0.7 are in bold.*

	pH	**EC**	**OC**	**CEC**	**$CaCO_3$**	**N_T**	**C_T**	**Fe_T**	**P_A**	**SR**
pH$_{(L)}$	0.771**	-0.042	0.109	0.227	0.175	-0.031	-0.017	-0.280	0.473**	0.480**
EC$_{(L)}$	0.156	0.725**	0.696**	0.735**	-0.595**	0.814**	0.782**	-0.238	0.179	-0.133
As$_{(L)}$	-0.009	0.766**	0.601**	0.672**	-0.582**	0.809**	0.840**	-0.170	0.001	-0.311
Ba$_{(L)}$	0.376*	0.172	0.697**	0.818**	-0.390*	0.646**	0.644**	0.232	-0.048	0.208
Be$_{(L)}$	0.220	0.648**	0.501*	0.794**	-0.533**	0.707**	0.865**	0.110	0.097	-0.054
Bi$_{(L)}$	-0.063	0.733**	0.178	0.614**	-0.469**	0.721**	0.799**	-0.213	-0.031	-0.430*
Cd$_{(L)}$	-0.008	0.715**	0.420*	0.553**	-0.712**	0.491**	0.596**	0.363*	0.129	-0.142
Co$_{(L)}$	-0.061	0.631**	-0.038	-0.012	-0.207	0.051	0.231	-0.059	0.085	-0.484**
Cr$_{(L)}$	0.110	0.774**	0.529**	0.694**	-0.723**	0.763**	0.758**	0.039	0.064	-0.250
Cu$_{(L)}$	0.179	0.831**	0.483**	0.664**	-0.537**	0.703**	0.853**	-0.200	0.182	-0.150
In$_{(L)}$	-0.005	0.750**	0.421*	0.623**	-0.637**	0.768**	0.780**	-0.124	-0.070	-0.448**
Mn$_{(L)}$	-0.025	0.539*	0.285	0.265	-0.282	0.198	0.340	0.260	0.273	-0.026
Mo$_{(L)}$	0.134	0.649*	0.498**	0.540**	-0.421*	0.583**	0.577**	-0.166	0.483**	0.198
Ni$_{(L)}$	-0.182	0.831**	-0.023	0.257	-0.498**	0.306	0.423*	0.021	0.236	-0.459**
Pb$_{(L)}$	0.012	0.675**	0.614**	0.743**	-0.643**	0.800**	0.852**	0.056	-0.060	-0.210
Sb$_{(L)}$	-0.087	0.843**	0.504**	0.775**	-0.608**	0.857**	0.878**	-0.061	0.249	-0.138
Sc$_{(L)}$	-0.048	0.523**	0.528**	0.829**	-0.677**	0.809**	0.864**	0.293	-0.092	-0.020
Sn$_{(L)}$	-0.113	0.654**	0.507**	0.713**	-0.596**	0.856**	0.851**	-0.051	-0.149	-0.399*
Th$_{(L)}$	0.211	0.782**	0.402*	0.642**	-0.507**	0.663**	0.830**	-0.154	0.102	-0.173
Tl$_{(L)}$	0.371*	0.418*	0.114	0.082	0.008	0.065	0.368*	-0.304	-0.140	-0.301
U$_{(L)}$	0.337	0.450*	0.260	0.515**	-0.264	0.472**	0.434*	-0.338	0.542**	0.214
V$_{(L)}$	0.077	0.784**	0.535**	0.695**	-0.750**	0.755**	0.780**	0.032	0.065	-0.245
Y$_{(L)}$	0.046	0.665**	0.596**	0.779**	-0.624**	0.759**	0.865**	0.119	0.033	-0.121
Zn$_{(L)}$	-0.008	0.622**	0.157	0.082	-0.398*	0.134	0.301	0.098	-0.023	-0.407*

** Significant correlation $p < 0.05$, ** Significant correlation $p < 0.01$*

- CHAPTER 4 -

Table S4.1. *Principal component analysis (PCA) after Varimax rotation with Kaiser normalisation, including soil properties [pH, electrical conductivity (EC), organic carbon (OC) and calcium carbonate ($CaCO_3$) content, total N and C (N_T, C_T)], total (T), water-soluble (W), and EDTA-bioavailable (E) concentrations of PHEs, soil enzyme activities [dehydrogenase (DHA), β-glucosidase (BGA), acid phosphatase (APA), cellulase (CA)], survival and biomass of T. campestre, seed germination (SG) and root elongation (RE) of L. sativa. Only coefficients above ± 0.5 were included.*

	Comp. 1	**Comp. 2**	**Comp. 3**
pH	0.834	-0.505	
EC		0.879	
OC			0.868
$CaCO_3$	0.892		
N_T			0.938
C_T	0.683		0.694
As_T		0.939	
Cu_T		0.649	0.655
Pb_T		0.943	
Sb_T		0.638	-0.654
Zn_T			0.684
As_W	0.836		0.534
Cd_W	-0.615	0.728	
Cu_W	-0.612	0.760	
Pb_W			0.799
Sb_W	0.954		0.502
Zn_W	-0.609	0.722	
As_E	0.748		
Cd_E	-0.881		
Cu_E	-0.662	0.658	
Pb_E	-0.555	-0.768	
Sb_E	0.945		
Zn_E	-0.825		
DHA			0.873
BGA		-0.760	-0.570
APA	0.517		
CA		-0.518	
Survival		-0.520	-0.533
Biomass		-0.655	-0.533
SG			-0.613
RE	0.648	-0.682	
% ac. ex. var.	39.76	65.61	81.44

- CHAPTER 5 -

Table S5.1. *Values of pH in the resulting leachates after the treatment of artificial AMD (as a function of increased added acidity/volume of AMD added) by polluted soil (PS), unpolluted soil (US), and Technosols (T1-T6) (mean ± SD, n = 3).*

Soils	**Volume of AMD added** (ml)	**Added acidity** (mmol H^+ kg^{-1})	**pH**
PS	*0*	*0*	3.53 ± 0.03 e
	1	*6.03*	2.56 ± 0.03 d
	1.5	*9.04*	2.39 ± 0.03 c
	2	*12.05*	2.31 ± 0.02 b
	3	*18.08*	2.23 ± 0.02 a
	5	*30.13*	2.21 ± 0.02 a
US	*0*	*0*	6.91 ± 0.08 d
	1	*6.03*	6.08 ± 0.03 c
	2	*12.05*	6.04 ± 0.03 c
	4	*24.10*	5.99 ± 0.08 c
	6	*36.15*	5.53 ± 0.06 b
	10	*60.26*	3.49 ± 0.41 a
T1	*0*	*0*	8.19 ± 0.04 d
	2	*12.05*	6.52 ± 0.04 c
	6	*36.15*	6.32 ± 0.05 b
	10	*60.26*	6.13 ± 0.02 a
	15	*90.38*	6.28 ± 0.03 b
	20	*120.51*	6.12 ± 0.01 a
T2	*0*	*0*	7.70 ± 0.02 c
	2	*12.05*	6.31 ± 0.02 a
	6	*36.15*	6.37 ± 0.01 a
	10	*60.26*	6.18 ± 0.01 a
	15	*90.38*	6.69 ± 0.26 b
	20	*120.51*	6.26 ± 0.03 a
T3	*0*	*0*	8.10 ± 0.03 f
	2	*12.05*	6.47 ± 0.01 e
	6	*36.15*	6.33 ± 0.01 d
	10	*60.26*	6.09 ± 0.01 a
	15	*90.38*	6.22 ± 0.01 c
	20	*120.51*	6.16 ± 0.04 b

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Soils	**Volume of AMD added** (ml)	**Added acidity** (mmol H^+ kg^{-1})	**pH**
T4	*0*	*0*	8.01 ± 0.05 f
	2	*12.05*	6.23 ± 0.03 e
	6	*36.15*	6.02 ± 0.01 d
	10	*60.26*	5.80 ± 0.01 a
	15	*90.38*	5.97 ± 0.01 c
	20	*120.51*	5.89 ± 0.01 b
T5	*0*	*0*	7.79 ± 0.01 e
	2	*12.05*	6.12 ± 0.02 d
	6	*36.15*	6.01 ± 0.01 c
	10	*60.26*	5.81 ± 0.01 a
	15	*90.38*	5.99 ± 0.02 c
	20	*120.51*	5.92 ± 0.02 b
T6	*0*	*0*	7.78 ± 0.04 e
	2	*12.05*	6.11 ± 0.01 d
	6	*36.15*	6.04 ± 0.01 c
	10	*60.26*	5.83 ± 0.01 a
	15	*90.38*	6.00 ± 0.01 c
	20	*120.51*	5.93 ± 0.02 b

Letters represent significant differences between the different added acidities in each treatment (Kruskal-Wallis and Mann-Whitney U tests; $p < 0.05$).

Table S5.2. *Concentrations of some PHEs (As, Cd, Cu, and Zn) in the resulting leachates after the treatment of artificial AMD (as a function of increased added acidity) by polluted soil (PS), unpolluted soil (US), and Technosols (T1-T6) (mean ± SD, n = 3).*

Soils	**Added acidity** (mmol H^{+} kg^{-1})	**As** (mg l^{-1})	**Cd** (mg l^{-1})	**Cu** (mg l^{-1})	**Zn** (mg l^{-1})
PS	*0*	0.009 ± 0.001a	0.214 ± 0.043 a	0.941 ± 0.105 a	11.99 ± 2.95 a
	6.03	0.153 ± 0.006 a	0.402 ± 0.010 ab	31.50 ± 0.78 c	94.57 ± 1.70 d
	9.04	0.488 ± 0.087 a	0.365 ± 0.007 b	33.02 ± 0.65 cd	86.19 ± 2.34 bcd
	12.05	1.740 ± 0.443 b	0.416 ± 0.043 bc	35.56 ± 3.41 d	93.82 ± 8.53 cd
	18.08	4.943 ± 0.073 c	0.418 ± 0.003 bc	30.62 ± 1.12 c	84.13 ± 2.06 bc
	30.13	8.902 ± 0.255 d	0.448 ± 0.006 c	26.21 ± 0.55 b	78.30 ± 1.65 b
US	*0*	0.005 ± 0.003	0.001 ± 0.001 a	0.015 ± 0.004 a	0.021 ± 0.011 a
	6.03	0.051 ± 0.006	0.002 ± 0.001 a	0.061 ± 0.019 a	0.411 ± 0.077 a
	12.05	0.124 ± 0.117	0.007 ± 0.004 a	0.048 ± 0.002 a	1.664 ± 0.440 a
	24.10	0.016 ± 0.028	0.010 ± 0.004 a	0.043 ± 0.009 a	2.481 ± 0.299 a
	36.15	0.062 ± 0.033	0.040 ± 0.005 b	0.091 ± 0.006 a	13.06 ± 0.92 b
	60.26	bdl	0.217 ± 0.013 c	6.212 ± 2.946 b	61.63 ± 3.21 c
T1	*0*	0.048 ± 0.008	0.001 ± 0.001 a	0.037 ± 0.004 a	0.014 ± 0.004 a
	12.05	0.035 ± 0.023	0.002 ± 0.002 ab	0.375 ± 0.082 c	0.395 ± 0.041 b
	36.15	0.027 ± 0.019	0.001 ± 0.001 a	0.326 ± 0.012 bc	1.006 ± 0.036 c
	60.26	0.031 ± 0.013	0.003 ± 0.001 bc	0.358 ± 0.23 bc	2.492 ± 0.042 d
	90.38	0.018 ± 0.012	0.005 ± 0.001 c	0.280 ± 0.006 b	2.819 ± 0.178 e
	120.51	0.043 ± 0.011	0.008 ± 0.001 d	0.307 ± 0.012 bc	4.992 ± 0.117 f
T2	*0*	0.037 ± 0.002 a	0.001 ± 0.001 a	0.077 ± 0.002 a	0.036 ± 0.003 a
	12.05	0.266 ± 0.115 b	0.007 ± 0.002 b	0.817 ± 0.080 d	1.158 ± 0.079 b
	36.15	0.074 ± 0.033 a	0.004 ± 0.002 ab	0.655 ± 0.006 b	1.294 ± 0.047 b
	60.26	0.025 ± 0.022 a	0.005 ± 0.001 ab	0.754 ± 0.025 c	2.475 ± 0.005 c
	90.38	0.023 ± 0.019 a	0.005 ± 0.001 ab	0.574 ± 0.012 b	1.311 ± 0.600 b
	120.51	0.043 ± 0.013 a	0.008 ± 0.001 b	0.603 ± 0.020 b	3.884 ± 0.114 d
T3	*0*	0.071 ± 0.003 b	bdl	0.024 ± 0.005 a	0.004 ± 0.001 a
	12.05	0.079 ± 0.017 b	0.004 ± 0.001 ab	0.160 ± 0.008 b	0.212 ± 0.047 a
	36.15	0.044 ± 0.026 ab	0.002 ± 0.001 a	0.140 ± 0.017 b	0.877 ± 0.046 a
	60.26	0.022 ± 0.025 a	0.005 ± 0.001 bc	0.236 ± 0.010 d	2.899 ± 0.179 b
	90.38	0.001 ± 0.001 a	0.006 ± 0.001 bc	0.205 ± 0.001 c	3.004 ± 0.072 b
	120.51	0.036 ± 0.015 ab	0.008 ± 0.002 c	0.247 ± 0.010 d	3.926 ± 1.030 b

Continued on next page

Soils	**Added acidity** (mmol H^+ kg^{-1})	**As** (mg l^{-1})	**Cd** (mg l^{-1})	**Cu** (mg l^{-1})	**Zn** (mg l^{-1})
T4	*0*	0.067 ± 0.016 a	0.001 ± 0.001 a	0.075 ± 0.021 a	0.039 ± 0.021 a
	12.05	0.372 ± 0.233 b	0.010 ± 0.005 b	0.640 ± 0.033 d	1.407 ± 0.114 b
	36.15	0.090 ± 0.038 a	0.007 ± 0.003 ab	0.265 ± 0.037 c	3.131 ± 0.188 c
	60.26	0.029 ± 0.016 a	0.012 ± 0.001 bc	0.190 ± 0.009 b	8.046 ± 0.127 d
	90.38	0.030 ± 0.012 a	0.013 ± 0.001 bc	0.121 ± 0.006 a	7.101 ± 1.204 d
	120.51	0.037 ± 0.016 a	0.018 ± 0.001 c	0.129 ± 0.002 a	9.961 ± 0.278 e
T5	*0*	0.039 ± 0.004 ab	0.001 ± 0.001	0.086 ± 0.010 a	0.051 ± 0.005 a
	12.05	0.183 ± 0.024 c	0.014 ± 0.013	0.474 ± 0.213 b	1.055 ± 0.767 b
	36.15	0.063 ± 0.008 b	0.004 ± 0.001	0.211 ± 0.012 a	1.359 ± 0.069 b
	60.26	0.034 ± 0.023 ab	0.007 ± 0.001	0.187 ± 0.011 a	4.051 ± 0.154 c
	90.38	0.010 ± 0.003 a	0.007 ± 0.001	0.164 ± 0.010 a	3.540 ± 0.105 c
	120.51	0.045 ± 0.018 ab	0.010 ± 0.001	0.165 ± 0.004 a	5.091 ± 0.711 d
T6	*0*	0.027 ± 0.001	bdl	0.014 ± 0.001 a	0.005 ± 0.001 a
	12.05	0.056 ± 0.033	0.001 ± 0.001 a	0.028 ± 0.003 b	0.633 ± 0.041 a
	36.15	0.056 ± 0.030	0.007 ± 0.001 b	0.030 ± 0.002 b	3.079 ± 0.114 b
	60.26	0.032 ± 0.013	0.017 ± 0.001 c	0.034 ± 0.011 b	6.849 ± 0.254 c
	90.38	0.026 ± 0.011	0.017 ± 0.001 c	0.031 ± 0.001 b	6.425 ± 0.179 c
	120.51	0.052 ± 0.009	0.022 ± 0.002 d	0.040 ± 0.002 b	7.751 ± 1.522 c

bdl – below detection limit. Letters represent significant differences between the different added acidities in each treatment (Kruskal-Wallis and Mann-Whitney U tests; $p < 0.05$).

- CHAPTER 7 -

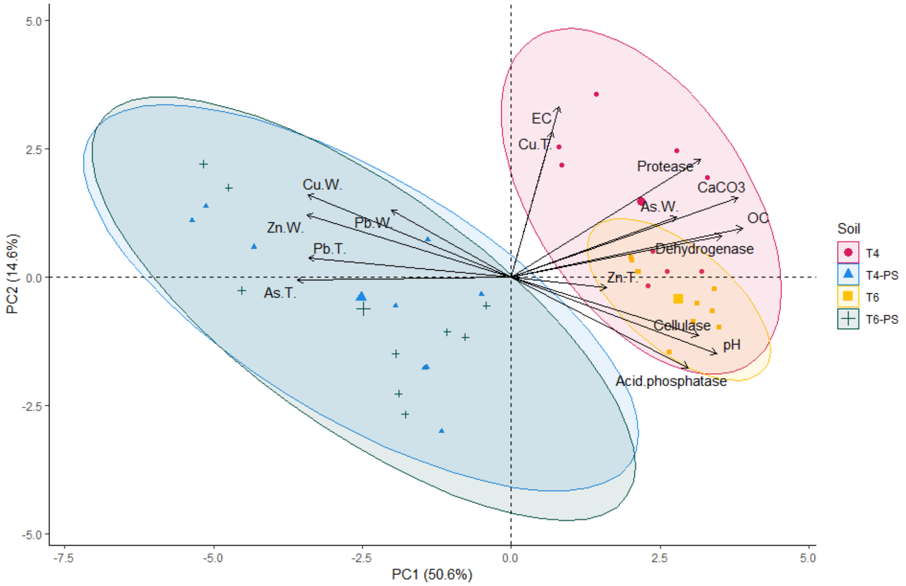

Figure S7.1. *Principal component analysis (PCA) including soil properties (pH, calcium carbonate [$CaCO_3$] content, electrical conductivity [EC], and organic carbon [OC] content), total (T) and water-soluble (W) contents of As, Cu, Pb, and Zn, and soil enzymatic activities [dehydrogenase, cellulase, acid phosphatase, and protease].*

Table S7.1. *List with the number of individuals of each plant species developed on Technosols T4 and T6 after a year of their installation.*

T4	**T6**
Replicate 1	
Spergularia rubra (L.) J. Presl & C. Presl. (51) *Anthemis arvensis* L. (48) *Brassica oxyrrhina* Willk. (Coss.) (33) *Vulpia membranacea* (L.) Dumort. (32) *Trifolium arvense* L. (28) *Lamarckia aurea* (L.) Moench (25) *Echium plantagineum* L. (8) *Ornithopus sativus* subsp. *isthmocarpus* (Coss.) Dostál (3) *Trifolium campestre* Schreb. (2) *Silene micropetala* Lag. (1) *Scorpiurus vermiculatus* L. (1)	*Brassica oxyrrhina* Willk. (Coss.) (98) *Echium plantagineum* L. (37) *Anthemis arvensis* L. (5)
Replicate 2	
Brassica oxyrrhina Willk. (Coss.) (45) *Anthemis arvensis* L. (38) *Trifolium arvense* L. (32) *Vulpia membranacea* (L.) Dumort. (25) *Echium plantagineum* L. (18) *Lamarckia aurea* (L.) Moench (9) *Hordeum leporinum* Link (5) *Trifolium campestre* Schreb. (4) *Ornithopus sativus* subsp. *isthmocarpus* (Coss.) Dostál (2) *Leontodon longirostris* (Finch & P. D. Sell) Talavera (1)	*Echium plantagineum* L. (46) *Anthemis arvensis* L. (34) *Brassica oxyrrhina* Willk. (Coss.) (27) *Vulpia membranacea* (L.) Dumort. (15) *Trifolium arvense* L. (7) *Hirschfeldia incana* (L.) Lagr.-Foss. (7) *Hordeum murinum* L. subsp. *leporinum* (Link) Arcang. (1)
Replicate 3	
Vulpia membranacea (L.) Dumort. (56) *Anthemis arvensis* L. (36) *Spergularia rubra* (L.) J. Presl & C. Presl. (29) *Lamarckia aurea* (L.) Moench (24) *Brassica oxyrrhina* Willk. (Coss.) (9) *Trifolium campestre* Schreb. (8) *Ornithopus sativus* subsp. *isthmocarpus* (Coss.) Dostál (7) *Echium plantagineum* L. (2) *Silene micropetala* Lag. (1)	*Vulpia membranacea* (L.) Dumort. (75) *Echium plantagineum* L. (54) *Anthemis arvensis* L. (38) *Lamarckia aurea* (L.) Moench (10) *Chrysanthemum coronarium* L. (8) *Trifolium arvense* L. (7) *Bromus hordeaceus* L. (6)

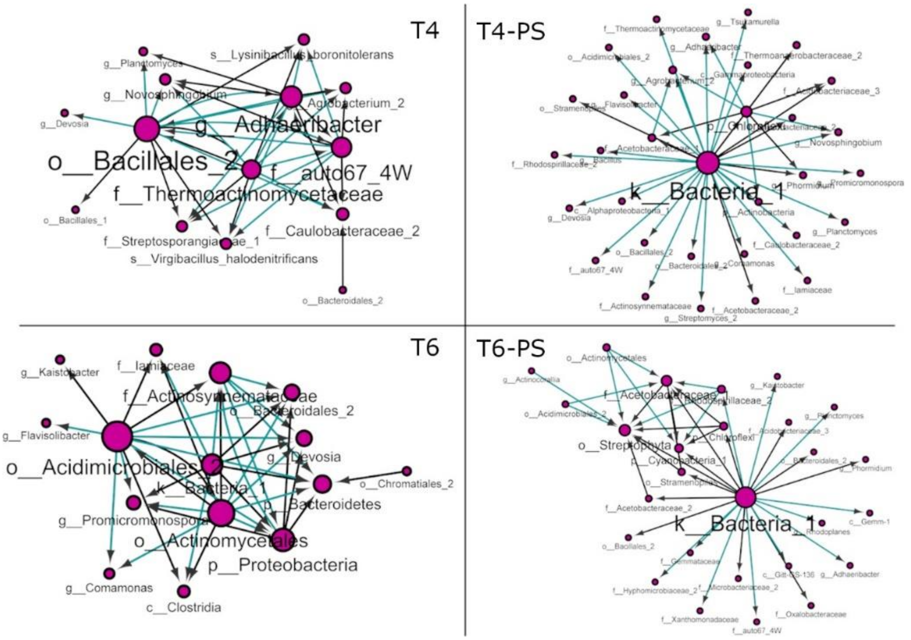

Figure S7.2. *Microbial-interaction consensus networks for Technosols (T4 and T6) and polluted soil samples (T4-PS and T6-PS) at the two sites. Inference of microbial interactions using Lotka-Volterra models for 16SrRNA microbial abundance profiles were calculated in METAMIS and consensus networks [P (concordant pairs) = 0.9]. Consensus networks were drawn and analysed in Cytoscape. The turquoise (or black) arrow represents a positive (or negative) correlation between the species.*


SCHOTT
DURAN
100 ml
APPROX. VOL.
100
75
50
Made in Germany
ENDO glassware
50mm
Lenz
DIN
NS 29/32

Soil organic matter determination: Sand bath heating, Department of Soil Science - UGR

Granada, Spain

June 2020

Antonio Aguilar Garrido

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COMMONLY USED ACRONYMS AND SYMBOLS

COMMONLY USED ACRONYMS

Abbreviation	Definition
PHEs / PHE	Potentially harmful elements / Potentially harmful element
IPB	Iberian Pyrite Belt
GGC	Guadiamar Green Corridor
AMD	Acid mine drainage
PS	Polluted soils
RS	Recovered soils
US	Unpolluted soils
GW	Gossan waste
IO	Dry sludge rich in iron oxyhydroxides
MS	Dry sludge from marble cutting and polishing
CW	Carbonated waste from peat bog mining
GS	Gypsum mining spoil
WS	Composted sewage sludge
BM	Bio-stabilised material from municipal solid waste
VC	Vermicompost from pruning and gardening waste
OW	Composted solid olive-mill by-product irrigated with drinking water
OL	Composted solid olive-mill by-product irrigated with liquid olive-mill waste
AW	Compost from agricultural greenhouse waste
T1	Technosol 1: 60% PS + 2% IO + 20% CW + 18% OL
T2	Technosol 2: 60% PS + 2% IO + 20% CW + 18% WS
T3	Technosol 3: 60% PS + 2% IO + 20% CW + 18% VC
T4	Technosol 4: 60% PS + 2% IO + 20% MS + 18% OL
T5	Technosol 5: 60% PS + 2% IO + 20% MS + 18% WS
T6	Technosol 6: 60% PS + 2% IO + 20% MS + 18% VC
TC50	Technosol TC50: Gossan waste + Waste mixture at 50 g kg^{-1}
TC75	Technosol TC75: Gossan waste + Waste mixture at 75 g kg^{-1}
EC	Electrical conductivity
OM	Organic matter
OC	Organic carbon
SOM	Soil organic matter
$CaCO_3$	Calcium carbonate
CEC	Cation exchange capacity
C_T	Total concentration of C
N_T	Total concentration of N

Continued on next page

COMMONLY USED ACRONYMS

Abbreviation	**Definition**
T	Total fraction (as subscript to refer to PHEs content)
W	Water-soluble fraction (as subscript to refer to PHEs content)
E	EDTA-extracted bioavailable fraction (as subscript to refer to PHEs content)
DHA	Dehydrogenase activity
BGA	β-glucosidase activity
CLA	Cellulase activity
PA	Protease activity
UA	Urease activity
APA	Acid phosphatase activity
SPAD	Soil plant analysis development
PAR	Photosynthetically active radiation
EDTA	Ethylenediaminetetraacetic acid
ICP-OES	Inductively coupled plasma optical emission spectrometry
ICP-MS	Inductively coupled plasma optical mass spectrometry
XRF	X-ray fluorescence
FW	Fresh weight
DW	Dry weight
PCA	Principal component analysis
SD	Standard deviation
n.a.	Not analysed
bdl	Below detection limit
FAO	Food and Agriculture Organization of the United Nations
ITPS	Intergovernmental Technical Panel on Soils
IUSS	International Union of Soil Sciences
WRB	World Reference Base
ISO	International Organization for Standardization
BOE	Boletín Oficial del Estado
BOJA	Boletín Oficial de la Junta de Andalucía
OECD	Organization for Economic Co-operation and Development
EEA	European Environment Agency
EPA	Environmental Protection Agency
SCS	Soil Conservation Service
USDA	United States Department of Agriculture

* More specific abbreviations are explained throughout the PhD thesis.

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CONTRIBUTIONS

Teaching | *Docencia*

Teaching in subjects of the Biology and Environmental Sciences Degrees.

- ☐ **Soil Science.** *Environmental Sciences (2° course).* Laboratory practices (soil analysis: sample treatment, pH, EC, OC, $CaCO_3$, texture, colour, structure, exchangeable bases and CEC, moisture, and useful water). Assigned hours: 20 (2019/2020), 20 (2020/2021), 60 (2021/2022).
- ☐ **The physical environment.** *Biology (1° course).* Laboratory practices (soil analysis: sample treatment, pH, EC, OC, $CaCO_3$, texture, colour, and structure). Assigned hours: 25 (2019/2020), 25 (2020/2021).
- ☐ **Soil evaluation, management, conservation, and restoration techniques.** *Environmental Sciences (3° course).* Field practices (description of recovery techniques for mining sites: erosion control, vegetation establishment); and measurement of PHEs in field by X-ray fluorescence) and computer-based practices on soil evaluation (USDA agrological classes, Riquier) and land use change assessment. Assigned hours: 8 (2019/2020), 8 (2020/2021).
- ☐ **Methods for the study of the natural environment.** *Biology (1° course).* Soil mapping studies by cabinet practices (photointerpretation, analysis of the soil-forming factors and description of different types of soils) and field practices (natural environment and soil profiles description). Assigned hours: 7 (2019/2020), 7 (2020/2021).

Workshops and seminars.

- ☐ Conducting the activity "Soil: the great unknown in the fight against climate change" within the programme of activities given to the students of the University of Granada selected in the **Arqus Challenge-Based Learning Programme "Rethinking Climate Change" – Pretraining 2020**. Organiser: Arqus European University Alliance. Date: November 2020.
- ☐ Short talk given in the subject **Soil Restoration of Master' Degree in Conservation, Management and Restoration of Biodiversity**, focused on the generation of Technosols from organic and inorganic waste for their application in remediation of soil and water affected by PHEs (As, Pb, Sb, and Zn). Date: February 2021.

Supervision of students' research | *Mentorización de TFG y TFM*

Course 2023/2024 (In progress)

- ☐ **Evaluación de la toxicidad potencial de Tecnosoles mediante la aplicación de un bioensayo con lombriz de tierra (*Eisenia andrei*).** *Degree in Biology.* Student: Elena Beltrán Gallego. Tutors: Ana Romero Freire, Francisco José Martín Peinado.

- ☐ **Fitorremediación de suelos contaminados mediante la aplicación de Tecnosoles.** *Master's Degree of Advances in Agricultural Biology and Aquaculture.* Student: Laura Mérida Sánchez. Tutors: Ana Romero Freire, Francisco José Martín Peinado.

Course 2022/2023

- ☐ **Evaluación de la carga crítica de Tecnosoles para el tratamiento de aguas ácidas mineras.** *Degree in Environmental Sciences.* Student: Laura Mérida Sánchez. Tutors: Ana Romero Freire, Francisco José Martín Peinado. Mark: 9.6 (Defence: 22/06/2023).
- ☐ **Evaluación de fitoextracción de elementos tecnológicamente críticos.** *Master's Degree in Conservation, Management and Restoration of Biodiversity.* Student: Jorge Fernández Garrido. Tutors: Ana Romero Freire, Francisco Javier Martínez Garzón. Mark: 9 (Defence: 20/09/2023).
- ☐ **Evaluación de Tecnosoles en la descontaminación de suelos y su influencia sobre la fitotoxicidad de metales pesados.** *Master's Degree of Advances in Agricultural Biology and Aquaculture.* Student: Mateo González Quero. Tutors: Begoña Blasco León, Manuel Sierra Aragón. Mark: 9.9 (Defence: 20/07/2023).

Course 2021/2022

- ☐ **Uso de Tecnosoles en la recuperación de suelos contaminados por actividades mineras.** *Master's Degree in Conservation, Management and Restoration of Biodiversity.* Student: Ignacio Montero Acosta. Tutors: Manuel Sierra Aragón, Ana Romero Freire. Mark: 9.7 (Defence: 20/07/2022).

Course 2020/2021

- ☐ **Aplicación de Tecnosoles para la recuperación de suelos contaminados.** *Master's Degree of Advances in Agricultural Biology and Aquaculture.* Student: Sheila Ocón Martín. Tutors: Francisco José Martín Peinado, Ana Romero Freire. Mark: 9.0 (Defence: 20/09/2021).

Course 2019/2020

- ☐ **Evolución de los suelos del Corredor Verde del Guadiamar dos décadas después del accidente de la mina de Aznalcóllar.** *Master's Degree in Advances in Food Quality and Technology.* Student: Habib Tayoub. Tutors: Manuel Sierra Aragón. Mark: 8.5 (Defence: 07/2020).
- ☐ **Revisión bibliográfica sobre la evolución de los suelos contaminado con As, desde el accidente medioambiental en Aznalcóllar.** *Degree in Environmental Sciences.* Student: Inmaculada Moreno Sáez. Tutor: Francisco Javier Martínez Garzón. Mark: Excellent (Defence: 12/2020).

Research stay | Estancia de investigación

- ☐ Short-term stay at the research centre **LEAF - Linking Landscape, Environment, Agriculture and Food, Instituto Superior de Agronomia, Universidade de Lisboa (Portugal)**. Length: 3 months (15/09/2021 – 14/12/2021). Funding: Spanish mobility grant FPU. Contact: Dr. Maria Manuela Abreu (manuelaabreu@isa.ulisboa.pt).
  Analysis of enzymatic activities in polluted soils treated with Technosols and study of the remediation process of mining waste and salinity-affected soils using Technosols in greenhouse experiments. Collaboration with an international research group.

Publications | Publicaciones

During this time, part of the results obtained in this thesis and in previous experiences have been published in the following research articles and book chapters.

Research articles

- ☐ González-Quero, Mateo*; **Aguilar-Garrido, Antonio***; Paniagua-López, Mario; García-Huertas, Carmen; Sierra-Aragón, Manuel; Blasco, Begoña. (2024). **Phytotoxicity reduction in polluted soils based on the use of Technosols and L. sativa as bioindicator.** Environmental Technology & Innovation (Under review) * Both are first authors.
  JIF (2022): 7.1 (Environmental Sciences: 45/275 – Q1)
- ☐ **Aguilar-Garrido, Antonio**; Purswani, Jessica; Romero-Freire, Ana; Martín-Peinado, Francisco José; Pozo, Clementina; Sierra-Aragón, Manuel. (2024). **Enhancing microbial biodiversity and vegetation growth in polluted soils by Technosols integration.** Pedosphere (Under review)
  JIF (2022): 5.7 (Soil Science: 9/37 – Q1)
- ☐ **Aguilar-Garrido, Antonio**; Romero-Freire, Ana; Paniagua-López, Mario; Martínez-Garzón, Francisco Javier; Martín-Peinado, Francisco José; Sierra-Aragón, Manuel. (2023). **Technosols Derived from Mining, Urban, and Agro-Industrial Waste for the Remediation of Metal(loid)-Polluted Soils: A Microcosm Assay.** Toxics, 11(10):854. doi: 10.3390/toxics11100854
  JIF (2022): 4.6 (Toxicology: 15/94 – Q1)
- ☐ **Aguilar-Garrido, A.**; Paniagua-López, M.; Sierra-Aragón, M.; Martínez Garzón, F. J.; Martín-Peinado, F. J. (2023). **Remediation potential of mining, agro-industrial, and urban wastes against acid mine drainage.** Scientific Reports, 13:12120. doi: 10.1038/s41598-023-39266-4
  JIF (2022): 4.6 (Multidisciplinary Sciences: 22/73 – Q2)

- **Aguilar-Garrido, Antonio**; Reyes-Martín, Marino Pedro; Vidigal, Patrícia; Abreu, Maria Manuela. (2023). **A green solution for the rehabilitation of lands: the case of Lablab purpureus (L.) Sweet grown in Technosols.** Plants, 12(14):2682. doi: 10.3390/plants12142682

  JIF (2022): 4.5 (Plant Sciences: 43/239 – Q1)

- **Aguilar-Garrido, Antonio**; Paniagua-López, Mario; Romero-Freire, Ana; Martínez Garzón, Francisco Javier; Fernández Ondoño, Emilia; Martín Peinado, Francisco José. (2022). **Short-term evolution of physico-chemical properties of Technosols made from contaminated soils by pyritic sludge.** Revista de Ciências Agrárias, 45(4):627-631. doi: 10.19084/rca.28744

  Journal of Sociedade de Ciências Agrárias de Portugal (not indexed in JCR)

- **Aguilar-Garrido, Antonio**; García-Carmona, Minerva; Sierra Aragón, Manuel; Martín Peinado, Francisco José; Martínez Garzón, Francisco Javier. (2022). **Carbonated waste valorisation from a peat bog exploitation in the treatment of arsenic-polluted waters.** International Journal of Environmental Science and Technology, 19:3457-3468. doi: 10.1007/s13762-021-03445-5

  JIF (2022): 3.1 (Environmental Sciences: 151/275 – Q3)

- **Aguilar-Garrido, A.**; Romero-Freire, A.; García-Carmona, M.; Martín Peinado, F.J.; Sierra Aragón, M.; Martínez Garzón, F.J. (2020). **Arsenic Fixation in Polluted Soils by Peat Applications.** Minerals, 10(11):968. doi: 10.3390/min10110968

  JIF (2020): 2.644 (Q2, Mining & Mineral Processing: 9/21 – Q2)

Book chapters

- **Aguilar-Garrido, A.**; Paniagua-López, M.; Martínez Garzón, F. J.; Martín Peinado, F. J. (2023). **Evaluación de la efectividad de Tecnosoles en la recuperación de suelos contaminados y el establecimiento de vegetación espontánea.** In Arricibita Videgain et al. (eds.). Libro de Actas de la XXXIII Reunión Nacional de Suelos de la Sociedad Española de la Ciencia del Suelo (pp. 18-19). Pamplona, Spain (September 2023): XXXIII Reunión Nacional de Suelos. ISBN 978-84-9769-395-0 [Chapter]

  Poster in XXXIII Reunión Nacional de Suelos (RENS23).

- **Aguilar-Garrido, Antonio**; Martínez Garzón, Francisco Javier; Paniagua-López, Mario; Sierra Aragón, Manuel; Martín Peinado, Francisco José. (2021). **Technosols made from organic/inorganic wastes for soil remediation: A microcosm assay.** In Pereira et al. (eds.). Solos e Desenvolvimento Sustentável. Desafios e Soluções (pp. 255-256). Porto, Portugal: Universidade do Porto Press. ISBN 978-989-746-313-6 [Chapter]

  Poster in Congresso Ibérico "Solo e Desenvolvimento Sustentável: Desafios e Soluções" (CISDS21).

- ❑ **Aguilar Garrido, Antonio**; Martín Peinado, Francisco José; Sierra Aragón, Manuel; Martínez Garzón, Francisco Javier. (2021). **Efectos sobre la capacidad de adsorción y la toxicidad del arsénico de estériles de mina ricos en óxidos de hierro.** In Almendro Candel, M.B. and Jordán Vidal, M.M. (eds.). El suelo: Clave para una gestión ambiental sostenible en un escenario de cambio global. Libro de resúmenes del IX Simposio Nacional sobre el Control de la Degradación y Recuperación de Suelos (pp. 507-510). Elche, Spain: IX Simposio Nacional sobre Control de la Degradación y Recuperación de Suelos. ISBN 978-84-18177-09-5. [Chapter]

  Oral communication in IX Simposio Nacional sobre Control de la Degradación y Recuperación de Suelos.

Participation in national and international conferences | Participación en congresos nacionales e internacionales

Likewise, results of the experiences developed have been regularly disseminated in different national and international congresses as posters and oral communications.

- ❑ **Aguilar-Garrido, A.**; Paniagua-López, M.; Sierra-Aragón, M.; Martínez Garzón, F. J.; Romero-Freire, A.; and Martín-Peinado, F. J. (2023). **Soil Enzyme Activity in Polluted Soils Treated With Waste-derived Technosols**. SETAC Europe 33rd Annual Meeting, Dublin, Ireland + Online, 30 April – 4 May 2023. 4.04.P-Th260. * [Paper, Poster]
- ❑ **Aguilar-Garrido, A.**; Paniagua-López, M.; Romero-Freire, A.; Sierra-Aragón, M.; Martínez Garzón, F. J.; and Martín-Peinado, F. J. (2023). **Biological assessment of in-situ rehabilitation of polluted soils by waste-derived Technosols**. EGU General Assembly 2023, Vienna, Austria, 23–28 April 2023, EGU23-226, doi: 10.5194/egusphere-egu23-226 ** [Oral Presentation]
- ❑ **Aguilar-Garrido, A.**; Paniagua-López, M.; Romero-Freire, A.; Martínez Garzón, F. J.; Fernández Ondoño, E.; and Martín-Peinado, F. J. (2022). **Short-term evolution of physico-chemical properties of Technosols made from contaminated soils by pyritic sludge.** In Sociedade Portuguesa da Ciência do Solo (SPCS) (eds.). Comunicações do IX Congresso Ibérico das Ciências do Solo. O solo, recurso estratégico para uma sociedade sustentável (pp. 486-489). Oeiras-Lisbon, Portugal (22-24 June 2022): IX Congresso Ibérico das Ciências do Solo. * [Paper, Poster]
- ❑ **Aguilar-Garrido, A.**; Vidigal, P.; Caperta, A. D.; and Abreu, M. M. (2022). **Development capacity of a biodiverse pasture on Technosols for the rehabilitation of marginal lands (saline soils and mining waste)**. EGU General Assembly 2022, Vienna, Austria, 23–27 May 2022, EGU22-121, doi: 10.5194/egusphere-egu22-121 ** [Oral Presentation]

- ☐ **Aguilar-Garrido, A.**; Romero-Freire, A.; Paniagua-López, M.; Martínez Garzón, F. J.; and Martín-Peinado, F. J. (2022). **Changes in the solubility and potential toxicity of metal(loid)s in soils treated with Technosols**. EGU General Assembly 2022, Vienna, Austria, 23-27 May 2022, EGU22-8337, doi: 10.5194/egusphere-egu22-8337 ** [Oral Presentation]
- ☐ **Aguilar-Garrido, A.**; Martínez Garzón, F. J.; Paniagua-López, M.; Sierra Aragón, M.; Fernández Ondoño, E.; Martín Peinado, F. J. (2021). **Potential of mining, agro-industrial, and urban wastes for the remediation of acidic mine water.** EGU General Assembly 2021, online, 19-30 April 2021, EGU21-7093, doi: 10.5194/egusphere-egu21-7093 ** [Oral Presentation]
- ☐ **Aguilar-Garrido, A.**; Martín-Peinado, F. J.; Sierra-Aragón, M.; García-Carmona, M.; Martínez-Garzón, F. J. (2019). **Decontamination of waters with arsenic through peat waste.** In Sociedad Española de la Ciencia del Suelo (SECS) (eds.). Libro de Resúmenes de la XXXII Reunión Nacional de Suelos (pp. 10). Sevilla, Spain (September 2019): XXXII Reunión Nacional de Suelos. *** [Paper, Oral presentation, Poster]

Note: * Poster presentation, ** Oral presentation, *** Poster and Oral presentation

Collaborations | Colaboraciones

Research projects

- ☐ Team member "**Restauración de suelos contaminados por metales pesados: Una estrategia basada en la revalorización de residuos y la biorremediación**". Funding: Spanish Ministry of Science, Innovation and Universities. Participants: Estación Experimental del Zaidín - CSIC and Department of Soil Science and Agricultural Chemistry - UGR. Main researchers: Inmaculada García Romera, Francisco José Martín Peinado. Amount financed: 217.800,00 €. Ref: RTI 2018-094327-B-I00.

Research articles

- ☐ Paniagua-López, Mario; García-Robles, Helena; **Aguilar-Garrido, Antonio**; Romero-Freire, Ana; Lorite, Juan; Sierra-Aragón, Manuel. (2024). **Vegetation establishment in soils polluted by heavy metals after assisted natural remediation.** Plant and Soil, 497:257-275. doi: 10.1007/s11104-024-06521-0

  JIF (2022): 4.9 (Plant Sciences: 37/239 – Q1)
- ☐ Pereira Melloni, Eliane Guimaraes; Melloni, Rogerio; Pastor-Jáuregui, Rocío; **Aguilar-Garrido, Antonio**; Martín Peinado, Francisco José. (2023). **Microbiological indicators as sensitive indicators in the assessment of areas contaminated by heavy metals.** Soil Research, 61(7):663-673. doi: 10.1071/SR23012

  JIF (2022): 1.6 (Soil Science: 33/37 – Q4)

- ☐ Paniagua-López, Mario; **Aguilar-Garrido, Antonio**; Contero-Hurtado, José; García-Romera, Inmaculada; Sierra-Aragón, Manuel; Romero-Freire, Ana. (2023). **Ecotoxicological Assessment of Polluted Soils One Year after the Application of Different Soil Remediation Techniques.** Toxics, 11(4):298. doi: 10.3390/toxics11040298

  JIF (2022): 4.6 (Toxicology: 15/94 – Q1)

- ☐ Paniagua-López, Mario; García-Robles, Helena; **Aguilar-Garrido, Antonio**; Romero-Freire, Ana; Lorite, Juan; Sierra-Aragón, Manuel. (2022). **Soil and vegetation recovery in an area affected by residual pollution after remediation measures.** Revista de Ciências Agrárias, 45(4):726-730. doi: 10.19084/rca.28864

  Journal of Sociedade de Ciências Agrárias de Portugal (not indexed in JCR).

- ☐ Pastor-Jáuregui, Rocío; Paniagua-López, Mario; **Aguilar-Garrido, Antonio**; Martínez-Garzón, Francisco Javier; Romero-Freire, Ana; Sierra-Aragón, Manuel. (2022). **Ecotoxicological risk assessment in soils contaminated by Pb and As years after a mining spill.** Journal of Contaminant Hydrology, 251:104100. doi: 10.1016/j.jconhyd.2022.104100

  JIF (2022): 3.6 (Environmental Sciences: 126/275 – Q2)

- ☐ Pastor-Jáuregui, Rocío; Paniagua-López, Mario; **Aguilar-Garrido, Antonio**; Martín-Peinado, Francisco José; Sierra-Aragón, Manuel. (2021). **Long-term assessment of remediation treatments applied to an area affected by a mining spill.** Land Degradation & Development, 32(18):2481-2492. doi: 10.1002/ldr.3911

  JIF (2021): 4.377 (Soil Science: 12/39 – Q2)

Book chapters

- ☐ Paniagua-López, M.; **Aguilar-Garrido, A.**; Martínez-Garzón, F. J.; Martín-Peinado, F. J.; Sierra-Aragón, M.; Romero-Freire, A. (2023). **Riesgos ambientales debido a la contaminación por gadolinio en suelos con cultivos de regadío.** In Arricibita Videgain et al. (eds.). Libro de Actas de la XXXIII Reunión Nacional de Suelos de la Sociedad Española de la Ciencia del Suelo (pp. 108-109). Pamplona, Spain (September 2023): XXXIII Reunión Nacional de Suelos. ISBN 978-84-9769-395-0 [Chapter]

  Poster in XXXIII Reunión Nacional de Suelos (RENS23).

Conference communications

- ☐ Abreu, M. M.; **Aguilar-Garrido, A.** ; Vidigal, P.; and Caperta, A. D. (2023). **Improvement of physical, chemical and biological properties of saline soils and gossan waste through integrated biotechnological approach: Technosols and pasture development**. EGU General Assembly 2023, Vienna, Austria, 23–28 April 2023, EGU23-10077, doi: 10.5194/egusphere-egu23-10077 * [Poster]

- ☐ Paniagua-López, M.; García-Robles, H.; **Aguilar-Garrido, A.**; Romero-Freire, A.; Lorite, J.; and Sierra-Aragón, M. (2022). **Soil and vegetation in an area affected by residual pollution after remediation measures.** In Sociedade Portuguesa da Ciência do Solo (SPCS) (eds.). Comunicações do IX Congresso Ibérico das Ciências do Solo. O solo, recurso estratégico para uma sociedade sustentável (pp. 518-521). Oeiras-Lisbon, Portugal (22-24 June 2022): IX Congresso Ibérico das Ciências do Solo. * [Paper]
- ☐ Paniagua-López, M.; Pastor-Jáuregui, R.; **Aguilar-Garrido, A.**; Romero-Freire, A.; and Sierra-Aragón, M. (2022). **Ecotoxicological risk assessment of the Guadiamar Green Corridor soils 20 years after the Aznalcóllar mining accident.** EGU General Assembly 2022, Vienna, Austria, 23–27 May 2022, EGU22-12397, doi: 10.5194/egusphere-egu22-12397 **
- ☐ Pastor-Jáuregui, R.; **Aguilar-Garrido, A.**; Martín-Peinado, F. J.; Sierra-Aragón, M. (2019). **Mobility of potentially harmful elements in soils affected by residual contamination.** In Sociedad Española de la Ciencia del Suelo (SECS) (eds.). Libro de Resúmenes de la XXXII Reunión Nacional de Suelos (pp. 8-9). Sevilla, Spain (September 2019): XXXII Reunión Nacional de Suelos. * [Paper, Poster]

Note: * Poster presentation, ** Oral presentation

Grants and awards | Ayudas y premios

- ☐ **Roland Schlich Travel Support 2023.**
  Registration grant to attend the General Assembly 2023 of the European Geosciences Union held in Vienna in Austria, 23–28 April 2023. Organiser: European Geosciences Union (EGU). Grant date: February 2023.
- ☐ **Roland Schlich Travel Support 2022.**
  Registration grant to attend the General Assembly 2022 of the European Geosciences Union held in Vienna in Austria, 23–27 May 2022. Organiser: European Geosciences Union (EGU). Grant date: April 2022.
- ☐ **Grants for Attendance at Conferences – UGR 2022.**
  Travel grant to attend the European Geosciences Union General Assembly 2022 (Vienna Austria, 23–27 May 2022). Organiser: School of Science, Technology and Engineering Doctoral Studies - UGR. Grant date: November 2022. Competitive concurrence.
- ☐ **Mobility grant of the University Teacher Training Program FPU 2021.**
  Short-term grant from the Spanish Ministry of Science, Innovation and Universities for a 3-month stay (September – December 2021) at LEAF - Linking Landscape, Environment, Agriculture and Food research centre (Instituto Superior de Agronomía, University of Lisbon). Grant date: October 2021. Competitive concurrence. Ref: EST21/00734.

- ☐ **Collaboration grant of the XXXII Reunión Nacional De Suelos 2019.**
  Registration grant to attend the XXXII Reunión Nacional De Suelos (RENS 2019) held in Seville in Spain, 10-13 September 2029. Organiser: Spanish Society of Soil Sciences (SECS). Grant date: September 2019.
- ☐ **Pre-doctoral contract of the University Teacher Training Program FPU 2018.**
  Support from the Spanish Ministry of Science, Innovation and Universities for the completion of this PhD thesis in the Soil Science and Agricultural Chemistry Department - UGR. Grant date: September 2019. Competitive concurrence. Ref: FPU18/02901.

Organization and participation in scientific committees | *Organización y participación en comités científicos*

- ☐ **EGU General Assembly 2023. Scientific session SSS7.7 - Challenges and solutions for assessment, prevention and mitigation of contamination of urban soils and mines as ecotoxicological and human health risks.** Convener: Carmen Pérez-Sirvent | Co-conveners: **Antonio Aguilar-Garrido**, Maria Manuela Abreu, Carolina Rosca, Annika Parviainen, Jaume Bech, Luz García-Lorenzo. https://meetingorganizer.copernicus.org/EGU23/session/46076
- ☐ **EGU General Assembly 2022. Scientific session SSS7.4/BG2.10/GI2.7 - Innovative and holistic approaches to prevent, assess and mitigate soil pollution: integrating natural solutions with advanced data analysis and digital tools.** Convener: Maria Manuela Abreu | Co-conveners: Selma Pena, Patrícia Vidigal, **Antonio Aguilar-Garrido**, Stefano Albanese. https://meetingorganizer.copernicus.org/EGU22/session/42321
- ☐ **XXXII National Meeting of Soils (RENS2019).** Assistance in the preparation of the XXXII National Meeting of Soils (documentation organization, accreditation of participants, preparation sessions) held in Seville in Spain, 10-13 September 2019.

Scientific outreach | *Divulgación científica*

- ☐ **European Researchers' Night 2018, 2019, 2021.**
  Collaboration in the activities "Discovering the world of the soil" and "Soil and Climate Change" for all audiences carried out in Granada and organized by the Soil Science and Agricultural Chemistry Department - UGR. Organiser: Marie Skłodowska-Curie Actions.
- ☐ **Sciences Week in Andalusia 2019, 2022, 2023.**
  Conducting the activity "With your feet on the soil" for high school and college student conducted in the Faculty of Sciences and organized by the Soil Science and Agricultural Chemistry Department - UGR. Organiser: Andalusia government.

CURRICULUM VITAE

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Antonio Aguilar-Garrido was born in Alfacar, Granada, Spain, on 28 July 1995. He has a Bachelor's Degree in Environmental Sciences (2018) and a Master's Degree in Conservation, Management and Restoration of Biodiversity (2019), both from the University of Granada (UGR). From October 2019 to October 2023, he held an FPU grant from the Spanish Ministry of Science, Innovation and Universities to perform his PhD thesis in Earth Sciences at the Department of Soil Science and Agricultural Chemistry - UGR supervised Prof. Dr. Francisco José Martín Peinado (fjmartin@ugr.es). During his PhD he carried out a short research stay at the research centre LEAF - Linking Landscape, Environment, Agriculture and Food (Instituto Superior de Agronomía, University of Lisbon, Portugal) under the supervision of Prof. Dr. Maria Manuela Abreu (manuelaabreu@isa.ulisboa.pt). His main interests are related to the fields of soil and water contamination/decontamination, mining restoration, toxicity assessment, and circular economy. He has worked with the main analyses of soil properties, enzymatic activities in soils, and toxicological bioassays to evaluate the toxicity on soils of different PHEs. Furthermore, he has teaching experience in the subjects of "The physical environment", "Soil science", "Methods for the study of the natural environment", and "Soil evaluation, management, conservation, and recovery techniques" of the degrees in Biology and Environmental Sciences; and has mentored some final degree/master's degree projects. He has been involved in the Spanish Ministry of Science research project "Restoration of soils contaminated by heavy metals: A strategy based on waste revalorisation and bioremediation (RTI 2018-094327-B-I00)". Aguilar-Garrido is the first author of five academic articles, and co-author of five others, which have all been published in JCR-indexed journals. He is also author of six book chapters and has presented several posters and oral communications in conferences at national and international level. He has also collaborated as a reviewer in prestigious JCR-indexed journals such as Science of the Total Environment, Environmental Geochemistry and Health, and Plant and Soil.

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