



Adsorption of antibiotics on bio-adsorbents derived from the forestry and agro-food industries

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ABSTRACT

Antibiotic consumption at high levels in both human and veterinary populations pose a risk to their eventual entry into the food chain and/or water bodies, which will adversely affect the health of living organisms. In this work, three materials from forestry and agro-food industries (pine bark, oak ash and mussel shell) were investigated as regards their potential use as bio-adsorbents in the retention of the antibiotics amoxicillin (AMX), ciprofloxacin (CIP) and trimethoprim (TMP). Batch adsorption/desorption tests were conducted, adding increasing concentrations of the pharmaceuticals individually (from 25 to 600 $\mu\text{mol L}^{-1}$), reaching maximum adsorption capacities of $\approx 12000 \mu\text{mol kg}^{-1}$ for the three antibiotics, with removal percentages of $\approx 100\%$ for CIP, 98–99% adsorption for TMP onto pine bark, and 98–100% adsorption for AMX onto oak ash. The presence of high calcium contents and alkaline conditions in the ash favored the formation of cationic bridges with AMX, whereas the predominance of hydrogen bonds between pine bark and TMP and CIP functional groups explain the strong affinity and retention of these antibiotics. The Freundlich's model provided the best prediction for AMX adsorption onto oak ash and mussel shell (heterogeneous adsorption), whereas the Langmuir's model described well AMX adsorption onto pine bark, as well as CIP adsorption onto oak ash (homogeneous and monolayer adsorption), while all three models provided satisfactory results for TMP. In the present study, the results obtained were crucial in terms of valorization of these adsorbents and their subsequent use to improve the retention of antibiotics of emerging concern in soils, thereby preventing contamination of waters and preserving environment quality.

Credit author statement

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1. Introduction

Anti-infective drugs, specifically antibiotics, are one of the most widely used pharmacological groups in therapy, not only for antimicrobial treatment but also as prophylactics to prevent the acquisition of transmissible diseases in humans, animals, or plants, and in certain countries even as growth promoters in animals (Castanon, 2007). However, due to related health risks, the European Union banned the use of antibiotics as growth promoters in livestock in 2006 (Casewell et al., 2003).

The massive use of antibiotics in recent years has led to their spreading and subsequent detection in water and cultivated soils, posing a great threat to humans and ecosystems (Zhang et al., 2017). As they are not fully absorbed in the intestine, a significant proportion of these biocides (up to 90%) is eliminated from the body through excretion in the feces and urine (Russell and Yost, 2021), (Duan et al., 2021). Therefore, they end up in solid excreta and effluents from veterinary consumption, and/or into wastewaters and the sludge generated from wastewater treatment plants (WWTP) after use in human medicine (Rodríguez-Mozaz et al., 2020). In relation to the latter, most WWTP are not efficient enough for the inactivation/removal of antibiotics (removal efficiencies ranging from 30% to 90%), which causes a large part of them are released in the effluents and/or accumulated in sludges (Gogoi et al., 2018), (Michael et al., 2013). The use of WWTP sludge and/or irrigation with wastewater effluents to improve soil fertility may involve the incorporation of these pollutants into crop soils (Pan and Chu, 2016), (Yang et al., 2016). In addition, the generation and proliferation of resistant bacteria, ecotoxicity and their entry into the food chain through water and crops are risks associated to the incorporation of antibiotics into soils. These compounds are considered emerging pollutants when reaching the environment, even at very low concentrations, due to their potential adverse effects on human and animal health (Rahbar Shahrouzi et al., 2019). The increase in bacterial resistance is causing higher medical costs, prolonged hospital stays and increased mortality (Brown and Wright, 2016)– (García, 2022).

Numerous investigations have focused on designing systems to remove antibiotics from soils (Ding et al., 2016). Furthermore, to note that advanced oxidation, hydrolysis, photodegradation, biodegradation and adsorption are among the main methods used to remove antibiotics from water. However, advanced oxidation processes can generate toxic by-products (Du and Liu, 2012) and membrane technologies do not offer sufficient reliability for the removal of antibiotics (Cheng et al., 2018). Conversely, adsorption is a simple, low-cost, highly efficient, non-polluting, and renewable process (Singh et al., 2020)– (Karoui et al., 2020). Adsorption of antibiotics onto soils could be of real help to find the best and affordable solution to control the entry of these pollutants in the food chain (Cela-Dablanca et al., 2022a). In this line, the use of (bio)adsorbent materials to retain antibiotics and prevent their entry into the food chain is a plausible alternative that could boost their retention in soils (Cela-Dablanca et al., 2022b), (Rodríguez-González et al., 2023). Among sorbent materials, activated carbon is widely used, but it has a very high cost with associated regeneration issues (Crisafulli et al., 2008). Other bio-adsorbents, such as mussel shells, waste and by-products of the forestry industry such as cork, pine needles or biomass ash are potential candidates for this purpose (Conde-Cid et al., 2019)– (Philippou et al., 2021). These residues/by-products could be added to the soil or used in modules specifically designed and implemented in WWTP to minimize the risk of dispersion of these pollutants into the environment. Additionally, this fact would imply the valorization of the by-products, reducing their impacts on the environment (Karić et al., 2022)– (González et al., 2023). A study by Conde-Cid et al. (2020) (Conde-Cid et al., 2020) showed that pine bark increases the adsorption and decreases the desorption of three types of sulfonamides. These materials have already been studied as adsorbents for heavy metals, inorganic anions, and antibiotics from the group of tetracyclines and sulfonamides, with very positive results (Ramírez-Pérez et al.,

2013)– (Quintáns-Fondo et al., 2019).

The present research aims to investigate the adsorption/desorption of three commonly used antibiotics in human medicine (Palacio et al., 2020), amoxicillin (AMX), trimethoprim (TMP), and ciprofloxacin (CIP) utilizing three different bio-adsorbents, namely pine bark, mussel shell, and oak ash. By focusing on these specific antibiotics and bio-adsorbents, this research offers novel insights into the mechanisms involved in the adsorption process. Furthermore, it builds upon previous findings (Cela-Dablanca et al., 2022b), (Cela-Dablanca et al., 2022c), (Rodríguez-López et al., 2022) and contributes to a deeper understanding of the role played by bio-adsorbents in retaining these pharmaceuticals of emerging concern. The outcomes of this study are significant as they can help minimize the environmental dispersion and associated risks of these pharmaceuticals.

2. Materials and methods

2.1. Selection of the bio-adsorbents

The biosorbents used in the study come from the forestry and agro-food industry. Specifically, oak ash comes from the combustion of oak logs used in a combustion boiler (Lugo, Spain); mussel shell was provided by the company Abonomar S.L. (Illa de Arousa, Pontevedra, Spain), with a particle size of less than 1 mm; and Geolia (Madrid, Spain) provided pine bark, which was crushed and sieved at the laboratory through a 0.63 mm mesh.

2.2. Chemicals and reagents

The antibiotics ciprofloxacin (CIP) and trimethoprim (TMP) were of 98% purity while amoxicillin trihydrate (AMX) was of >95% purity, all of them supplied by Sigma-Aldrich (Madrid, Spain).

2.3. Characterizations of the bio-adsorbents

The pH was measured in water and in a KCl solution using a 1:2.5 soil:liquid ratio (Guitian Ojea and Carballas, 1976), with 10 min and 2 h of contact time, respectively, using a pH-meter CRISON model 2001 (Crison, Barcelona, Spain). Carbon (and then Organic matter -OM) and nitrogen contents were measured by elemental analysis using a TRUSPEC CHNS equipment, LECO model (USA). Exchange cations were displaced from the bio-adsorbents using a 1 M NH₄Cl extractant (Peech, 1947), in a 1:10 adsorbent:solution ratio, for 12 h. Atomic adsorption/emission spectrophotometry (Analyst 200, PerkinElmer, USA) was used to quantify Al, Ca, Mg, Na and K, adding 1% of LaCl₃ to avoid interferences. The sum of these exchange cations was made to obtain the effective cation exchange capacity (eCEC) (Fox and Kamprath, 1970). The non-crystalline iron (Fe_{ox}) and aluminum (Al_{ox}) fractions were extracted with an ammonium oxalate buffered solution at pH = 3 (v Blackmore, 1978). These fractions were measured with the atomic absorption spectrophotometer referenced above. Table S1 (Supplementary Material) shows the values of these physicochemical parameters for the three bio-adsorbents, as done in previous publications dealing with the same sorbent materials (Cela-Dablanca et al., 2021).

2.4. Adsorption/desorption experiments and antibiotics quantification

Batch tests were carried out in three simple systems at 25 °C (including just one of the antibiotics, three replicates for each adsorption test) for seven increasing concentrations (0, 25, 50, 100, 200, 400 and 600 μmol L⁻¹) of the individual antibiotics AMX, CIP or TMP. So, 0.5 g of each of the individual bio-adsorbents were stirred with 10 mL of a 0.005 M CaCl₂ solution (used as background electrolyte to keep constant the ionic strength) with each of the different antibiotic concentrations (for each individual antibiotic independently) for 48 h, time enough to reach equilibrium, based on previous kinetics experiments (Cela-Dablanca

et al., 2022b), (Rodríguez-López et al., 2022). After ending the adsorption step, the samples were centrifuged at 4000 rpm for 15 min and then the resulting supernatants were filtered through 0.45 μm nylon-type syringe filters. Antibiotic concentrations were measured by HPLC, using a Thermo-Fisher Model LPG 3400 SD equipment (USA) coupled with a HPG-3400 quaternary pump (USA), a WPS3000 autosampler (USA), a thermostated compartment for the TM-120 column, and an ultraviolet-visible detector of the UltiMate 3000 series (USA). To process the data, a computer with the Chromeleon software was connected. The column used was a Luna C18 (150 mm long; 4.6 mm internal diameter; 5 μm particle size) provided by Phenomenex (Madrid, Spain) and a pre-column (4 mm long; 2 mm internal diameter; 5 μm particle size) packed with the same material as the column. The injection volume was 50 μL and the flow rate was 1.5 mL min^{-1} .

In the case of AMX, the mobile phase was acetonitrile (phase A) and 0.01 M phosphoric acid, pH = 2 (phase B). The linear gradient had a variation from 5% to 15% of phase A and from 95% to 85% of phase B for 4 min. With respect to TMP and CIP, the phases were the same as for AMX, but with a different linear gradient, varying from 5 to 32% of phase A and from 95 to 68% of phase B for 10.5 min. The wavelengths used for the quantification were: 212 nm for TMP and CIP and 230 nm for AMX.

After the adsorption step, subsequent desorption tests were conducted at 25 $^{\circ}\text{C}$ (three replicates for each desorption test, as for adsorption). First, the remaining material after the adsorption process was weighed and 10 mL of 0.005 M CaCl_2 was introduced, letting the samples stir for 48 h. After this time, they were centrifuged and filtered under the same conditions as in the adsorption process. In the equilibrium solution, the concentration of the corresponding antibiotic was determined, analogously to that indicated in the adsorption tests.

2.5. Adsorption modelling and data treatment

To describe adsorption data, Freundlich (Eq. (1)), Langmuir (Eq. (2)), and the Linear model (Eq. (3)) were applied:

$$q_e = K_f C_e^n \quad (1)$$

$$q_e = \frac{q_{max} K_L C_e}{1 + K_L C_e} \quad (2)$$

$$K_d = \frac{q_e}{C_e} \quad (3)$$

Where q_e ($\mu\text{mol kg}^{-1}$) is the adsorbed amount of antibiotic per unit mass of the adsorbent (difference between what is added and what remains in the equilibrium solution); C_e ($\mu\text{mol L}^{-1}$) is the concentration in the equilibrium solution of the antibiotic of study; K_f ($\text{L}^n \text{kg}^{-1} \mu\text{mol}^{1-n}$) is the Freundlich constant related to the adsorption capacity; n (dimensionless) is a parameter related to the intensity of adsorption (Sukul et al., 2008); K_L ($\text{L } \mu\text{mol}^{-1}$) is the Langmuir constant related to the adsorption energy; q_{max} ($\mu\text{mol kg}^{-1}$) represents the Langmuir's maximum adsorption capacity, and K_d (L kg^{-1}) is the partition coefficient in the Linear model.

The SPSS software (version 18) was used for the fitting of these models, as well as for performing bivariate statistical Pearson correlations between adsorption and desorption parameters and bio-adsorbent's physicochemical characteristics.

3. Results and discussion

3.1. Adsorption of antibiotics onto bio-adsorbents

Fig. 1 shows results corresponding to adsorption/desorption of the different antibiotics onto the bio-adsorbents, expressed both in $\mu\text{mol kg}^{-1}$ and as percentage. Regarding AMX, the best bio-adsorbent was oak ash, with adsorption increasing as the concentration of added antibiotic

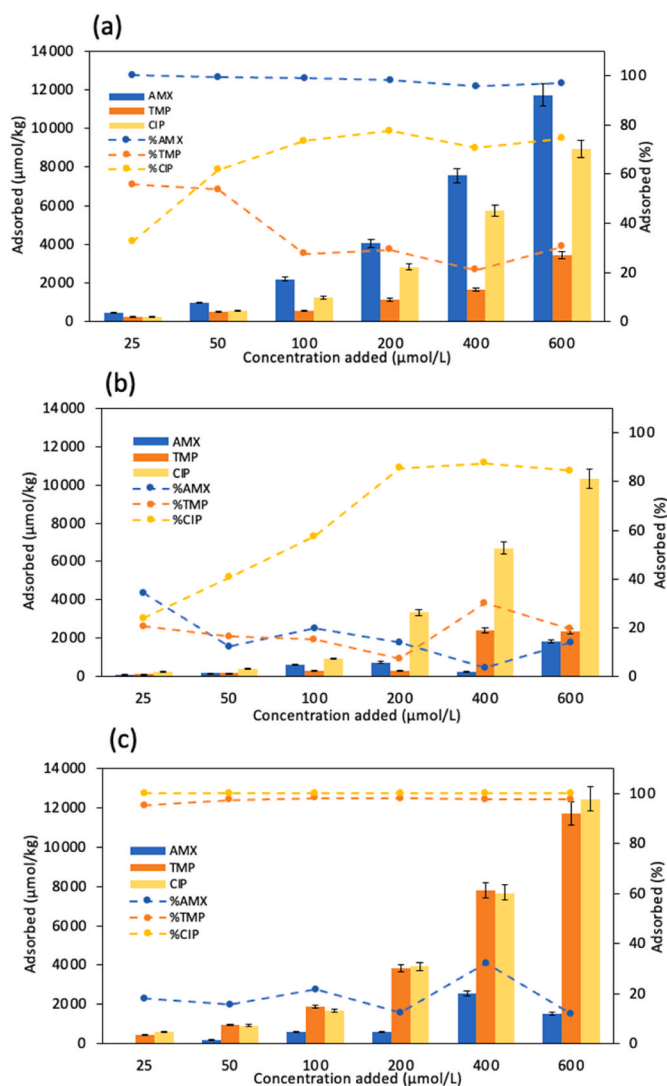


Fig. 1. Adsorption in $\mu\text{mol kg}^{-1}$ (left y-axis) and percentage (right y-axis) for AMX, TMP and CIP onto (a): oak ash; (b): mussel shell, and (c): pine bark.

grew, obtaining the highest value at a added concentration of 600 $\mu\text{mol kg}^{-1}$. In any case, the percentages of this antibiotic adsorbed on the ash were very close to 100% for any of the concentrations added. Pine bark and mussel shell showed a clearly lower capacity to adsorb AMX, generally reaching less than 20%.

Contrary, for CIP and TMP the best adsorbent turned out to be pine bark, with adsorption percentages very close to 100% for any of the concentrations added (Fig. 1). Moreover, for CIP, oak ash and mussel shell also have considerable adsorption potential, especially when the highest concentrations of antibiotic were added, with percentages close to 70% and 80%, respectively. Contrary, in the case of TMP, oak ash could only retain 50% of that antibiotic when the two lowest concentrations of this pollutant were added, and did not exceed 30% at the highest, while mussel shell generally adsorbed less than 20% of the antibiotic for any of the concentrations added.

Regarding the processes of AMX adsorption, it must be taken into account that this antibiotic has three pK_a values, so that it can behave as a cation, anion or as a zwitterion. The carboxyl group has a pK_{a1} of 2.67, the amine group has a pK_{a2} of 7.11, and the phenolic hydroxyl has a pK_{a3} of 9.63 (de Oliveira et al., 2018). Consequently, at a pH below pK_{a1} , the positive charge of the amino group of AMX determines the charge of the molecule. When the pH values are between pK_{a1} and pK_{a2} , the zwitterionic form predominates, while for pH values between pK_{a2} and pK_{a3}

the deprotonated form of the carboxyl and amine groups prevails. For pH values greater than pK_{a3} , AMX acquires a double negative charge since the deprotonation of the phenolic hydroxyl is favored (Pezoti et al., 2016).

The retention mechanisms of this antibiotic are different in the various bio-adsorbents used since these materials have different characteristics. The main mechanisms taking place are electrostatic interactions, cationic bridges, and hydrogen bonds (Anastopoulos et al., 2020).

As regards oak ash, its basic pH (11.31) and high concentrations of Fe and Al non-crystalline minerals (Table S1, Supplementary Material) make that this bio-adsorbent has negative charge. In fact, when AMX is at a pH of 11.31 its amino, carboxyl and phenol groups are deprotonated, acquiring a high negative charge. These characteristics added to the high calcium content of the ash (Table S1, Supplementary) explain the high adsorption of AMX onto this bio-adsorbent, since calcium would act as a cationic bridge between the adsorbent and adsorbate. This explanation could be extrapolated to mussel shell, although its adsorption capacity is much lower (Fig. 1). This can be due to its lower content in non-crystalline Fe and Al, as well as in exchangeable calcium, and to the fact that mussel shell has a pH not as alkaline as oak ash (9.39 vs 11.31). Therefore, there are fewer reactive surfaces in this bio-adsorbent, as well as fewer negative charges in the antibiotic (because the prevailing pH is below its pK_{a3}), and lower content of exchangeable Ca that could act as a cationic bridge. Although other adsorption mechanisms may also be possible, such as electrostatic attraction between the protonated groups of AMX (which at this pH behaves as a zwitterionic species) and the negative charges of the adsorbent material, it has a low content of variable charge components such as organic matter or non-crystalline minerals.

The low adsorption of AMX onto pine bark may be due to the fact that it has a very acidic pH (3.99) and at that pH the organic components and non-crystalline minerals are positively charged. At that pH value, AMX would act as a zwitterion, with protonated amino groups and deprotonated carboxyl groups, the former possibly predominating given the acidity of the medium, with electrostatic repulsions existing that would justify this low adsorption. When, in a previous study, Cela-Da-Blanca et al. (2022b) added low concentrations of AMX ($<50 \mu\text{mol kg}^{-1}$) to different soils and adsorbent materials, these authors also found greater adsorption when using oak ash (89.71%) than on mussel shell (48.15%), and much lower adsorption for pine bark (29.38%).

In the case of CIP, it has pK_a values of 5.90 ± 0.15 (for the carboxylic acid group) and 8.89 ± 0.11 (for the N-basic group) so it can exist as cation, zwitterion and anion depending on soil and water pH (Genc et al., 2013). The bio-adsorbent with the highest adsorption capacity for this antibiotic is pine bark, which has a pH lower than the pK_{a1} of CIP, so that the adsorbent will have positive charges and so will CIP. Thus, electrostatic forces would prevent adsorption to occur. Pine bark has a high organic matter content, which is made up of a large amount of carboxyl and hydroxyl groups that can form hydrogen bridges with the carbonyl groups and the hydroxyl groups of CIP. In addition, this antibiotic possesses benzene rings that can act as an electron acceptors, particularly due to the high electronegativity of the available fluorine contained in its structure (Movasaghi et al., 2019), receiving electrons donated by the hydroxyl groups of the pine bark's organic components. In addition, under those acidic conditions, free H^+ in the medium can contribute to the formation of hydrogen bonds between the adsorbent surface and CIP molecules (Peng et al., 2015). Some authors also pointed out that the maximum adsorption efficiency for this antibiotic occurs between pH 4 and 7 (Chandrasekaran et al., 2020). However, in the current study oak ash and mussel shell showed a considerable adsorption capacity for CIP (close to 80% for the highest added concentrations) (Fig. 1). In the case of oak ash, the most likely mechanism is cationic bridges established by exchangeable calcium, as in the case of AMX, since at the pH of oak ash (11.31) CIP acts as an anion, and oak ash will be negatively charged. Finally, in the case of mussel shell, the adsorption

mechanisms will be similar to those that occur for the adsorption of AMX onto this bio-adsorbent. In addition, it is possible that at the pH of mussel shell (9.39), CIP still has some positive charge so that there could also be some electrostatic attractions, although this might not be the main mechanism. Studies conducted by Chandrasekaran et al. (2020) with *Prosopis juliflora* wood powder showed that there is a greater adsorption in a simple system of CIP than of AMX, as it happens in the current study for pine bark and mussel shell, but not for oak ash.

In the present research, the only effective bio-adsorbent to retain TMP was pine bark, presenting the other two sorbent materials poor retention, particularly when the highest concentrations of the antibiotic were added (in the case of oak ash) (Fig. 1). This antibiotic has just one pK_a , with a value of 7.3, having only cationic and zwitterionic form, being mainly cation (80%) and in a smaller percentage zwitterion (20%) (de Oliveira et al., 2018). Its high adsorption on pine bark could be mainly due to hydrogen bonding, since pine bark has a large number of carboxyl and hydroxyl groups that bind to the NH_2 groups in the heterocyclic aromatic ring of TMP (Li and Zhang, 2017). In addition, in acidic conditions, as in the present case, the free H^+ in the medium can contribute to the formation of hydrogen bonds between the surface of the adsorbent and TMP molecules, as happened with CIP (Peng et al., 2015). Considering all the bio-adsorbents used in this work in relation to TMP adsorption, a positive and significant correlation ($p < 0.05$) was found with carbon content. Thus, as pine bark has the highest content in organic compounds, it exhibited the highest percentage of TMP adsorption compared to the rest of the bio-adsorbents.

3.1.1. Adsorption modelling

Fig. 2 shows the adsorption curves representing the experimental results obtained in this research for the set of antibiotics and bio-adsorbents tested.

In addition, Table 1 shows the values corresponding to the fitting of the experimental data to the Freundlich, Langmuir and Linear models for each of the antibiotics and bio-adsorbents.

Restricting the comments to those cases where error values associated to the fitting are not too high, comparing R^2 values it can be observed that for TMP all the models have rather similar scores in all the bio-adsorbents (0.80–0.99), while the Freundlich model describes better the adsorption of AMX in oak ash and mussel shell (0.94 and 0.69, respectively), while Freundlich and the Linear models fit similarly for AMX adsorption onto pine bark (0.69 and 0.64, respectively). For CIP, Freundlich and the Linear models show the best fits for oak ash ($R^2 = 0.73$ and 0.71 , respectively), and for mussel shell ($R^2 = 0.81$ and 0.76 , respectively). CIP adsorption onto pine bark was 100% for all the antibiotic concentrations studied, therefore its modelling was not possible. It is important to mention that Langmuir model shows significantly high errors when predicting most parameters and for most of the sorbents. This could be due to the fact that the thermodynamic saturation condition of the bio-adsorbents was not achieved as a sufficiently high concentration of the antibiotics was not used to ensure the saturation state (Tran et al., 2016).

In TMP adsorption, considering the Linear model, the partition coefficient values (K_d) ranged between 5.3 and 833 L kg^{-1} , with the highest level corresponding to the adsorption onto pine bark, characterized by adsorption percentages close to 100% for all the concentrations (Fig. 1). Comparing these results with previous studies, K_d values for TMP adsorption onto agricultural soils were in the range of $10\text{--}48 \text{ L kg}^{-1}$ (Rodríguez-López et al., 2022), while in Australian soils the scores were $9\text{--}311 \text{ L kg}^{-1}$ (Williams et al., 2009), in accordance with what was obtained in the present study. Also, K_f and K_L corresponding to TMP adsorption onto pine bark showed values (which was not possible for oak ash and mussel shell due to too high error values associated to the fitting), evidencing a better affinity of pine bark towards the adsorption of this antibiotic. Previously reported for TMP adsorption on agricultural soils, K_f levels were in the range of $29\text{--}125 \text{ L}^n \text{ kg}^{-1} \mu\text{mol}^{1-n}$ (Rodríguez-López et al., 2022), being comparable but significantly lower

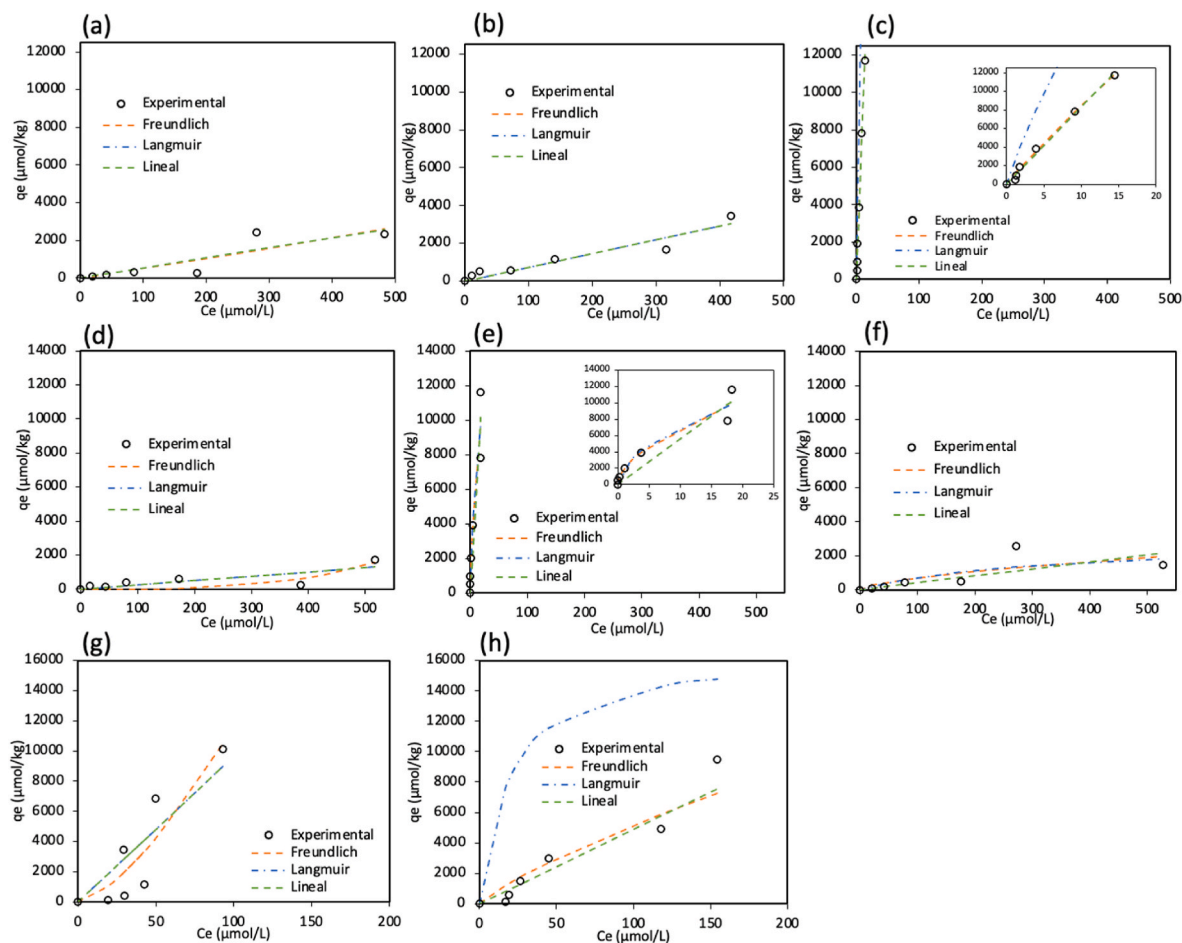


Fig. 2. Adsorption curves for trimethoprim (a–c) onto (a): mussel shell, (b): oak ash, and (c): pine bark; amoxicillin (d–f) onto (d): mussel shell; (e): oak ash, and (f): pine bark; ciprofloxacin (g,h) onto (g): mussel shell, and (h): oak ash.

*CIP adsorption onto pine bark was 100% for all the concentrations tested, therefore it was not possible to be modelled.

Table 1

Parameters of the Freundlich (K_f , expressed in $L^n kg^{-1} \mu mol^{1-n}$, and n , dimensionless), Langmuir (K_L , expressed in $L \mu mol^{-1}$, and q_{max} , expressed in $\mu mol kg^{-1}$) and Linear (K_d , expressed in $L kg^{-1}$) models for amoxicillin (AMX), ciprofloxacin (CIP) and trimethoprim (TMP) adsorption onto the three bio-adsorbents of study; -: error value too high for fitting.

Bio-adsorbent	Antibiotic	Freundlich			Langmuir			Linear model						
		K_f	Error	n	Error	R^2	K_L	Error	q_{max}	Error	R^2	K_d	Error	R^2
Oak ash	AMX	1824.03	685.92	0.58	0.14	0.94	0.104	0.076	14909	4359	0.93	555.28	55.84	0.89
	CIP	–	–	0.81	0.36	0.73	0.050	0.009	–	–	0.75	48.81	8.82	0.71
	TMP	–	–	1.02	0.28	0.91	–	–	–	–	–	7.26	0.66	0.91
Mussel shell	AMX	$2.767 \cdot 10^{-6}$	0	3.22	2.00	0.69	–	–	–	–	–	2.50	0.52	0.64
	CIP	–	–	1.47	0.42	0.81	–	–	–	–	–	96.56	16.25	0.76
	TMP	–	–	1.03	0.40	0.80	–	–	–	–	–	5.30	0.81	0.80
Pine bark	AMX	–	–	0.62	0.38	0.58	–	–	–	–	–	4.05	0.49	0.49
	CIP	–	–	–	–	–	–	–	–	–	–	–	–	–
	TMP	981.88	137.41	0.93	0.06	0.99	0.030	0.009	76429	$4.6 \cdot 10^4$	0.99	833.48	23.10	0.99

than those obtained in the present investigation for pine bark ($981 L kg^{-1} \mu mol^{1-n}$). A value of the Freundlich n parameter lower than 1 (specifically 0.93) was found for TMP adsorption on pine bark, indicating the presence of heterogeneous adsorption sites and a concave adsorption curve (Cela-Dablanca et al., 2022a), whereas for the other two adsorbent the values were slightly higher than 1. Additionally, the n parameter was strongly negatively correlated with adsorption capacities of TMP ($r = 1.00$; $p < 0.01$).

As for AMX, oak ash exhibited the highest values of K_f ($1824 L^n kg^{-1} \mu mol^{1-n}$), followed by mussel shell, with a low score ($2.767 \cdot 10^{-6} L^n kg^{-1} \mu mol^{1-n}$), while no value was available for pine bark, due to being

affected by too high error values when fitting. In previous studies focused on AMX retention in corn and vineyard soils amended with bio-adsorbents, K_f values were in the range of 1–139 $L^n kg^{-1} \mu mol^{1-n}$ (Cela-Dablanca et al., 2022b). The higher values obtained for K_f in the current work (especially for oak ash) indicate the stronger affinity of these bio-adsorbents towards the retention of this antibiotic. Indeed, the incorporation of oak ash in a corn soil increased the K_f level by 2 times (Cela-Dablanca et al., 2022b). The n parameter of the Freundlich model was lower than 1 for oak ash and pine bark, resulting in concavity for the AMX adsorption curve.

In the Linear model, the K_d value corresponding to AMX adsorption

onto oak ash was 2 orders of magnitude higher compared to those of mussel shell and pine bark (555 L kg^{-1} vs. 2.5 L kg^{-1} and 4.0 L kg^{-1} , respectively) as expected. These values are higher to those previously obtained for vineyard soils ($5.93\text{--}112.34 \text{ L kg}^{-1}$) and lower than for corn soils ($1\text{--}1525.76 \text{ L kg}^{-1}$) (Cela-Dablanca et al., 2022b), but comparable with the results presented in this study.

The value of the K_L Langmuir parameters was $0.104 \text{ L } \mu\text{mol}^{-1}$ for oak, with no fitting possible for the other adsorbent due to too high errors associated to the estimation (Table 1). Again, the value is comparable to those found by Cela et al. (Cela-Dablanca et al., 2022b) in vineyard soils ($0.037\text{--}0.975 \text{ L } \mu\text{mol}^{-1}$) for AMX adsorption. A positive correlation of adsorption capacities with K_f , K_L and K_d was obtained ($p < 0.05$), in agreement with the discussion provided before.

In the case of CIP, the modelling of its adsorption onto pine bark was not possible as the material was able to adsorb all the antibiotic at each concentration, denoting its very high affinity. No fitting was possible to Freundlich for CIP, due to too high errors associated to the estimation. As a reference, the value of the Freundlich K_f found in agricultural soils in a previous study was $3334 \text{ L}^n \text{ kg}^{-1} \mu\text{mol}^{1-n}$ (Rodríguez-López et al., 2022), while Rath et al. (2019) reported scores in the range $230\text{--}1366 \text{ mL}^n \mu\text{g}^{1-n} \text{ g}^{-1}$ for CIP adsorption in subtropical soils.

The value of the Langmuir constant (K_L) was only available for oak ash ($0.050 \text{ L } \mu\text{mol}^{-1}$), in accordance with the mean score of $K_L = 0.080 \text{ L } \mu\text{mol}^{-1}$ reported by Rodríguez-López et al. (Rodríguez-López et al., 2022) for CIP adsorption in agricultural soils.

3.2. Desorption of the three antibiotics from the bio-adsorbents used

Fig. 3 depicts the desorption of the different antibiotics from the bio-adsorbents used in the study, expressed in $\mu\text{mol kg}^{-1}$ and as percentage. In general, AMX and CIP are the antibiotics with the lower desorption from oak ash and mussel shell, especially when high antibiotic concentrations were added, while TMT was the antibiotic showing least desorption from pine bark. AMX presented the highest levels of desorption from pine bark, with a value of $839 \mu\text{mol kg}^{-1}$ when a concentration of $600 \mu\text{mol L}^{-1}$ was added. CIP showed the highest desorption from pine bark, with a value of $966.78 \mu\text{mol kg}^{-1}$ for an added concentration of $100 \mu\text{mol L}^{-1}$. TMP desorption levels were especially noticeable for mussel shell, with values of 2380.186 and $2294.927 \mu\text{mol kg}^{-1}$ for added concentrations of 400 and $600 \mu\text{mol L}^{-1}$, respectively. For oak ash, desorption values were intermediate between those of mussel shell and pine bark, obtaining maximum TMP desorption of $883.45 \mu\text{mol kg}^{-1}$ for an added concentration of $600 \mu\text{mol L}^{-1}$, while pine bark desorbed just $189.60 \mu\text{mol kg}^{-1}$ for an added concentration of $400 \mu\text{mol L}^{-1}$.

Expressing the values in percentage, AMX showed the highest desorption from mussel shell for an added concentration of $50 \mu\text{mol L}^{-1}$ (80%), followed by pine bark for an added concentration of $600 \mu\text{mol L}^{-1}$ (60%), being oak ash the adsorbent that most irreversibly adsorbed this antibiotic, with desorption close to 0% (Fig. 3). CIP achieved desorption percentages close to 100% at the lowest concentrations added, for both oak ash and mussel shell, reaching medium values as regards desorption percentages from pine bark at those concentrations; however, for the two highest concentrations added, CIP desorption was always less than 10% for the three adsorbent materials.

To note that, for this antibiotic, a higher percentage of adsorption is observed (on oak ash and mussel shell), as well as lower desorption (for the three bio-adsorbents) when the added concentrations are higher (Figs. 1 and 3), suggesting that, as the concentration added of this antibiotic increases, adsorption is favored and the bonds with the adsorbent are stronger. This can be related to the cooperative model of adsorption pointed out by several authors (Foo and Hameed, 2010), (Ringot et al., 2007) in which it is assumed that a solute, when it is retained by a site of a homogeneous adsorbent surface, can influence the consecutive active sites of that surface, favoring new adsorptions. In the case of pine bark, for the concentration added of $200 \mu\text{mol L}^{-1}$,

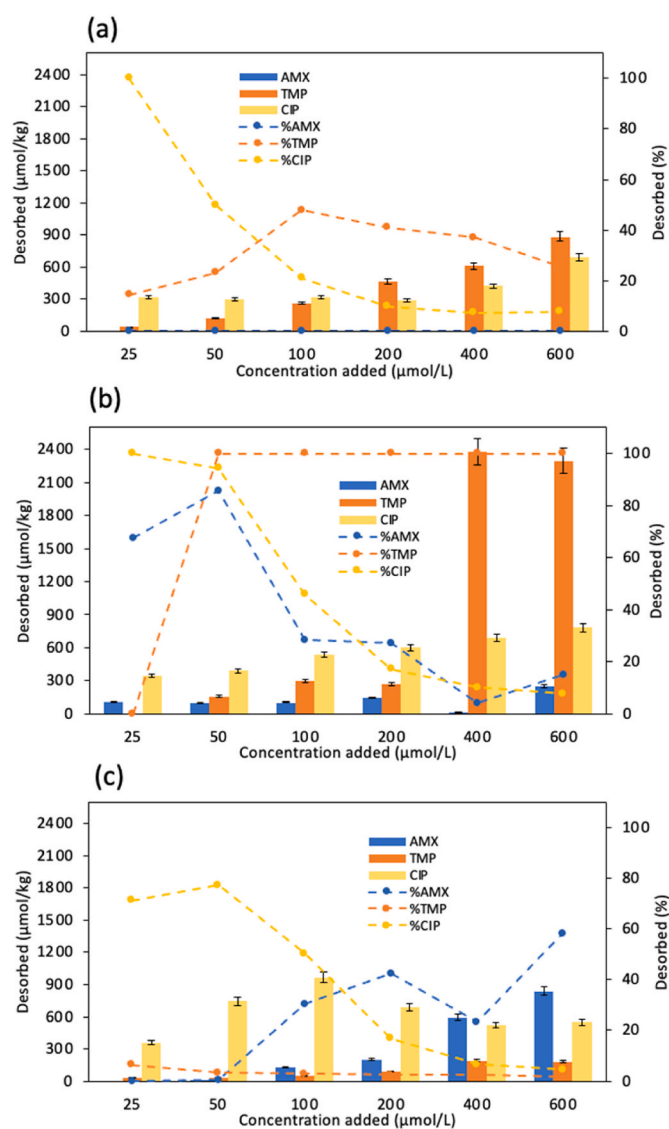


Fig. 3. Desorption in $\mu\text{mol kg}^{-1}$ (left y-axis) and percentage (right y-axis) for AMX, TMP and CIP in (a): oak ash; (b): mussel shell, and (c): pine bark.

desorption (expressed in $\mu\text{mol kg}^{-1}$) is lower for AMX than for CIP. However, in terms of percentage, the trend is the opposite. This occurs because the value of the desorption percentage is based on the amount adsorbed in $\mu\text{mol kg}^{-1}$ and this is significantly higher in the case of CIP, therefore, although the desorbed amount of CIP (in $\mu\text{mol kg}^{-1}$) is higher than that of AMX, the percentage desorbed in relation to the adsorbed is lower (Fig. 3). With respect to TMP, a desorption of practically 100% is observed for mussel shell, while it is minimal for pine bark, reaching a maximum of 6.29% desorption for an added concentration of $25 \mu\text{mol L}^{-1}$.

Mussel shell and pine bark have a low AMX adsorption capacity (always less than 30% of what is added) (Fig. 1) and a desorption that can reach up to 80% for mussel shell and up to 60% for pine bark (Fig. 3). On the contrary, oak ash is the bio-adsorbent that adsorbed the most AMX while desorbing negligible amounts. This occurs since oak ash has the highest content in Fe and Al non-crystalline minerals, and the highest amount of calcium, so that the cationic bridges formed during adsorption are stronger than those formed in the case of mussel shell. A previous work carried out by Cela-Dablanca (Cela-Dablanca et al., 2022b) also determined that the desorption of AMX from oak ash was minimal, highlighting its suitability for the retention of this antibiotic.

CIP showed high desorption percentages in general for all the bio-adsorbents, especially when low concentrations were added, while at higher concentrations the desorption percentages fall to values below 10%. So, when the two highest concentrations of this antibiotic were added, the three biosorbents showed a high adsorption capacity (between 75% and 100%) (Fig. 1) and a low desorption (<10%), especially pine bark (Fig. 3). However, in terms of CIP amount desorbed (in $\mu\text{mol kg}^{-1}$), the values are similar for the whole range of concentrations. This may indicate that a constant amount of CIP is being desorbed from the bio-adsorbents, possibly due to the fact that several adsorption mechanisms might be involved in the adsorption process, as described in a previous section.

With respect to TMP, Fig. 3 shows that the levels of $\mu\text{mol kg}^{-1}$ desorbed increase as the added concentration increases, being this fact especially noticeable in the case of mussel shell, since it is the bio-adsorbent desorbing the largest amount of this antibiotic. This trend was also observed in a study about TMP adsorption on (Li and Zhang, 2017). For this antibiotic, pine bark could be used as effective retention-material, since it adsorbs practically all of the added antibiotic and its desorption is very low (<5%), so the retention process is rather irreversible.

Conde-Cid et al. (2019) found that these same three bio-adsorbents (oak ash, pine bark and mussel shell) were able to retain high amounts of tetracyclines (oxytetracycline, chlortetracycline, and tetracycline) being pine bark and oak ash the ones showing the best performances. In other study, sulfonamides (sulfadiazine, sulfamethazine, and sulfachloropyridazine) were also highly retained by pine bark (almost 100%) while oak ash and mussel shell did not show favorable results in the retention of these antibiotics (Conde-Cid et al., 2021). On the other hand, wood ash and mussel shell were the bio-adsorbents showing the highest sorption capacity and retention for the antibiotic cefuroxime (CFX) (Cela-Dablanca et al., 2021).

4. Conclusions

The differences found in the adsorption of the three antibiotics of study can be related to the specific properties of these substances (polarity, hydrophobicity, molecular structure, speciation with pH), which determine their interaction with the reactive surfaces of the bio-adsorbents used. Pine bark was found to be the most suitable bio-adsorbent for the retention of CIP and TMP, adsorbing almost 100% of CIP at all the concentrations tested. In addition, the release of these two pharmaceuticals from pine bark was the lowest compared to the other two sorbent materials, probably due to the formation of strong hydrogen bonding with the functional groups of the drugs, favored by the acidic conditions in TMP and the presence of benzene rings as electron acceptor groups in CIP. Oak ash was the best material in the retention of AMX, also exhibiting the lowest level of AMX desorption, due to the formation of strong cationic bridges between the material and the antibiotic, favored by the alkaline pH and the high content of calcium and non-crystalline substances in the bio-adsorbent. Having in mind the results derived from this investigation, the use of pine bark and oak ash as remediation agents to be added to agricultural soils for the retention of AMX, CIP and TMP seems to be a viable, environmentally friendly, and effective way to face the entry of these pollutants of emerging concern into the food chain. It would be interesting to program further research into the use of binary and tertiary adsorption systems with soil/bio-adsorbent samples to determine whether the inclusion of these green adsorbents is useful for their valorization and as barriers against antibiotics mobilization into water resources.

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

No data was used for the research described in the article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envres.2023.116360>.

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