

ORGANIC AMENDMENTS APPLICATION EFFECT ON A CONTAMINATED SOIL WITH ARSENIC

Efecto de la aplicación de enmiendas orgánicas en un suelo contaminado con arsénico

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Key words: Remediation, lettuce, compost, biochar.

ABSTRACT

Soil is a non-renewable natural resource essential for obtaining food. Anthropogenic activities threaten this resource by exposing it to different pollutants, such as metals and metalloids, which can alter its functionality and threaten human health through the intake of food contaminated with these elements. The aim of this study was to evaluate the effect of two organic amendments, compost (CP) and biochar (BC) in a phytoremediation process of a soil contaminated with arsenic (As) at a dose of 60 mg/kg. For this purpose, germination tests and a greenhouse assay were carried out applying two doses of each amendment, 5 and 10% to As-contaminated soil using *Lactuca sativa* L. as control plant. The application of CP showed a decrease in the As bioavailability in the soil (from 1.95 mg/kg in control soil to 1.77 in CP 5% and 1.45 in CP 10%), decreasing the translocation to the aerial part and enhancing the development of plants, being this effect dose-dependent. The treatments with BC caused an increase in As bioavailability in the soil, allowing its translocation to the aerial part of the plants and affecting its development. In soil, both amendments increased organic matter content observing the greatest increases with CP. Moreover, using CP, an increase in the nutrients analyzed (N, P, K, Ca, Mg, and Na) was observed, as well as an increase in the total As content at the 10% dose. The enzymatic activity of the soils treated with CP increased significantly, whereas inhibition of most of the evaluated activities was observed with BC treatments. The application of CP led to an As immobilizing effect, decreasing As availability and preventing its translocation to plants. At the same time, it contributed to soil remediation, improving its fertility. Finally, the used BC mobilized As, which negatively affected the development of the lettuce plants, as well as the biological activity of the soil.

Palabras clave: remediación, lechuga, compost, biocarbón.

RESUMEN

El suelo es un recurso natural no renovable esencial para la producción de alimentos. Las actividades antrópicas amenazan este recurso exponiéndolo a diferentes contaminantes, como los metales y metaloides, que pueden alterar su funcionalidad y poner en riesgo la salud humana por el consumo de alimentos contaminados con estos elementos. El objetivo de este trabajo fue evaluar el efecto de dos enmiendas orgánicas, compost (CP) y biocarbón (BC) en un proceso de fitorremediación de un suelo contaminado con arsénico (As) a la dosis de 60 mg/kg. Se realizaron ensayos de germinación y un ensayo en invernadero en el que se trató el suelo contaminado con las enmiendas a dos dosis, 5 y 10 %, utilizando *Lactuca sativa* L. como planta testigo. La aplicación de CP mostró una disminución en la biodisponibilidad del As en el suelo (de 1.95 mg/kg en el suelo testigo a 1.77 en CP 5 % y 1.45 en CP 10 %), disminuyendo la translocación a la parte aérea y favoreciendo el desarrollo de las plantas, siendo el efecto dosis-dependiente. Los tratamientos con BC provocaron un aumento en la biodisponibilidad del As en el suelo, permitiendo su translocación a la parte aérea de la planta y afectando a su desarrollo. En el suelo, ambas enmiendas incrementaron la materia orgánica, observándose los mayores incrementos con CP. Con este último se observó un incremento en los nutrientes analizados, así como del contenido de As total a la dosis del 10 %. La actividad enzimática de los suelos tratados con CP aumentó significativamente observándose inhibición en la mayoría de las actividades analizadas en los tratamientos con BC. La aplicación de CP tuvo un efecto inmovilizador del As, impidiendo su translocación a la planta. Al mismo tiempo contribuyó a la remediación del suelo, mejorando su fertilidad. Finalmente, el BC utilizado movilizó el As, lo que afectó negativamente al desarrollo de las plantas de lechuga, así como a la actividad biológica del suelo.

INTRODUCTION

Soil is a non-renewable natural resource since the formation and regeneration processes are slow compared to the degradation ones. Globalization and anthropogenic activities threaten soil health by exposing it to different contaminants such as metals and metalloids, which leads to soil degradation. Metals and metalloids, such as arsenic (As), have a wide distribution in the environment, which can generate different soil alterations such as low fertility, micronutrient imbalance, toxicity, low availability of nutrients, high electrical conductivity and changes in pH, as well as loss of biodiversity (Lwin et al. 2018). As is highly toxic, widespread in the environment can proceed from both natural and anthropogenic sources (Moreno-Jiménez et al. 2012). Inorganic As is present in soil, water, air, and food. Therefore, humans are constantly exposed to this contaminant, posing a risk to human health due to the fact that this metalloid is considered a carcinogen by the International Agency for Research on Cancer (IARC) and by the United States Environmental Protection Agency (USEPA) (Yadav et al. 2021). Arsenic concentration in edible plants depends largely on the availability of As in the soil and the capacity of the plant to absorb and transfer it to the aerial parts of the plant. The presence

of this metalloid in plants can affect their growth and productivity because it can induce different morphological, physiological, biochemical, and molecular alterations (Abbas et al. 2018).

Phytoremediation is a biological technique based on the use of plants and their associated microorganisms to extract, remove or reduce the bioavailability of contaminants present in the soil (Yan et al. 2020). The addition of organic amendments is commonly used in phytoremediation due to the organic matter's relevant effect on the redox transformations of the transformations of the contaminants present in the soil. In addition, its use allows the immobilization or elimination of metalloids present in the soil (Ashraf et al. 2019, Tang et al. 2020, Verbeeck et al. 2020). This study evaluated the effect of two organic amendments (compost and biochar) in a phytoremediation process of a soil contaminated with arsenic.

MATERIALS AND METHODS

Experimental design

Germination test

To evaluate the effect of the amendments on the tolerance of the seeds to As-contaminated soils,

a phytotoxicity test was carried out following the Zucconi germination test (Zucconi et al. 1985) using a hot distilled water extract of contaminated soil. Soil was artificially contaminated with As at 60 mg/kg using $\text{Na}_2\text{HAsO}_4 \cdot 7\text{H}_2\text{O}$. After a stabilization period of 30 days in an incubation chamber (25 °C, 65% humidity), two types of amendments were applied to the soil: sewage sludge compost with pruning residues (CP) and olive biochar (BC). Two doses of the amendments were used (5 and 10%). An unamended contaminated soil was used as a control (SC). Soil and amendments characteristics are shown in **Table I**. The 15 watercress (*Lepidium sativum*) seeds were placed on each plate and three replicates were made for each treatment. A control treatment with distilled water was used. The plates with the watercress seeds were incubated at 25 °C for 48 hours. A second trial was carried out using lettuce (*Lactuca sativa* L.) seeds. In this case, the germination period was maintained for 72 hours. Subsequently, the number of germinated seeds and root length were evaluated and the germination index (GI) was determined (eq. 1).

$$\text{GI} = \%G \times \frac{L_m}{L_c} \quad (1)$$

where %G represents the percentage of seeds germinated with respect to control, L_m the root length in

the treatment (cm), and L_c the mean root length of the control seedlings.

Greenhouse assay

The study was performed in a greenhouse, using the described As-contaminated soil. The plant material used was lettuce (*Lactuca sativa* L. Maravilla de Verano variety). The contaminated soil was disposed in pots (1.1 kg soil per pot). Four replicates were used. 10 lettuce seeds were sown in each pot. Until germination, pots were kept in growth chambers at 24 °C with a relative humidity of 70%. Then, pots were transferred to a greenhouse where they remained for 30 days. After this time, plants were harvested, and soils were collected for analysis.

Plant analysis

The aerial part of each plant was weighed after harvest. Afterwards, lettuces were oven-dried at 65 °C and dry weight was determined. The dried plant material was ground in a mill (IKA Labortechnik A10) for analysis. The content of nutrients, metals and As were measured after acid digestion of dried material (30 mg) with 1 ml of HNO_3 and 1 mL of HClO_4 at 130 °C in a Techne Dri-Block DB-3D (Camlab, Cambridge, UK) for 2.5 hours. In the aerial part of the plant, macroelements (Ca, K, Mg, and Na) were quantified by Flame Atomic Absorption Spectrometer (FAAS) (AA240FS, Varian)

TABLE I. SOIL AND AMENDMENTS CHARACTERIZATION.

Parameter	Soil	Compost	Biochar
pH	8.54	6.57	8.12
C.E (dS/m)	0.35	12	0.37
CaCO_3 (%)	4.56	—	13.5
N (%)	0.09	3.15	0.59
MO (%)	1.24	52.3	7.33
P (mg/kg)	28	1800	145
Ca (mg/kg)	4882	4017	9127
Mg (mg/kg)	507	1547	591
Na (mg/kg)	92	411	111
K (mg/kg)	237	4298	1745
Fe (mg/kg)	28494	25725	4453
As (mg/kg)	78	27	2.8

and P content was determined by inductively coupled plasma spectrometry (ICP-OES) (5110, Agilent). Additionally, bioaccumulation factor (BCF) and translocation factor (TF) were calculated using the equations 2 and 3, respectively:

$$\text{BCF} = \frac{C_{\text{shoots}}}{C_{\text{soil}}} \quad (2)$$

$$\text{TF} = \frac{C_{\text{shoots}}}{C_{\text{roots}}} \quad (3)$$

where C_{shoots} is the concentration of metal(loid)s in the shoots, C_{soil} is the concentration of metal(loid)s in soil and C_{roots} is the concentration of metal(loid)s in the plant roots.

Soil and amendment analysis

Soil and amendments (air-dried and sieved (<2mm)) were analyzed before and after harvest following the official soil analysis methods. In brief, organic matter was determined using the Walkley-Black method. pH and EC were measured in a 1:2.5 soil:water ratio except for the compost that was determined in saturated paste. Total N content was quantified using the Kjeldahl method, and available nutrients (Ca, K, Mg, Na) were extracted with 0.1 N ammonium acetate and quantified using FAAS. Heavy metal concentrations in the samples were determined after acid digestion in a microwave reaction system (Multiwave Go, Anton Paar GmbH). In the digestion extract, the concentrations of Cd, Cr, Cu, Ni, Pb, and Zn were quantified by FAAS and As by ICP-OES. The availability of As in soil samples before planting was evaluated using the diethylenetriaminepentaacetic acid (DTPA) method proposed by Lindsay and Norvell (Lindsay and Norvell 1978) and by TCLP (leaching potential) methodology (USEPA, 1992). As content in soil solution was obtained using rhyzon probes. Briefly, Ryzon probes were introduced into the soil and connected to a 10 mL syringe which maintained the suction for 24 hours. After this time, the extracted pore water was collected and As was quantified by ICP-OES.

Soil respiration in samples collected after harvest was analyzed by the glucose-induced method (Fernández et al. 2004), monitoring the CO₂ production for 24 hours on 5 g of soil (n = 3), using the µ-Trac 4200 system (SY-LAB, GmbH, Pukersdorf, Austria).

The potential activity of soil enzymes involved in the C, N, P, and S cycles were evaluated: β-glucosidase (EC 3.2.1.21) and β-galactosidase (EC 3.2.1.23) activity (C cycle); urease activity (EC 3.5.1.5) (N cycle); alkaline and acid phosphatase activity (EC 3.1.3.1 and EC 3.1.3.2) (P cycle); aryl-sulfatase activity (EC 3.1.6.1) (S cycle). The activity of these enzymes was measured using colorimetric substrates in 96-well plates following the ISO 20130:2018 methodology. Soil enzyme activity was expressed as nmol of p-nitrophenol or ammonium chloride released per minute and gram of dry soil.

Statistical Analysis

The data were analyzed using version 4.2.1 of the R program. Differences between treatments were evaluated with one-way analysis of variance (ANOVA) at a significance level of $p < 0.05$. The mean values of the replicates were compared using Duncan's test.

RESULTS

Germination test

The GI in As-contaminated soil (SC) was higher in lettuce than in watercress seeds. BC treatment increased GI values for watercress and maintained high values for lettuce. CP treatment induced a decrease in GI values for both seed species (**Table II**).

Greenhouse experiment

Plant analysis

Plants grown in SC and those treated with BC showed reduced shoot development and leaf wilting symptoms (**Fig. 1**). The CP treatments showed a normal plant development, with a significant dose-dependent increase in biomass compared to the other treatments (**Table III**).

Nutrients in the aerial part of plants are shown in **Table IV**. Both organic treatments significantly increased the K and P content in plant in comparison to the untreated soil, corresponding the higher values to CP treatments. No significant differences were observed for Ca and Mg. In relation to Na, CP treatments showed the lower concentration of this element. CS and BC treatments (5 and 10%) presented the highest BCF values whereas the application of CP to the soil reduced this factor significantly, being this reduction dose-dependent. Regarding TF values, BC application significantly increased As translocation to the aerial part compared to untreated contaminated soil (SC), while the lowest TF values are observed with the CP treatment (**Table V**).

TABLE II. ZUCCONI GERMINATION INDEX (GI) IN WATERCRESS (*Lepidium sativum*) AND LETTUCE (*Lactuca sativa* L.).

Treatment	Watercress (%)	Lettuce (%)
SC	65	100
CP 5%	56	65
CP 10%	47	97
BC 5%	67	100
BC 10%	76	100

SC: Control contaminated soil; CP: compost; BC: biochar.

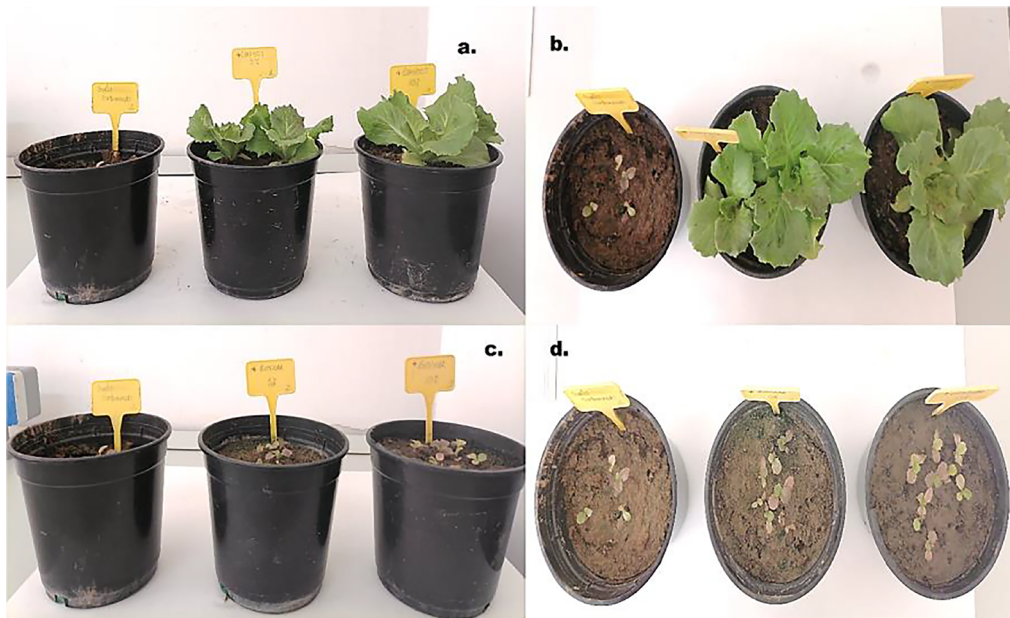


Fig. 1. Lettuce (*Lactuca sativa* L.) plant development at the end of the greenhouse assay. a-b. From left to right: Control contaminated soil, Compost 5 and 10%, respectively. c-d. From left to right: Control contaminated soil, biochar 5 and 10%, respectively.

Soil analysis

Soil treated with CP at both doses (5% and 10%) showed lower pH values and higher conductivity than SC and BC. Both organic treatments increased significantly organic matter values, showing the higher contents in CP treatments (**Table VI**). Moreover, CP and BC increased the nutrient content of the soil being this increase greater when CP was used, especially at the high dose. As levels was significantly higher in soils treated with the higher dose of CP.

CP treatments reduced As bioavailability in soil (DTPA) and in pore water being this decrease dose-

dependent (**Table VII**). This effect was not observed when BC was applied, showing As values similar to those observed in the untreated soil (SC). Regarding to leaching capacity (TCLP), the 10% BC treatment showed higher values than the CP treatment, even higher than the SC.

A dose-dependent decrease in enzyme activity was observed in soils treated with BC, with values even lower than those of contaminated soil without amendments. CP-treated soils showed increases in enzyme activities; higher activities were observed at the 10% dose (**Fig. 2**).

TABLE III. FRESH AND DRY WEIGHT (g) OF LETTUCE (AERIAL PART) IN THE DIFFERENT TREATMENTS.

Treatments	Fresh weight (g)	Dry weight (g)
SC	0.10 ± 0.07 a	0.02 ± 0.01 a
CP 5%	14.84 ± 1.56 b	0.84 ± 0.24 b
CP 10%	19.67 ± 5.62 b	1.08 ± 0.21 b
BC 5%	0.32 ± 0.20 a	0.05 ± 0.03 a
BC 10%	0.40 ± 0.37 a	0.04 ± 0.03 a

SC: Control contaminated soil; CP: compost; BC: biochar. Values expressed as mean ± standard deviation. Different letters indicate significant differences ($p < 0.05$, Duncan's test).

TABLE IV. NUTRIENT CONTENT IN LETTUCE (AERIAL PART) OBTAINED IN THE DIFFERENT SOILS.

Treatments	Ca	Mg	Na	K	P
	mg/kg				
SC	21692 ± 916 a,b	5500 ± 135	16470 ± 1656 b	8074 ± 984 a	300 ± 91 a
CP 5%	33205 ± 4302 c	5704 ± 881	5731 ± 1246 a	53135 ± 4921 c	3368 ± 623 c
CP 10%	26451 ± 1692 b	4914 ± 331	4238 ± 684 a	73124 ± 7355 d	4485 ± 720 d
BC 5%	23175 ± 4560 a,b	5967 ± 1789	17680 ± 1943 b	12471 ± 1861 b	992 ± 267 b
BC 10%	17202 ± 4986 a	5683 ± 724	20930 ± 6466 b	19326 ± 240 b	661 ± 345 b

SC: Control contaminated soil; CP: compost; BC: biochar. Values expressed as mean ± standard deviation. Different letters indicate significant differences ($p < 0.05$, Duncan's test).

TABLE V. BIOACCUMULATION FACTOR (BCF) AND TRANSLOCATION FACTOR (TF).

Treatments	BCF	TF
SC	1.82 ± 0.07 a	0.17 ± 0.01 a
CP 5%	0.76 ± 0.83 b	0.05 ± 0.02 a,b
CP 10%	0.17 ± 0.09 b	0.04 ± 0.03 b
BC 5%	2.22 ± 0.71 a	0.30 ± 0.12 a
BC 10%	1.96 ± 0.30 a	0.20 ± 0.02 a

SC: Control contaminated soil; CP: compost; BC: biochar. Values expressed as mean ± standard deviation. Different letters indicate significant differences ($p < 0.05$) between treatments.

TABLE VI. SOIL PARAMETERS OF THE DIFFERENT TREATMENTS AFTER HARVEST.

Parameter	SC	CP 5%	CP 10%	BC 5%	BC 10%
pH	8.37 ± 0.08a	7.74 ± 0.04b	7.47 ± 0.09c	8.37 ± 0.04a	8.42 ± 0.1a
CE (dS/m)	0.44 ± 0.07a	1.60 ± 0.05b	2.00 ± 0.00c	0.36 ± 0.0a.d	0.33 ± 0.05d
N (%)	0.09 ± 0.00a	0.24 ± 0.01b	0.35 ± 0.02c	0.13 ± 0.00d	0.16 ± 0.01d
MO (%)	1.27 ± 0.05a	3.23 ± 0.22b	4.63 ± 0.12c	1.63 ± 0.08d	2.06 ± 0.09e
P (mg/kg)	30 ± 1a	130 ± 8b	247 ± 16c	40 ± 1a.d	48 ± 4a.d
Ca (mg/kg)	3875 ± 131a	3989 ± 121a.b	4289 ± 64c	3249 ± 100d	2955 ± 99e
Mg (mg/kg)	497 ± 5a	558 ± 6b	628 ± 5c	473 ± 10d	446 ± 11e
K (mg/kg)	264 ± 12a	370 ± 7b	466 ± 10c	304 ± 4d	359 ± 18b
Na (mg/kg)	105 ± 7a	124 ± 3b	137 ± 1c	105 ± 16d	131 ± 4b.c
Pb (mg/kg)	19 ± 0.3a	19 ± 0.6a.b	20 ± 0.7b	17 ± 0.6a	17 ± 1a
Cu (mg/kg)	14 ± 0.2a	22 ± 1.2b	34 ± 3.2c	14 ± 0.6a.d	14 ± 0.3a.d
Ni (mg/kg)	16 ± 0.4a	16 ± 0.5a.b	17 ± 0.5ab	15 ± 0.2b.c	14 ± 0.5c
Zn (mg/kg)	83 ± 1.7a	116 ± 2.8b	154 ± 0.5c	81 ± 0.5a.d	78 ± 0.4d
Cr (mg/kg)	24 ± 0.9a	24 ± 0.5a.b	26 ± 1.2b	21 ± 0.7c	19 ± 0.2c
As (mg/kg)	73 ± 2.1a	78 ± 3.4a.b	82 ± 6.1b	72 ± 0.9a	71 ± 0.5a

SC: Control contaminated soil; CP: compost; BC: biochar. Values expressed as mean ± standard deviation. Different letters indicate significant differences ($p < 0.05$) between treatments.

TABLE VII. BIOAVAILABILITY IN PORE WATER AND IN SOIL BY DTPA AND TCLP EXTRACTION.

Treatments	Pore water (mg/L)	DTPA (mg/kg)	TCLP (mg/kg)
SC	3.47 ± 0.40 a	1.95 ± 0.12 a	14.75 ± 1.10 a
CP 5%	1.25 ± 0.29 b	1.77 ± 0.08 b	15.18 ± 0.91 a.b
CP10%	1.14 ± 0.08 b	1.45 ± 0.02 c	16.47 ± 0.79 a.b
BC 5%	3.54 ± 0.14 a	2.00 ± 0.02 a	16.67 ± 0.56 b.c
BC10%	3.23 ± 0.16 a	2.08 ± 0.04 a	18.49 ± 1.06 c

SC: Control contaminated soil; CP: compost; BC: biochar. Values expressed as mean ± standard deviation. Different letters indicate significant differences ($p < 0.05$) between treatments.

DISCUSSION

The GI in the SC was higher in lettuce than in watercress, which suggests a high tolerance of lettuce to As concentration in the soil. The BC application increased the GI in the case of watercress and maintained the high values in the case of lettuce where as

CP treatment induced a decrease in the GI values for both crops probably due to the high conductivity of compost. Seed germination is one of the most critical stages of plant growth and determines the failure or success of their development being salinity a limiting factor (Uçarlı 2021). Some authors have shown that the high concentrations of sodium and chloride

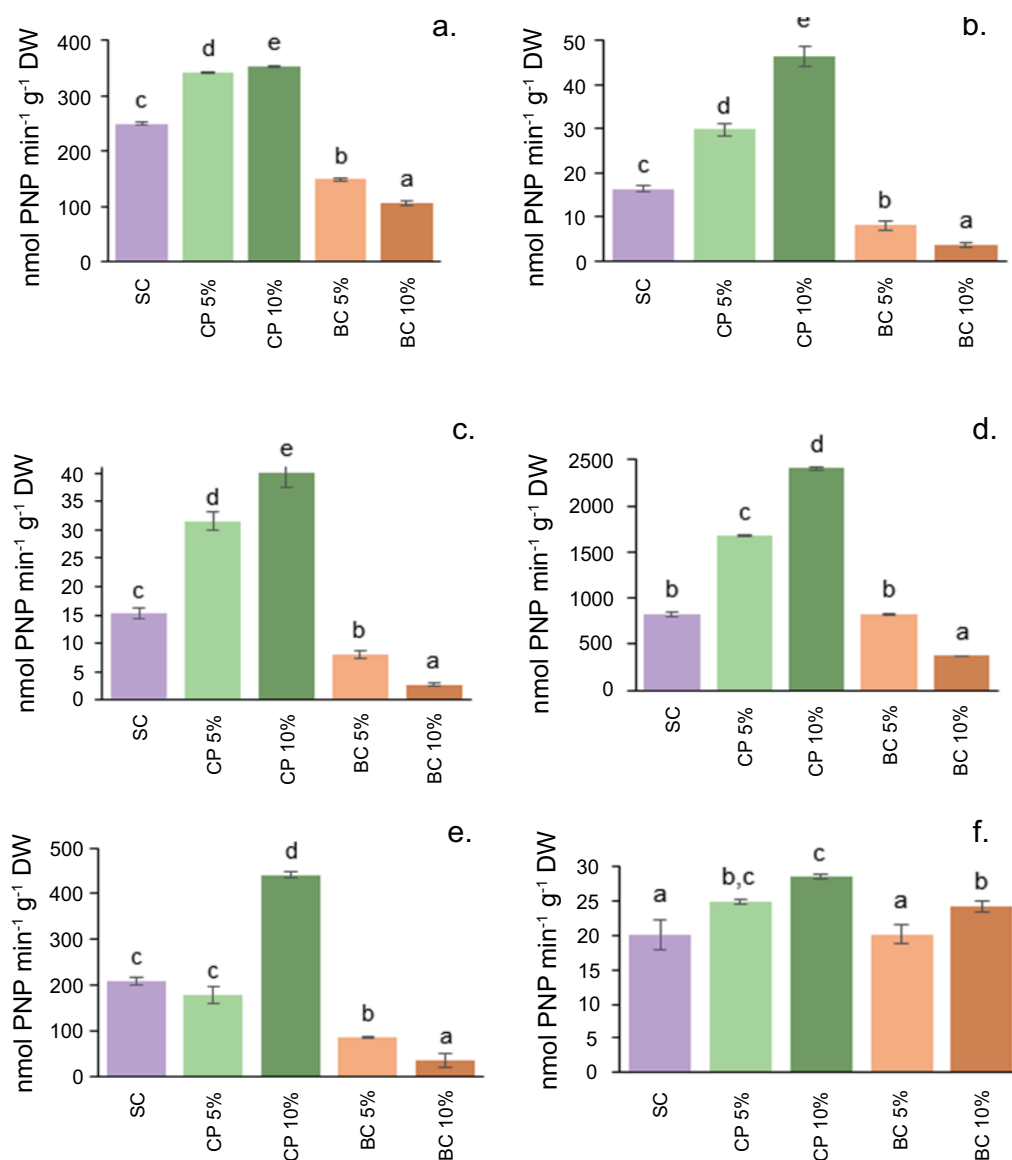


Fig. 2. Soil a. β -Glucosidase, b. β -Galactosidase, c. Arysulfatase, d. Alkalyne phoshatase, e. Acid phosphatase, f. Urease. Bars with different letters indicate significant differences ($p < 0.05$) between treatments. SC: Control contaminated soil; CP: compost; BC: biochar.

present in some amendments can be toxic to seeds (Guo et al. 2020). In this sense, the management of As contaminated soil by applying BC could be adequate to favor the germination of both types of seeds, while the application of CP worsens it at the applied doses.

In the greenhouse assay, BC and CP application affected lettuce plants differently. The development of the lettuce plants was inhibited in the BC and SC treatments according to the higher BCF and TF values. According to these results, it is shown that

BC does not favour the development of lettuce plants in As-contaminated soils under the conditions of this study. The ability of BC for As removal from wastewaters has been observed (Mohan and Pittman 2007) and other studies have obtained positive results with the application of BC in combination with other amendments such as activated carbon and coffee residues in As-contaminated soils (Oliveira et al. 2017). In the case of BC and coffee residues, increases in the biomass of *Pteris vittata* and *Lactuca*

sativa plants were obtained, which were attributed to the high percentage of organic matter in these amendments. However, other authors have reported absence of any positive effect of BC application on the development of *Miscanthus* plants in As-contaminated soils (Hartley et al. 2009), similar to that observed in this study. It must be taken into account that the behaviour of BC will be determined by its characteristics that will depend on its origin. In contrast, CP treatment showed adequate lettuce development, which was reflected in an increase in plant biomass with respect to the other treatments, being the effect dose-dependent. Additionally, in CP treatments, low BCF and TF were observed, favouring the plant development. Several studies have shown the efficacy of compost in reducing the mobility and availability of heavy metals (Van Herwijnen et al. 2007, Liu et al. 2009). However, the effect of composted organic amendments on the mobility and bioavailability of metal(loid)s are controversial and depends on several factors as soil and amendment characteristic and metal(loid)s (Lwin et al. 2018). Thus, there are some reports that suggest that amending contaminated soils with compost may actually increase the mobility of some metal(loid)s, especially As (Hartley et al. 2010). Wheat and barley studies on As-contaminated soils did not show a positive effect on biomass production after compost application and a different behaviour among both species, was observed. As translocation to aerial parts was enhanced in barley which lead to a reduction of biomass (González et al. 2019). However, in agreement with our results, Cao and Ma (2004) reported a positive effect on As adsorption that reduced As uptake when using compost to remediate an As spiked contaminated soil. Moreover, it has also been observed biomass increase in *Agrostis capillaris* L. growth in a soil contaminated with metals and metalloids (As) and treated with two composts derived from green waste and from urban solid waste (Farrell et al. 2010). The same effect was observed by McBride et al. (2015) using of 10% of compost as amendment to treat As and Pb contaminated soils which reduced the concentration of both elements in lettuce, which agrees with the data obtained in our study.

Regarding the effects of the amendments on the soil, both provided nutrients and organic matter that contribute to soil fertility and improve its physical properties, as has been observed in other studies (Lobo et al. 2012, Tang et al. 2020) with a major contribution with the use of CP, being the effect dose-dependent. A significant increase in total As

was observed in the case of the high dose of CP with respect to the SC and BC soils, which is due to the fact that the compost used presents As in its composition due to its origin. The CP-treated soils also showed a significantly lower pH. However, although total As levels were higher in the CP treatments, As bioavailability, expressed both as solubilized As content in the pore water and extracted with DTPA, was significantly lower. In addition, the As leaching capacity estimated by TCLP was lower than that of the other treatments. These data are consistent with the low bioavailability and translocation to the aerial part of the plant found in the CP treatment which confirms the As immobilizing effect of this amendment, especially at the highest dose, as it has been described by other authors (González et al. 2019).

Regarding BC application, in our study, the results recorded were not positive and resulted in a high As bioavailability, translocation and leaching capacity. Other studies, in contrast to our results, showed that the application of BC reduces the bioavailability of metal(loid)s (Rong et al. 2020, Ullah et al. 2022). The differences with these studies could be due to the BC characteristics (pH, porosity, organic matter content, surface area, functional groups amount, etc.) that depend on its origin and will condition As behaviour in soil and plant.

In this study, a reduction in the enzymatic activity, except urease, was observed in BC treated soils. Similar results were observed in relation to the enzyme activity inhibition due to BC application (Tang et al. 2020). This negative effect might be due to the fact that BC addition directly affects the microorganisms by limiting enzyme production (Huang et al. 2016). In addition, BC can adsorb different organic and inorganic molecules and thus can inhibit certain enzymes or substrates by adsorption or blocking the reaction sites (Bailey et al. 2011, Elzobair et al. 2016). Different studies have reported that the reduction of soil biological activity depends on the availability of metals, as well as their characteristics (Giller et al. 2009, Martínez-Iñigo et al. 2009), which is consistent with the lower biological activity in the BC treatments where As availability increased significantly.

In CP-treated soils all enzyme activities evaluated increased significantly, indicating the beneficial effect of CP on the soil biological activity. The ability of compost to promote soil enzymatic activity has been reported by several authors. Its effect has been associated to the fact that this amendment can generate a greater availability of nutrients and improve soil fertility, as well as to the immobilizing effect of metals (Lobo et al. 2012, Mackie et al. 2015, Tang et al. 2020).

In general, the contribution of organic amendments to the remediation of contaminated soils will depend on multiple factors, such as type and concentration of the contaminant, soil characteristics and tolerance of the species of plants used, as well as the characteristics of the amendment.

CONCLUSION

Compost application as an amendment to As-contaminated soil contributed to the immobilization of As in the soil, decreasing its bioavailability to plants and, therefore, its translocation, which favoured biomass production. Although the 10% CP dose produced the highest biomass values, an increase in total As levels in the soil was also observed (due to its composition). Therefore, the most appropriate dose to carry out a soil remediation process would be 5%. The biochar used in this study did not have a positive effect on As immobilization, contributing to an increase in its availability and translocation to the aerial part, which led to a considerable reduction in plant biomass. Under the conditions of our study, the use of an adequate dose of compost would be a useful strategy to favour the phytostabilization of As, promoting the remediation of the contaminated soil.

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REFERENCES

- Abbas, G., Murtaza, B., Bibi, I., Shahid, M., Niazi, N.K., Khan, M.I., Amjad, M., Hussain, M. y Natasha. (2018). Arsenic Uptake, Toxicity, Detoxification, and Speciation in Plants: Physiological, Biochemical, and Molecular Aspects. *International Journal of Environmental Research and Public Health* 15. MDPI. <https://doi.org/10.3390/ijerph15010059>.
- Ashraf, S. Ali, Q., Zahir, Z.A., Ashraf, S. y Asghar, H.N.. (2019). Phytoremediation: Environmentally Sustainable Way for Reclamation of Heavy Metal Polluted Soils. *Ecotoxicology and Environmental Safety* 174 (February): 714-27. <https://doi.org/10.1016/j.ecoenv.2019.02.068>.
- Bailey, V.L., Fansler, S.J., Smith, J.L. y Bolton, H. (2011). Reconciling Apparent Variability in Effects of Biochar Amendment on Soil Enzyme Activities by Assay Optimization. *Soil Biology and Biochemistry* 43 (2): 296-301. <https://doi.org/10.1016/j.soilbio.2010.10.014>.
- Cao, X., Ma, L.Q. y Shiralipour, A. (2004). Effects of Compost and Phosphate on Plant Arsenic Accumulation from Soils near Pressure-Treated Wood. *Environmental Pollution* 132 (3): 435-42. <https://doi.org/10.1016/j.envpol.2004.05.019>.
- Elzobair, K.A., Stromberger, M.E., Ippolito, J.A. y Lentz, R.D. (2016). Contrasting Effects of Biochar versus Manure on Soil Microbial Communities and Enzyme Activities in an Aridisol. *Chemosphere* 142: 145-52. <https://doi.org/10.1016/j.chemosphere.2015.06.044>.
- Farrell, M., Perkins, W.T., Hobbs, P.J., Griffith, G.W. y Jones, D.L. (2010). Migration of Heavy Metals in Soil as Influenced by Compost Amendments. *Environmental Pollution* 158 (1): 55-64. <https://doi.org/10.1016/j.envpol.2009.08.027>.
- Fernández, C., Alonso, C., Babín, M.M., Pro, J. Carbonell, G. y Tarazona, J.V. (2004). Ecotoxicological Assessment of Doxycycline in Aged Pig Manure Using Multispecies Soil Systems. *Science of the Total Environment* 323 (1-3): 63-69. <https://doi.org/10.1016/j.scitotenv.2003.10.015>.
- Giller, K.E., Witter, E. y McGrath, S.P. (2009). Heavy Metals and Soil Microbes. *Soil Biology and Biochemistry* 41 (10): 2031-37. <https://doi.org/10.1016/j.soilbio.2009.04.026>.
- González, Á., García-Gonzalo, P., Gil-Díaz, M.M., Alonso, J. y Lobo, M.C. (2019). Compost-Assisted Phytoremediation of As-Polluted Soil. *Journal of Soils and Sediments*. <https://doi.org/10.1007/s11368-019-02284-9>.
- Guo, J., Du, M., Tian, H. y Wang, B. (2020). Exposure to High Salinity During Seed Development Markedly Enhances Seedling Emergence and Fitness of the Progeny of the Extreme Halophyte Suaeda salsa. *Frontiers in Plant Science* 11 (August): 1-11. <https://doi.org/10.3389/fpls.2020.01291>.
- Hartley, William, Nicholas M. Dickinson, Philip Riby, Elizabeth Leese, Jackie Morton, and Nicholas W. Lepp. (2010). Arsenic Mobility and Speciation in a Contaminated Urban Soil Are Affected by Different Methods of Green Waste Compost Application. *Environmental Pollution* 158 (12): 3560-70. <https://doi.org/10.1016/j.envpol.2010.08.015>.
- Hartley, W., Dickinson, N.M., Riby, P. y Lepp, N.W. (2009). Arsenic Mobility in Brownfield Soils Amended with Green Waste Compost or Biochar and Planted with Miscanthus. *Environmental Pollution* 157 (10): 2654-62. <https://doi.org/10.1016/j.envpol.2009.05.011>.
- Van Herwijnen, R., Hutchings, T.R., Al-Tabbaa, A., Moffat, A.J., Johns, M.L. y Ouki, S.K. (2007). Remediation of Metal Contaminated Soil with Mineral-Amended Composts. *Environmental Pollution* 150 (3): 347-54. <https://doi.org/10.1016/j.envpol.2007.01.023>.

- Huang, M., Zhu, Y., Li, Z., Huang, B., Luo, N., Liu, C. y Zeng, G. (2016). Compost as a Soil Amendment to Remediate Heavy Metal-Contaminated Agricultural Soil: Mechanisms, Efficacy, Problems, and Strategies. *Water, Air, and Soil Pollution* 227 (10). <https://doi.org/10.1007/s11270-016-3068-8>.
- Lindsay, W.L., y Norvell, W.A.. (1978). Development of a DTPA Soil Test for Zinc, Iron, Manganese, and Copper. *Soil Science Society of America Journal* 42 (3): 421-28. <https://doi.org/10.2136/sssaj1978.03615995004200030009x>.
- Liu, L., Chen, H., Cai, P., Liang, W. y Huang, Q. (2009). Immobilization and Phytotoxicity of Cd in Contaminated Soil Amended with Chicken Manure Compost. *Journal of Hazardous Materials* 163 (2): 563-67. <https://doi.org/10.1016/j.jhazmat.2008.07.004>.
- Lobo, M.C., Martínez-Inigo, M.J., Pérez-Sanz, A., Cabezas, G., Plaza, A.; Vicente, M.A. y Sastre-Conde, I. (2012). Evaluation of the Biological Activity in a Gypsiferous Soil Co-Amended with Residues. In: edited by S. Trasar-Cepeda, C., Hernandez, T., Garcia, C., Rad, C., Gonzalez Carcedo, 195–210. New York: *Soil Enzymology in the Recycling of Organic Wastes and Environmental Restoration*. Springer. New York.
- Lwin, C.S., Seo, B.H., Kim, H.U., Owens, G. y Kim, K.R. (2018). Application of Soil Amendments to Contaminated Soils for Heavy Metal Immobilization and Improved Soil Quality—a Critical Review. *Soil Science and Plant Nutrition* 64 (2): 156-67. <https://doi.org/10.1080/00380768.2018.1440938>.
- Mackie, K.A., Marhan, S., Ditterich, F., Schmidt, H.P. y Kandler, E. (2015). The Effects of Biochar and Compost Amendments on Copper Immobilization and Soil Microorganisms in a Temperate Vineyard. *Agriculture, Ecosystems and Environment* 201: 58-69. <https://doi.org/10.1016/j.agee.2014.12.001>.
- Martínez-Iñigo, M.J., Pérez-Sanz, A., Ortiz, I., Alonso, J., Alarcón, R., García, P. y Lobo, M.C.. (2009). Bulk Soil and Rhizosphere Bacterial Community PCR-DGGE Profiles and Beta-Galactosidase Activity as Indicators of Biological Quality in Soils Contaminated by Heavy Metals and Cultivated with *Silene Vulgaris* (Moench) Garcke. *Chemosphere* 75 (10): 1376-81. <https://doi.org/10.1016/j.chemosphere.2009.03.014>.
- McBride, M.B., Shayler, H.A., Russell-Anelli, J.M., Spliethoff, H.M. y Marquez-Bravo, L.G. (2015). Arsenic and Lead Uptake by Vegetable Crops Grown on an Old Orchard Site Amended with Compost. *Water, Air, and Soil Pollution* 226 (8). <https://doi.org/10.1007/s11270-015-2529-9>.
- Mohan, D. y Pittman, C.U. (2007). Arsenic Removal from Water/Wastewater Using Adsorbents-A Critical Review. *Journal of Hazardous Materials* 142 (1-2): 1-53. <https://doi.org/10.1016/j.jhazmat.2007.01.006>.
- Moreno-Jiménez, E., Esteban, E. y Peñalosa, J.M. (2012). The Fate of Arsenic in Soil-Plant Systems. *Reviews of Environmental Contamination and Toxicology*. Vol. 215. https://doi.org/10.1007/978-1-4614-1463-6_1.
- Oliveira, L.M. De, Suchismita, D., Gress, J., Rathinasabapathi, B., Chen, Y. y Ma, L.Q. (2017). Arsenic Uptake by Lettuce from As-Contaminated Soil Remediated with *Pteris Vittata* and Organic Amendment. *Chemosphere*. <https://doi.org/10.1016/j.chemosphere.2017.02.124>.
- Rong, Q., Zhong, K., Li, F., Huang, H., Li, C. y Nong, X. (2019). Combined Effect of Ferrous Ion and Biochar on Cadmium and Arsenic Accumulation in Rice. *Applied Sciences*. 10(1), 300. <https://doi.org/10.3390/app10010300>.
- Tang, J., Zhang, L., Zhang, J., Ren, L., Zhou, Y., Zheng, Y., Luo, L., Yang, Y., Huang, H. y Chen, A. (2020). Physico-chemical Features, Metal Availability and Enzyme Activity in Heavy Metal-Polluted Soil Remediated by Biochar and Compost. *Science of the Total Environment* 701: 134751. <https://doi.org/10.1016/j.scitotenv.2019.134751>.
- Uçarlı, C. (2021). Effects of Salinity on Seed Germination and Early Seedling Stage. *Abiotic Stress in Plants*, October. <https://doi.org/10.5772/intechopen.93647>.
- Ullah, F., Wang, X., Farooq, M., Hussain, S., Alam, S., Latif, A., Ejaz, M., Janyshova, U. y Liqun, C. (2022). Ecotoxicology and Environmental Safety Biochar Application for the Remediation of Trace Metals in Contaminated Soils : Implications for Stress Tolerance and Crop Production. *Ecotoxicology and Environmental Safety* 230 (January): 113165. <https://doi.org/10.1016/j.ecoenv.2022.113165>.
- USEPA, US. (1992). Environmental Protection Agency. Method 1311: toxicity characteristic leaching procedure. <https://www.epa.gov/hw-sw846/sw-846-test-method-1311-toxicity-characteristic-leaching-procedure>.
- Verbeeck, M., Thiry, Y. y Smolders, E. (2020). Soil Organic Matter Affects Arsenic and Antimony Sorption in Anaerobic Soils. *Environmental Pollution* 257 (February). <https://doi.org/10.1016/j.envpol.2019.113566>.
- Yadav, M.K., Saidulu, D., Gupta, A.K., Ghosal, P.S. y Mukherjee, A. (2021). Status and Management of Arsenic Pollution in Groundwater: A Comprehensive Appraisal of Recent Global Scenario, Human Health Impacts, Sustainable Field-Scale Treatment Technologies. *Journal of Environmental Chemical Engineering* 9 (3): 105203. <https://doi.org/10.1016/j.jece.2021.105203>.
- Yan, A., Wang, Y., Tan, S.N., Mohd Yusof, M.L., Ghosh, S. y Chen, Z. (2020). Phytoremediation: A Promising Approach for Revegetation of Heavy Metal-Polluted Land. *Frontiers in Plant Science* 11 (April): 1-15. <https://doi.org/10.3389/fpls.2020.00359>.
- Zucconi, F., Monaco, A., Forte, M. y Bertoldi, M. de. (1985). *Phytotoxins during the Stabilization of Organic Matter. Composting of Agricultural and Other Wastes* / Edited by J.K.R. Gasser. London: Elsevier Applied Science Publishers, c1985.