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Effects of fresh and field-aged holm-oak biochar on As, Cd and Pb bioaccumulation in different rice growing environments

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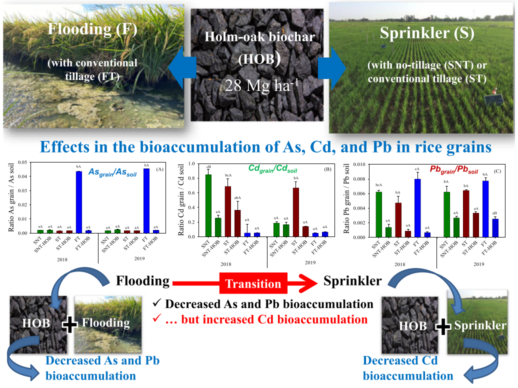
HIGHLIGHTS

- Biochar acted as an important soil passivator of As in flooding irrigation systems.
- Pb bioaccumulation in rice grain decreased in all production systems with biochar.
- Increased bioaccumulation of Cd in sprinkler irrigation was counteracted by biochar.
- Biochar simultaneously avoids metals and metalloids bioaccumulation in rice grain.

GRAPHICAL ABSTRACT



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ABSTRACT

Arsenic, Cd, and Pb environmental fate is influenced when the traditional permanent flooding rice production systems are replaced by water-saving and soil conservation practices, urging for additional strategies that avoid their bioaccumulation in rice grain. The aim of this two-years field study was to evaluate the effects of fresh and field-aged biochar on As, Cd, and Pb bioaccumulation, and on As speciation, in rice grain produced in different growing environments (flooding *versus* sprinkler and conventional tillage *versus* direct seeding). Biochar produced from holm-oak pruning residues (pyrolysis at 550 °C, 48 h), in a single application (28 Mg ha^{-1}), reduced As bioaccumulation in rice grain in the permanent flooding system to non-quantifiable concentrations (*e.g.*, from 0.178 mg kg^{-1} to <0.04 mg kg^{-1}, for inorganic-As, respectively), an effect which remained under field-aging conditions, increasing rice commercial value. When adopting sprinkler irrigation, the undesirable increase in Cd bioaccumulation in rice, relatively to the anaerobic system, was counteracted by biochar application, reducing its bioaccumulation in kernels between 32 and 80 %, allowing a simultaneous control of metals and metalloids bioaccumulation in rice. The bioaccumulation of Pb was also prevented with biochar application, with a reduction in its concentration four- to 13-times, in all the management systems, relatively to the non-amended plots, under fresh biochar effects. However, Pb immobilization decreased with biochar field-aging, indicating that the biochar application may have to be repeated to maintain the same beneficial effect. Therefore, the present study shows that the implementation of sprinkler irrigation with holm-oak biochar could reduce the risk of heavy metals(loids) bioaccumulation in rice grains and, thereby, ensuring food safety aspects, particularly under fresh biochar effects.

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1. Introduction

Rice (*Oryza sativa* L.) provides staple food for >3.5 billion people, about half of the world population (Mandal et al., 2019), turning it is a crucial crop to ensure world's food safety (Wu et al., 2018). The continuous flooding irrigation is the most widely used method for rice production, with >60 % of the total rice area irrigated through this method (Rao et al., 2017). In fact, Ariani et al. (2022) indicated that rice consumes about 40 % of the total world irrigation water, which represents >25 % of the total freshwater resources, thus competing with the demand for freshwater by other sectors of the economy. This situation could be even more critical in the Mediterranean environment, where shortage of water resources do not allow guarantee of water supply for rice fields. Therefore, agricultural practices to increase sustainability and productivity of rice crop are urgent (Peña et al., 2022). In this sense, several water-saving systems have been developed to reduce unproductive losses of water (*e.g.*, seepage, percolation, evaporation), increasing water productivity, relatively to continuous flooding irrigation (Poddar et al., 2022). These water-saving methods, whose application in rice fields have increased in recent years, include alternate wetting and drying (Carrijo et al., 2017), sprinkler (Alvarenga et al., 2022; Spanu et al., 2022), drip (Abd El-Mageed et al., 2022), among others irrigation systems (Poddar et al., 2022).

The implementation of water-saving irrigation methods could affect the bioavailability of toxic elements, such as arsenic (As), cadmium (Cd) and lead (Pb), which have been ranked first, seventh and second, respectively, as hazardous substances by the Agency for Toxic Substances and Disease Registry (ATSDR, 2019), and whose bioaccumulation in rice grains could lead to adverse effects on human health (Carrijo et al., 2022; Wen et al., 2021). This is especially important in rice, since, apart from being a staple food for a large part of the world's population, is more effective than other cereals in accumulating these toxic elements (Khanam et al., 2020). Thus, rice could act as an important source of dietary intake of these elements (As, Cd, and Pb), whose concentrations were regulated, in the case of European Union, by the Commission Regulation in 2006 and 2015 (Spanu et al., 2020; Alvarenga et al., 2022). In particular, rice is one of the most important sources of As to the human diet (Moreno-Jiménez et al., 2014; Oberoi et al., 2019), whose toxicity is mainly influenced by its chemical speciation in rice grain (Meharg and Hartley-Whitaker, 2002). Inorganic As (As_{inorg}), the sum of arsenite, As(III), and arsenate, As (V), corresponds to the most toxic species, while the organic forms are considered to be of low risk. Due to the reduction conditions associated with continuous flooding irrigation, As is reduced from As(V) to As(III), which is more mobile, leading to increased phytoavailability and uptake by rice plants (Islam et al., 2020). Previous studies have demonstrated an increase in grain As(III) concentration under continuous flooding irrigation, whereas a significant decrease was recorded under water saving methods, such as alternate wetting and drying and sprinkler irrigation (*e.g.*, Honma et al., 2016; Alvarenga et al., 2022). This observation was explained because As(V), the more abundant As_{inorg} form under oxidizing environmental conditions, is strongly sorbed to soil colloids (Honma et al., 2016). However, Cd, whose predominant form taken up by rice plants is Cd^{2+}, can be stabilized as sulfide (CdS), under reducing environmental conditions produced by permanent flooding irrigation, causing the decrease of Cd solubility and phytoavailability (Wen et al., 2021). Previous studies have indicated that the effects of water management on Cd and As bioaccumulation in rice grains were opposite, due to their contrasting biogeochemical behaviours (Moreno-Jiménez et al., 2014; Honma et al., 2016; Alvarenga et al., 2022). However, literature diverge in results about the effects of water management on As and Cd bioaccumulation in rice grains (Carrijo et al., 2022). Indeed, the effects on As bioaccumulation, in rice grain under water-saving systems, ranged from no decrease to 90 % decrease, in reference to permanent flooding irrigation (Acharjee et al., 2021; Norton et al., 2017). Likewise, there are studies that reported a high variability on the effects of water-saving methods on Cd bioaccumulation in rice grain and even contradictory trends across studies (da Silva et al., 2020; Spanu et al., 2018; Xu et al., 2019). The wide variability in results could be due to differences between water-saving managements, rice genetics, soil properties and rice growing environments, showing the need to develop further studies.

There are few published studies about the effects of rice water management on Pb bioaccumulation in rice grains (Spanu et al., 2020). The availability of Pb in soils could be affected by several factors, being soil pH a crucial aspect. Thus, the mobility of Pb under acidic conditions can be favoured due to its higher solubility (Khanam et al., 2020). Therefore, it is very difficult to, simultaneously, reduce the bioaccumulation of different toxic elements in rice grain using single management options (Wu et al., 2021).

Biochar, a carbon-rich organic material produced by pyrolysis, is very effective to improve rice yields under water-saving irrigation (Liu et al., 2022), increasing the sustainability and productivity of rice in areas with high water stress, such as in the Mediterranean (López-Piñeiro et al., 2022). As biochar application to rice soils could lead to improved soils properties, such as pH control, increase in porosity, surface charges and exchange sites, it may also play an important role in reducing plants' toxic element uptake (Kumar et al., 2022).

However, further studies are needed, because most studies have been developed: (i) with modified biochar, which makes it very costly, and not economically attractive; (ii) in soils affected by high concentrations of toxic elements, when most of the rice is cultivated under no-contaminated soil; and (iii) under greenhouse or laboratory conditions (*e.g.*, Yu et al., 2017; Diao et al., 2022; Majumdar et al., 2023; Yang et al., 2021, 2023), constraining the inference of the effects to the whole growth period of rice, under real field conditions.

Biochar can be obtained from a wide variety of organic materials. That obtained from holm oak pruning, holm-oak biochar (HOB), has high total organic carbon content as well as greater water holding capacity than those produced from other organic materials (Takaya et al., 2016; López-Cano et al., 2018). Furthermore, HOB is extensively produced at a reasonable price in different Mediterranean countries, thus making it practicable to use it as an organic amendment (López-Piñeiro et al., 2022). Despite, to our best knowledge, the effects of HOB application, under different water irrigation practices, on As, Cd and Pb rice bioaccumulation in Mediterranean conditions, has never been studied. Likewise, different tillage managements should also be considered since they can modify soils properties and cause changes in the phytoavailability of toxic elements. Therefore, the objective of the present field study was to analyse the effects of HOB application on the bioaccumulation of As, Cd, and Pb, as well as on As speciation in rice grain, under different rice growing environments (flooding *versus* sprinkler and conventional tillage *versus* direct seeding). Considering that fresh HOB properties may be altered by weathering and aging processes under these different rice growing environments, which in turn could also affect the availability and bioaccumulation of As, Cd and Pb, aging effects are also intended to be measured, after two years of HOB application to soil.

2. Materials and methods

2.1. Site description

A 2-year field experiment (2018 and 2019) was carried out in a paddy field at Gévora, south-western Spain (38°55′N; 6°57′W), under a semi-arid Mediterranean climate with rainfall of 450 mm and mean annual temperature of 17 °C. The experimental area has been dedicated to rice production for >10 years, with deep ploughing and flooding techniques. However, in a part of the experimental area, the sprinkler irrigation method was implemented since 2015. According to FAO (2006), the soil of the experimental area is classified as Hydragic Anthrosol, with a particle size distribution of 50.3 % sand, 28.9 % silt and 20.8 % clay (loam texture) and 12.5 g kg^{-1} total carbon. Total As, Cd, and Pb concentrations in soil samples throughout the study were ≤ 6.30 mg kg^{-1}, 0.275 mg kg^{-1} and 21.8 mg kg^{-1}, respectively, which indicates that the soil is not contaminated by these elements, as further discussed in Section 3.1.

2.2. Biochar

The commercial biochar applied in the experimental area was purchased from Carylevere Co., Ltd. (Zahinos, Spain). It was produced by slow pyrolysis of the pruning residues of holm oak (*Quercus ilex* L.), a typical tree of the Mediterranean agroforestry system, at a temperature of 550 °C for 48 h. Before application to soil, the HOB was ground to pass through a sieve (2 mm), and analysed for: total carbon (TC), total hydrogen (TH), total nitrogen (TN), total oxygen (TO), ash, H/C and O/C ratios, pH, electrical conductivity (EC), water-soluble organic carbon (WSOC), specific surface area (SSA) and total pore volume, according to the methods previously described by López-Piñeiro et al. (2022). The biochar physicochemical characterization was made in its fresh (before soil application) and aged status (after two years of aging processes under different management and natural field conditions) (Table S1). The toxic trace elements (Cr, Ni, As, Cd, Hg, and Pb) were extracted from the HOB according to the European Biochar Certificate (EBC) analytical methods (EBC, 2022), and analysed using inductively coupled plasma optical atomic emission spectrophotometer (ICP-OES, ICAP 6500 Duo Thermo®) (Table 1). Considering the metals/metalloids concentrations in the biochar, and the standards of the European Biochar Certificate-Version 10.2 from 8th Dec 2022 of EBC (EBC, 2022), the HOB can be labelled as EBC-FeedPlus, which meet all European Union regulations and whose use as a soil amendment is allowed.

2.3. Experimental design and field management

Six different management systems were implemented in the experimental area, with three replicates per treatment, in a completely randomized design: (*i*) sprinkler irrigation under no-tillage management (SNT), (*ii*) sprinkler irrigation under no-tillage management with HOB application (SNT- HOB), (*iii*) sprinkler irrigation under conventional tillage (ST), (*iv*) sprinkler irrigation under conventional tillage with HOB application (ST-HOB), (*v*) permanent flooding irrigation under conventional tillage (FT), and (*vi*) permanent flooding irrigation under conventional tillage with HOB application (FT- HOB). In all the amended treatments (SNT- HOB, ST- HOB, and FT- HOB) the dosage of HOB used was 28 Mg ha^{-1}, which was applied only in April 2018 (single application, before rice sowing).

In October of each year (after rice harvest), a composite soil sample of four subsamples was collected from each plot to a depth of 0–20 cm. Therefore, the measurements done in the year 2018 corresponded to the "fresh" effects, whereas the measurements done in the year 2019 corresponded to the "aged" effects. To improve planning and efficient irrigation management, the SNT, SNT- HOB, ST, and ST- HOB treatments were irrigated by sprinkler at a frequency of 6 days per week, throughout the rice growing season, considering crop's evapotranspiration. However, the FT and FT-HOB treatments were permanent flooded, maintaining the water level about 10 cm above ground. Sprinkler irrigation allowed an overall considerable water saving, since the amount of water applied in the sprinkler irrigation ranged from 53 to 58 % of the total water applied in the permanent flooding treatments. The water used for irrigation, regardless of sprinkler irrigation or permanent flooding, was the same, and was supplied from the Montijo Dam. During the two years of the study, toxic metals and metalloids concentrations in the irrigation water were always below the thresholds set for drinking water by the World Health Organization (WHO), which are 10 μg L^{-1}, 3 μg L^{-1} and 10 μg L^{-1} for As, Cd and Pb, respectively (WHO, 2022). The concentrations ranged from 2.00 to 9.30 μg L^{-1} for As, from <0.02 to 0.08 μg L^{-1} for Cd, and from <0.3 to 2.21 μg L^{-1} for Pb.

Table 1
Trace element concentrations in the holm-oak biochar (HOB) applied to the soil in the field study (mean values, $n = 3$). Concentrations are reported on a dry matter basis.

Potentially toxic trace elements	HOB	Limit value of European Biochar Certificate[a]
Cr (mg kg^{-1})	0.70	70
Ni (mg kg^{-1})	2.12	25
As (mg kg^{-1})	0.26	2
Cd (mg kg^{-1})	<0.10	0.8
Hg (mg kg^{-1})	<0.10	0.1
Pb (mg kg^{-1})	1.19	10

[a] European Biochar Certificate - Guidelines for a Sustainable Production of Biochar (Version 10.2 from 8th Dec 2022).

In both years of the study, the rice was sown in the first seven days of May at a dosage of 160 kg ha^{-1} seeds of *Oryza sativa* var. Sirio, with a Semeato 320 Disc Seeder for the sprinkler irrigated treatments, and with a broadcast seed drill for the permanent flooding irrigation treatments. The harvest of rice was done at the end of September. Regardless of the seeding and irrigation system, the rest of agricultural practices, such as mineral fertilization and pesticides application, were the same and repeated during both years of the study (2018 and 2019).

The physico-chemical properties of soils (total carbon (TC), water-soluble organic carbon (WSOC), electrical conductivity (EC, saturation extract), soil pH (soil:water suspension 1:1 v/v) were determined by methods previously described by López-Piñeiro et al. (2022). Specific UV-absorbance at 254 nm (SUVA$_{254}$), an indicator of the aromaticity of the dissolved organic compounds in soil (dependent on the C═O and C═C bonds in aromatic compounds and humic-like substances; Sun et al., 2021), was obtained, by measuring the absorbance coefficient at 254 nm of the $CaCl_2$ 0.01 M extract of soil (1:5 w:v soil:solution ratio), normalized by dividing its value by the WSOC content (Sun et al., 2021). SUVA$_{254}$, and other selected properties of the unamended and HOB-amended soils, for 2018 and 2019, are listed in Table S2, adapted from López-Piñeiro et al. (2022).

2.4. Analytical procedures

The soil samples were air-dried and passed through a sieve (2 mm). Then, this fraction was further milled in an agata mortar to achieve particles with a lower size (<0.2 mm). Similarly, after harvest, hand-dehusked rice grain samples were dried for 2 days (60 °C), milled in an agata mortar, and sieved using a 0.2 mm sieve. Arsenic, Cd, and Pb total concentrations in soil and rice grain were determined by ICP-OES (ICAP 6500 Duo Thermo®) after digestion with HNO_3:H_2O_2 (80:20, v/v) in a microwave digestor (MARSXpress, CEM), at 220 °C for 20 min.

Furthermore, soil available As, Cd, and Pb concentrations were determined, assuming that they can be predicted using a single-step extraction procedure, with $NaHCO_3$ 0.5 M (1:10, w:v) for As, after Pardo et al. (2014), and with DTPA 0.005 M (diethylenetriaminepentaacetic acid) (1:2, w:v), to predict the available Cd and Pb, after Lindsay and Norvell (1978).

All samples were analysed in triplicates and blanks (deionized water) were measured in parallel, to check for possible contamination. Trace element concentrations, in soil and plant material, were reported on a dry matter basis. Quantification limits for all elements in soil, total and extractable, were 0.005 mg kg^{-1}. Quantification limits for total As, Cd, and Pb in rice grain were 0.010 mg kg^{-1}.

Arsenic speciation, As(III) and As(V), in the rice grains, was determined as described by Alvarenga et al. (2022), using extracts of the samples (1:20 w:v, with HNO_3 0.17 M, at 95 °C for 90 min, in a microwave digestion system (Milestone ETHOS-1)), after centrifugation (10,000 rpm for 15 min, at 4 °C), and filtration through a 0.45 μm membrane. Quantification was performed by high-performance liquid chromatography (HPLC) (Varian Prostar, Spectralab Scientific, Toronto, Canada), coupled to an inductively coupled plasma – mass spectrometer system (ICP-MS) (Varian 820-MS). All samples were analysed in triplicates and concentrations were reported on a dry matter basis. Analytical quantification limits determined were 0.040 mg kg^{-1}, for As(III), and 0.050 mg kg^{-1}, for As(V). Furthermore, to validate the analytical methods, including digestion and extraction procedures, certified reference materials were analysed: soil standards SRM® 2709a, SRM® 2710a and SRM® 2711a (NIST, USA) were used, and the rate of recovery of As, Cd and Pb was >94 %. For the rice grain samples, the extraction efficiency was verified with SRM® 1568b certified rice flour (NIST, Gaithersburg, USA), and the mean recoveries of this standard reference material were 91–107 % for As, Cd, Pb and As$_{\text{inorg}}$.

2.5. Statistical analyses

Statistical analyses were performed using the SPSS (version 22) software. Data on soils properties, As, Cd and Pb concentrations in soils and rice grains were checked for normality distribution and homogeneity of variances and then analysed by two-way ANOVA with repeated measures on the factor "year" was use. All pairwise multiple comparisons were performed using the *post hoc* Tukey test. For statistical analyses purposes, results below the quantification limit, were assumed to be equal to the quantification limit.

Pearson correlation coefficients were calculated to study possible correlations between different properties. Correlations were considered significant at two levels of significance: $p < 0.05$, statistically significant, and at $p < 0.01$, with a higher level of statistical significance.

3. Results

3.1. Effects of the treatments on As, Cd, and Pb concentrations and extractability in the soil

The soil in the experimental field was not contaminated with Cd or Pb (Table 2), and that fact can be ascertained by comparing total Cd and Pb concentrations in the soil with the maximum allowed concentrations for agricultural soils in the Spanish legislation (Royal Decree 1051/2022, of December 27, which establishes standards for sustainable nutrition in agricultural soils). Maximum allowed concentration for Cd is 1.0 or 1.5 mg kg^{-1}, while for Pb is 50 or 100 mg kg^{-1}, depending on the soil pH, if it is <7 or >7, respectively (Royal Decree 1051/2022, of December 27). Therefore, taking the concentrations for Cd and Pb in the soil of the non-amended plots (Table 2), all with soil pH values <7 (Table S2), it is possible to see that Cd concentrations were approximately 5-times lower than the maximum allowed concentration, while Pb concentrations were approximately 2.5-times below the limit.

Regarding As, its maximum allowed concentration was not established for agricultural soils in Spain (Royal Decree 1051/2022, of December 27), but it is possible to use regulations from other countries, namely the Canadian Soil Quality Guidelines, with a limit value for inorganic As in agricultural soils of 12 mg kg^{-1} (CCME, 2006), or the less conservative value of 50 mg kg^{-1}, established by the Ministry of Environment of Finland, for all types of soils (Tóth et al., 2016). Therefore, total As concentration in the soil was also below limit values established, allowing it to be considered a soil non-contaminated with As. Despite, since the irrigation regime, soil management, and the application of amendments may affect As, Cd, and Pb availability in soil, and bioaccumulation in rice grain, it is important to evaluate how these values changed.

Total concentrations of As, Cd, and Pb in the soil were not affected by the management regimes (Table 2), with values, in all the treatments, that did not differ significantly between treatments and years. That fact was somehow expected, because the biochar used, HOB, had low concentrations of toxic trace elements (Table 1), and the water used for irrigation was not contaminated. Biochar produced from lignocellulosic waste materials as feedstocks is a good option, not only because its production does not correspond to a competition for land use to food and feed production, but also because its toxic trace elements concentrations are low, when compared with biochar produced from other residues, like urban organic waste (López-Cano et al., 2018). Several authors have characterized biochar produced from raw holm oak residues, like López-Cano et al. (2018), stating that it has characteristics like wood biochar, suitable for agricultural use, but alerted for the fact that the agronomical benefits and impacts on soil biogeochemical cycles need to be thoroughly assessed. In fact, despite the potential importance of this agricultural practice to improve soil quality in Mediterranean countries, Teutscherova et al. (2018) have evaluated the impact of HOB on biological properties of two contrasting Mediterranean soils, and they have found that, besides the amelioration on soil aggregation, the beneficial effects were not evident, and depended on the soil initial characteristics.

Arsenic-extractable concentrations in soil (with $NaHCO_3$ 0.5 M, 1:10 w:v; Pardo et al., 2014), a chemical surrogate measure of its bioavailable fraction, were very low in all the plots, and were not affected by the treatment, ranging from 0.8 to 1.8 % of the total (Table 2). The same was true for Cd-extractable concentrations in soil (with 0.005 M DTPA, 1:2 w:v; Lindsay and Norvell, 1978), with values below the quantification limit of the analytical technique (<0.005 mg kg^{-1}), which corresponds to a maximum of 3.4 % bioavailable fraction, taking the worst case scenario.

On the contrary, the management regime affected extractable Pb in soil ($p < 0.001$ for the ANOVA factor) (Table 2), and the most evident effect was a decrease of the extractable Pb in the soil of the biochar-treated plots, when compared with their non-amended counterparts (approximately, a

Table 2
Effect of the different treatments on trace element concentrations in the soils, total and extractable (mean values, n = 3). All results are reported on a dry matter basis.

	Total concentrations			$NaHCO_3$-extractable	DTPA-extractable	
	As (mg kg^{-1})	Cd (mg kg^{-1})	Pb (mg kg^{-1})	As (mg kg^{-1})	Cd (mg kg^{-1})	Pb (mg kg^{-1})
2018						
SNT	4.79aA	0.190aA	14.8aA	0.064aA	<0.005	0.153abA
SNT- HOB	5.04aA	0.187aA	17.7aA	0.061aA	<0.005	0.078aA
ST	6.30aA	0.275aA	21.8aA	0.059aA	<0.005	0.181abA
ST- HOB	6.16aA	0.214aA	20.9aA	0.059aA	<0.005	0.088aA
FT	5.83aA	0.194aA	19.7aA	0.045aA	<0.005	0.212bA
FT- HOB	5.46aA	0.191aA	17.9aA	0.058aA	<0.005	0.147abA
2019						
SNT	5.73aA	0.203aA	20.0aA	0.063aA	<0.005	0.176abcA
SNT- HOB	4.38aA	0.149aA	15.5aA	0.078aA	<0.005	0.094abA
ST	5.77aA	0.212aA	20.2aA	0.062aA	<0.005	0.193bcA
ST- HOB	5.56aA	0.190aA	18.0aA	0.062aA	<0.005	0.106abA
FT	6.15aA	0.207aA	21.0aA	0.060aA	<0.005	0.234cA
FT- HOB	5.05aA	0.156aA	17.7aA	0.044aA	<0.005	0.080aA
Y	0.716NS	3.82NS	0.01NS	0.456NS	–	0.084NS
M	2.26NS	2.08NS	3.16NS	2.83NS	–	23.9***
Y x M	1.94NS	1.17NS	2.83NS	0.663NS	–	1.07NS

ANOVA factors: Y, year; M, management regime; Y × M, interaction year × management regime; significant at $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$, respectively; NS, not significant. Results in the same column marked with different letters indicate significant differences ($p < 0.05$, Tukey HSD test) between management regimes in the same year (lower case letters) and between years within the same management regime (upper case letters). HOB: holm-oak biochar: SNT: sprinkler irrigation under no-tillage management; ST: sprinkler irrigation under conventional tillage; FT: permanent flooding irrigation under conventional tillage; SNT- HOB, ST- HOB, and FT- HOB: the same management regimes, with HOB application.

2-fold decrease). This effect may have been the consequence of two additive factors: not only the adsorptive capacity of biochar for metals, including Pb (Zheng et al., 2012), but also the fact that HOB application led to an increase in the soil pH value (Table S2), a very important effect that may have caused Pb precipitation at higher pH values (Cao et al., 2011; Zheng et al., 2012; Khanan et al., 2020). In fact, the immobilization of Pb was considered, by Zheng et al. (2012), to be mostly attributed to the iron plaque on root surface, with a high adsorptive capacity for metals, and that increased because of biochar application.

3.2. Effects of the treatments on As, Cd, and Pb concentrations in the rice grain

3.2.1. Arsenic

The reduction of inorganic As (As_{inorg}) in rice grain under a sprinkler irrigation regime, identified by other authors (Moreno-Jiménez et al., 2014; Spanu et al., 2020; Alvarenga et al., 2022), was also evident in this study, with a considerable lower As_{inorg} concentration in rice grain in all the treatments subjected to that irrigation regime (Table 3). Flooded irrigation, on the contrary, lead to As_{inorg} concentration in the rice grain >250-fold higher, when compared to the sprinkler irrigation, without significant differences in consecutive years.

The maximum allowed concentrations of inorganic As is set at 0.20 mg kg^{-1} in the rice grain (fresh material) in the European Union (EU), or at a lower limit of 0.10 mg kg^{-1} in white polished rice when is intended to produce food for infants (European Commission, 2006, 2015). Therefore, the transition from the flooded irrigation to the sprinkler irrigation regime allowed a clear beneficial effect in this food security aspect for rice consumption, allowing a decrease of As_{inorg} concentration in rice to below the limit value of 0.10 mg kg^{-1}, in all the treatments.

On the other hand, the application of biochar had a very marked effect on As_{inorg} concentration in rice when using flooding irrigation, allowing a decrease of As_{inorg} concentration in rice to below the limit value of 0.10 mg kg^{-1}, even when using that irrigation regime, granting the safe consumption of rice, and increasing its commercial value. More important, that effect of the biochar application did not decrease under field-aging conditions.

This aspect is very evident in Fig. 1-A, where the bioaccumulation factors for As (As_{grain}/As_{soil}) were calculated for all the treatments. Higher values for As bioaccumulation factors were obtained for the TF treatment, ranging between 0.04 and 0.05, but hose ratios decreased, very markedly, for the TF treatment when biochar was applied, to values similar to those found for all other management systems evaluated (<0.05), without significant differences between treatments within each year, an effect that was maintained from one year to the other.

This aspect is very important, and it was a critical point in this study, because the liming effect of biochar may have an important role on metal immobilization, in addition to its sorbent capacity, but may, undesirably, mobilise some elements, like As (Beesley et al., 2011). In fact, unlike cationic metals, As is present in soil solution in oxy-anionic forms, whose mobility increases with increasing soil pH (Beesley et al., 2011; Yin et al., 2016). Therefore, the acidity alleviation proportioned by biochar application in the flooding irrigation environment (from pH 5.53 to pH 6.40 in 2018, and from pH 5.65 to pH 6.50 in 2019) could have promoted an increase in As mobility, regardless of the capacity of biochar as a sorbent, as other authors have mentioned (Zheng et al., 2012; Yin et al., 2016, 2017). That was not a fact in this study, and it is possible to say that holm-oak biochar evidenced as an important soil passivator for As, under anaerobic conditions, those of the traditional rice production, avoiding As bioaccumulation in rice grain, in either fresh and field-aging conditions.

3.2.2. Cadmium

For Cd, the flooding irrigation regime, with or without biochar application, was the one which allowed lower concentrations of Cd in rice. The transition to the sprinkler irrigation regime led to an undesirable increase in Cd concentration in rice, already identified by other authors (Moreno-Jiménez et al., 2014; Alvarenga et al., 2022; Peera Sheikh Kulsum et al., 2023), but the values were below the maximum allowed concentration for Cd in rice grain of the EU regulations, set at 0.20 mg kg^{-1} (European Commission, 2006), in all the treatments. Biochar application led to a decrease of Cd concentration in rice for each treatment, relatively to the non-amended plots, reducing its bioaccumulation in kernels between 32 and 80 %. This effect of biochar on Cd immobilization on rice production was reported by other authors, although more effective in acid soils (Meng et al., 2023; Li et al., 2023). Awad et al. (2018) have published a meta-analysis about the effects of biochar application on different aspects of rice production, and they reported that, irrespective of the biochar type and application rate, a significant decrease of available Cd in soil and bioaccumulation in rice grains was observed, compared to the untreated soil.

Table 3
Effect of different treatments on trace element concentrations in the rice grains (mean values, n = 3). All results are reported on a dry matter basis.

	Arsenic speciation (mg kg^{-1})		Total concentrations (mg kg^{-1})		
	As(III)	As(V)	As	Cd	Pb
2018					
SNT	<0.04aA	<0.05aA	<0.010aA	0.159cB	0.093bA
SNT-HOB	<0.04aA	<0.05aA	<0.010aA	0.049bA	0.024aA
ST	<0.04aA	<0.05aA	<0.010aA	0.184cA	0.096bA
ST-HOB	<0.04aA	<0.05aA	<0.010aA	0.070bB	0.018aA
FT	0.088bA	0.052bA	0.253bA	<0.010	0.156cA
FT-HOB	<0.04a	<0.05aA	<0.010aA	<0.010	0.012aA
2019					
SNT	<0.04aA	<0.05aA	<0.010aA	0.037aA	0.124bA
SNT-HOB	<0.04aA	<0.05aA	<0.010aA	0.025aA	0.061aA
ST	<0.04aA	<0.05aA	<0.010aA	0.141bA	0.130bA
ST-HOB	<0.04aA	<0.05aA	<0.010aA	0.027aA	0.089aB
FT	0.114bB	0.064bA	0.274bA	<0.010	0.162bA
FT-HOB	<0.04aA	<0.05aA	<0.010aA	<0.010	0.044aB
Y	5.11*	4.45NS	0.554NS	153***	23.8***
M	551***	15.9***	105***	97.6***	122***
Y x M	5.11*	4.45*	0.554NS	34.9***	0.937NS

ANOVA factors: Y, year; M, management regime; Y × M, interaction year × management regime; significant at *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$, respectively; NS, not significant. Results in the same column marked with different letters indicate significant differences ($p < 0.05$, Tukey HSD test) between management regimes in the same year (lower case letters) and between years within the same management regime (upper case letters). HOB: holm-oak biochar: SNT: sprinkler irrigation under no-tillage management; ST: sprinkler irrigation under conventional tillage; FT: permanent flooding irrigation under conventional tillage; SNT- HOB, ST- HOB, and FT- HOB: the same management regimes, with HOB application.

Bioaccumulation factors for Cd in rice grain (Fig. 1-B) corroborated these observations, and the beneficial effect of biochar application on Cd immobilization in soil was not lost from one year to the other, maintaining low concentration in rice grain (SNT- HOB and ST- HOB, Table 3), as well as low concentration for bioaccumulation factors for Cd in rice grain in the second year of the study (Fig. 1-B), where aged effects were analysed.

3.2.3. Lead

Lead concentrations in rice grains were always below the maximum allowed concentration of the EU regulations, set at the commonly accepted safety threshold level of 0.2 mg kg^{-1} (European Commission, 2006). However, the concentrations were generally above the medium value reported by Norton et al. (2014), for samples collected in Spain (0.015 ± 0.011 mg kg^{-1}, $N = 89$), a situation that may be a consequence of the fact that grain Pb concentrations present high genotypic differences (Norton et al., 2014). In this study, Pb behaved differently from Cd, evidencing a bioaccumulation in rice grain produced with sprinkler irrigation decreased, relatively to the flooded irrigation treatments, although only with statistically significant differences in the first year of the study (Table 3). This behaviour was also reported by Spanu et al. (2020), that reported a 50 % decrease in Pb bioaccumulation in rice kernels when using sprinkler irrigation. Nevertheless, contradictory results were also reported by other authors, and Wu et al. (2021), when evaluating the impact of

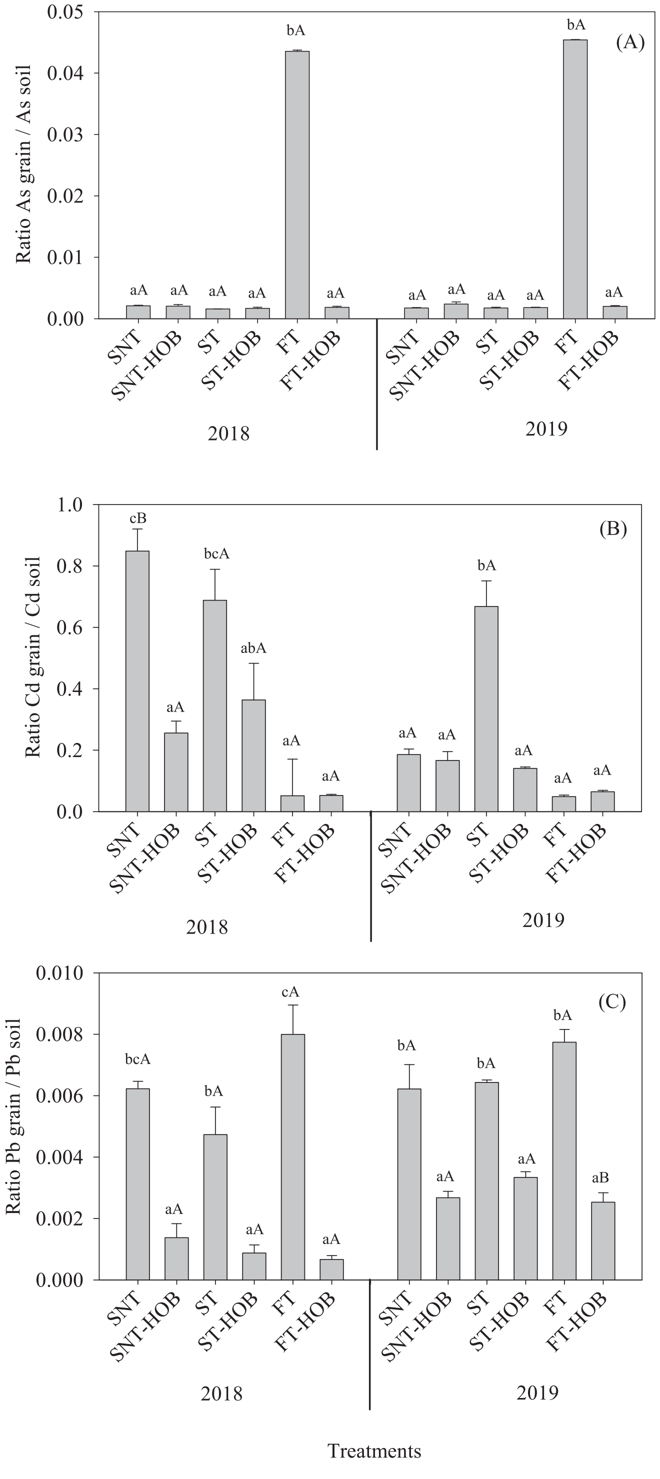

Fig. 1. Effect of different treatments on bioaccumulation factors in the rice grains. Bars marked with different letters indicate significant differences between management regimes in the same year (lower case letters) and between years within the same management regime (upper case letters) ($p < 0.05$, Tukey HSD test). HOB: holm-oak biochar: SNT: sprinkler irrigation under no-tillage management; ST: sprinkler irrigation under conventional tillage; FT: permanent flooding irrigation under conventional tillage; SNT- HOB, ST- HOB, and FT- HOB: the same management regimes, with HOB application.

alternate wetting and drying irrigation system on Pb bioaccumulation in rice, reported an increase in Pb bioaccumulation in a water saving irrigation system, which they have tried to avoid by applying phosphate to soil.

In all management systems, biochar application allowed a significant decrease in Pb concentration in rice grain, relatively to the non-amended plots (between 4- to 13-times, under fresh effects, and between 1.5- to 3.7-times, under field-aging conditions), offering a good solution to produce rice in Pb contaminated soils, as Norton et al. (2014) have identified in their study. Similar results were reported by other authors, who have also emphasised the importance of biochar application to immobilize Pb in soil (Zheng et al., 2012; Amin et al., 2023), in their case in a multi-element contaminated soil. Cao et al. (2011), explained Pb immobilization in soil by its reaction with phosphorus, contained in biochar, leading to the formation of insoluble hydroxypyromorphite $Pb_5(PO_4)_3(OH)$, which they have identified by X-ray diffraction.

Nevertheless, in traditional seeding plots with biochar application (ST- HOB and FT- HOB), in the second year of the study, Pb concentration in the grain raised, when compared with that obtained in the previous year, an effect which needs to be evaluated in the future, because it can mean that, to obtain the desired effect, biochar application needs to be repeated from time to time. That same effect was obvious from Fig. 1-C, with an increase in the bioaccumulation factors for Pb, from the 2018 to 2019, in the treatment where biochar was applied (ST-HOB and FT- HOB).

4. Discussion

To have a better idea on how the physicochemical soil properties that were evaluated (soil pH, EC, TC, WSOC, and $SUVA_{254}$; Table S2), influenced As, Cd, and Pb behaviour in soil, and their bioaccumulation in the rice grains (Tables 2 and 3), Pearson's correlation coefficients were calculated between these properties, considering two levels of significance, $p < 0.05$ and $p < 0.01$ ($n = 36$; Table 4). Arsenic, Cd, and Pb total concentrations in soil, as well as extractable As and Cd were not used, because their values were not affected by the treatments, or were below the analytical quantification limits (Table 2). In fact, when considering the values obtained for the chemical extraction of As and Cd (Table 2), that were interpreted as plant-available, it is fair to say, when comparing with the concentrations obtained for As and Cd in the rice grain (Table 3), that the effects of the treatments on the bioavailability of these elements, were not well-predicted. The opposite was true for Pb, for which the extraction procedure was able to predict the trend on its bioavailability. In fact, the influence of the management regime on DTPA-extractable Pb in the soil was marked ($p < 0.001$; Table 2), with a decrease on extractable Pb in all the treatments, because of HOB application, indicating a beneficial effect on the immobilization of Pb. That effect was corroborated by the Pearson's correlation coefficient between Pb_{ext} and other soil physico-chemical parameters that were influenced by biochar application (soil pH, TC, and $SUVA_{254}$), all with negative correlations, at $p < 0.01$ (Table 4). That only happened for Pb, indicating that its availability was correctly predicted by the extraction procedure with DTPA, but the opposite was true for As and Cd, whose extracted concentrations were not influenced by the management regimes (Table 2), or whose concentrations were far too low to evidence a specific trend.

The application of HOB led to a significant increase in the TC content, without a significant increase in the WSOC (Table S2). This fact may have contributed to an increase in the capacity of the biochar-amended soil to immobilize toxic trace elements, by sorption, without the risk of their transference to the soil solution, by complexation with soluble organic compounds, avoiding their bioaccumulation in rice. This is a risk with specific types of biochar, as Yin et al. (2017) have reported in their study, where they have applied rice-straw biochar and, while Cd diminish its concentration in soil pore-water, As solubility was enhanced.

On the other hand, the alkaline nature of HOB (pH = 9.08; Table S1), allowed a correction of soil acidity (Table S2), which can also be an important factor to immobilize metals by precipitation (*i.e.*, Cd and Pb; Cao et al., 2011; Zheng et al., 2012; Li et al., 2023), especially in the flooding irrigation treatments (FT), whose pH is acid (pH 5.53 and 5.65 for FT, in 2018 and 2019, respectively), increasing approximately one

Table 4

Pearson's correlation coefficients between trace element concentrations that were affected by the treatments and their correlation with soil physico-chemical properties (n = 36).

	pH	EC	TC	WSOC	$SUVA_{254}$	As_{soil}	Cd_{soil}	Pb_{soil}	As_{ext}	Cd_{ext}	Pb_{ext}	As_{grain}	iAs_{grain}	Cd_{grain}	Pb_{grain}	As_{grain}/As_{soil}	Cd_{grain}/Cd_{soil}
Pb_{ext}	−0.762**	0.076	−0.648**	−0.290	−0.355*	0.351*	0.339*	0.256	−0.167	−0.618**	–	–	–	–	–	–	–
As_{grain}	−0.783**	−0.070	−0.398*	−0.440**	−0.546**	0.234	0.043	0.197	−0.178	−0.578**	0.561**	–	–	–	–	–	–
As_{inorg}	−0.783**	−0.061	−0.400*	−0.431**	−0.577**	0.271	0.056	0.234	−0.152	−0.597**	0.583**	0.981**	–	–	–	–	–
Cd_{grain}	0.086	−0.360*	−0.508**	0.221	−0.069	0.147	0.461**	0.038	0.171	0.002	0.174	−0.363*	−0.364*	–	–	–	–
Pb_{grain}	−0.770**	0.162	−0.829**	−0.216	−0.425**	0.281	0.267	0.298	−0.085	−0.505**	0.777**	0.666**	0.666**	0.134	–	–	–
As_{grain}/As_{soil}	−0.770**	−0.069	−0.390*	−0.431**	−0.534**	0.197	0.027	0.169	−0.187	−0.558**	0.541**	0.996**	0.963**	−0.362*	0.656**	–	–
Cd_{grain}/Cd_{soil}	0.156	−0.363*	−0.480**	0.252	−0.061	0.102	0.250	−0.091	0.220	0.060	0.115	−0.379*	−0.380*	0.963**	0.088	−0.377*	–
Pb_{grain}/Pb_{soil}	−0.707**	0.130	−0.838**	−0.152	−0.440**	0.190	0.176	0.087	−0.029	−0.450**	0.745**	0.628**	0.617**	0.179	0.971**	0.625**	0.168

pH, EC, and TC: soil pH, electrical conductivity, and total carbon; WSOC: Water-Soluble Organic Carbon; $SUVA_{254}$: Specific UV-absorbance at 254 nm; As_{soil}, Cd_{soil} and Pb_{soil}: total concentration of As, Cd, and Pb in soil; As_{ext}, Cd_{ext} and Pb_{ext}: extractable concentration of As, Cd, and Pb in soil; As_{grain}, Cd_{grain} and Pb_{grain}: total concentration of As, Cd, and Pb in the grain; iAs_{grain}: concentration of inorganic As in the grain; As_{grain}/As_{soil}, Cd_{grain}/Cd_{soil} and Pb_{grain}/Pb_{soil}: concentration ratios of the element total concentration in the grain to its concentration in the soil; Marked correlations are significant at (*) $p < 0.05$ and (**) $p < 0.01$.

unit of pH by the application of biochar (to pH 6.40 and 6.50 for FT-HOB, in 2018 and 2019, respectively; Table S2).

In fact, the immobilization mechanisms are diverse, including ion exchange, sorption, complexation, and precipitation (Paz-Ferreiro 2014; Yang et al., 2021), and the contribution of each of these mechanisms to the immobilization of a specific element is difficult to ascertain, because they are dependent on a wide range of factors, like the properties of each trace element, soil properties, biochar characteristics (feedstock material and pyrolysis conditions), as well as the application doses (Diao et al., 2022). For instance, Li et al. (2023) have reported that the same type of biochar and doses reduced the bioaccumulation of Cd and Pb in rice shoots grown in acid soils but had a contrary effect on an alkaline soil.

Considering Cd, its concentration in rice grains obtained in the aerobic systems were significantly affected by the treatment, decreasing with the HOB application, and by the year, with an overall decrease from 2018 to 2019, and by the interaction of both properties. Although some authors (Bian et al., 2013; Meng et al., 2023), considered that Cd immobilization in biochar-amended soils is mostly a liming effect, their results were not corroborated in this study, since there were no negative correlation coefficients between Cd parameters in the grain and soil pH (they are positive, and without significance; Table 4). On the other hand, considering the highly significant negative Pearson's correlation coefficients between TC in soil and Cd parameters in the grain, it is possible that the sorption capacity of HOB had a crucial role in the immobilization of Cd in this field study. This is a very important result, providing a strategy to counteract Cd increased mobility when adopting improved irrigation practices (*e.g.*, sprinkler irrigation systems).

The application of HOB to each soil management system did not promote an increase in the WSOC in soil (Table S2), relatively to the non-treated counterpart, in most of the treatments (the exception was SNT, in 2019). Because of that, there was a risk of an increase on As mobility, as a consequence of an increase in the biochar-derived dissolved organic compounds, already pointed out by other authors (Kim et al., 2018). That did not happen even in the anaerobic environment (FT), in contradiction with the results reported by Wang et al. (2017), who observed an increase in As release from an anaerobic paddy soil, contaminated with As, as a consequence of biochar application (biochar application enhanced microbial reduction of As(V) to As(III), increasing its mobility). The conditions of the reported studies were different, not only because of the biochar was different, but also because the soil was contaminated with As, and the conditions used were mostly lab-scale incubation experiments (Kim et al., 2018; Wang et al., 2017). In the case of this study, it is fair to say that in a field scale scenario, in a two-years experiment in a non-contaminated soil, the bioavailability of As was reduced, as a consequence of HOB application to soil, decreasing its bioaccumulation in the rice grain in all the management systems (Table 3). These considerations are also supported by the highly significant negative Pearson's correlation coefficients ($p < 0.01$) between grain-As parameters (total As, As_{inorg}, and As_{grain}/As_{soil}, and WSOC and $SUVA_{254}$ in the soil (Table 4). In this study, the HOB-derived WSOC, and the aromaticity of their soluble organic compounds, contributed positively to a decrease in the risk of As bioaccumulation in the rice grain.

The immobilization of Pb in soil, and the decrease of its bioaccumulation in rice grain, may have had a liming-derived effect, which is obvious from the highly significant negative Pearson's correlation coefficients between Pb_{ext}, Pb_{grain}, and Pb_{grain}/Pb_{soil} and soil pH ($p < 0.01$; Table 4), but may have also had a contribution for its immobilization of the type of WSOC compounds which were brought to soil by the HOB, which can be inferred from the significant negative Pearson's correlation coefficients between $SUVA_{254}$, and Pb_{ext}, Pb_{grain}, and Pb_{grain}/Pb_{soil}.

Sun et al. (2021) have highlighted that biochar-derived dissolved organic matter, despite being a small proportion of the whole mass of biochar applied (biochar is mostly a refractory soil amendment), plays an important role in the immobilization of inorganic, as well as of organic, contaminants in soil. An increase in $SUVA_{254}$ index values indicates and increase in the aromaticity of the dissolved organic compounds, with molecules of larger molecular weight, richer in humic-acid like substances (Sun et al., 2021), and that happened in all the management systems where HOB was applied (Table S2), which may have contributed to the decrease in the bioavailability of both As and Pb, as indicated by the lowering in these elements concentrations in rice grains.

The immobilization of As, Cd, and/or Pb in biochar-treated soils may occur by different biochar-induced processes (Paz-Ferreiro et al., 2014; Yang et al., 2021; Diao et al., 2022), which may have contradictory results regarding specific elements. Because of that, mostly to optimize simultaneous As, Cd, and Pb immobilization in different irrigation systems, several studies have evaluated the use of modified biochar (*e.g.*, iron-enriched biochar, magnetic biochar, biochar-based composites preparation; Yu et al., 2017; Yin et al., 2016, 2017; Khum-in et al., 2020; Tang et al., 2020; Wan et al., 2020; Islam et al., 2021; Diao et al., 2022). Despite the successful use of these materials, it is important when a raw biochar can tackle with the immobilization of all elements, which was the case of this study, to increase the sustainability of its production and the possibility of using only wastes as feedstocks.

5. Conclusions

Biochar produced from holm-oak pruning residues reduced As bioaccumulation in rice grain in the permanent flooding system. When adopting sprinkler irrigation as a water-saving system, the undesirable increase in Cd bioaccumulation in rice was counteracted by biochar application. The bioaccumulation of Pb in rice was also prevented by biochar application, reducing its concentration in all the management systems, relatively to the non-amended plots.

HOB application can be a good option to avoid, simultaneously, metals and metalloids bioaccumulation in rice grains, increasing its safe consumption and commercial value. At least in the lifespan of this experiment (two-years), the immobilization of As, Cd, and Pb in soil was successfully achieved, avoiding their bioaccumulation in rice grain, but the need to reapply biochar in extended periods of time still needs to be further explored.

CRediT authorship contribution statement

Carmen Martín-Franco: Formal analysis, Investigation, Methodology, Writing – original draft. **Jaime Terrón Sánchez:** Formal analysis, Investigation, Methodology, Writing – original draft. **Paula Alvarenga:** Conceptualization, Data curation, Formal analysis, Writing – original draft, Writing – review & editing. **David Peña:** Conceptualization, Data curation, Formal analysis, Methodology, Validation, Writing – review & editing. **Damián Fernández-Rodríguez:** Investigation, Methodology, Validation, Writing – review & editing. **Luis Andrés Vicente:** Formal analysis, Investigation. **Ángel Albarrán:** Investigation, Methodology, Validation, Writing – review & editing. **Antonio López-Piñeiro:** Conceptualization, Investigation, Methodology, Validation, Supervision, Project administration, Resources, Writing – review & editing.

Data availability

Data will be made available on request.

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.

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Appendix A. Supplementary data

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