Environmental Pollution 322 (2023) 121161

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Direct toxicity of six antibiotics on soil bacterial communities affected by the addition of bio-adsorbents☆

Laura Rodríguez-González [a], Avelino Núñez-Delgado [b], Esperanza Álvarez-Rodríguez [b], Montserrat Díaz-Raviña [c], Manuel Arias-Estévez [b], David Fernández-Calviño [a], Vanesa Santás-Miguel [a,*]

[a] Área de Edafoloxía e Química Agrícola, Departamento de Bioloxía Vexetal e Ciencia do Solo, Facultade de Ciencias, Universidade de Vigo, Campus As Lagoas, s/n, 32004, Ourense, Spain
[b] Departamento de Edafoloxía e Química Agrícola, Escola Politécnica Superior de Enxeñaría, Universidade de Santiago de Compostela, Campus Univ, 27002, Lugo, Spain
[c] Departamento de Bioquímica del Suelo, Misión Biológica de Galicia (MBG-CSIC), Apartado 122, 15780, Santiago de Compostela, Spain

ARTICLE INFO

Keywords:
Bacterial growth
Waste material
Mussel shell
Biomass ash
Pine bark

ABSTRACT

Reducing the toxicity caused by antibiotics on bacterial communities in the soil is one of the great challenges of this century. For this, the effectiveness of amending the soil with different bioadsorbents such as crushed mussel shell (CMS), pine bark (PB) and biomass ash (BA), as well as combinations of them (CMS + PB and PB + BA) was studied at different doses (0 g kg^{-1} to 48 g kg^{-1}). Soil samples were spiked, separately, with increasing doses (0–2000 mg kg^{-1}) of cefuroxime (CMX), amoxicillin (AMX), clarithromycin (CLA), azithromycin (AZI), ciprofloxacin (CIP) and trimethoprim (TMP). Their toxicity on bacterial growth was estimated using the tritium-labeled leucine (^{3}H) incorporation method. Toxicity was observed to behave differently depending on the antibiotic family and bioadsorbent, although in different magnitude and at different doses. The toxicity of β-lactams (AMX and CXM) was reduced by up to 54% when the highest doses of bio-adsorbents were added due to the increase in pH (CMS and BA) and carbon (PB) contribution. Macrolides (CLA and AZI) showed slight toxicity in un-amended soil samples, which increased by up to 65% with the addition of the bio-adsorbents. The toxicity of CIP (a fluoroquinolone) increased with the dose of the bio-adsorbents, reaching up to 20% compared with the control. Finally, the toxicity of TMP (a diaminopyrimidine) slightly increased with the dose of bio-adsorbents. The by-products that increase soil pH are those that showed the highest increases of CLA, AZI, CIP and TMP toxicities. These results could help to prevent/reduce environmental pollution caused by different kinds of antibiotics, selecting the most appropriated bio-adsorbents and doses.

1. Introduction

The use of antibiotics to treat a variety of infectious diseases has widely spread over the last few decades, and specifically in human medicine. In fact, global antibiotic consumption increased by 65% between 2000 and 2015, reaching a total annual use of 42 billion DDD (defined-daily-doses), as indicated by Klein et al. (2018). Antibiotics are essential for the treatment of bacterial infections, however, their degradation rate in the human intestine is low, causing that around 70% of the doses are excreted as an original compound or as a metabolite (Koch et al., 2021; Kümmerer, 2009). This fact causes that domestic and hospital effluents are frequently loaded with too high concentrations of antibiotics for human use, such as amoxicillin, azithromycin, cefuroxime, ciprofloxacin, clarithromycin and trimethoprim, all of them reported as detected in wastewater (Hartmann et al., 1999; Iatrou et al., 2014; Aydın et al., 2022). To note that these antibiotics are included in the list of the most prescribed and associated to higher concern at global level (Adriaenssens et al., 2011; European Commission, 2015; Collignon et al., 2016).

Treatments carried out in wastewater treatment plants hardly eliminate antibiotics, while the persistent use of liquid effluent for irrigation, and sewage sludge to fertilize crop fields, disseminate these emerging pollutants in the environment (Michael et al., 2013) and cause the accumulation of antibiotics in soils (Kemper, 2008; Pan and Chu, 2017).

☆ This paper has been recommended for acceptance by Jiayin Dai.

* Corresponding author.
E-mail address: vsantas@uvigo.es (V. Santás-Miguel).

https://doi.org/10.1016/j.envpol.2023.121161
Received 30 September 2022; Received in revised form 14 January 2023; Accepted 26 January 2023
Available online 28 January 2023

Some of the main risks arising from the arrival of antibiotics to soils lie on the effect they may cause on the structure and function of soil microbial communities, due to the bioactive/biocide nature of these pollutants (Cycoń et al., 2019), which can affect the maintenance of biological activity in soils, considered a key feature for sustainable soil productivity and for ensuring ecosystem functions (Pan and Chu, 2017; Zhao et al., 2022). The pressure exerted by human medicine antibiotics on soil bacterial communities may cause increases in antibiotic resistance genes in soils, and these resistances can be transmitted, by horizontal transfer, to human pathogens (Huddleston, 2014), causing serious problems in human and environmental health (Levy and Marshall, 2004).

There is a variety of methods for removing antibiotics, such as membrane separation, advanced oxidation or adsorption to synthetic materials. However, these methods have a high cost and generate a large amount of waste (Ata et al., 2017; Ding et al., 2016; Juela, 2021; Russell and Yost, 2021). Focusing on soils, an alternative treatment to address this problem is the addition of low-cost waste/by-products, to be used as bio-adsorbents. In fact, bio-adsorbents derived from waste or by-products have numerous advantages compared to synthesized material. On the one hand, its low cost should be highlighted, as well as the simultaneous promotion of circular economy when recycling these waste materials. On the other hand, these bio-adsorbents are usually degradable and non-polluting materials.

In spite of their potential advantages, there are still few studies on how the application of adsorbents to soil contaminated with antibiotics could affect the microbiota (Rousk et al., 2008; Santás-Miguel et al., 2020, 2021), although their effectiveness had been widely proven in soils contaminated with heavy metals (Fernández-Calviño et al., 2015; Fernández-Calviño et al., 2018; Yu et al., 2022). It would be of main importance to provide new scientific data on the effects that antibiotics reaching the soil as pollutants have on soil bacterial communities, and specifically after being amended with bio-adsorbents derived from waste/by-products. This kind of research could contribute to obtain key information applicable in the protection of environmental and public health on the basis of the One Health approach.

In view of all the previous background, the main objective of this work is assessing the following hypothesis: the amendments consisting in different doses (0, 6, 12, 24 and 48 g kg^{-1}) of the bio-adsorbents pine bark (PB), crushed mussel shell (CMS) and biomass ash (BA), and two mixtures (CMS + PB and PB + BA), applied on soils contaminated with antibiotics belonging to the families of β-Lactams (amoxicillin, AMX, and cefuroxime, CXM), Macrolides (azithromycin, AZI, and clarithromycin, CLA), Fluoroquinolones (ciprofloxacin, CIP), and Diaminopyrimidines (trimethoprim, TMP), could decrease the negative effects on the growth of microbial communities due to the toxicity of these compounds. This is the first time that such an investigation is carried out, and it could provide relevant results as regards environmental and public health preservation.

2. Materials and methods

2.1. Chemicals and reagents

The 6 antibiotics used had a purity >95% and were provided by Sigma-Aldrich (Steinheim, Germany): AMX (CAS: 61336-70-7), AZI (CAS: 117772-70-0), CIP (CAS: 85721-33-1), CLA (CAS: 81103-11-9), CXM (CAS: 55268-75-2) and TMP; (CAS: 738-70-5), as well as talc (CAS: 14807-96-6). The main characteristics of each antibiotic are shown in Table 1.

2.2. By-products/bio-adsorbents

In this study, two by-products from the forestry industry (Pine Bark (PB) and Biomass Ash (BA)) and one from the food industry (Crushed Mussel Shell (CMS)) were used. PB was provided by Geolia (Madrid, Spain), CMS by Abonomar SL (Illa de Arousa, Spain), and BA was from a combustion boiler in Lugo (Spain). The three by-products/bio-adsorbents have been characterized previously by Quintáns-Fondo et at. (2017), and their main details are included in Table 2.

A theoretical calculation was carried out to estimate the total carbon content added after amending the soil with the different doses of the bio-adsorbents. The addition of CMS contributed to increase the total carbon content (in this case being mainly inorganic) from 0.69 g kg^{-1} (for the dose of 6 g kg^{-1}) to 5.49 g kg^{-1} (for the dose of 48 g kg^{-1}). The

Table 2
Main chemical characteristics of the bio-adsorbents studied. CMS: crushed mussel shell; PB: pine bark; BA: biomass ash.

	CMS	PB	BA
C (%)	11.4	47.0	11.7
N (%)	0.21	0.32	0.21
pH_{water}	9.4	4.0	11.3
Ca_e ($cmol_{(+)}$ kg^{-1})	24.8	5.4	95.0
Mg_e ($cmol_{(+)}$ kg^{-1})	0.7	2.7	3.3
Na_e ($cmol_{(+)}$ kg^{-1})	4.4	0.5	12.2
K_e ($cmol_{(+)}$ kg^{-1})	0.4	4.6	250.7
Al_e ($cmol_{(+)}$ kg^{-1})	0.03	1.8	0.1
$eCEC_e$ ($cmol_{(+)}$ kg^{-1})	30.25	14.92	361.2
P_T (mg kg^{-1})	101.5	<0.01	663.7
Ca_T (mg kg^{-1})	280168	2319	136044
Mg_T (mg kg^{-1})	980.6	473.6	23171
Na_T (mg kg^{-1})	5173	68.92	2950
K_T (mg kg^{-1})	202.1	737.8	99515
As_T (mg kg^{-1})	1.1	<0.001	8.4
Cd_T (mg kg^{-1})	0.1	0.1	19.3
Cr_T (mg kg^{-1})	4.5	1.9	36.3
Cu_T (mg kg^{-1})	6.7	<0.001	146.3
Ni_T (mg kg^{-1})	8.2	1.9	69.3
Zn_T (mg kg^{-1})	7.7	7.0	853.0
Mn_T (mg kg^{-1})	33.75	30.19	10554
Al_T (mg kg^{-1})	433.2	561.1	14966
Fe_T (mg kg^{-1})	1855	169.8	12081
Al_o (mg kg^{-1})	178.3	315.0	8323
Fe_o (mg kg^{-1})	171.0	74.02	4233

X_e: exchangeable concentration of the element; X_T: total concentration of the element; Al_o, Fe_o: extracted with ammonium oxalate.

Table 1
Main chemical characteristics of the antibiotics used. K_{OW}: distribution coefficient octanol-water. pK_a: negative log of the acid dissociation constant.

Antibiotic	Chemical group	Chemical formula	Molecular weight (g mol^{-1})	Water solubility (mg L^{-1}) [a]	Log K_{OW}	pK_a [b]
Amoxicillin	β-Lactams	$C_{16}H_{19}N_3O_5S$ $3H_2O$	419.45	3430	0.87	3.23–7.22
Azithromycin	Macrolide	$C_{38}H_{72}N_2O_{12}$	785.02	2.37	4.02	12.53–9.57
Ciprofloxacin	Fluoroquinolone	$C_{17}H_{18}FN_3O_3$	331.34	36,000	0.28	6.09–8.74
Clarithromycin	Macrolide	$C_{38}H_{69}NO_{13}$	747.95	2	3.16	12.46–9
Cefuroxime	β-Lactams	$C_{16}H_{16}N_4O_8S$	424.39	145	−0.16	2.5
Trimethoprim	Diaminopyrimidines	$C_{14}H_{18}N_4O_3$	290.32	400	0.91	7.16

[a] Ghirardini et al. (2020), Solliec et al. (2016).
[b] Ghirardini et al. (2020), Hanamoto and Ogawa (2019), Talaiekhozani et al. (2020).

amendment with BA increased total soil carbon (mainly inorganic) from 0.70 g kg^{-1} (at dose of 6 g kg^{-1}) to 5.59 g kg^{-1} (for the dose of 48 g kg^{-1}). As for the addition of PB to soils, the increase in the total carbon content (mainly organic) ranged from 2.81 g kg^{-1} to 22.54 g kg^{-1}, for the doses of 6–48 g kg^{-1}, respectively. The soil amendment with PB caused the highest increase in total carbon content.

For the mixtures used, the total carbon added to the soil with CMS + PB ranged from 1.75 g kg^{-1} to 14.01 g kg^{-1}, for the doses from 6 to 48 g kg^{-1}, respectively. Finally, the addition of PB + BA caused an increase in the total carbon content of 1.76 g kg^{-1} for the lowest dose added (6 g kg^{-1}), while reached 14.06 g kg^{-1} when the maximum dose (48 g kg^{-1}) was added.

2.3. Soil

The soil used in this research was sampled at a vineyard plot situated in Galicia (Northwest of the Iberian Peninsula) and resulted from the combination of different sub-samples from the surface horizon of the soil (0–20 cm), which were collected using an Edelman probe. The soil sampled was transported to the laboratory, where it was air dried, sieved through a 2 mm mesh and stored in polyethylene bottles until analysis. Standard methods were used to carry out soil analyses (Tan, 1996).

The main characteristics of the soil are shown in Table 3. Briefly, the pH value measured in water was 5.4, while pH in 0.1 M KCl was 3.8. The total carbon and nitrogen contents were 1.2% and 0.1%, with C/N ratio of 12. As for the sand, silt and clay contents, they were 45.5%, 40.0%, and 14.5%, respectively, showing a loam texture. In addition, the effective cation exchange capacity (CEC) was 7.9 $cmol_{(+)}$ kg^{-1}, while Cu_T and As_T concentrations were similar to those found in uncontaminated soils in the study area (Macías and Calvo, 2008).

2.4. Experimental design

Aliquots consisting of 24 g of dry soil, introduced in 500 mL polyethylene canisters, were added separately with 0, 0.29, 0.58, 1.15 and 2.30 g of each of the three bio-adsorbents, to obtain doses of bio-adsorbents of 0, 6, 12, 24 and 48 g per kg of soil (Fig. S1; Supplementary Material). The bio-adsorbents used were those indicated above: pine bark (PB), crushed mussel shell (CMS) and biomass ash (BA), separately and in two mixtures (PB + CMS and PB + BA), added in the quantities required to obtain a bio-adsorbent concentration equal to that of those added separately (adding half the weight of each of both bio-adsorbents in these mixtures). This was done for each antibiotic and each bio-adsorbent (separately and its mixtures) in triplicate, obtaining a total number of 450 canisters (5 doses x 5 bio-adsorbents x 6 antibiotics x 3 replicates). Once the samples were weighed, a homogenization of the soil and the bio-adsorbents was performed, and subsequently the samples were moistened to reach between 60 and 80% of their field capacity. Then they were incubated for 30 days at 22 °C in the dark, which is time enough to allow that the growth of bacterial communities stabilize under the new soil conditions (Meisner et al., 2013). After this time, the samples of the canisters were subdivided into 3 g of dry soil, obtaining from each sample 8 different 50-mL Falcon tubes.

Subsequently, these 8 Falcon tubes were separately spiked with 8 different antibiotic concentrations, obtaining a concentration gradient of 0, 0.49, 1.95, 7.81, 31.25, 125, 500 and 2000 mg of antibiotic per kg of soil sample. This was performed for the 6 antibiotics investigated: AMX, AZI, CIP, CLA, CXM and TMP. The soil samples obtained were incubated for 1 day (direct toxicity) at 22 °C in the dark. After this incubation period, each soil sample (3 g) was subdivided into 3 different 15-mL Falcon tubes (1 g of soil in each), resulting in a total of 24 tubes. This was performed for the 5 bio-adsorbents, 5 doses and 6 antibiotics, obtaining a total of 3600 microcosms (Fig. S1; Supplementary Material). In each of these samples the bacterial growth was estimated by the method of incorporating leucine marked with tritium (^3H) (Bååth, 1994; Bååth et al., 2001). In addition, the pH values (w/v ratio 1:10) of the bacterial suspensions obtained were measured for each of the samples. Briefly, aliquots of 1 g of soil were placed in 15-mL centrifuge tubes, then adding 10 mL of distilled water. After 3 min of vortex agitation and 10 min of centrifugation at 1000×*g*, a 1 mL aliquot of the bacterial suspension was taken and transferred to a 2 mL microcentrifuge tube, to which a volume of 20 μL of a tritium-marked leucine dilution was added ([^3H] Leu (3.7 MBq mL^{-1} and 5.74 TBq $mmol^{-1}$; Amersham, UK). The samples were incubated for 2 h at 22 °C in the dark. At the end of the incubation period, the bacterial growth was stopped by adding 75 μL of 100% trichloroacetic acid. Finally, the bacteria in the microcentrifuge tubes were washed and treated using the method described by Bååth et al. (2001). The amount of Leu incorporated in bacterial cells was determined by the per-minute decays obtained for each sample using a liquid scintillation counter (Tri-Carb 2810 TR, PerkinElmer, USA).

Table 3
Main chemical characteristics of the soil used in the study.

Parameter	Soil
%C	1.2
%N	0.1
%sand	45.5
%silt	40.1
%clay	14.5
pH_W	5.4
pH_{KCl}	3.8
K_e ($cmol_{(+)}$ kg^{-1})	3.0
Ca_e ($cmol_{(+)}$ kg^{-1})	0.6
Mg_e ($cmol_{(+)}$ kg^{-1})	0.2
Na_e ($cmol_{(+)}$ kg^{-1})	3.2
Al_e ($cmol_{(+)}$ kg^{-1})	1.0
eCEC ($cmol_{(+)}$ kg^{-1})	7.9
Cu_T (mg kg^{-1})	41
As_T mg kg^{-1}	34

pH_W is pH measured in water; pH_{KCl} is pH measured in 0.1 M KCl; eCEC is the effective Cation Exchange Capacity ($cmol_{(+)}$ kg^{-1}); X_e: exchangeable concentration of the element; X_T: total concentration of the element.

2.5. Data analysis

Bacterial growth data were normalized with respect to the control (without antibiotic) for each dose of bio-adsorbent and antibiotic, in order to allow comparison of different dose-response curves.

Dose-response curves (bacterial growth relative to logarithmic antibiotic concentration) were plotted using the data obtained for each dose of bio-adsorbent and antibiotic. From each inhibition curve, the logarithm of the concentration that inhibits 50% of bacterial growth (Log IC_{50}) was obtained, calculated by means of the following logistic model:

$$Y = c \,/\, \left[1 + e^{b\,(a-x)}\right] \quad (1)$$

where Y is the incorporation of leucine (bacterial community growth) observed at each added antibiotic concentration, x is the logarithmic value of the added antibiotic concentration, a is logarithm of IC_{50} (Log IC_{50}), b is a parameter related to the slope of the inhibition curve, and c is the bacterial growth rate observed in the control sample (without antibiotic). High values obtained for Log IC_{50} indicate low antibiotic toxicity, as a high concentration is needed to inhibit 50% of the bacterial population, while low Log IC_{50} values indicate high antibiotic toxicity. Figures were drawn and calculations performed using Synergy Software KaleidaGraph.

3. Results and discussion

3.1. Changes in soil characteristics after adding the bio-adsorbents

The amendment of soils with PB, CMS, and BA caused changes in the physicochemical characteristics of the soil. The pH value of the bacterial suspension was 6.0 in the control sample (the dose of bio-adsorbents being 0 mg kg^{-1}). The pH variations measured after amending the soil with different doses of the bio-adsorbents are shown in Table S1 (Supplementary Material).

The addition of CMS (pH = 9.4) caused increases of 0.5 units when the dose of this bio-adsorbent was 6 g kg^{-1}, and of 1.6 units when the dose was 48 g kg^{-1}. The addition of PB (pH = 4.0) caused that the pH value decreased 0.2 units when the dose was 6 g kg^{-1} and 0.5 units when the maximum PB dose (48 g kg^{-1}) was added. Finally, the amendment with BA (pH = 11.3) caused the highest increases in pH, reaching 1.4 units for the dose of 6 g kg^{-1} and of 4.1 units for the dose of 48 g kg^{-1}. As for the mixtures of bio-adsorbents, the increases in pH were lower than when the bio-adsorbents were applied individually. Specifically, the CMS + PB amendment increased pH from 0.4 to 1.5 units for doses of 6–48 g kg^{-1} respectively. Finally, the addition of PB + BA to soils increased pH values from 0.8 units (with the dose of 6 g kg^{-1}) to 2.9 units (with the dose of 48 g kg^{-1}). As a result, the sequence of highest to lowest pH values when the concentration of bio-adsorbent added was 48 g kg^{-1} was as follows: BA > PB + BA > CMS ≥ CMS + PB > PB.

3.2. Effect of PB, CMS, and BA amendments on antibiotic toxicity affecting bacterial community growth

In general, sigmoidal dose-response curves were obtained, indicating that, as the concentration of antibiotics added increased, the growth of soil bacterial communities decreased. Regarding the dose-response curves, 3 different behaviors can be observed: The dose-response curve shifts to the right with respect to the control, this means that the toxicity of the antibiotic on the bacterial communities of the soil decreases. On the other hand, if the displacement of the curves is to the left with respect to the control, the toxicity that the antibiotic exerts on the bacterial communities of the soil increases. Finally, there may not be a displacement of the dose-response curves, therefore, the effect exerted by the antibiotics on the bacterial communities of the soil with respect to the control does not vary. The dose-response curves obtained show a different behavior depending on the antibiotics family, as detailed in the subsections below.

3.2.1. β-Lactams: amoxicillin and cefuroxime

The dose response curves obtained for AMX and CXM after the addition of the different doses of the bio-adsorbents are shown in Fig. 1. In general, two different behaviors can be observed depending on the bio-adsorbent added. On the one hand, the dose-response curves obtained for these antibiotics move to the right with respect to the control in response to the addition of PB and BA, meaning that the toxicity exerted by AMX and CXM decreases with the addition of these bio-adsorbents, showing a further decrease in toxicity for the highest dose tested (48 g kg^{-1}). On the other hand, the addition of CMS does not cause a clear shift in the dose-response curves, indicating that this bio-adsorbent does not modify the toxicity exerted by AMX and CXM on soil bacterial communities with respect to the control.

Fig. 1 shows the dose-response curves corresponding to the treatments with two mixtures of bio-adsorbents (CMS + PB and PB + BA). The addition of CMS + PB and PB + BA to the soil caused similar effects to those observed after adding PB and BA separately, with AMX and CXM toxicities decreasing due to these two mixtures, and the highest reduction associated to their maximum concentrations added (48 g kg^{-1}).

The dose-response curves were well described by the logistic model (Eq. (1)), obtaining R^2 values between 0.993 and 0.999 (mean = 0.997) for AMX and CXM. From the dose-response curves, estimated Log IC_{50} values were obtained for each antibiotic, bio-adsorbent and dose. The magnitude in the reduction of the toxicity exerted by AMX and CXM depending on the by-product is given by the values of Log IC_{50} obtained for each of the doses and antibiotic studied, with data presented in Table S2 (Supplemtary Material). The Log IC_{50} values obtained for the control sample (without addition of bio-adsorbent, dose 0 g kg^{-1}) were 2.10 ± 0.07 for AMX, and 1.63 ± 0.08 for CXM, indicating that CXM caused higher toxicity on soil bacterial communities than AMX.

The addition of different doses of CMS to the soil did not cause significant differences in the estimated Log IC_{50} values for AMX and CXM with respect to the control, meaning that the addition of CMS hardly affects the toxicity of AMX and CXM on the growth of soil bacterial communities. Furthermore, the decrease in toxicity of AMX and CXM following the amendment of PB takes place at doses ≥12 g kg^{-1} for AMX and ≥24 g kg^{-1} for CXM, with percentage decrease ranging 15–30% for AMX and 21–38% for CXM. As for the addition of BA to soil, the decrease in AMX toxicity occurs from the lowest BA doses tested (≥6 g kg^{-1}), reaching a value of percentage decrease ≥17%. For CXM toxicity, the only significant decrease corresponded to the highest dose of BA tested (48 g kg^{-1}), and reached 52%.

For the mixtures (Table S2 (Supplemtary Material)), the estimated Log IC_{50} values for CMS + PB showed a decrease in AMX toxicity from 7% to 24% for doses ≥24 g kg^{-1}, while for CXM the decrease was 35% for the dose of 48 g kg^{-1}. The mixture PB + BA was the most effective to reduce the toxicity of AMX and CXM, since doses ≥6 g kg^{-1} decreased their toxicities decreases from 18 to 34% for AMX, and from 19 to 54% for CXM.

These two behaviors had already been observed by Santás-Miguel et al. (2020) and Santás-Miguel et al. (2021), who studied the effect of PB, CMS and BA on the growth of soil bacterial communities after the addition of the antibiotics tetracycline, oxytetracycline and chlortetracycline. These studies concluded that the addition of PB and BA decreased the toxicity of tetracycline antibiotics on bacterial growth, while CMS did not decrease toxicity.

The reduction of AMX and CXM toxicities on soil bacterial communities can be attributed to the increase in organic matter content due to PB (which has C = 47%). In fact, organic matter has a fundamental role in the adsorption of some antibiotics, such as tetracyclines (Gu et al., 2007; Zhao et al., 2011; Fernández-Calviño et al., 2015) and β-lactams onto soils (Kim et al., 2012; Balarak et al., 2017; Cela-Dablanca et al., 2021, 2022a; 2022b). This increased adsorption reduces the bioavailability of the antibiotics in the soil and therefore their toxicity.

As for BA (with pH = 11.3), the change in pH after the addition of this bio-adsorbent (Table S1; Supplemtary Material) may affect the chemical speciation of antibiotics present in the soil (Table 4). After the addition of different doses of BA, the species are predominantly in anionic for AMX (AMX^{-1}), and as zwitterion for CXM (CXM^0). Anionic forms hinder the passage of the antibiotics through the cellular membrane, due to the existence of Donnan equilibrium (Stock et al., 1977), causing lesser toxicity of the molecules as high is the presence of these anionic species in soils. In addition, zwitterion species can interact more easily with soil carbon and with carbon atoms provided by BA (Cela-Dablanca et al., 2021, 2022a; 2022b), decreasing the bioavailability of these antibiotics and their toxicity to soil bacterial communities, which is more pronounced at higher doses of BA.

However, CMS (with pH = 9.4) did not reduce the toxicity of the antibiotics regardless of its dose added to the soil. The addition of CMS to soil caused a lower increase in pH than that observed for BA (Table S1; Supplemtary material), and the AMX species that predominate are zwitterion (AMX^0) for doses ≤12 g kg^{-1}, and anionic (AMX^{-1}) for doses >12 g kg^{-1} (Table 4). In addition, for CXM, the predominant chemical species is zwitterion (CXM^0) (Table 4). These changes in chemical species generated by CMS were not sufficient to modify the toxicities on soil bacterial communities caused by AMX and CXM.

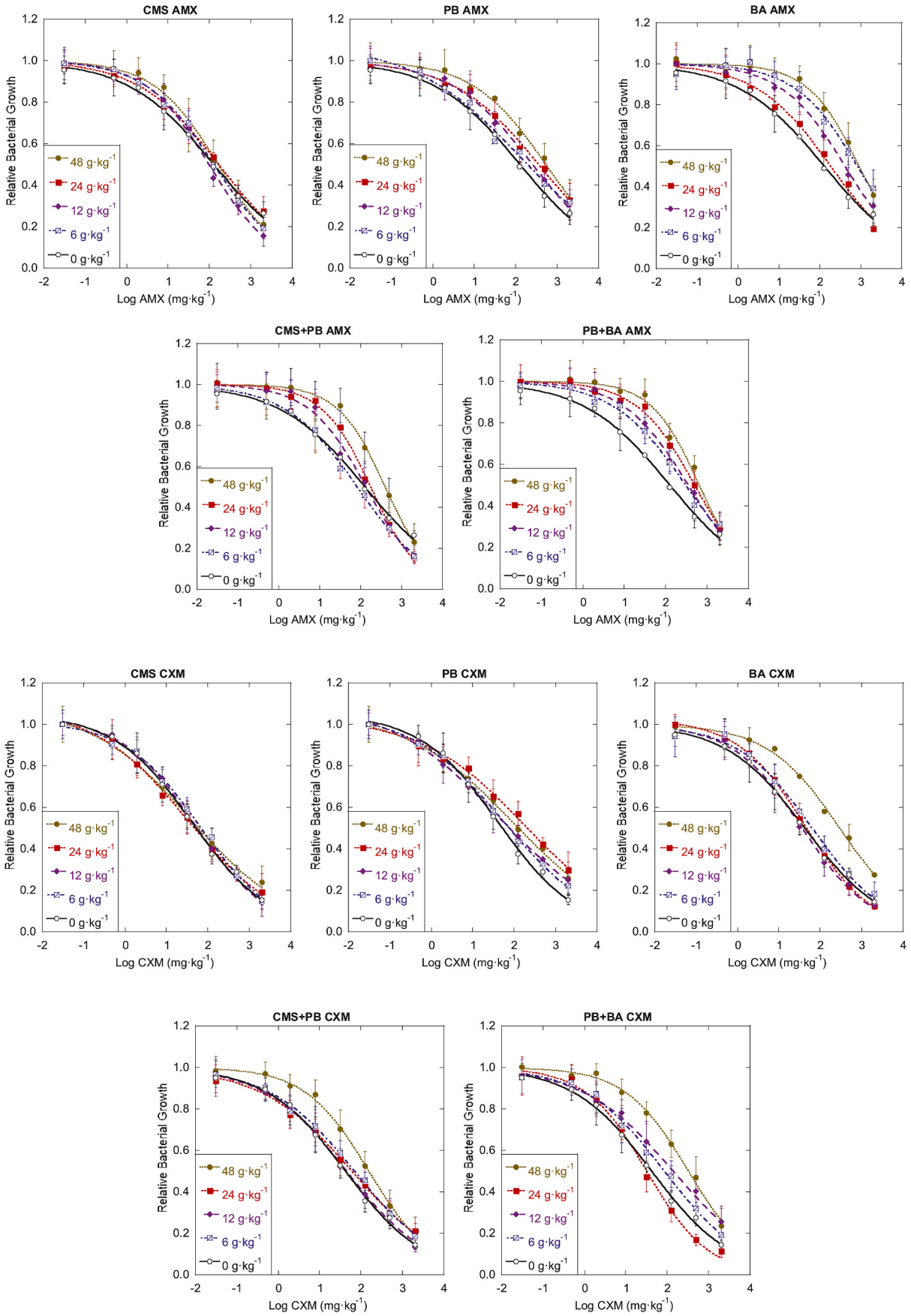

Fig. 1. Dose-response curves as regards the effects exerted by the antibiotics amoxicillin (AMX) and cefuroxime (CXM) on soil bacterial communities in the presence of the three bio-adsorbents studied: crushed mussel shell (CMS), pine bark (PB), and biomass ash (BA), added separately and mixtures.

Table 4
Percentage of amoxicillin (AMX) and cefuroxime (CXM) species for different pH values measured in the soil samples amended with crushed mussel shell (CMS), pine bark (PB), and biomass ash (BA), and their mixtures (CMS + PB and PB + BA). Average values (n = 3) with coefficients of variation always <5%. The values were calculated using pK_a 3.23–7.22 for AMX and using pK_a 2.5 for CXM.

	Dose (g kg^{-1})	pH	AMX^{+1}	AMX^{0}	AMX^{-1}	CXM^{+1}	CXM^{0}
	0	6.0	0.20	94.20	5.70	0.03	**99.97**
CMS	6	6.5	0.05	**83.96**	16.00	0.01	**99.99**
	12	7.1	0.01	**56.86**	**43.13**	0.00	**100.00**
	24	7.5	0.00	34.42	**65.58**	0.00	**100.00**
	48	7.7	0.00	24.88	**75.12**	0.00	**100.00**
PB	6	5.8	0.26	**96.09**	3.65	0.05	**99.95**
	12	5.8	0.26	**96.09**	3.65	0.05	**99.95**
	24	5.7	0.33	**96.75**	2.92	0.06	**99.94**
	48	5.5	0.52	**97.62**	1.86	0.10	**99.90**
BA	6	7.4	0.00	39.78	**60.21**	0.00	**100.00**
	12	8.5	0.00	4.99	**95.01**	0.00	**100.00**
	24	9.2	0.00	1.04	**98.96**	0.00	**100.00**
	48	10.1	0.00	0.13	**99.87**	0.00	**100.00**
CMS + PB	6	6.5	0.05	**83.96**	16.00	0.01	**99.99**
	12	6.8	0.02	**72.44**	27.54	0.01	**99.99**
	24	7.1	0.01	**56.86**	**43.13**	0.00	**100.00**
	48	7.5	0.00	34.42	**65.58**	0.00	**100.00**
PB + BA	6	6.9	0.01	**67.62**	32.37	0.00	**100.00**
	12	7.4	0.00	39.78	**60.21**	0.00	**100.00**
	24	8.0	0.00	14.23	**85.77**	0.00	**100.00**
	48	8.9	0.00	2.05	**97.95**	0.00	**100.00**

3.2.2. Macrolides: azithromycin and clarithromycin

The results of the dose-response curves obtained corresponding to AZI and CLA for the different bio-adsorbents and doses are shown in Fig. 2. In general, no inhibition in the growth of soil bacterial communities took place for the antibiotic AZI in the control sample (dose of bio-adsorbent 0 g kg^{-1}). The addition of CLA to control soil (dose of bio-adsorbent 0 g kg^{-1}) caused a slight inhibition of the bacterial growth.

The dose-response curves corresponding to the soil samples amended with CMS and BA (Fig. 2) show increased toxicities on soil bacterial communities due to AZI and CLA. In general, AZI and CLA toxicities increase with the dose of the bio-adsorbents, evidenced by higher shift to the left in these curves (Fig. 2). In contrast, the PB amendment did not cause changes in CLA toxicity for any of the doses added to the soil. A similar behavior is observed for AZI, although with a slight inhibition of bacterial growth taking place at the highest dose of PB (48 g kg^{-1}) added to the soil.

For the two mixtures of bio-adsorbents (Fig. 2), both CMS + PB and PB + BA increased the toxicity of AZI and CLA on soil bacterial communities. In general, AZI and CLA toxicities increased with their doses, causing higher displacement to the left in the dose-response curves as the doses added increased (Fig. 2).

The dose-response curves obtained were well described by the logistic model (Eq. (1)), obtaining R^2 values between 0.987 and 0.999 (mean = 0.995) for AZI and CLA. Log IC_{50} values were estimated from the dose-response curves obtained for each antibiotic, bio-adsorbent, and dose (Table S3; Supplementary material). The Log IC_{50} value of the control sample (0 g kg^{-1}) contaminated with AZI could not be estimated, since the soil bacterial communities were not affected by the presence of AZI. However, the Log IC_{50} value estimated for CLA was 4.2 ± 0.1. In view of that, CLA showed higher toxicity than AZI to soil bacterial communities.

The amendment of the soil with the different doses of the by-products increased the toxicity exerted by AZI and CLA on the bacterial communities of the soil and, therefore, the values of Log IC_{50} decreased with the dose (Table S3; Supplementary material).

When the soil samples were amended with BA, the Log IC_{50} values estimated for AZI showed an increase in the toxicity for the lowest dose tested (6 g kg^{-1}), with Log IC_{50} reaching 3.5 ± 0.04. These Log IC_{50} values decreased progressively with the dose of BA, reaching 1.7 ± 0.08 when the highest dose (48 g kg^{-1}), indicating that the maximum dose of BA added to the soil was associated to the highest toxicity of AZI. The amendment with CMS increased toxicity when added at doses of 12 g kg^{-1}, with Log IC_{50} values achieving 3.6 ± 0.06. Log IC_{50} values corresponding to AZI decreased after the addition of CMS, meaning that AZI toxicity to soil bacterial communities increased, reaching 2.5 ± 0.04 for the highest dose (48 g kg^{-1}) of CMS of. Finally, the addition of PB to the soil samples only increased the toxicity of AZI on soil bacterial communities when added at the highest dose tested (48 g kg^{-1}), with a Log IC_{50} value of 3.7 ± 0.09.

Regarding the bio-adsorbent mixtures (Table S3; Supplementary material), the toxicity of AZI after the addition of PB + BA was similar to that observed for BA, with AZI showing an increase in its toxicity for the dose of 6 g kg^{-1} of the mixture (Log IC50 value of 3.8 ± 0.08), and then progressively decreasing up to 1.7 ± 0.12 (for the dose of 48 g kg^{-1}). Finally, the CMS + PB mixture caused increases in the toxicity of AZI on the soil bacterial communities rising with the dose, obtaining Log IC_{50} values of 4.3 ± 0.16 at doses of 12 g kg^{-1}, and reaching 3.0 ± 0.04 for the dose of 48 g kg^{-1}.

As for the Log IC_{50} values estimated for CLA (Table S3; Supplementary material), the amendment with the lowest dose tested (6 g kg^{-1}) increased the toxicity of CLA on soil bacterial communities, with very similar Log IC_{50} values for CMS (3.7 ± 0.08), for PB (3.8 ± 0.04), and for BA (3.3 ± 0.05). The toxicity exerted by CLA on the bacterial communities increased as a function of the dose of any of the bio-adsorbents added. Specifically, when the highest dose of the bio-adsorbents (48 g kg^{-1}) was added, the following lowest Log IC_{50} values were reached: 2.0 ± 0.09 for CMS, 3.6 ± 0.04 for PB, and 1.5 ± 0.09 for BA.

The effects of the addition of the CMS + PB and PB + BA were similar to that observed for the bio-adsorbents added separately, showing an increase in CLA toxicity from the lowest concentration tested (6 g kg^{-1}) (Table S3; Supplementary material). For that dose, the Log IC_{50} values obtained were 3.4 ± 0.05 for CMS + PB, and 3.1 ± 0.05for PB + BA. These Log IC_{50} values decreased to 2.8 ± 0.07 for CMS + PB and to 2.3 ± 0.12 for PB + BA when the dose added was 48 g kg^{-1}.

This increase in the toxicity exerted by AZI and CLA with the dose of the bio-adsorbent added separately and in mixtures may be due to the reduction in the degree of degradation affecting to these antibiotics as a result of increasing the pH of the solution (Sayadi et al., 2019; Rodríguez-López et al., 2021), which would be caused by the bio-adsorbents. Sayadi et al. (2019) studied the effects of changing pH values on the degradation rate of azithromycin in the presence of nanoparticles, and concluded that the degradation of AZI was greater by decreasing the pH (maximum degradation of AZI at pH = 3). Rodríguez-López et al. (2021) studied the degradation of CLA in water at different pH values (4, 5.5, and 7), and also concluded that CLA degradation was lower when the pH increased. Therefore, increasing the pH of the solution due to the bio-adsorbent amendments that cause such kind of effect (increasing the pH of the soil) will decrease the degradation of AZI and CLA in the soils receiving these amendments. In addition, AZI adsorption suffers a slightly decrease with the rise in pH (Sayadi et al., 2019), causing that, at high pH, AZI will be more bioavailable for soil bacterial communities and therefore its toxicity will increase. In this sense, AZI and CLA toxicities on bacteria at different pH was studied by several authors (Neu, 1991; Heifets et al., 1992; Rastogi and Goh, 1992), who observed that the minimal inhibitory concentration (MIC) of AZI and CLA decreased with the increase in pH, indicating that the toxicity of AZI and CLA increased with the increase in pH.

3.2.3. Fluoroquinolones: ciprofloxacin

Fig. 3 shows the results corresponding to the dose-response curves obtained after the addition of CIP to the soil, for the different doses of the individual bio-adsorbents and mixtures. In general, these curves shift to the left with respect to control in the case of all the bio-adsorbents added

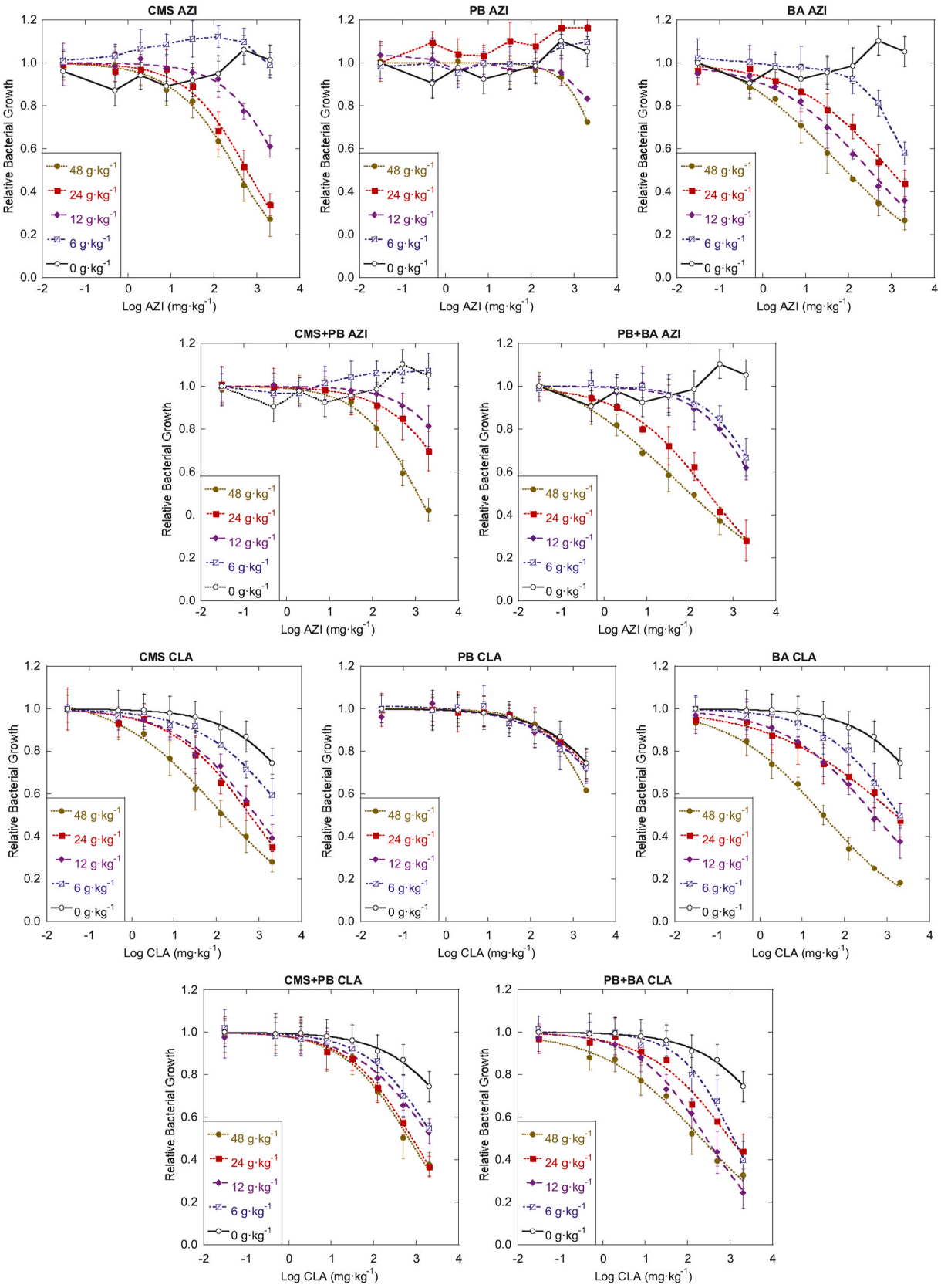

Fig. 2. Dose-response curves as regards the effects exerted by the antibiotics azithromycin (AZI) and clarithromycin (CLA) on the soil bacterial community in the presence of the three bio-adsorbents studied: crushed mussel shell (CMS), pine bark (PB), and biomass ash (BA), added separately and mixtures.

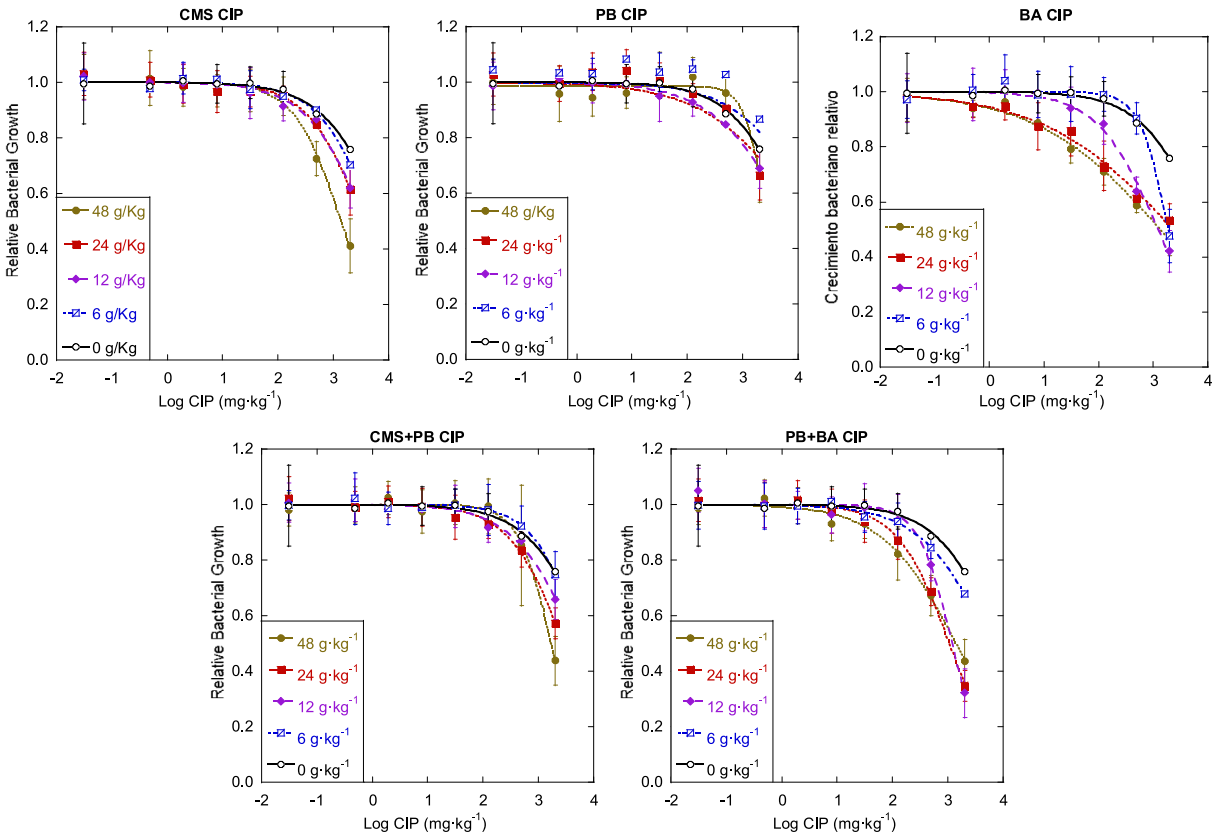

Fig. 3. Dose-response curves as regards the effects exerted by the antibiotic ciprofloxacin (CIP) on the soil bacterial community in the presence of the three bio-adsorbents studied: crushed mussel shell (CMS), pine bark (PB), and biomass ash (BA), added separately and mixtures.

separately (CMS, PB and BA) and their mixtures (CMS + PB and PB + BA), indicating that the toxicity of CIP increases with the dose of these materials.

The dose-response curves were well described by the logistic model (Eq. (1)), obtaining R^2 values from 0.910 to 0.995 (mean = 0.990). The dose-response curves were used to estimate the Log IC_{50} values obtained for all the bio-adsorbents and doses tested (Table S4; Supplementary Material). The Log IC_{50} value estimated for the control sample (0 g kg^{-1}) was 3.9 ± 0.04.

In general, the values of Log IC_{50} obtained show that the addition of doses ≥6 g kg^{-1} of CMS and BA increase the toxicity of CIP on soil bacterial communities. These increases in CIP toxicity with respect to the control ranged from 5% to 20% for CMS, and from 14% to 21% for BA. The soil amendment with PB also caused increases in CIP toxicity at doses ≥6 g kg^{-1}, although these increases were lower than those observed for CMS and BA, and ranged from 2% to 6%. As regards the mixtures (CMS + PB and PB + BA), their addition to the soil samples caused effects similar to those observed after amending with the bio-adsorbents separately, namely the doses of ≥6 g kg^{-1} increased CIP toxicity from 4% to 18% in the case of CMS + PB, and from 4% to 23% for PB + BA.

The effects of CIP on soil bacterial communities after the addition of the bio-adsorbents can be attributed to two fundamental reasons: i) The first one is that CIP is highly adsorbed at pH between 4 and 7–8, with maximum adsorption at pH between 5.5 and 6 (Vasudevan et al., 2009; Jalil et al., 2015); therefore, those bio-adsorbents that cause a modification of soil pH can modify the adsorption of CIP on soil colloids; in addition, the mechanisms of CIP adsorption in soils are intimately linked to the pH of the solution, and this will condition the solubility of the antibiotic; it should be noted that CIP has higher solubility at pH < 5 and pH > 9 (Jalil et al., 2015), compared to intermediate pH values; as for the high adsorption shown by CIP, it has also been observed in bio-adsorbents such as nanotubes (Li et al., 2014), oat hulls (Movasaghi et al., 2019) and in mineral components of soils such as clays (Jalil et al., 2015); therefore, the higher the solubility of CIP, the lower the adsorption on the soil colloids and the more bioavailable is for the soil bacterial communities, showing a higher toxicity. ii) The second reason is that the increased toxicity of CIP on soil bacterial communities with the dose of bio-adsorbents added could also be due to the fact that at extreme pH values (where CIP is more soluble) the degradation of CIP can also be increased (De Bel et al., 2009) and, therefore, secondary metabolites can be generated (Girardi et al., 2011) that may be more toxic than the original CIP compound; the toxicity of secondary metabolites on organisms has been tested for other antibiotics by several authors (Cáceres et al., 2008; Baumann et al., 2015; Xu et al., 2019).

3.2.4. *Diaminopyrimidines: trimethoprim*

Fig. 4 shows the results of the response dose curves obtained after the addition of TMP to the soil, for the different doses of the bio-adsorbents added separately and in mixtures. The control sample (0 g kg^{-1}) did not show inhibition of bacterial growth as a function of the increase in the TMP concentration, therefore no dose-response curve was obtained for this sample.

In general, the addition of CMS and BA to the soil samples caused toxicities that increased with the dose of bio-adsorbent, obtaining maximum inhibition for the highest dose, and a displacement of the dose-response curves to the left. Specifically, the PB amendment caused a shift to the left for doses of PB ≥ 24 g kg^{-1}, with slight inhibition on the growth of soil bacterial communities due to TMP.

With regard to the mixtures (CMS + PB and PB + BA), the dose-response behavior was similar to that shown for CMS and BA amendments added separately to the soil samples, with a shift to the left of the curves, although the magnitude of this displacement was lesser than that found for CMS and BA added individually.

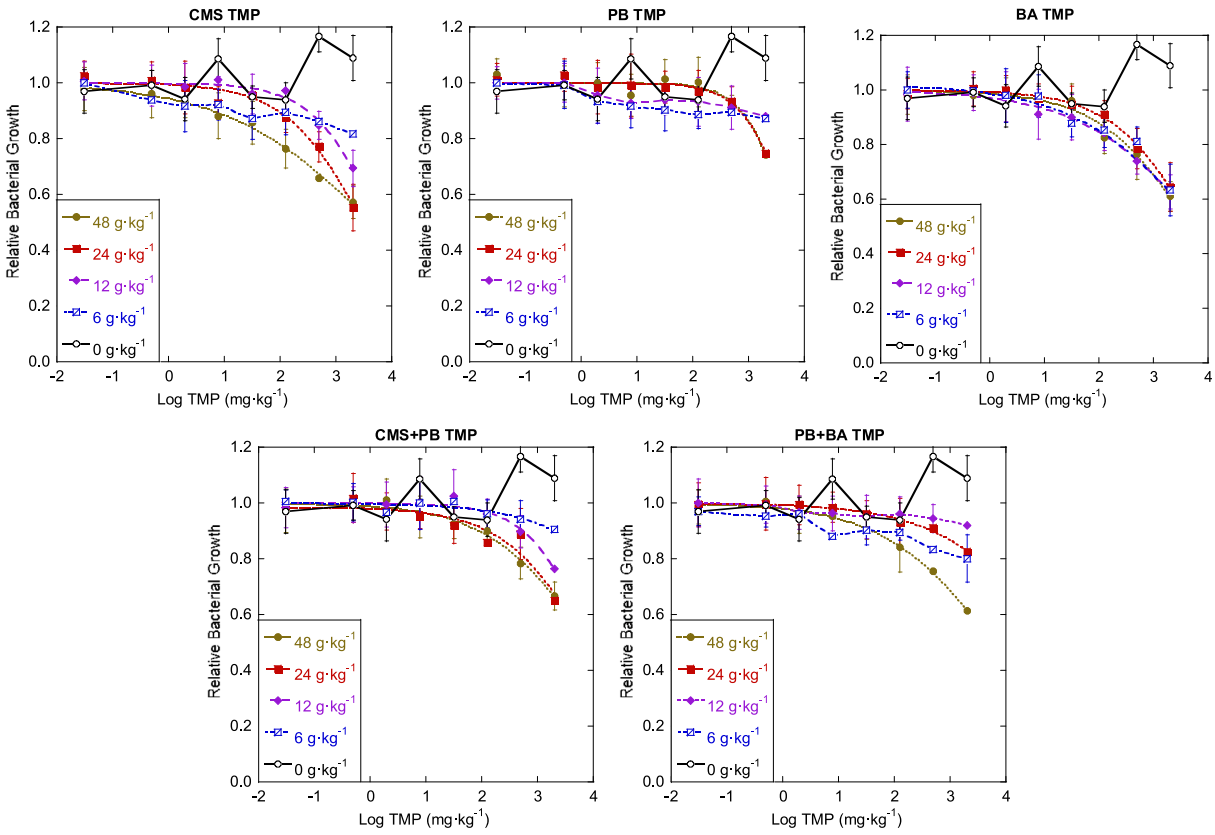

Fig. 4. Dose-response curves as regards the effects exerted by the antibiotic trimethoprim (TMP) on the soil bacterial community under the presence of the three bio-adsorbents: crushed mussel shell (CMS), pine bark (PB), and biomass ash (BA), added separately and mixtures.

The dose-response curves obtained were well described by the logistic model (Eq. (1)), with R^2 values of 0.987–0.998 (mean = 0.989). The Log IC_{50} values obtained for TMP from the dose-response curves, after the addition of the different doses of bio-adsorbents and their mixtures are shown in Table S5 (Supplementary Material). As stated above, the addition of TMP to the soil did not generate inhibition of soil bacterial communities in the control sample, and therefore the Log IC_{50} of the control sample (0 g kg^{-1}) could not be estimated.

The magnitude of the increase in the toxicity exerted by TMP on the bacterial growth was dependent on the type and dose of bio-adsorbent added. The amendment with CMS caused increased toxicity of TMP at doses ≥12 g kg^{-1}, with a Log IC_{50} value of 3.8 ± 0.10. This value of Log IC_{50} was progressively lowering with the dose of CMS added, up to 3.6 ± 0.07 (for dose of 48 g kg^{-1}), indicating that the toxicity of TMP on soil bacterial communities increased with the dose of that bio-adsorbent. Furthermore, the PB amendment caused a slight increase in TMP toxicity for the two highest doses tested (24 and 48 g kg^{-1}), with Log IC_{50} values of 3.8 ± 0.10 and 3.7 ± 0.14. As for the BA amendment, it caused an increase in the toxicity due to TMP at the lowest dose tested (6 g kg^{-1}), with a Log IC_{50} value of 3.8 ± 0.06. The Log IC_{50} value estimated for the maximum dose of BA tested (48 g kg^{-1}) was 3.7 ± 0.12.

Regarding the bio-adsorbent mixtures, the CMS + PB amendment caused increased toxicity on bacterial communities due to TMP when added at doses ≥12 g kg^{-1}, reaching a value of 3.9 ± 0.15 for Log IC_{50}, and being 3.9 ± 0.14 for the dose of 48 g kg^{-1}. Finally, the amendment with PB + BA caused increased toxicity due to TMP for the highest doses added (24 and 48 g kg^{-1}), with estimated Log IC_{50} values of 4.9 ± 0.22 and 3.8 ± 0.07, respectively.

The increased TMP toxicity on soil bacterial communities after the addition of some of the amendments may be due to the TMP species present at different pH values (Table 5). The amendment with PB caused a decrease in soil pH, and it should be noted that the TMP species that predominate at pH between 5.8 and 5.5 is cationic (TMP^{+1}) (Table 5). Cationic species show reduced permeation across cellular membrane (Fresta et al., 1996), with just favored passage through certain porins located in the membrane (Hancock and Bell, 1989), as previously

Table 5
Percentage of trimethoprim (TMP) species for different pH values measured in the soil amended with crushed mussel shell (CMS), pine bark (PB) and biomass ash (BA), and their mixtures (CMS + PB and PB + BA). Average values (n = 3) with coefficients of variation always <5%. The values were calculated using pK_a 7.16

	Dose (g kg^{-1})	pH	TMP^{+1}	TMP^{0}
	0	6.0	93.5	6.5
CMS	6	6.5	82.05	17.95
	12	7.1	53.45	46.55
	24	7.5	31.37	68.63
	48	7.7	22.38	77.62
PB	6	5.8	95.82	4.18
	12	5.8	95.82	4.18
	24	5.7	96.65	3.35
	48	5.5	97.86	2.14
BA	6	7.4	36.53	63.47
	12	8.5	4.37	95.63
	24	9.2	0.90	99.10
	48	10.1	0.11	99.89
CMS + PB	6	6.5	82.05	17.95
	12	6.8	69.61	30.39
	24	7.1	53.45	46.55
	48	7.5	31.37	68.63
PB + BA	6	6.9	64.54	35.46
	12	7.4	36.53	63.47
	24	8.0	12.63	87.37
	48	8.9	1.79	98.21

described for tetracyclines.

In addition, the amendment with the bio-adsorbents characterized by having alkaline pH (CMS and BA) increased the presence of the TMP zwitterionic species (TMP^0) compared to the cationic ones (Table 5). Zwitterionic species are those that show greater penetration through the cellular membrane of bacteria (Fresta et al., 1996) and, therefore, TMP shows greater toxicity on soil bacterial communities in the presence of these chemical species. Therefore, bio-adsorbents that increase soil pH also increase the toxicity exerted by TMP on soil bacterial communities, which is more marked at higher doses added, as the proportion of zwitterionic species in solution will be higher.

4. Conclusions

Variations in toxicity to soil bacterial communities as a function of the added bio-adsorbent show similar behaviors as a function of the antibiotic family studied. In this research, the amendment of soil samples with pine bark (PB) and biomass ash (BA) was effective in reducing the toxicity on soil bacterial communities exerted by the β-lactam antibiotics studied (AMX and CXM), whereas the amendment with crushed mussel shell (CMS) did not decrease the toxicity of these antibiotics. The amendment with bio-adsorbent mixtures (CMS + PB and PB + BA) reduced the toxicity of AMX and CXM more effectively than the addition of the bio-adsorbents separately at high doses. The increase in carbon in the soil and the variation in the chemical species of AMX and CXM, caused by the increase of the pH, could be the cause of the decrease in its toxicity on the soil bacterial communities. Soil amendment with by-products that increase the soil pH (CMS, BA, CMS + PB and PB + BA), increased the toxicity on soil bacterial communities of the macrolide antibiotics studied (AZI and CLA), as a function of the dose added, while the PB amendment only caused a slight increase in the toxicity of AZI and CLA when added at the highest dose (48 g kg^{-1}). The toxicity on soil bacterial communities due to CIP (a fluoroquinolone) increased with the addition of all the bio-adsorbents and mixtures tested (CMS, PB, BA, CMS + PB and PB + BA), although the mixtures caused higher toxicity. The increase of the pH in the soils reduces the sorption of CIP in the soils, being bioavailable for the microorganisms of the soil. Finally, the toxicity on soil bacterial communities due to TMP (a diaminopyrimidines) was slightly increased with the addition of the bio-adsorbents and their mixtures and was higher with rising doses. For the future, it would be interesting to investigate other low-cost by-products such as biochar o microalgae, which could be assessed as potential bio-adsorbents, studying their effectiveness to reduce the harmful effects that antibiotics and/or other emerging pollutants may cause in the environment and in human health.

Credit author statement

Avelino Núñez-Delgado: Conceptualization, Data curation, Visualization, Writing- Reviewing and Editing, Validation, Supervision, Visualization, Esperanza Álvarez-Rodríguez: Conceptualization, Data curation, Validation, Visualization, Montserrat Díaz-Raviña: Conceptualization, Methodology, Data curation, Validation, Supervision, Visualization, Writing – original draft preparation: Manuel Arias-Estévez, Validation, Conceptualization, Supervision, Visualization, Methodology: David Fernández-Calviño: Conceptualization, Methodology, Data curation, Validation, Supervision, Visualization, Writing – original draft preparation, Vanesa Santás-Miguel: Validation, Supervision, Investigation, Visualization, Writing – original draft preparation, Data curation, Software, Conceptualization, Laura Rodríguez-González: Software, Writing – original draft preparation, Visualization, Investigation

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.

Acknowledgements

This study has been funded by the Spanish Ministry of Science, Innovation and Universities through the projects RTI2018-099574-B-C21 and RTI2018-099574-B-C22 (FEDER Funds). David Fernández Calviño holds a Ramón y Cajal contract (RYC-2016-20411) financed by the Spanish Ministry of Economy, Industry and Competitiveness. Vanesa Santás Miguel holds a postdoctoral fellowship (ED481B-2022-081) financed by Xunta de Galicia. Laura Rodríguez González holds a predoctoral fellowship (ED481A-2021/309) financed by Xunta de Galicia. Funding for open access charge: Universidade de Vigo/CISUG.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2023.121161.

References

Adriaenssens, N., Coenen, S., Versporten, A., Muller, A., Minalu, G., Faes, C., Vankerckhoven, V., Aerts, M., Hens, N., Molenberghs, G., Goossens, H., 2011. European surveillance of antimicrobial consumption (ESAC): outpatient quinolone use in europe (1997–2009). J. Antimicrob. Chemother. 66 https://doi.org/10.1093/jac/dkr457 suppl_6), vi47-vi56.vi47-vi56.

Ata, R., Sacco, O., Vaiano, V., Rizzo, L., Tore, G.Y., Sannino, D., 2017. Visible light active N-doped TiO2 immobilized on polystyrene as efficient system for wastewater treatment. J. Photochem. Fotobiol. A: Chemistry 348, 255–262. https://doi.org/10.1016/j.jphotochem.2017.08.054.

Aydın, S., Ulvi, A., Bedük, F., Aydın, M.E., 2022. Pharmaceutical residues in digested sewage sludge: occurrence, seasonal variation and risk assessment for soil. Sci. Total Environ., 152864 https://doi.org/10.1016/j.scitotenv.2021.152864.

Bååth, E., 1994. Thymidine and leucine incorporation in soil bacteria with different cell size. Microb. Ecol. 27, 267–278. https://doi.org/10.1007/BF00182410, 1994.

Bååth, E., Pettersson, M., Söderberg, K.H., 2001. Adaptation of a rapid and economical microcentrifugation method to measure thymidine and leucine incorporation by soil bacteria. Soil Biol. Biochem. 3, 1571–1574. https://doi.org/10.1016/S0038-0717(01)00073-6, 2001.

Balarak, D., Joghatayi, A., Mostafapour, F.K., Azarpira, H., 2017. Biosorption of amoxicillin from contaminated water onto palm bark biomass. International journal of life science & pharma research 7 (1), 9–16.

Baumann, M., Weiss, K., Maletzki, D., Schüssler, W., Schudoma, D., Kopf, W., Kühnen, U., 2015. Aquatic toxicity of the macrolide antibiotic clarithromycin and its metabolites. Chemosphere 120, 192–198. https://doi.org/10.1016/j.chemosphere.2014.05.089.

Cáceres, T., Megharaj, M., Naidu, R., 2008. Toxicity and transformation of fenamiphos and its metabolites by two micro algae Pseudokirchneriella subcapitata and Chlorococcum sp. Sci. Total Environ. 398 (1–3), 53–59. https://doi.org/10.1016/j.scitotenv.2008.03.022.

Cela-Dablanca, R., Barreiro, A., López, L.R., Santás-Miguel, V., Arias-Estévez, M., Núñez-Delgado, A., Álvarez-Rodríguez, E., Fernández-Sanjurjo, M.J., 2022a. Relevance of sorption in bio-reduction of amoxicillin taking place in forest and crop soils. Environ. Res. 208, 112753 https://doi.org/10.1016/j.envres.2022.112753.

Cela-Dablanca, R., Barreiro, A., Rodríguez-López, L., Santás-Miguel, V., Arias-Estévez, M., Fernández-Sanjurjo, M.J., Álvarez-Rodríguez, E., Núñez-Delgado, A., 2022b. Amoxicillin retention/release in agricultural soils amended with different bio-adsorbent materials. Materials 15 (9), 3200. https://doi.org/10.3390/ma15093200.

Cela-Dablanca, R., Nebot, C., Rodríguez-López, L., Fernández-Calviño, D., Arias-Estévez, M., Núñez-Delgado, A., Fernández-Sanjurjo, M.J., Álvarez-Rodríguez, E., 2021. Efficacy of different waste and by-products from forest and food industries in the removal/retention of the antibiotic cefuroxime. Processes 9 (7), 1151. https://doi.org/10.3390/pr9071151.

Collignon, P.C., Conly, J.M., Andremont, A., McEwen, S.A., Aidara-Kane, A., , World Health Organization Advisory Group, Bogotá Meeting on Integrated Surveillance of Antimicrobial Resistance (WHO-AGISAR), Ninh, T.D., Donado-Godoy, P., Fedorka-Cray, P., Fernandez, H., Galas, M., Irwin, R., Karp, B., Mater, G., , McDermott, Mitema, E., Reid-Smith, R., Stelling, J., Toleman, M., Watanabe, H., Woo, G.J., 2016. World Health Organization ranking of antimicrobials according to their importance in human medicine: a critical step for developing risk management strategies to control antimicrobial resistance from food animal production. Clin. Infect. Dis. 63 (8), 1087–1093. https://doi.org/10.1093/cid/ciw475.

Cycoń, M., Mrozik, A., Piotrowska-Seget, Z., 2019. Antibiotics in the soil environment—degradation and their impact on microbial activity and diversity. Front. Microbiol. 10, 338. https://doi.org/10.3389/fmicb.2019.00338.

De Bel, E., Dewulf, J., De Witte, B., Van Langenhove, H., Janssen, C., 2009. Influence of pH on the sonolysis of ciprofloxacin: biodegradability, ecotoxicity and antibiotic activity of its degradation products. Chemosphere 77 (2), 291–295. https://doi.org/10.1016/j.chemosphere.2009.07.033.

Ding, H., Wu, Y., Zou, B., Lou, Q., Zhang, W., Zhong, J., Lu, L., Dai, G., 2016. Simultaneous removal and degradation characteristics of sulfonamide, tetracycline, and quinolone antibiotics by laccase-mediated oxidation coupled with soil adsorption. J. Hazard Mater. 307, 350–358. https://doi.org/10.1016/j.jhazmat.2015.12.062.

European Commission, 2015. Commission Implementing Decision (EU) 2015/495 of 20 March 2015 establishing a watch list of substances for Union-wide monitoring in the field of water policy pursuant to Directive 2008/105/EC of the European Parliament and of the Council. Off. J. Eur. Union 78, 20–30. C (2015) 1756.

Fernández-Calviño, D., Garrido-Rodríguez, B., Arias-Estévez, M., Díaz-Raviña, M., Álvarez-Rodríguez, E., Fernández-Sanjurjo, M.J., Nuñez-Delgado, A., 2015. Effect of crushed mussel shell addition on bacterial growth in acid polluted soils. Appl. Soil Ecol. 85, 65–68. https://doi.org/10.1016/j.apsoil.2014.09.010.

Fernández-Calviño, D., Cutillas-Barreiro, L., Nóvoa-Muñoz, J.C., Díaz-Raviña, M., Fernández-Sanjurjo, M.J., Álvarez-Rodríguez, E., Núñez-Delgado, A., Arias-Estévez, M., Rousk, J., 2018. Using pine bark and mussel shell amendments to reclaim microbial functions in a Cu polluted acid mine soil. Appl. Soil Ecol. 127, 102–111. https://doi.org/10.1016/j.apsoil.2018.03.010.

Fresta, M., Furneri, P.M., Mezzasalma, E., Nicolosi, V.M., Puglisi, G., 1996. Correlation of trimethoprim and brodimoprim physicochemical and lipid membrane interaction properties with their accumulation in human neutrophils. Antimicrob. Agents Chemother. 40 (12), 2865–2873. https://doi.org/10.1128/AAC.40.12.2865.

Girardi, C., Greve, J., Lamshöft, M., Fetzer, I., Miltner, A., Schäffer, A., Kästner, M., 2011. Biodegradation of ciprofloxacin in water and soil and its effects on the microbial communities. J. Hazard Mater. 198, 22–30. https://doi.org/10.1016/j.jhazmat.2011.10.004.

Gu, C., Karthikeyan, K.G., Sibley, S.D., Pedersen, J.A., 2007. Complexation of the antibiotic tetracycline with humic acid. Chemosphere 66 (8), 1494–1501. https://doi.org/10.1016/j.chemosphere.2006.08.028.

Ghirardini, A., Grillini, V., Verlicchi, P., 2020. A review of the occurrence of selected micropollutants and microorganisms in different raw and treated manure–environmental risk due to antibiotics after application to soil. Sci. Total Environ. 707, 136118 https://doi.org/10.1016/j.scitotenv.2019.136118.

Hanamoto, S., Ogawa, F., 2019. Predicting the sorption of azithromycin and levofloxacin to sediments from mineral and organic components. Environ. Pollut. 255, 113180 https://doi.org/10.1016/j.envpol.2019.113180.

Hancock, R.E.W., Bell, A., 1989. Antibiotic uptake into gram-negative bacteria. In: Jackson, G.G., Schlumberger, H.D., Zeiler, H.J. (Eds.), Perspectives in Antiinfective Therapy. Bayer Centenary Symposium Washington, D.C. Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-642-46666-3_6. Aug. 31.–Sept. 3, 1988.

Hartmann, A., Golet, E.M., Gartiser, S., Alder, A.C., Koller, T., Widmer, R.M., 1999. Primary DNA damage but not mutagenicity correlates with ciprofloxacin concentrations in German hospital wastewaters. Arch. Environ. Contam. Toxicol. 36 (2), 115–119. https://doi.org/10.1007/s002449900449.

Heifets, L.B., Lindholm-Levy, P.J., Comstock, R.D., 1992. Clarithromycin minimal inhibitory and bactericidal concentrations against Mycobacterium avium. Am. J. Respir. Crit. Care Med. 145 (4), 856–858. https://doi.org/10.1128/AEM.05352-11.

Huddleston, J.R., 2014. Horizontal gene transfer in the human gastrointestinal tract: potential spread of antibiotic resistance genes. Infect. Drug Resist. 7, 167. https://doi.org/10.2147/IDR.S48820.

Iatrou, E.I., Stasinakis, A.S., Thomaidis, N.S., 2014. Consumption-based approach for predicting environmental risk in Greece due to the presence of antimicrobials in domestic wastewater. Environ. Sci. Pollut. Res. 21 (22), 12941–12950. https://doi.org/10.1007/s11356-014-3243-7.

Jalil, M.E.R., Baschini, M., Sapag, K., 2015. Influence of pH and antibiotic solubility on the removal of ciprofloxacin from aqueous media using montmorillonite. Appl. Clay Sci. 114, 69–76. https://doi.org/10.1016/j.clay.2015.05.010.

Juela, D.M., 2021. Promising adsorptive materials derived from agricultural and industrial wastes for antibiotic removal: a comprehensive review. Separ. Purif. Technol., 120286 https://doi.org/10.1016/j.seppur.2021.120286.

Kemper, N., 2008. Veterinary antibiotics in the aquatic and terrestrial environment. Ecol. Indicat. 8 (1), 1–13. https://doi.org/10.1016/j.ecolind.2007.06.002.

Kim, Y., Lim, S., Han, M., Cho, J., 2012. Sorption characteristics of oxytetracycline, amoxicillin, and sulfathiazole in two different soil types. Geoderma 185, 97–101. https://doi.org/10.1016/j.geoderma.2012.03.016.

Klein, E.Y., Van Boeckel, T.P., Martinez, E.M., Pant, S., Gandra, S., Levin, S.A., Goossens, H., Laxminarayan, R., 2018. Global increase and geographic convergence in antibiotic consumption between 2000 and 2015. Proc. Natl. Acad. Sci. USA 115 (15), E3463–E3470. https://doi.org/10.1073/pnas.171729511.

Koch, N., Islam, N.F., Sonowal, S., Prasad, R., Sarma, H., 2021. Environmental antibiotics and resistance genes as emerging contaminants: methods of detection and bioremediation. Curr. Res. Microb. Sci. 2, 100027 https://doi.org/10.1016/j.crmicr.2021.100027.

Kümmerer, K., 2009. Antibiotics in the aquatic environment–a review–part I. Chemosphere 75 (4), 417–434. https://doi.org/10.1016/j.chemosphere.2008.11.086.

Levy, S.B., Marshall, B., 2004. Antibacterial resistance worldwide: causes, challenges and responses. Nat. Med. 10 (12), S122–S129. https://doi.org/10.1038/nm1145.

Li, H., Zhang, D., Han, X., Xing, B., 2014. Adsorption of antibiotic ciprofloxacin on carbon nanotubes: pH dependence and thermodynamics. Chemosphere 95, 150–155. https://doi.org/10.1016/j.chemosphere.2013.08.053.

Macías, F., Calvo, R., 2008. Niveles genéricos de referencia de metales pesados y otros elementos traza en suelos de Galicia (Reference values for heavy metals and other trace elements in soils in Galicia). In: Consellería de Medio Ambiente e Desenvolvemento Sostible. Xunta de Galicia, Santiago de Compostela, p. 229.

Meisner, A., Bååth, E., Rousk, J., 2013. Microbial growth responses upon rewetting soil dried for four days or one year. Soil Biol. Biochem. 66, 188–192. https://doi.org/10.1016/j.soilbio.2013.07.014.

Michael, I., Rizzo, L., McArdell, C.S., Manaia, C.M., Merlin, C., Schwartz, T., Dagot, C., Fatta-Kassinos, D.J.W.R., 2013. Urban wastewater treatment plants as hotspots for the release of antibiotics in the environment: a review. Water Res. 47 (3), 957–995. https://doi.org/10.1016/j.watres.2012.11.027.

Movasaghi, Z., Yan, B., Niu, C., 2019. Adsorption of ciprofloxacin from water by pretreated oat hulls: equilibrium, kinetic, and thermodynamic studies. Ind. Crop. Prod. 127, 237–250. https://doi.org/10.1016/j.indcrop.2018.10.051.

Neu, H.C., 1991. Clinical microbiology of azithromycin. Am. J. Med. 91 (3), S12–S18. https://doi.org/10.1016/0002-9343(91)90395-E.

Pan, M., Chu, L.M., 2017. Transfer of antibiotics from wastewater or animal manure to soil and edible crops. Environ. Pollut. 231, 829–836. https://doi.org/10.1016/j.envpol.2017.08.051.

Quintáns-Fondo, A., Fernández-Calviño, D., Nóvoa-Muñoz, J.C., Arias-Estévez, M., Fernández-Sanjurjo, M.J., Álvarez-Rodríguez, E., Núñez-Delgado, A., 2017. As (V) sorption/desorption on different waste materials and soil samples. Int. J. Environ. Res. Publ. Health 14 (7), 803. https://doi.org/10.3390/ijerph14070803.

Rastogi, N., Goh, K.S., 1992. Effect of pH on radiometric MICs of clarithromycin against 18 species of mycobacteria. Antimicrob. Agents Chemother. 36 (12), 2841–2842. https://doi.org/10.1128/AAC.36.12.2841.

Rodríguez-López, L., Cela-Dablanca, R., Núñez-Delgado, A., Álvarez-Rodríguez, E., Fernández-Calviño, D., Arias-Estévez, M., 2021. Photodegradation of ciprofloxacin, clarithromycin and trimethoprim: influence of pH and humic acids. Molecules 26 (11), 3080. https://doi.org/10.3390/molecules26113080.

Rousk, J., Demoling, L.A., Bahr, A., Bååth, E., 2008. Examining the fungal and bacterial niche overlap using selective inhibitors in soil. FEMS Microbiol. Ecol. 63 (3), 350–358. https://doi.org/10.1111/j.1574-6941.2008.00440.x.

Russell, J.N., Yost, C.K., 2021. Alternative, environmentally conscious approaches for removing antibiotics from wastewater treatment systems. Chemosphere 263, 128177. https://doi.org/10.1016/j.chemosphere.2020.128177.

Santás-Miguel, V., Fernández-Sanjurjo, M.J., Núñez-Delgado, A., Álvarez-Rodríguez, E., Díaz-Raviña, M., Arias-Estévez, M., Fernández-Calviño, D., 2020. Use of biomass ash to reduce toxicity affecting soil bacterial community growth due to tetracycline antibiotics. J. Environ. Manag. 269, 110838 https://doi.org/10.1016/j.jenvman.2020.110838.

Santás-Miguel, V., Fernández-Sanjurjo, M.J., Núñez-Delgado, A., Álvarez-Rodríguez, E., Díaz-Raviña, M., Arias-Estévez, M., Fernández-Calviño, D., 2021. Use of waste materials to prevent tetracycline antibiotics toxicity on the growth of soil bacterial communities. Environ. Res. 193, 110404 https://doi.org/10.1016/j.envres.2020.110404.

Sayadi, M.H., Sobhani, S., Shekari, H., 2019. Photocatalytic degradation of azithromycin using GO@ Fe3O4/ZnO/SnO2 nanocomposites. J. Clean. Prod. 232, 127–136. https://doi.org/10.1016/j.jclepro.2019.05.338.

Solliec, M., Roy-Lachapelle, A., Gasser, M.O., Coté, C., Généreux, M., Sauvé, S., 2016. Fractionation and analysis of veterinary antibiotics and their related degradation products in agricultural soils and drainage waters following swine manure amendment. Sci. Total Environ. 543, 524–535. https://doi.org/10.1016/j.scitotenv.2015.11.061.

Stock, J.B., Rauch, B., Roseman, S., 1977. Periplasmic space in Salmonella typhimurium and Escherichia coli. J. Biol. Chem. 252, 7850–7861. https://doi.org/10.1016/S0021-9258(17)41044-1.

Talaiekhozani, A., Banisharif, F., Eskandari, Z., Talaei, M.R., Park, J., Rezania, S., 2020. Kinetic investigation of 1, 9-dimethyl-methylene blue zinc chloride double salt removal from wastewater using ferrate (VI) and ultraviolet radiation. J. King Saud Univ. Sci. 32 (1), 213–222. https://doi.org/10.1016/j.jksus.2018.04.010.

Tan, K.H., 1996. Soil Sampling, Preparation, and Analysis. Marcel Dekker, New York, USA.

Vasudevan, D., Bruland, G.L., Torrance, B.S., Upchurch, V.G., MacKay, A.A., 2009. pH-dependent ciprofloxacin sorption to soils: interaction mechanisms and soil factors influencing sorption. Geoderma 151 (3–4), 68–76. https://doi.org/10.1016/j.geoderma.2009.03.007.

Xu, D., Xiao, Y., Pan, H., Mei, Y., 2019. Toxic effects of tetracycline and its degradation products on freshwater green algae. Ecotoxicol. Environ. Saf. 174, 43–47. https://doi.org/10.1016/j.ecoenv.2019.02.063.

Yu, H., Ma, J., Chen, F., Zhang, Q., Wang, Y., Bian, Z., 2022. Effective remediation of electronic waste contaminated soil by the combination of metal immobilization and phytoremediation. J. Environ. Chem. Eng. 10 (3), 107410 https://doi.org/10.1016/j.jece.2022.107410.

Zhao, Y., Geng, J., Wang, X., Gu, X., Gao, S., 2011. Tetracycline adsorption on kaolinite: pH, metal cations and humic acid effects. Ecotoxicology 20 (5), 1141–1147. https://doi.org/10.1007/s10646-011-0665-6.

Zhao, F., Yang, L., Li, G., Fang, L., Yu, X., Tang, Y.T., Li, M., Chen, L., 2022. Veterinary antibiotics can reduce crop yields by modifying soil bacterial community and earthworm population in agro-ecosystems. Sci. Total Environ. 808, 152056 https://doi.org/10.1016/j.scitotenv.2021.152056.