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Integrating waste valorization and symbiotic microorganisms for sustainable bioremediation of metal(loid)-polluted soils

Mario Paniagua-López [a,b,*], Gloria Andrea Silva-Castro [b], Ana Romero-Freire [a], Francisco José Martín-Peinado [a], Manuel Sierra-Aragón [a], Inmaculada García-Romera [b]

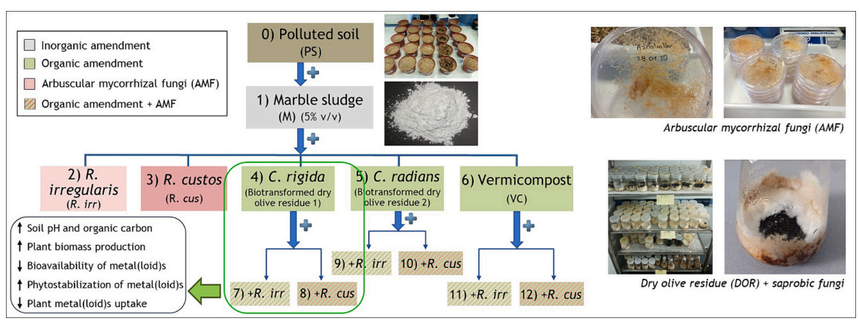
[a] *Departamento de Edafología y Química Agrícola, Facultad de Ciencias, Universidad de Granada, Campus de Fuentenueva, s/n, Granada, 18071, Spain*

[b] *Departamento de Microbiología del Suelo y la Planta, Estación Experimental del Zaidín, Consejo Superior de Investigaciones Científicas (EEZ-CSIC), C/ Profesor Albareda, 1, Granada, 18008, Spain*

HIGHLIGHTS

- Biological remediation strategy of metal (loid)-polluted soil is evaluated.
- Remediation treatments using agro-industrial by-products and symbiotic microorganisms.
- Treatments were effective in improving main soil properties and reducing toxicity.
- Biotransformed dry olive residue highlighted as the most effective amendment used.
- Mycorrhizal inoculation enhanced the beneficial effects of the amendments applied.

GRAPHICAL ABSTRACT



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ABSTRACT

Remediation strategies for metal(loid)-polluted soils vary among the wide range of approaches, including physical, chemical, and biological remediation, or combinations of these. In this study, we assessed the effectiveness of a set of soil remediation treatments based on the combined application of inorganic (marble sludge) and organic amendments (vermicompost, and dry olive residue [DOR] biotransformed by the saprobic fungi *Coriolopsis rigida* and *Coprinellus radians*) and inoculation with arbuscular mycorrhizal fungi (AMFs) (*Rhizophagus irregularis* and *Rhizoglomus custos*). The treatments were applied under greenhouse conditions to soil residually polluted by potentially toxic elements (PTEs) (Pb, As, Zn, Cu, Cd, and Sb), and wheat was grown in the amended soils to test the effectiveness of the treatments in reducing soil toxicity and improving soil conditions and plant performance. Therefore, we evaluated the influence of the treatments on the main soil properties and microbial activities, as well as on PTE availability and bioaccumulation in wheat plants. Overall, the results showed a positive influence of all treatments on the main soil properties. Treatments consisting of a combination of marble and organic amendments, especially biotransformed DOR amendments, showed the greatest effectiveness in improving the soil biological status, promoting plant growth and survival, and reducing PTE availability and plant uptake. Furthermore, AMF inoculation further enhanced the efficacy of DOR amendments by promoting the immobilization of PTEs in soil and stimulating the phytostabilization mechanisms induced by AMFs, thus

* Corresponding author at: Departamento de Edafología y Química Agrícola, Facultad de Ciencias, Universidad de Granada, Campus de Fuentenueva, s/n, Granada, 18071, Spain.

E-mail address: mpaniagua@ugr.es (M. Paniagua-López).

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playing an important bioprotective role in plants. Therefore, our results highlight that biotransformed DOR may represent an efficient product for use as a soil organic amendment when remediating metal(loid)-polluted soils, and that its application in combination with AMFs may represent a promising sustainable bioremediation strategy for recovering soil functions and reducing toxicity in polluted areas.

1. Introduction

Soil, which is an essential element of ecosystems, has great regulatory potential to respond to all types of pollution and protect other ecosystem elements such as water, air, and living organisms (Adhikari and Hartemink, 2016). However, the resilience and protective capacity of soil are finite, and intense or continuous degradation processes over time can exceed its buffering capacity and prevent the performance of one or more of these functions, leading to serious damage to ecosystems and humans (Lal, 1997; Seybold et al., 1999). Soil pollution has become an increasingly pressing issue in recent decades, with the main cause being associated with human activities, such as mining, which have resulted in the accumulation of pollutants in soils to levels that may be of concern (Hu et al., 2013; Cachada et al., 2018). Active soil remediation is required in scenarios involving extensive and severe pollution.

The revalorization of waste materials through their use as soil amendments can represent an effective, low-cost, and ecological solution to remediate polluted soils (González-Núñez et al., 2015), as it converts waste into essential resources, thereby improving soil properties. This may simultaneously help mitigate a major global problem representing the increasing generation of waste from human activities, which constitutes a serious environmental and management challenge (Abdel-Shafy and Mansour, 2018; Ferronato and Torretta, 2019). Techniques based on the addition of inorganic and organic amendments to facilitate the natural remediation processes of soils damaged or polluted with potentially toxic elements (PTEs) represent a viable and widely recognized strategies for soil remediation (Bolan and Duraisamy, 2003; Guo et al., 2006; Raklami et al., 2021).

A wide variety of materials and by-products have been used as soil amendments (Adriano et al., 2004; Pérez-de-Mora et al., 2006). Marblestone waste sludge generated during processing has been used and tested by several authors, showing promising results as an amendment for assisting the natural remediation of heavily polluted acidic mine soils and for reducing PTE toxicity risks (Pérez-Sirvent et al., 2007; Fernández-Caliani and Barba-Brioso, 2010; González et al., 2017). Considering that marble is the most widely produced natural stone in the world and that at least 20–30 % of marble is turned into powder during cutting, large quantities of this low-cost waste material are produced annually and must be sustainably managed (Alyousef et al., 2019). Moreover, organic wastes, such as compost or those produced by the agri-food industry, can be used as soil amendments owing to their high organic matter (OM) content, which enhances the soil pH and microbial structure as well as reduces PTE bioavailability in metal-polluted soils, thereby restoring soil quality and functionality (García-Sánchez et al., 2015, 2019). Among the different organic amendments used in recovering metal-polluted soils, the promotion of dry olive residue (DOR) is of great interest because this residue is produced by the olive oil industry in very large quantities, which is a major concern for this sector, especially in Mediterranean countries where this industry is of great importance at social and economic levels (Tortosa et al., 2012). However, the direct application of DOR to soil can also increase metal availability and negatively impact soil microorganisms and plant growth owing to its phytotoxicity, which is mainly caused by the high presence of phenolic compounds and other substances such as fatty acids (García-Sánchez et al., 2012; Siles et al., 2014a). Therefore, transformation of DOR prior to its application to soil is required. The inoculation with saprobe fungi, which are able to both stabilize waste and degrade phytotoxic compounds such as phenols, is a rapid and effective technique for reducing the phytotoxic effects of DOR, which facilitates its use as an organic amendment (Sampedro et al., 2005, 2009; Aranda et al., 2006). The application of DOR mycoremediated by these fungi has possitive effects on plant growth, increases microbial diversity, and decreases metal bioavailability (Siles et al., 2014b; Hovorka et al., 2016; Reina et al., 2017). However, García-Sánchez et al. (2017) highlighted the need for further research on the potential remediation effectiveness of biotransformed DOR in polluted soils, as well as on the combined application of this material with other element-stabilizing materials, as its potential effect on the mobility and plant availability of risk elements has not been widely investigated.

The application of arbuscular mycorrhizal fungi (AMFs) is another strategy for the remediation of soils polluted by PTEs, because AMFs can provide plant protection against adverse elements through their immobilization, extraction, and concentration in their tissues (Arriagada et al., 2009; Meier et al., 2012; Leung et al., 2013). AMFs occur extensively in polluted soils, and evidence suggests that they improve plant nutrition and tolerance to excess trace elements (Janoušková et al., 2005; Cabral et al., 2015). In particular, the toxicity of PTEs in plants and the environment may be mitigated by the high metal sorption capacity of mycorrhizal mycelia and their ability to transfer and sequester excess elements into vacuoles and root cell walls, along with the retention of metal ions in chemically inactive complexes (Colpaert et al., 2011; Shi et al., 2019). In addition, the combination of AMFs with organic amendments may facilitate the successful establishment of the soil microbial community and full restoration of soil function, while also improving AMF PTE stabilization (García-Sánchez et al., 2017, 2019). Consequently, AMFs represent an important tool used for the recovery of polluted sites (Leung et al., 2006; Cabral et al., 2015), and bioremediation approaches based on microorganisms should be considered, as they may complement or replace conventional methods by enhancing the effectiveness of the processes involved (Sepehri et al., 2020).

We hypothesized that the combined application of amendments and AMF inoculation is a promising remediation strategy for the recovery of soil function in PTE-polluted areas. Therefore, the general objective of this study was to evaluate the effectiveness of soil remediation treatments based on inorganic (marble sludge) and organic (vermicompost and DOR amendments biotransformed by the saprobic fungi *Coriolopsis rigida* and *Coprinellus radians*) combined with the inoculation of AMFs (*Rhizophagus irregularis* and *Rhizoglomus custos*) applied to metal(loid)-polluted soil (Pb, As, Zn, Cu, Cd, and Sb).

2. Material and methods

2.1. Soil sampling and materials

Residual polluted soil from the Aznalcóllar metal mining spill affected area (Seville, SW Spain) was sampled for use in the experimental set up (37°29′36″N, 6°13′14″W). The spill accident occurred in 1998 after the collapse of the tailings dam of the mine, which produced a spillage of approximately 4,5 × 10^6 m^3 of acidic water and toxic tailings containing high concentrations of PTEs), affecting an area of over 43 km^2 (Grimalt et al., 1999; Simón et al., 1999). A detailed map of the sampling locations is available in Fig. S1. The area is characterized by heterogeneously distributed unvegetated soil patches of different sizes with high concentrations of several PTEs (especially Pb, As, Zn, Cu, Cd, and Sb). Composite soil samples were obtained from these unvegetated patches by mixing 200 g of topsoil (0–10 cm) from each corner and the center of a square of 1 m per side. Individual soil samples were thoroughly mixed and homogenized to obtain a single representative soil sample. Finally, the soil was air-dried at room temperature in the laboratory, sieved (<2 mm), and stored until its use.

Polluted soil was amended with inorganic and organic waste materials. Marble sludge, from the mining area of Macael (Almería, SE Spain), which consisted of waste material from the cutting and polishing of marble stones, was used as an inorganic amendment. Two organic amendments were used:commercial vermicompost provided by Lombricor SCA (Algallarín, Córdoba, Spain), and DOR, which was used as an organic amendment after transformation with two saprobic fungi. This last amendment was supplied by the olive oil manufacturer Aceites Sierra Sur S.A. (Granada, Spain), sieved, autoclaved over three cycles (121 °C for 20 min), and stored at 4 °C prior its biological transformation.

2.2. Biological transformation of DOR (mycoremediation)

The DOR used as an organic amendment was transformed by two different saprobic fungal species: *Coriolopsis rigida* (EEZ-92), and *Coprinellus radians* (EEZ-84). The fungi were pre-cultured on a 2 % malt extract agar plates for two weeks at 28 °C to maintain a fresh inoculum. Fungal transformation of the DOR was performed under solid-state fermentation conditions to accelerate the ability of the fungi to undergo DOR transformation, as described by Reina et al. (2013). For this purpose, the fungi were first pre-cultured in barley-based media (18 g of barley seeds and 30 mL of sterile water) for one week. Subsequently, sterilized DOR (50 % *w*/w) was added to a barley-seed media inoculated with fungi, moistened with sterile water, and incubated at 28 °C for four weeks. Non-inoculated barley-based media with sterilized DOR were also incubated and used as controls. The incubation was heat-inactivated by autoclaving and the DOR samples were sieved (<5 mm). Finally, the remaining barley seeds were manually removed, and the samples were manually homogenized and stored at 4 °C.

2.3. Arbuscular mycorrhizal fungi (AMFs) inocula

The AMFs used in this experiment were *R. irregularis* DAOM 197198 (*Glomus irregulare* DAOM 197198), formerly known as *Glomus intraradices*, and *R. custos* MUCL47214. Both are known for their ability to support plant survival and development in mining soils (Silva-Castro et al., 2022). The model fungus *R. irregularis* was used as the control. *R. custos* was originally isolated from the banks of the Tinto River near Nerva (Huelva, southern Spain), a mining area located in the Iberian Pyrite Belt, which also comprised our study site, where high concentrations of PTEs occur naturally (Cano et al., 2009). AMF inoculation was carried out by adding a 1-cm^3 cube of AM-in-vitro-issued inoculum (Cano et al., 2008) containing high amounts of AM propagules (spores, active extraradical hyphae, and aseptic root pieces colonized by AM intraradical mycelia) from the different AM used. AM inoculation was performed during seedling transplantation, and special care was taken to ensure that fungal propagules were in close contact with the roots, thus inducing rapid colonization.

2.4. Experimental design and set up

A model pot experiment was set up to evaluate the role of the selected amendments and AMF and their different combinations, as a potential strategy for immobilizing PTEs present in soil and for improving soil properties and quality. The experiment was set up in a series of identical polypropylene pots, each with a volume of 0.3 L. Approximately 300 g of polluted soil was placed in each pot. The experimental design consisted of a randomized factorial system with three variation factors. The first experimental factor consisted of two levels: polluted-soil application with either the inorganic amendment (marble sludge) or its application with both the inorganic amendment and an organic amendment or AMF. The second factor was the organic amendment type and comprised three levels: DOR mycoremediated by *C. rigida*, DOR mycoremediated by *C. radians*, and vermicompost. The third factor included AMF inoculation comprising two levels: soil non-inoculated and soil inoculated with the two AMF (*R. irregularis* and *R. custos*). In total, thirteen treatments (n = 13), resulting from the combinations of the polluted soil with the different amendments and AMF (Fig. 1), were established and tested as follows: 0) polluted soil (PS); 1) polluted soil+marble sludge addition (M); 2) M + inoculation with *R. irregularis* (*R.irr*); 3) M + inoculation with *R. custos* (*R.cus*); 4) M + DOR mycoremediated by *C. rigida* (D1); 5) M + DOR mycoremediated by *C. radians* (D2); 6) M + vermicompost (VC); 7) M + D1 + inoculation with *R. irregularis* (D1-*R.irr*); 8) M + D1 + inoculation with *R. custos* (D1-*R.cus*); 9) M + D2 + inoculation with *R. irregularis* (D2-*R.irr*); 10) M + D2 + inoculation with *R. custos* (D2-*R.cus*); 11) M + VC + inoculation with *R. irregularis* (VC-*R.irr*); 12) M + VC + inoculation with *R. custos* (VC-*R.cus*). Five replicates were performed for each treatment.

The inorganic and organic amendments were applied and manually mixed with the soil. The marble and vermicompost amendments were applied at a rate of 5 % (*v*/v) following previous studies (Paniagua-López et al., 2023), whereas the DOR amendments were applied at a rate of 8 % (v/v) according to preliminary tests. The soil moisture was brought to 60 % of the soil water-holding capacity. A 10-day-old wheat plant (*Triticum aestivum* L.) was planted in each pot. The experiment was carried out for 45 d under greenhouse conditions (supplementary light at 25/19 °C and relative humidity at 50 %), and the plants were regularly watered to maintain the same initial moisture conditions.

After 45 d, soil samples from each pot were collected individually, sieved (<2 mm), homogenized, and subdivided into three subsamples. The first subsample was air-dried at room temperature and used for chemical analyses, the second was maintained at 4 °C for the analysis of the soil enzymatic activity, and the third was finely ground and used for the determination of the PTE concentrations. Wheat plants were individually harvested, divided into roots and shoots, and then divided into different subsamples to determine the plant biomass, percentage of root mycorrhization, and PTE concentrations in each plant part after homogenization.

2.5. Analytical methods

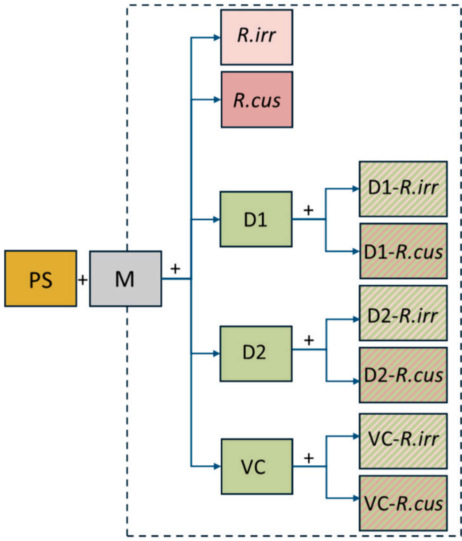
2.5.1. Soil analysis

The main chemical properties of the soil in each sample were characterized. Soil pH was determined at a soil:water ratio 1:2.5 with a 914 pH/Conductometer Metrohm (Herisau, Switzerland); a soil:water extract (1:5) was prepared to determine the electrical conductivity (EC, dS/m) using a Eutech CON700 conductivity meter (Oakton Instruments, Vernon-Hills, IL, USA); organic carbon (%OC) was determined according to Tyurin (1951); available phosphorous was analyzed according to Olsen and Sommers (1982); and total nitrogen (%N) was analyzed by dry combustion using an elemental analyzer TruSpec CN LECO® (St. Joseph, MI, USA).

Dehydrogenase activity was quantified using a method by Camiña et al. (1998). To determine urease activity, a method described by Kandeler and Gerber (1988) was adopted. Phosphatase and β-glucosidase activities were assessed followingmethods described by Eivazi and Tabatabai (1977, 1988), respectively.

2.5.2. Total and bioavailable concentrations of PTEs in soil samples

The total concentrations of the main PTEs present in the studied soil (Pb, As, Zn, Cu, Cd, and Sb), expressed in mg kg^{-1}, were determined by X-ray fluorescence with a NITON XL3t-980 GOLDD + Analyzer (Thermo Fisher Scientific, Tewksbury, MA, USA). The precision and accuracy of the method were confirmed by analyzing a certified reference material (CRM052–050 RT-Corporation Limited, Salisbury, UK; n = 6). The average concentrations of the studied elements ranged from 90 % to 110 % of the reference material. Moreover, selective extraction of PTEs was performed for all treatments to assess the bioavailability of these elements in the soils. The bioavailable fraction was extracted using 0.05-M



	ACRONYM	TREATMENT DESCRIPTIONS
0	PS	Polluted soil (control -)
1	M	Marble sludge addition (control +)
2	*R.irr*	Inoculation with *R. irregularis*
3	*R.cus*	Inoculation with *R. custos*
4	D1	DOR mycoremediated by *C. rigida*
5	D2	DOR mycoremediated by *C. radians*
6	VC	Vermicompost
7	D1-*R.irr*	D1+inoculation with *R. irregularis*
8	D1-*R.cus*	D1+inoculation with *R. custos*
9	D2-*R.irr*	D2+inoculation with *R. irregularis*
10	D2-*R.cus*	D2+inoculation with *R. custos*
11	VC-*R.irr*	VC+inoculation with *R. irregularis*
12	VC-*R.cus*	VC+inoculation with *R. custos*

Fig. 1. Flow diagram of treatments used and descriptions of their compositions and acronyms. DOR (dry olive residue).

EDTA (pH = 7) at a soil:solution ratio of 1:10 (*w/v*) and shaken for 1 h at room temperature, as described by Quevauviller et al. (1998). The PTE concentrations for this extracted form were measured by Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) in a PerkinElmer Avio® 500 spectrometer (Shelton, CT, USA).

2.5.3. Plant analysis

After harvesting, the plants were washed, divided into roots and shoots, dried (70 °C for 24 h), and used for the different determination methods. Plant biomass was recorded and expressed as the root and shoot dry weights. The percentage of mycorrhization in the roots was estimated according to the staining methodology described by Phillips and Hayman (1970), and colonization was quantified using a method by Giovannetti and Mosse (1980). Additionally, dry plant materials were finely ground and used to measure the concentrations of PTEs in both the roots and shoots. Acid digestion was performed in a Mars® XP1500 Plus microwave in a HNO_3:H_2O_2 (1:1) mixture for 1 h at 200 °C and 800 W. Thereafter, PTE concentrations were measured using an Avio® 500 ICP-optical emission spectrometer (Shelton, CT, USA).

The bioaccumulation factor (BAF) in the shoots and bioconcentration factor (BCF) in the roots were calculated to determine the uptake of PTEs by wheat plants under each of the applied treatments, which allowed for the determination of whether the PTEs were transported to the aboveground part of the plants or accumulated in the roots. For this purpose, the bioavailable (EDTA-extractable) PTE fraction in the soil samples of each treatment was selected, and both the BAF and BCF were calculated as the ratio between the PTE concentration measured in each part of the plant (shoot or root) (mg kg^{-1} dry weight) and EDTA-the extractable PTE concentration in the soils (mg kg^{-1} dry soil). Finally, the translocation factor (TF) within the plant was estimated as the ratio between the concentration of PTEs in shoots and their concentration in roots, to determine the degree of element migration from the roots to the aboveground part of the plants.

2.6. Statistical analysis

Mean values and the corresponding standard deviations of the parameters were calculated for each sample set. After verifying that the data fit a normal distribution, differences between the individual means of the samples were analyzed by one-way analysis of variance as well as post-hoc analysis using Duncan's test. Levene's test was used to check for homogeneity of variance. All analyses were performed at a 95 % confidence level using SPSS v.28.0 (SPSS Inc., Chicago, USA). Principal component analysis (PCA) was performed to determine the relationships between the treatments and the PTE concentrations in both the soils and plants by using the Canoco 5.04 software. When necessary, that is, to meet normality assumptions, the data were log-transformed.

3. Results and discussion

3.1. Effect of amendment and AMF treatments on soil properties

3.1.1. Description of soils and amendments

The main chemical properties of the polluted soil and those of the inorganic and organic amendments used in the experiment, were analyzed (Table S1). The control polluted soil (PS) was characterized by being highly acidic (pH 3.4) and having a high EC (3.5 dS/m), as previously discussed in studies carried out in the same study area (Romero-Freire et al., 2016; Álvarez-Mateos et al., 2019). The marble and vermicompost amendments showed an alkaline and slightly basic pH (9.1 and 7.7), respectively, while the DOR showed a distinctive slightly acidic pH after mycoremediation with *C. rigida* (6.3) (Hovorka et al., 2016), and a more acidic pH after mycoremediation with *C. radians* (5.0). The organic amendments showed elevated ECs in all cases (> 4 dS/m), although the EC was significantly lower in the DOR mycoremediated with *C. rigida*. Very low contents of (< 1 %) and N were detected in PS, whereas these were negligible in the marble amendment. In contrast, high OC and N contents were found in the vermicompost and DOR amendments, especially in the second amendment, in which the OC content was three-to-four-fold higher than that in the vermicompost. The organic amendments were also characterized by high available P levels (>200 mg kg^{-1} P), with that in the DOR mycoremediated with *C. rigida* being approximately two-fold higher (>400 mg kg^{-1} P) than those with *C. radians* and the vermicompost. In addition, high K levels were detected in DOR amendments, which were approximately twice as high as those measured in vermicompost. This is an important finding, considering the important role of this element in the stress tolerance of plants (Siles et al., 2015; Johnson et al., 2022).

3.1.2. Changes in soil chemical properties

The application of the inorganic and organic amendments and AMF inoculation strongly changed the soil chemical properties (Table 1). All treatments induced a sharp increase in the soil pH compared to the control polluted soil (PS), from strongly acidic (pH 3.4) to neutral (pH 7–7.5), mainly because of the great potential of the marble amendment to neutralize soil acidity (Tozsin et al., 2014; Fernández-Caliani et al.,

Table 1
Soil chemical properties after the different amendments and AMF treatments application. PS (control polluted soil); M (marble addition); D1 (dry olive residue mycoremediated by *C. rigida*); D2 (dry olive residue mycoremediated by *C. radians*); VC (vermicompost); *R.irr* (*R. irregularis*); *R.cus* (*R. custos*). EC (electrical conductivity); OC (organic carbon); N (total nitrogen); Pav (available phosphorus). Data are presented as the mean ± standard deviation, $n = 5$.

Treatment	pH	EC (dS/m)	OC (%)	N (%)	Pav (mg kg^{-1})	K (mg kg^{-1})
PS	3.40 ± 0.01 *a*	3.48 ± 0.07 *g*	0.72 ± 0.11 *a*	0.102 ± 0.006 *de*	8.2 ± 2.0 *a*	12.13 ± 0.14 *b*
M	7.06 ± 0.09 *b*	3.25 ± 0.05 *f*	0.72 ± 0.10 *ab*	0.074 ± 0.010 *ab*	19.6 ± 10.3 *abc*	11.13 ± 0.38 *a*
R.irr	7.50 ± 0.04 *d*	3.00 ± 0.03 *b*	0.67 ± 0.17 *a*	0.067 ± 0.007 *a*	34.0 ± 15.7 *bc*	13.00 ± 0.29 *bcd*
R.cus	7.51 ± 0.04 *d*	3.03 ± 0.03 *bc*	0.71 ± 0.13 *a*	0.063 ± 0.003 *a*	25.6 ± 6.2 *abc*	12.56 ± 0.18 *cd*
D1	7.10 ± 0.01 *b*	3.20 ± 0.11 *ef*	1.11 ± 0.23 *cd*	0.092 ± 0.007 *cd*	29.6 ± 13.7 *bc*	12.19 ± 0.62 *b*
D2	7.35 ± 0.05 *c*	3.08 ± 0.06 *bcd*	0.97 ± 0.04 *bc*	0.084 ± 0.011 *bc*	30.0 ± 14.0 *bc*	12.90 ± 0.07 *cd*
VC	7.55 ± 0.03 *d*	3.15 ± 0.02 *def*	0.81 ± 0.11 *ab*	0.074 ± 0.005 *ab*	29.2 ± 10.5 *bc*	12.88 ± 0.13 *d*
D1-*R.irr*	7.29 ± 0.04 *c*	3.14 ± 0.07 *de*	1.00 ± 0.10 *bcd*	0.085 ± 0.006 *bc*	35.9 ± 20.7 *bc*	12.28 ± 0.32 *b*
D1-*R.cus*	7.28 ± 0.12 *c*	3.11 ± 0.05 *cde*	1.10 ± 0.14 *cd*	0.078 ± 0.011 *abc*	38.9 ± 16.6 *c*	12.80 ± 0.14 *cd*
D2-*R.irr*	7.14 ± 0.10 *b*	3.17 ± 0.01 *def*	1.00 ± 0.16 *bcd*	0.077 ± 0.010 *abc*	23.9 ± 17.5 *abc*	12.28 ± 0.15 *b*
D2-*R.cus*	7.28 ± 0.10 *c*	2.76 ± 0.19 *a*	1.19 ± 0.15 *d*	0.084 ± 0.010 *bc*	28.2 ± 8.8 *bc*	12.75 ± 0.58 *cd*
VC-*R.irr*	7.52 ± 0.04 *d*	3.20 ± 0.06 *ef*	0.70 ± 0.11 *a*	0.088 ± 0.018 *bc*	21.7 ± 5.6 *abc*	12.73 ± 0.23 *cd*
VC-*R.cus*	7.46 ± 0.08 *d*	3.18 ± 0.02 *def*	0.70 ± 0.11 *a*	0.106 ± 0.014 *e*	17.0 ± 4.1 *ab*	12.51 ± 0.32 *bc*

Lowercase letters indicate significant differences among treatments according to Duncan's post hoc test ($p < 0.05$).

2022). However, the organic and AMF treatments reached a slightly higher pH than the addition of marble alone (M). The treatments also induced a change in EC, which was significantly reduced in all cases compared with that of PS, even though the EC of the organic amendment materials was higher than that of PS (Table S1). This general decrease in EC could be attributed to the marble addition, for which a potential in decreasing EC values in soil has been previously reported (Jain et al., 2020a). This is possibly related to a reduction, induced by this fine material, in the porosity of the soil, which then increases its bulk density, thus inversely correlating with the soil EC (Jain et al., 2020b). Among the treatments, the AMF treatments (*R.irr* and *R.cus*), especially D2-*R.cus*, exhibited the largest EC decrease, displaying the capability of AMF to lower the EC in the mycorrhizosphere when high levels of salinity are present (Giri et al., 2003). In relation to the OC content in the soil, the DOR and DOR + AMF treatments, especially those including the AMF *R. custos*, significantly increased the OC content in relation to PS and M, mainly because of the elevated OC content of the DOR amendment. In contrast, treatments consisting of only AMF addition (*R.irr* and *R.cus*), as well as the vermicompost treatments, were not capable of increasing the OC content in the soil. A decrease in N content was observed in most of the treatments compared to that in PS, with the most remarkable decrease being observed under treatments with AMFs alone, as no input of N from organic amendment was added, which also indicated that overall N consumption by biological activity occurred in the treated soils. AMFs may promote the decomposition and subsequent uptake of organic N from organic sources and transfer the N to plants (Leigh et al., 2009; Whiteside et al., 2009). They also enhance the plant uptake of inorganic N from soil and increase N demand owing to the increased amounts of bacteria in the soil (Hawkins et al., 2000; Saia et al., 2014). Meanwhile, significant increases in available P and, to a lesser extent, the K content under the treatments compared to those in PS was also observed. For available P, treatments with DOR application, and especially the D1 combined with both AMFs, induced a greater increase. This could be directly related to the high contents of these elements in the selected organic amendments, especially in the case of the P in DOR1.

3.1.3. Soil enzymatic activity

Soil enzymatic activity measured at the end of experiments have been used as a bioindicator of soil quality change and biological activity in soils under the different treatments (Paz-Ferreiro et al., 2014; Silva-Castro et al., 2022). Enzymatic activity plays a key role in soil biochemical processes, controls the decomposition rate of organic matter and nutrient cycling (Kaschuk et al., 2010; Singh et al., 2019), and is highly sensitive to PTE pollution stress (Hu et al., 2014). For this purpose, soil dehydrogenase activity was measured since it reflects the oxidative activity of soil microflora and is a good indicator of overall soil microbiological activity (Burgos et al., 2002; López-Piñeiro et al., 2011), while β-glucosidase activity is involved in the C cycle, playing an important role in the degradation of cellulose to glucose, and thus in the recycling of soil organic matter and the bioavailability of soil C (Cañizares et al., 2011). Moreover, phosphatase is an enzyme involved in the mineralization of organic P and plays a key role in increasing the concentrations of inorganic phosphates in soil, while urease is an important enzyme in soil N transformation (Cui et al., 2013; Yang et al., 2016).

In this regard, the greatest enzymatic activities in all cases were found in treatments with DOR application (either alone or when combined with AMFs especially), which sharply increased all activities measured (Fig. 2). This general enhancement in enzymatic activity after the application of this amendment has also been reported in other studies, which might be related to the high nutrient content and low amount of toxic compounds in this material after biotransformation with saprobic fungi (Siles et al., 2014b; García-Sánchez et al., 2019). In contrast, AMFs alone and vermicompost treatments (both alone and in combination with AMF) did not induce an increase in the soil enzymatic activities in general when compared to M; vermicompost only produced a significant increase in dehydrogenase activity, but to a lesser extent than the DOR treatments. This contrasts with what the findings of other studies that reported an increase in soil enzymatic activity following the application of vermicompost amendments (Tejada and Benítez, 2011; Przemieniecki et al., 2021). The type of vermicompost used, its humic substances, and OC rates are significantly correlated with its influence on soil enzyme activity (Pramanik et al., 2010; García et al., 2012; Aranda et al., 2015). In our case, vermicompost showed a significantly lower OC rate than the DOR amendments, which could be related to the slight effect of vermicompost treatments on the studied enzymatic activities. With regard to the increases observed in the enzymatic (both dehydrogenase and urease) activities under the DOR treatments, all three treatments consisting of D1 highlighted as the ones that most significantly increased these activities, as the treatment with D1 alone in the case of glucosidase. Furthermore, the addition of *R. custos* to DOR enhanced the activity of dehydrogenase to a greater extent than the addition of *R. irregularis*, which is consistent with results obtained by Silva-Castro et al. (2022) in similar highly PTE-polluted soil. The general enhancement in β-glucosidase activity under the DOR treatments (both with and without AMF inoculation), might have been derived from the added amendment of stabilized organic matter via the biotransformed DOR (García-Sánchez et al., 2019), while the generally higher values obtained for phosphatase under the treatments combining DOR + AMF might have been related to the role of AMFs in the mobilization of soil phosphorus (Smith and Read, 2008).

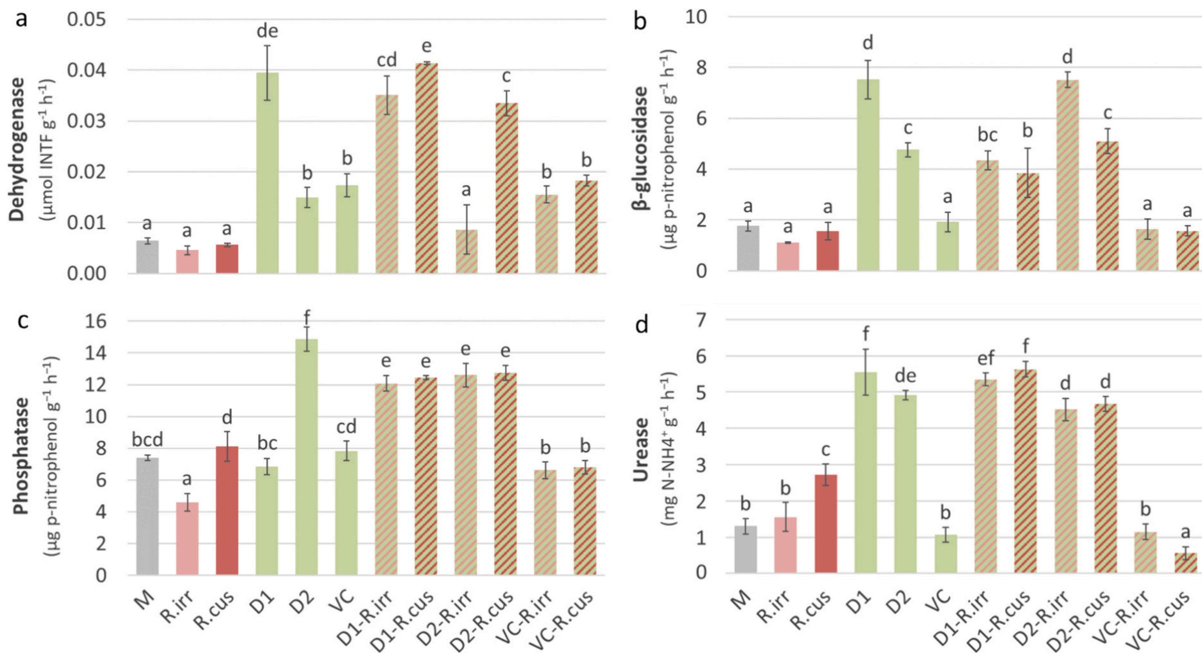


Fig. 2. Soil enzymatic activities [dehydrogenase (a), β-glucosidase (b), phosphatase (c), and urease (d)] measured in polluted soil after the different amendment and AMF treatment applications. M (marble addition); D1 (dry olive residue mycoremediated by *C. rigida*); D2 (dry olive residue mycoremediated by *C. radians*); VC (vermicompost); *R.irr* (*R. irregularis*); *R.cus* (*R. custos*). Error bars represent the standard deviation from the mean ($n = 5$). Lowercase letters indicate significant differences among treatments according to Duncan's post hoc test ($p < 0.05$).

3.2. Effect of amendment and AMF treatments on PTE mobility

3.2.1. Total concentrations of PTEs

The total PTE concentration decreased in all treatments in PS (Fig. 3; Table S2). However, when comparing the measured values with the current guidelines set by the Regional Government of Andalusia (BOJA, 2015), the concentrations of Pb and As were still very high in all treatments, exceeding the established regulatory thresholds for these elements for natural and urban soils (275 mg kg^{-1} for Pb, 36 mg kg^{-1} for As). Among all the treatments, D1-*R.cus* produced a greater decrease in the total concentrations of all PTEs in soil, whereas the PTE concentrations in VC-*R.cus* showed the slightest decreases, reflecting the contrasting responses of both organic amendments under the influence of the same AMF. Marble addition alone (M) also produced significant reductions in the PTE concentration compared to PS, although generally less than those caused by the addition of organic amendments and AMF inoculation. These reductions might have been partly caused by a dilution effect induced by the mixture of amendments with the polluted soil, even though their application was not expected to significantly affect the total concentration of these elements (Madejón et al., 2021; Paniagua-López et al., 2023). Moreover, it might have been caused by an enhancement in the metal-accumulating ability of the plants and the increased biomass caused by the AMF and organic amendments (Jing et al., 2007; Nadeem et al., 2014).

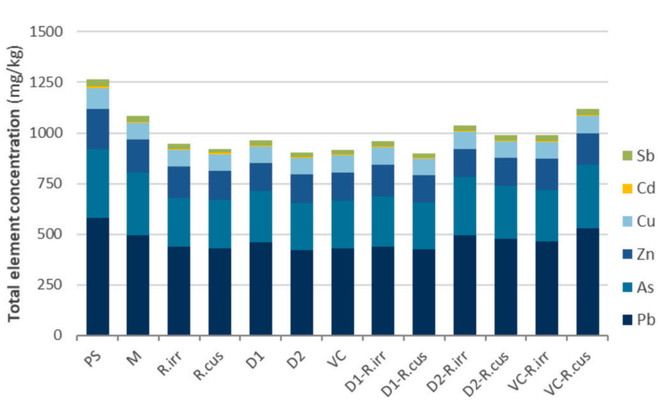


Fig. 3. Total PTE concentrations measured in polluted soil after different amendment and AMF treatment applications (see Table S2 for detailed concentration values by individual element and treatment). PS (control polluted soil); M (marble addition); D1 (dry olive residue mycoremediated by *C. rigida*); D2 (dry olive residue mycoremediated by *C. radians*); VC (vermicompost); *R.irr* (*R. irregularis*); *R.cus* (*R. custos*).

3.2.2. Bioavailable concentrations of PTEs

Bioavailability is a key indicator of the potential risk that pollutants pose to the environment and living organisms and governs the ecotoxicology of pollutants in soil (Naidu et al., 2008; Zhang et al., 2013). In this study, the EDTA-extractable fraction of PTEs was measured to assess their potential bioavailability, as this is considered an accurate method for determining the bioavailable fraction of PTEs and is suitable for acidic soils (Feng et al., 2005; Wilson et al., 2010; Alibrahim and Williams, 2016). Furthermore, compared to total concentrations, this fraction more accurately represents the proportion of PTEs in soil that are potentially available for uptake by plants and other living organisms (Kidd et al., 2007; Marguí et al., 2007) and that pose the greatest risk to terrestrial ecosystems (Bagherifam et al., 2019). The treatments led to different responses in the soil PTE bioavailability according to the different elements (Table 2). The bioavailability of elements such as Pb and Sb increased after treatment in comparison to that in PS, where the available fraction of these elements was very low. The higher EDTA-extractable concentrations of these less mobile elements under the different treatments compared with that of PS might have corresponded to the EDTA extraction capacity of the carbonate-bound and organically bound fractions of metals developed by forming strong chelates (Nakamaru and Martín-Peinado, 2017). Consequently, high carbonate and OC contents in the treatments could lead to an increase in the bioavailable fraction measured for these specific elements, as previously observed in similar scenarios where carbonated and organic amendments were used (Nakamaru and Martín-Peinado, 2017; García-Robles et al., 2022). However, compared to M, all organic and AMF treatments,

Table 2
Bioavailable PTE concentrations (B) measured in polluted soil after different amendment and AMF treatment applications. PS (control polluted soil); M (marble addition); D1 (dry olive residue mycoremediated by *C. rigida*); D2 (dry olive residue mycoremediated by *C. radians*); VC (vermicompost); *R.irr* (*R. irregularis*); *R.cus* (*R. custos*). Data are presented as the mean ± standard deviation, $n = 5$.

Treatment	Pb_B	As_B	Zn_B	Cu_B	Cd_B	Sb_B
PS	0.04 ± 0.04 a	0.81 ± 0.05 bc	80.62 ± 1.25 g	38.24 ± 1.10 d	0.41 ± 0.01 g	0.11 ± 0.05 a
M	0.49 ± 0.07 g	0.74 ± 0.15 abc	42.43 ± 1.76 f	27.12 ± 1.12 bc	0.32 ± 0.02 f	0.26 ± 0.11 bcd
R.irr	0.19 ± 0.11 bcd	0.47 ± 0.10 a	24.43 ± 2.16 bc	24.34 ± 1.64 a	0.26 ± 0.01 bc	0.24 ± 0.07 bcd
R.cus	0.31 ± 0.09 def	0.56 ± 0.14 ab	23.48 ± 2.31 ab	28.33 ± 2.31 c	0.24 ± 0.02 ab	0.20 ± 0.05 b
D1	0.22 ± 0.07 bcde	0.94 ± 0.09 cd	28.10 ± 1.83 cd	27.45 ± 1.92 bc	0.26 ± 0.01 bc	0.26 ± 0.01 bcd
D2	0.23 ± 0.12 cde	0.70 ± 0.11 abc	22.10 ± 1.36 ab	26.26 ± 1.46 abc	0.23 ± 0.01 a	0.27 ± 0.05 bcd
VC	0.35 ± 0.07 ef	0.94 ± 0.37 cd	25.04 ± 1.90 bc	28.44 ± 1.82 c	0.26 ± 0.01 bc	0.23 ± 0.06 bc
D1-*R.irr*	0.20 ± 0.06 bcd	0.59 ± 0.08 ab	27.28 ± 0.99 c	25.33 ± 0.98 ab	0.27 ± 0.01 cd	0.29 ± 0.04 cd
D1-*R.cus*	0.13 ± 0.09 abc	0.48 ± 0.14 a	19.95 ± 1.92 a	25.62 ± 2.07 ab	0.22 ± 0.01 a	0.32 ± 0.06 d
D2-*R.irr*	0.10 ± 0.05 ab	0.68 ± 0.13 abc	33.70 ± 1.73 e	26.21 ± 1.17 abc	0.29 ± 0.01 de	0.23 ± 0.05 bc
D2-*R.cus*	0.11 ± 0.09 abc	0.64 ± 0.23 bc	28.17 ± 6.36 cd	26.38 ± 0.26 abc	0.25 ± 0.01 bc	0.20 ± 0.03 b
VC-*R.irr*	0.37 ± 0.11 f	1.11 ± 0.18 d	32.00 ± 2.20 e	27.18 ± 1.56 bc	0.30 ± 0.02 ef	0.24 ± 0.04 bcd
VC-*R.cus*	0.24 ± 0.08 cde	0.67 ± 0.19 ab	31.56 ± 3.41 de	26.78 ± 2.26 abc	0.29 ± 0.01 de	0.26 ± 0.04 bcd

Lowercase letters indicate significant differences among treatments according to Duncan's post hoc test ($p < 0.05$).

both independently and in combination, led to significant decreases in Pb availability, especially in treatments consisting of a DOR amendment combined with AMFs, which might have been related to the higher increase in pH than by M alone. Moreover, As bioavailability remained similar to that of PS in M and the treatments with organic amendment (D1, D2, and VC), although a significant decrease was observed in most (with the exception of VC-*R.irr*) treatments where AMFs were present, leading to an increase in As availability. The general decrease in As and Pb bioavailability in the presence of AMFs might have been related to the influence of AMFs in reducing their mobility through mechanisms that include the secretion of chelating substances into soil or the superficial immobilization of these elements in their tissues by physical and chemical binding processes (Meier et al., 2012). In addition, all treatments applied to PS induced sharp reductions in the bioavailable forms of elements such as Zn, Cu, and Cd. These are highly mobile elements under acidic conditions; therefore, the large increases in pH and OC induced by all treatments strongly reduced their mobility, highlighting the role of these soil properties in controlling the mobility of these elements (Sierra-Aragón et al., 2019). It has been observed that AMFs can directly influence the mobility of these elements by changing their speciation from bioavailable to non-bioavailable forms in the rhizosphere (Jing et al., 2007). In summary, a combination of factors, including changes in the soil properties (increased pH and OC) and AMF immobilization mechanisms, were the main effects induced by the treatments, leading to the observed changes in PTE bioavailability under their influence.

3.3. Effect of amendments and AMF treatments on wheat plants

3.3.1. Plant growth and mycorrhizal colonization

The application of treatments led to a significant general increase in wheat plant biomass compared to that in PS, where plant growth and survival were greatly restricted owing to the acidic pH (3.4) and high EC (3.5) present in this soil (Fig. 4). A significant increase in soil pH after the application of the marble treatment (M) enabled plant survival and growth to a certain extent. Treatments consisting of AMF inoculation (*R. irr* and *R.cus*) barely induced a further increase in plant growth compared with those with the addition of marble alone (M). No significant increase in plant biomass after inoculation with *R. irregularis* has been observed previously (Bissonnette et al., 2010). Multiple experiments have indicated that a plant's reaction to AMF is not uniformly favorable, contingent upon both the plant and fungal genotypes, as well as environmental factors (Berger and Gutjahr, 2021). However, treatments incorporating organic amendments, with or without AMF inoculation, demonstrated superior promotion of plant biomass, particularly shoot growth, compared to treatments focused solely on mineral amendments. In contrast to M, which contributed mainly to pH neutralization, the organic amendments provided significant amounts of OC and nutrients (e.g., N, P, and K) that are naturally present in these amendments, as shown in Table S1, and are crucial for promoting plant growth to a greater extent. Moreover, the addition of AMFs to these organic amendments augmented this promotion in some cases, particularly in root development, as corroborated by previous studies (Alguacil et al., 2008; Curaqueo et al., 2014). The presence of AMFs can promote plant growth in polluted environments by improving plant tolerance to PTEs and their nutrition status through an increased absorption of nutrients (Nadeem et al., 2014; Bhantana et al., 2021). AMFs also provide protection to plants against PTEs through different physiological mechanisms, such as the accumulation of these elements in their structures, including spores and vesicles. In addition, the extraradical mycelia of AMFs can contribute to the immobilization of PTEs in soil through mechanisms such as the production of glomalin, a glycoprotein that acts as a chelating agent and has the potential to sequester large amounts of these elements (González-Chávez et al., 2004; Lenoir

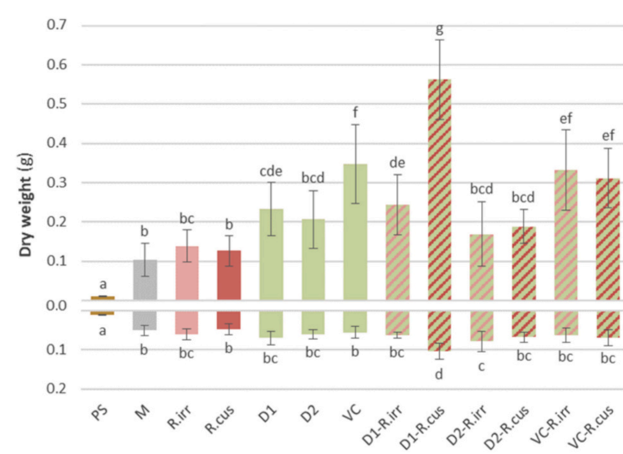


Fig. 4. Total biomass (dry weight) of wheat plant roots (down) and shoots (up) in polluted soil after different amendment and AMF treatment applications. PS (control polluted soil); M (marble addition); D1 (dry olive residue mycoremediated by *C. rigida*); D2 (dry olive residue mycoremediated by *C. radians*); VC (vermicompost); *R.irr* (*R. irregularis*); *R.cus* (*R. custos*). Error bars represent the standard deviation from the mean ($n = 5$). Lowercase letters indicate significant differences among treatments according to Duncan post hoc test ($p < 0.05$).

et al., 2016), and increases in the synthesis of plant phytochelatins, which also enable the chelation of PTEs (Garg and Chandel, 2012; Riaz et al., 2021). Both these mechanisms promote the binding capacity of pollutants in soil, reducing their potential uptake by plants and the toxicity stress induced to them (Garg et al., 2017). Among the different treatments, D1 + *R.cus* most significantly promoted both root and shoot growth. Furthermore, the vermicompost treatments led to higher shoot growth compared with the other treatments, which might have corresponded to the higher P levels detected in the shoots of plants grown under these treatments (data not shown). This might have been because vermicompost greatly increases the available P by inhibiting the fixation of labile P by soil particles, leading to an increase in shoot P concentrations (Sáinz et al., 1998; Zhang et al., 2020). Therefore, the combination of AMF inoculation with organic amendments may be beneficial for increasing the potential of these fungi to enhance plant growth, as a synergistic effect is produced by the positive effect of organic matter on the growth of the external mycelia of AMFs (Wang et al., 2013; Kohler et al., 2015). In addition, their combined application contributes to an increased diversity of soil microbial populations, which may improve the performance of these bioremediation-based treatments by promoting collaborative interactions between the different microbial communities present (Sepehri and Sarrafzadeh, 2018 and 2019).

Moreover, mycorrhization in wheat roots, accounted as the degree to which plant roots were colonized by AMF, was measured in the treatments in which AMF were inoculated (Fig. S2). No significant differences were observed between AMF treatments, consistent with the findings reported by Silva-Castro et al. (2022), who studied various AMF strains in soil polluted with PTEs. In the organic amendment treatments, the percentage of mycorrhization decreased, particularly under vermicompost treatments, an effect also detected in other studies (Beck, 2012; Pierart et al., 2019). The DOR amendments with AMFs did not induce greater mycorrhization of the roots than AMF inoculation alone, as previously observed by García-Sánchez et al. (2019). In all cases, root colonization ranged from 5 % to 10 %. However, *R. custos* showed slightly higher mycorrhization rates than *R. irregularis* when combined with the different organic amendments, particularly D2. This might have been due to the better mycorrhization capacity of *R. custos* under pollution stress conditions, as this AMF was isolated from a naturally PTE-polluted environment (Cano et al., 2009).

3.3.2. Concentration of PTEs in plants

The very limited plant growth in PS soil did not allow for the analysis of the PTE content in plants growing in this soil, as the plant material harvested from it was too scarce. However, the analysis of PTE content in plants grown under the different treatments showed significantly higher concentrations in the roots than in the shoots across all treatments (Fig. S3). This contrast was particularly notable for Pb, with concentrations in the roots being in the range of approximately 50-to-200-fold higher than those in the shoots, followed by As (30-to-100-fold) and Cd (up to 90-fold under specific treatments). Conversely, elements such as Cu and Sb exhibited concentrations in roots that were 5-to-20-fold higher than those in shoots. Finally, Zn exhibited more equilibrated concentrations between the roots and shoots, although they were still slightly higher in the roots. The significantly higher accumulation of PTEs in the roots than in the shoots, enhanced by the influence of treatments, might have interesting implications for phytoremediation programs intended for the remediation of PTE-polluted sites. The promotion of PTE immobilization at the root level contributes to the phytostabilization of pollutants, thus reducing the potential toxicity risk to the ecosystem (Yang et al., 2014).

The PTE concentrations measured in the plants also highlighted the overall effectiveness of all treatments tested in decreasing the concentration of most of the PTEs studied in plant tissues compared with sole marble application (M), with the exception of Cu in the roots and Cd in the shoots (Fig. 5). This is consistent with the higher concentrations measured in M for both the total and, especially, bioavailable PTE fractions compared to the other treatments, which directly modulate plant uptake. The most significant reductions in PTEs concentrations driven by the treatments were observed for Pb, As, and Sb in both roots and shoots, as well as for Cu and Zn, especially in shoots, showing the greatest deviations from most of the treatments in the PCA analysis. Meanwhile, M showed a strong direct correlation with these elements in the PCA, as the highest plant uptake for all of them occurred under this treatment. In contrast, treatment with D1 combined with AMFs (D1-*R.irr* and D1-*R.cus*) generally displayed a lower uptake of most of the PTEs in the plants, both in the roots and shoots, as evidenced by the PCA results, where these treatments were positioned furthest from most elements and the M treatment. This was particularly significant for Pb in both plant parts, and for As, Cd, and Sb in the roots (Fig. S3). However, the general influence observed for all treatments tested, leading to a reduced uptake of PTEs by plants to different extents compared to that in M, highlights that the combined application of organic amendments with AMFs was effective in promoting survival and conferring resistance to plants in polluted soils by different mechanisms.

As previously discussed, the treatments improved PTE immobilization in the soil through different mechanisms, which reduced the PTE availability. Treatments that showed greater decreases in plant uptake compared to that of M (DOR combined with AMF treatments) coincided with those for which lower bioavailable fractions of PTEs were measured in soil (Table 2). Therefore, the influence of treatments on the

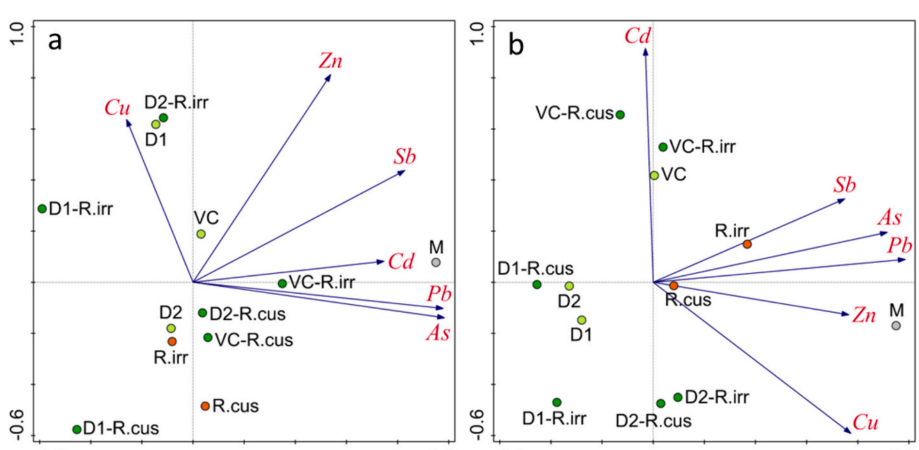


Fig. 5. Principal component analysis results for different treatments and their correlations with PTEs concentrations in plants roots (a) and shoots (b). The first two principal components explained over 90 % of the variance in both cases. M (marble addition); D1 (dry olive residue mycoremediated by *C. rigida*); D2 (dry olive residue mycoremediated by *C. radians*); VC (vermicompost); *R.irr* (*R. irregularis*); *R.cus* (*R. custos*).

immobilization of pollutants in the soil was evidenced as a primary process for their protective effect on the plants growing in polluted soils. AMFs play an important role in reducing PTE transfer to plants, as they can enhance the phytostabilization of these elements by increasing their retention in the hyphae and roots, thus reducing their translocation to the shoots (Soares and Siqueira, 2008; Meier et al., 2012; Cabral et al., 2015). This is consistent with the highest PTEs reduction rates observed in the shoots of the plants, especially in treatments with AMFs in combination with DOR amendments (Fig. S3). García-Sánchez et al. (2021) documented an enhancement of mycorrhizostabilization mechanisms through the application of organic amendments, primarily attributed to the AMF promotion of glomalin and phytochelatin production, consequently stimulating these mechanisms. In the present study, this was evidenced by the fact that AMF treatments without organic amendments (i.e., *R.irr* and *R.cus*), especially *R.irr*, were less effective in reducing the concentrations of Pb and As in the shoots. Moreover, increases in PTE concentrations in plants under the treatments were also found in some cases, with the most remarkable being the increase in Cd concentration in the shoots under the vermicompost treatments, which showed a sharp increase compared with that of M and the other treatments. This was indicated by the strong positive correlation shown in the PCA results (Fig. 5b). A higher accumulation of Cd in the leaves of plants growing in soil amended with vermicompost was previously identified by Sebastian and Prasad (2013), although in our study, the exceptional Cd accumulation in shoots under the vermicompost treatments was not related to a negative effect on their growth.

3.3.3. Transfer of PTEs from soil to plant

Concentrations extracted by EDTA were selected for BAF and BCF calculation, since this fraction better represents the bioavailable forms of PTEs for plants (Marguí et al., 2007; Parra et al., 2014). In general, the applied treatments were effective in significantly reducing the shoot uptake of PTEs compared to M, especially for Pb, As, Cu, and Sb, leading to significantly lower BAFs for these elements under most treatments (Table 3). Nevertheless, a slight accumulation of Pb, As, Zn, and Sb in wheat shoots was still observed (BAF >1), although to a significantly lesser extent than in M. Moreover, treatments with only AMF inoculation showed lower BAF reductions for these elements, especially in the case of Pb and As with *R. irregularis* inoculation, which remained at levels similar to those measured in M. Conversely, the DOR amendments, especially the combination of D1 with both AMFs, showed the lowest accumulations of certain elements (i.e. Pb and Sb) in shoots. No accumulation in the shoots (BAF <1) was found for Cu and Cd, although for the latter, BAF >1 was observed for the vermicompost treatments.

Regarding PTE accumulation in roots, concentrations measured in this part of the plant for all studied elements were significantly higher than their bioavailable fraction in soils (BCF >1) in all treatments, being especially high for Pb and As, but also for Cd and Sb (Table 3). In contrast, the accumulation of Zn and Cu in the roots was much lower, and low accumulation was also observed in the shoots for both elements. These elements are considered to have low accumulation capacity in different grass plants (Bhatti et al., 2016; Andrejić et al., 2018). However, only for Zn did shoot concentrations approximate the values measured in the roots, exhibiting a TF closer to one. In any case, no translocation from the roots to shoots was estimated for this element under any treatment (TF <1) (Table S3). For all other elements, the minimal uptake in shoots compared to the substantial accumulation in roots led to a very low TF under all treatments, especially for Pb and As (TF <0.01). This highlighted the effectiveness of the treatments in preventing the transfer of pollutants to the aboveground parts of the plants. In fact, the significantly higher accumulation of PTEs in the root tissues was attributed to AMF mechanisms, which immobilized them in the mycorrhizosphere and fungal structures, thereby restricting their translocation to shoots and consequently promoting the phytostabilization process (Singh et al., 2019). This process can be further improved by the addition of organic amendments (García-Sánchez et al., 2017), as observed in our results, particularly for DOR-based amendments. Among other factors promoting phytostabilization that can be attributed to organic amendments is their direct effect in promoting plant growth and, more specifically, root development. This results in increased soil colonization by roots and greater biomass in this part of plants, leading

Table 3
Transfer of PTEs from soil to plant (BAF: bioaccumulation factor; BCF: bioconcentration factor) in each of the different studied treatments. M (marble addition); D1 (dry olive residue mycoremediated by *C. rigida*); D2 (dry olive residue mycoremediated by *C. radians*); VC (vermicompost); *R.irr* (*R. irregularis*); *R.cus* (*R. custos*). Data are presented as the mean ± standard deviation, $n = 5$.

Treatment	Pb_BAF	As_BAF	Zn_BAF	Cu_BAF	Cd_BAF	Sb_BAF
M	10.12 ± 2.30 *c*	5.09 ± 3.44 *b*	2.14 ± 0.23 *cde*	0.58 ± 0.32 *b*	0.22 ± 0.03 *a*	8.95 ± 4.66 *e*
R.irr	12.31 ± 2.93 *c*	5.23 ± 1.52 *b*	3.05 ± 0.40 *f*	0.33 ± 0.05 *a*	1.09 ± 0.15 *c*	2.82 ± 0.95 *abc*
R.cus	4.34 ± 1.64 *ab*	2.15 ± 0.72 *a*	2.60 ± 0.41 *e*	0.36 ± 0.19 *a*	0.46 ± 0.08 *ab*	6.31 ± 3.03 *d*
D1	2.98 ± 1.62 *ab*	0.71 ± 0.24 *a*	2.09 ± 0.24 *cd*	0.23 ± 0.03 *a*	0.38 ± 0.13 *ab*	2.26 ± 0.28 *ab*
D2	2.91 ± 1.46 *ab*	1.14 ± 0.67 *a*	2.33 ± 0.26 *cde*	0.21 ± 0.02 *a*	0.39 ± 0.21 *ab*	1.93 ± 1.06 *ab*
VC	3.73 ± 0.77 *ab*	1.12 ± 0.14 *a*	2.36 ± 0.30 *cde*	*0.23* ± 0.04 *a*	2.07 ± 0.98 *d*	5.89 ± 1.99 *d*
D1-*R.irr*	1.32 ± 0.65 *a*	1.26 ± 0.55 *a*	2.22 ± 0.34 *cde*	*0.31* ± 0.16 *a*	0.30 ± 0.15 *a*	1.05 ± 0.79 *a*
D1-*R.cus*	2.46 ± 2.01 *ab*	1.73 ± 0.62 *a*	2.54 ± 0.40 *de*	0.20 ± 0.02 *a*	0.76 ± 0.20 *bc*	1.17 ± 0.09 *a*
D2-*R.irr*	9.27 ± 4.30 *c*	2.09 ± 0.36 *a*	1.41 ± 0.24 *a*	0.23 ± 0.04 *a*	0.16 ± 0.05 *a*	2.79 ± 0.71 *abc*
D2-*R.cus*	5.68 ± 2.36 *b*	1.54 ± 1.36 *a*	2.51 ± 0.49 *de*	0.38 ± 0.17 *a*	0.38 ± 0.15 *ab*	5.48 ± 1.52 *cd*
VC-*R.irr*	3.84 ± 2.14 *ab*	1.23 ± 0.44 *a*	1.93 ± 0.16 *bc*	0.24 ± 0.02 *a*	2.32 ± 0.22 *d*	5.12 ± 1.40 *cd*
VC-*R.cus*	3.42 ± 2.41 *ab*	2.41 ± 0.63 *a*	1.64 ± 0.15 *ab*	0.19 ± 0.02 *a*	2.14 ± 0.19 *d*	3.98 ± 0.85 *bcd*
	Pb_BCF	**As_BCF**	**Zn_BCF**	**Cu_BCF**	**Cd_BCF**	**Sb_BCF**
M	615.9 ± 97.1 *bc*	189.9 ± 29.2 *d*	2.57 ± 0.47 *a*	2.99 ± 0.39 *ab*	15.16 ± 3.99 *ab*	61.68 ± 29.38 *d*
R.irr	589.0 ± 253.8 *bc*	150.1 ± 35.2 *cd*	3.30 ± 0.49 *abc*	3.28 ± 0.96 *ab*	15.14 ± 1.80 *ab*	34.29 ± 12.24 *abc*
R.cus	369.0 ± 110.1 *ab*	148.7 ± 45.4 *cd*	3.08 ± 0.52 *ab*	2.34 ± 0.25 *a*	19.96 ± 2.60 *c*	47.45 ± 17.38 *cd*
D1	433.8 ± 174.3 *abc*	66.0 ± 18.1 *a*	3.32 ± 0.62 *bc*	4.45 ± 0.68 *c*	12.94 ± 1.34 *a*	31.76 ± 8.53 *abc*
D2	426.5 ± 78.7 *abc*	103.4 ± 18.3 *abc*	3.44 ± 0.66 *bc*	3.43 ± 0.58 *b*	15.76 ± 1.97 *ab*	29.84 ± 7.02 *abc*
VC	322.3 ± 88.1 *a*	95.0 ± 52.3 *abc*	3.85 ± 0.44 *c*	2.92 ± 0.91 *ab*	16.81 ± 3.22 *b*	42.17 ± 13.77 *bc*
D1-*R.irr*	268.8 ± 68.2 *a*	76.2 ± 21.9 *ab*	3.07 ± 0.38 *ab*	3.42 ± 0.33 *b*	12.36 ± 1.22 *a*	26.66 ± 5.46 ab
D1-*R.cus*	517.9 ± 185.6 *abc*	132.0 ± 59.4 *bc*	3.05 ± 0.23 *ab*	3.59 ± 0.79 *bc*	12.67 ± 1.11 *a*	18.74 ± 8.45 *a*
D2-*R.irr*	596.8 ± 297.3 *bc*	100.6 ± 32.9 *abc*	3.14 ± 0.34 *ab*	3.59 ± 1.14 *bc*	14.31 ± 3.87 *ab*	46.47 ± 14.28 *bcd*
D2-*R.cus*	670.8 ± 134.8 *c*	133.8 ± 39.9 *c*	3.19 ± 0.47 *abc*	3.11 ± 0.49 *ab*	14.92 ± 1.08 *ab*	41.81 ± 9.27 *bc*
VC-*R.irr*	439.4 ± 130.1 *abc*	94.0 ± 28.9 *abc*	2.94 ± 0.58 *ab*	3.20 ± 0.23 *ab*	14.74 ± 1.98 *ab*	41.54 ± 10.06 *bc*
VC-*R.cus*	495.1 ± 126.4 *abc*	127.0 ± 35.8 *bc*	2.85 ± 0.41 *ab*	2.80 ± 0.59 *ab*	13.26 ± 2.70 *ab*	30.32 ± 5.36 *abc*

Lowercase letters indicate significant differences among treatments according to Duncan's post hoc test ($p < 0.05$).

to a higher capacity of plants to accumulate and immobilize greater amounts of PTEs.

4. Conclusions

The application of amendments to polluted soil led to an overall improvement in the main soil properties and reduced soil toxicity by decreasing the concentrations of some PTEs. The treatments tested showed different degrees of effectiveness in recovering soil function and reducing pollution levels. The treatment based only on an inorganic liming amendment (marble sludge) was effective in neutralizing the soil pH, thereby reducing the availability of highly mobile elements such as Zn, Cu, and Cd and allowing plants to survive in polluted soil. In contrast, it was less effective in promoting plant growth and soil enzymatic activities and in providing protection to plants against PTE uptake. However, the combined application of inorganic and organic amendments increased their effectiveness in promoting plant growth and survival, and improving soil biological status. Vermicompost-based treatments led to significant increases in the plant shoots, but their performance in reducing PTE toxicity and providing plants with protection mechanisms against pollutant uptake was less effective than that of treatments based on DOR biotransformed by saprobic fungi. Treatments incorporating this amendment led to the greatest increase in the wheat plant biomass and soil enzymatic activity, especially when combined with the inoculation of AMFs. In this regard, the treatment consisting of a combination of marble with DOR biotransformed by *C. rigida* (DOR1) and inoculated with AMFs showed the highest potential for reducing PTE concentrations in plants and inducing growth, thus playing an important bioprotective role in the plants. Therefore, the joint implementation of DOR and AMFs further enhanced their efficacy through the synergistic effects produced between them, promoting the immobilization of PTEs in soil and stimulating the phytostabilization mechanisms induced by AMFs. Among the AMFs tested, *R. custos* showed slightly better performance than *R. irregularis*, suggesting the potential benefits of using indigenous, locally adapted fungi from polluted sites, as they may perform better under pollution conditions and consequently show higher remediation potential.

Our results demonstrate that mycoremediated DOR may be an efficient product for use as a soil organic amendment for remediating metal (loid)-polluted soils. Furthermore, our findings suggest that the application of biotransformed DOR in combination with AMFs may enhance the remediation potential of soils polluted with PTEs, representing a promising remediation strategy for the recovery of soil function in polluted areas.

CRediT authorship contribution statement

Mario Paniagua-López: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation. **Gloria Andrea Silva-Castro:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation. **Ana Romero-Freire:** Writing – review & editing, Visualization, Validation, Methodology. **Francisco José Martín-Peinado:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Manuel Sierra-Aragón:** Writing – review & editing, Visualization, Validation, Supervision, Methodology, Investigation, Data curation, Conceptualization. **Inmaculada García-Romera:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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