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ORIGINAL PAPER

Evaluating the capability of tannic acid-modified biochar in sequestering metal ions and in enhancing soil health

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Abstract

Pollution of soils is one of the currently significant environmental concerns. Heavy metals that contaminate the soil can have harmful effects on crops, enter the food chain, and pose a threat to human health. Various approaches have been employed in the past to remediate heavy metal-contaminated soils for agricultural use, including the use of biochar and its modified forms through physical or chemical methods. In the present study, the suitability of tannic acid as a biochar modifier was evaluated for the first time with the aim of enhancing its ability to adsorb Cd and Pb, thereby reducing their transfer from soil to plants. A mesocosm trial was set-up using three treatments: control, unmodified biochar, and tannic acid-modified biochar and their impact was evaluated on soil characteristics and plant physiology. The FT-IR spectra demonstrate that tannic acid-modified biochar considerably improved its capability to absorb Cd but worsened those for Pb and this occurred due to an increase in oxygen-containing functional groups and a reduction in total pore volume, respectively. Tannic acid-modified biochar application increased the carbon and nitrogen concentrations in the soil, as well as the microbial biomass and activities. Lettuce plants grown on soil amended with modified biochar accumulated a low Cd amount in roots but a high Pb amount in leaves that reduced the photosynthetic pigments and the photosynthetic rates and increased the antioxidant response. It is found that tannic acid treatment of biochar is effective for the remediation of Cd-contaminated soils but not for those polluted by Pb.

Keywords Cadmium and lead · Modified biochar · Plant physiology · Polluted soils · Soil biological activities · Tannic acid

Introduction

Heavy metal contamination in soil have gained the attention due to their high toxicity and long-term bioaccumulation capability (Wuana and Okieimen 2014). Large areas in the world are affected by heavy metal accumulation due to anthropogenic activities such as industrial, mining, and intensive agricultural processes. In Europe, about 137,000 km^2 of agricultural lands representing almost 8% of the total agricultural area (Tóth et al. 2016) are affected by heavy metal pollution, mainly Cd, Co, Cr, Cu, Mn, Ni, Pb, and Zn, which often tend to accumulate in the soil threatening human health and food security. Cadmium (Cd) and lead (Pb) are the most common heavy metal contaminants in soils, which concentrations produce negative effects on plants, reducing photosynthesis and chlorophyll synthesis, compromising plant water relations and hormonal and/or nutritional balances (Collin et al. 2022).

Different strategies have been employed to reclaim polluted soil, including the use of amendments. Biochar—the

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solid material obtained from the thermochemical conversion of biomass in an oxygen-limited environment—has gained attention as potential and effective mean for the removal of various contaminants, including heavy metals. Biochar immobilizes heavy metals from soil through adsorption (Bashir et al. 2020), which effectiveness is directly proportional to biochar physicochemical properties such as surface area, functional groups, cation exchange capacity (Yaashikaa et al. 2020). These properties can be improved through physic, chemical, and biological-induced modifications (Bao et al. 2022). Chemical modification is one most used method at present; it usually includes the use of acid, alkaline, oxidizing-agents, metal salts and oxides (Wang and Wang 2019). Acid modification introduces acidic functional groups on biochar surface, improving biochar surface acidity and changing its porous structure; furthermore, it improves the heavy metals adsorption by ion exchange and complexation (Wang and Wang 2019; Bao et al. 2022). Inorganic and organic acid agents are currently used to ameliorate the biochar adsorption capability for Cd and Pb (Bao et al. 2022). These two heavy metals are complexed by biochar through precipitation, surface complexation with oxygen-containing functional groups, such as –OH as well as –COOH, and cation-π interactions involving aromatic systems, C=C, and C=O (Chi et al. 2019).

Recently, the tannic acid (TA) has been used to modify biochar (Chen et al. 2021). This is the lone study which reports the tannic acid as biochar-modifying agent, and it was aimed to evaluate the modified biochar in removing antibiotics from water. To date, there has been no research on the potential of biochar to adsorb heavy metals in the presence of TA, nor on the effect of TA-modified biochar on the translocation of heavy metals from soil to plants.

Tannic acid comprises many oxygen-containing functional groups (Chen et al. 2021); thus, using it to modify biochar and enhancing its chelating affinity for heavy metal pollutants could be of great significance. This if you consider also that the tannic acid is an environmentally friendly substance and easy to find. For example, the grape pomace is rich in tannins and could be easily extracted using modern techniques and eventually used to modify biochar.

In this study–carried out from spring to summer 2022 at CNR-ISAFoM (Portici, Italy)—it was tested, for the first time, the suitability of tannic acid in modifying biochar to improve its capability in Cd and Pb adsorption with the goal to use this modified biochar for reclaiming contaminated soil. In the present study, the TA-modified biochar was utilized to remediate soil contaminated with Cd and Pb, aiming to reduce the translocation of these pollutants into plants. Specifically, we assessed: 1) the physicochemical properties of the tannic acid-modified biochar; 2) its effectiveness in mitigating ecotoxicity on key soil biological activities; and 3) its capacity to reduce Cd and Pb uptake by plants. It is hypothesized that the tannic acid treatment enhances oxygen-containing functional groups on modified biochar, so increasing the sorption properties of biochar for both studied ions and limit their uptake by lettuce plants.

Materials and methods

Experimental set-up

A mesocosm trial was set-up in spring 2022 using three treatments, each in four replicates: control (CTRL), unmodified (B) biochar and tannic acid-modified (TA-B) biochar. Unmodified and modified biochar were added (2.5% w/w) to soil contaminated by Cd and Pb which was put in 4 L pots whereas CTRL consisted in unamended soil. The soil showed the following physical–chemical properties: texture (%): clay, silt, and sand respectively 8, 12, and 80; pH_{H2O}: 7.01; EC (mS m^{-1}): 14.9; C_{tot} (% d.w.): 2.5; N_{tot} (% d.w.): 0.2; organic matter (% d.w.): 2.7; total Cd (10.6 mg kg^{-1}) and Pb (85.0 mg kg^{-1}). After the addition of B and TA-B, the soil was analysed, every 15 days, for pH, total carbon (C_{tot}) and nitrogen (N_{tot}), microbial biomass and soil basal respiration, urease, and β-glucosidase activities until 60 days since soil amendment (DAA) and was in unplanted soil. During this time, soil was fertilized by nutrient solution containing micro and macronutrients and urea (N_{tot} 20%, N-NO_3 3.6%, N-NH_4 3.6%, N-CH_4N_2O 12.1%, P_2O_5 40%, K_2O 20%, Cu 0.02%, B 0.05%, Mn 0.2%, Fe 0.4%, Zn 0.02%, Mo 0.02%, AGROFILL, Ponso, Padua, Italy). At 60 DAA, seedlings of lettuce (*Lactuca sativa* L. cv Ranger) two weeks old were transplanted (one seedling *per* pot) and grown until 90 days after transplanting (DAT) under white light provided by a LED lighting system. Plants were grown under 200 µmol photons m^{-2} s^{-1} at 16 h d^{-1} photoperiod. At 90 DAT, the photosynthetic activity was measured, and the same leaves were sampled for Cd and Pb and bioactive substance content. Thereafter plants were harvested and roots cleaned.

Chemical modification of biochar and its physical–chemical properties

Commercial biochar (Nera biochar, Settimo Vittone, Torin, Italy), obtained from pyrolysis of woody biomass, was used in this experiment (Table S1) and chemically modified by using tannic acid. Tannic acid-modified (TA-B) biochar solid was prepared by precipitation method. In detail, 2.0 g of tannic acid (Sigma-Aldrich) was dissolved into 50 mL of 1 M NaOH. Then, 4.6 g of biochar was added into the solution and stirred for 12 h. Finally, 60 mL of 1 M HCl solution was added with vigorous stirrings. The solid was washed by distilled water and dried at 60 °C.

The textural properties of the adsorbents were determined by N_2 adsorption at − 196 °C with an Autosorb iQ apparatus (Quantachrome Instruments). In particular, the Brunauer–Emmett–Teller (BET) method was adopted for the calculation of the specific surface area (S_{BET}), while the total pore volume (V_p) was evaluated using the Gurvitsch's rule applied to the N_2 amount adsorbed at relative pressure $P/P_0 = 0.99$.

The pH of biochar samples was measured by suspending 0.5 g (with dimensions between 58 µm and 180 µm) of biochar in distillated water in a 1:10 ratio. After 1 h of shaking, suspensions were allowed to stand for 30 min before pH measurements were taken using a pH meter (Hanna HI2002-02). The pH meter has been calibrated using pH = 4.00, pH = 7.00, and pH = 10.00 buffers. Electrical specific conductivity (EC) was measured on the same samples using suspensions prepared for pH measurement by a conductivity meter (ISTEK 430C model). The analyses of pH and EC were performed in triplicate. The determination of the cation exchange capacity (CEC) of biochar is based on displaced NH_4^+ by KCl, after washing with an organic solvent. 0.5 g of biochar was treated with 20 mL of 1 M NH_4OAc, and the tubes were shaken at 50 rpm during 24 h. After extraction with NH_4OAc, the biochar slurry was washed three times with 20 mL 99% isopropanol. 20 mL of 2 M KCl were added subsequently, and the tubes were shaken during 24 h at 30 rpm. The suspensions were filtered, and the supernatants were analysed for NH_4^+ (Munera-Echeverr et al. 2018).

Acid–base properties of the solid were studied by potentiometric titrations at 25 °C in 0.1 M $NaClO_4$ as ionic medium. The biochar suspensions (TS) examined were the following composition: C_H M $HClO_4$, C_{OH} M NaOH, $(0.1 - C_H)$ M $NaClO_4$, a g dm^{-3} biochar (where a = 1.50 g dm^{-3}).

The free hydrogenionic concentration was measured by a glass electrode and reference electrode, respectively. By the experimental data collected, the quantity Q_H is evaluated, as the number of moles of protons exchanged per g of solid (mmol g^{-1}):

$$Q_H = \left(\left(C_H - C_{OH} - h + K_W/h\right)\right)/a$$

Assuming that on the surface are present BOH basic sites and AOH acidic sites, the protolytic equilibria on the surface are:

$$> BOH + H^+ => BOH_2^+ K_B$$

$$> AOH => AO^- + H^+ K_A$$

Considering the mass balances of the two sites the surface charge is expressed by the equation:

$$Q_H = \left[> BOH_2^+\right] - [> AO^-]$$

Substituting the mass balances into this equation, we get:

$$Q_H = Q_B\left(K_B h\right)/\left(1 + K_B h\right) - Q_A\left(K_A h^{-1}\right)/\left(1 + K_A h^{-1}\right)$$

Q_H as a function of pH is represented in Fig. S1 where shows an inflection point: the pH at this point represents the point of zero charge (ZCP).

The FT-IR spectra, performant to detect any functional groups induced by chemical treatment, were recorded using an FT-IR 4700 model JASCO (Tokyo, Japan) in absorbance mode. The biochar sample was prepared at a 1:100 ratio with anhydrous KBr, and the blank was recorded using anhydrous KBr as a reference. The KBr before use was kept in an oven at 105 °C overnight.

Adsorption of Pb(II) and Cd(II) ions onto biochar was studied in 0.1 M $NaClO_4$ as ionic medium. Test suspensions (TS) of biochar (with a (g dm^{-3}) solid concentration) had the following composition: C_0 mg dm^{-3} Pb(II) or Cd(II), 0.1 M $NaClO_4$, a g dm^{-3}. C_0 is the initial concentration of metal (Pb(II) or Cd(II)) ranged from 10.0 to 100.0 mg dm^{-3} and all suspensions were prepared to be at pH = 5.50. Test suspensions were kept in an air thermostat, at (25.00 ± 0.05) °C, stirred using a rotating system for test tubes, at a speed of 30 rpm. The stirred time was sufficient to reach the balance between the phases. The pH of the suspensions was measured using the potentiometric cell described. When the equilibrium between phases was reached, the specific adsorption capacity Q_E was evaluated as:

$$Q_E = \frac{C_0 - C_E}{a}$$

where C_E is equilibrium concentration of Pb(II) or Cd(II).

Soil biological activities determination

The biological analyses (microbial biomass and respiration, β-glucosidase, and Urease activities) were carried out on soil samples stored at 4 °C within three days from the soil sampling. The microbial metabolic quotient (qCO_2) was calculated from basal respiration per unit microbial biomass carbon.

The microbial biomass (MB) and the basal microbial respiration (MR) were evaluated as CO_2 evolution measured by a non-dispersive IRGA (Qubit S151 CO_2 analyser, Qubit systems Inc., Canada) and incubating soil samples for 4 h in darkness at 25 °C. MB was measured after addition of 2 ml D–glucose solution (75 mM) to fresh soil, equivalent to 1 g dry mass, whereas MR was evaluated on fresh soil after addition of 2 ml of distilled water.

The activity of β-glucosidase (β-glu) was assessed by incorporating 4 mL of modified universal buffer (MUB) and 1 mL of 0.025 M p-nitrophenyl β-D-glucopiranoside (PNP) into 1 g of soil. This mixture was incubated at 37 °C

for one hour, followed by a rapid cooling on ice for 15 min to halt the enzyme reaction. Subsequently, 1 mL of 0.5 M $CaCl_2$ and 4 mL of 0.1 M Tris-hydroxymethylaminomethane-sodium hydroxide (THAM-NaOH) were added. For the control samples, the substrate PNP was introduced prior to the addition of $CaCl_2$ and NaOH. The absorbance of the resulting supernatant was measured at a wavelength of 420 nm. The enzyme activity was quantified as mmol of PNP produced per gram of dry soil per minute, based on methods established by Tabatabai (1982).

Urease activity (Ure) was quantified following the methodology protocols established by Alef and Nannipieri (1995). To 1 g of fresh soil, 0.5 mL of 0.1 M urea and 4 mL of 0.1 M borate buffer were added, and the mixture was incubated at 37 °C for 2 h. After that, 10 mL of a solution containing 1.35 M potassium chloride in 0.1 M hydrochloric acid (KCl–HCl) was added and the samples were then shaken and centrifuged. The clear extract from each sample was mixed with 2.5 mL of buffer, 4 mL of salicylate solution, and 2.5 mL of hypochlorite solution. This final mixture was incubated once more at 37 °C for 30 min. The absorbance of the supernatant was measured at 660 nm, and urease activity was reported as mmol of NH_4^+ produced per gram of dry soil per minute.

C, N, Cd, and Pb content in soil

The soil samples were analysed to determine their pH levels, total C and N contents, and for total Cd and Pb contents as well as their relative bioavailable fractions. Soil pH was measured in a soil: distilled water (1:2.5 = v:v) suspension; total C and N contents were determined on oven-dried (105 °C) and grounded (Fritsch Analysette Spartan 3 Pulverisette 0) soil samples by using a CNS Analyzer (Thermo Finnigan, Italy).

The soil samples were dehydrated in oven at 40 °C until reaching a constant weight, then ground in an agate mortar. The pulverized samples, weighing between 1 and 2 g, were then digested in a solution containing 20 mL of 65% HNO_3 (hyperpure, Carlo Erba) and 10 mL of 30% (w/w) H_2O_2 (hyperpure, Sigma Aldrich) for total metal analysis. Finally, they were made up to volume in a 50 cm^3 flask. The samples were diluted to 25 mL with Millipore water and filter. The free concentration of Cd and Pb was determined using an Atomic Absorption Spectrometer (AAS, model ZEEnit 700P- Analytik Jena, Germany) with flame (air/acetylene) at appropriate wavelengths of 228.8 nm for Cd and at 283.3 nm for Pb. The calibration curve is obtained by measures of standard solutions of Cd and Pb that were prepared in 0.1 M HNO_3 by diluting the stock solutions of $Pb(NO_3)_2$ and $Cd(NO_3)_2$, respectively. The stock solutions of lead (II) and cadmium (II) nitrate were prepared by dissolving lead and cadmium metal, 5N8 (Metal Research) in a hyperpure stock solution of nitric acid. The concentration of metal in both solutions was determined using EDTA through complexometric titration. The blank for the instrument was recorded with a 5% (w/w) HNO_3 solution.

The available Cd and Pb fractions were extracted according to Lindsay and Norwell (1969) method. Briefly, to 25 g of oven-dried (75 °C) soil samples were added 50 mL of diethylenetriamine pentacetic acid (DTPA), $CaCl_2$ and triethanolamine (TEA) solution at pH 7.3 ± 0.05. The soil suspensions were shaken for 2 h and filtered with Whatman 42 filter. The ions concentration in extracts was determined as described for the total metals.

Biometrical determination and photosynthetic activity

At 90 DAT plants were collected and weight of root, stem, and leaves was determined. Samples were oven dried at 75 °C until reaching a constant weight to evaluate the standing biomass. Leaf area was measured by using a leaf area meter (Li-3000, Licor, USA).

Gas exchange and fluorescence measurements were simultaneously performed on mature fully expanded leaves through the portable photosynthesis system LI-6400 integrated with LI-6400–40 leaf chamber fluorometer (Li-Cor, Lincoln, Nebraska, USA). Photosynthetic light-response curves were carried out illuminating leaves with a red:blue LEDs light source at 25 °C, 400 µmol CO_2 mol^{-1}, and 50% air relative humidity (RH) to determine the parameters according to von Caemmerer and Farquhar (1981). The following three-parameter exponential function was considered to describe the response of net assimilation rate to light intensity:

$$A_N = R_d + A_{Nmax}\left(1 - e^{-(Q_{app}/A_{Nmax})Nmax}\right)$$

where R_d is the dark respiration, A_{Nmax} is the maximum photosynthetic rate, Q_{app} is the "apparent" light efficiency representing the maximum quantum yield, whereas PPFD is the incident light intensity.

The steady-state fluorescence yield (F_s) and the maximal fluorescence yield in the light-adapted state (F'_m) were measured at each irradiance level by applying a 0.8 s saturating flash of 8000 µmol photon m^{-2} s^{-1}. These parameters were used to calculate the effective quantum yield of PSII photochemistry (Φ_{PSII}) and the photochemical quenching (q_P) as well as the non-photochemical quenching (NPQ), this latter by also using the fluorescence parameters in the dark-adapted state obtained by darkening the leaf for 30 min (Maxwell and Johnson 2000).

C and N content, Cd and Pb, and bioactive substances content in leaves

The same leaves used for gas exchange measurements were collected for C and N content, heavy metals, and for bioactive substance determinations.

The C and N content in leaves were evaluated on oven-dried (105 °C) and grounded (Fritsch Analysette Spartan 3 Pulverisette 0) samples by a CNS Analyzer (Thermo Finnigan, Italy). The plant samples were divided into leaves and roots for total element content analysis. Subsequently, they were dehydrated in an oven at 40 °C until a constant weight was achieved and then weighed. To prepare the dehydrated plant materials for digestion, they were ground in an agate mortar. The powdered samples, weighing between 100 and 250 mg, were then digested in a solution containing 5 mL of 65% HNO_3 (hyperpure, Carlo Erba) and 2.5 mL of 30% (w/w) H_2O_2 (hyperpure, Sigma Aldrich) for metal analysis. Cd and Pb content in vegetables was determined by following the same procedure as previously described.

Chlorophylls and carotenoids were determined spectrophotometrically according to Lichtenthaler (1987) and expressed on a surface basis. Total polyphenols, anthocyanins, flavonoids, antioxidant capacity—expressed as FRAP, and soluble proteins were determined as reported in Costanzo et al. (2022) and Vitale et al. (2021), whereas H_2O_2 was determined as reported in Vitale et al. (2020). The above-mentioned parameters were expressed on a weight basis.

The crystallinity of materials was identified by X-ray diffraction (using Malvern PANanalytical Empyrean Series 3 X-ray diffractometer operating with Ni-filtered CuKα1 radiation, λ = 0.154059 nm).

Statistical analysis

Differences in soil C and N content and biological characteristics were statistically checked by using a two-way ANOVA followed by the Tukey test as post-hoc and using treatments (CTRL, B and TA-B) and time (T0 and T5 for C and N, and T0, T1, T2, T3, T4, T5 for biological characteristics) as main factors, whereas differences in plant biometry and leaf photosynthetic-related parameters were checked by one-way ANOVA. Correlations among plant characteristics were checked by Pearson' test.

Results and discussion

Effects of acid treatment on physical and chemical properties of biochar

The treatment with acid tannic induced a drastic reduction in the specific surface area (S_{BET}), in the total pore volume (V_p), pH, and in acid type sites (Table S2). S_{BET} changed from 172 $m^2\ g^{-1}$ in unmodified (B) biochar to 21 $m^2\ g^{-1}$ in tannic acid-modified (TA-B) biochar, whereas V_p from 0.125 $cm^3\ g^{-1}$ in B to 0.039 $cm^3\ g^{-1}$ in TA-B. pH and the acid type sites decreased to 5.5 and to 0.5 in TA-B, respectively. No change occurred in cationic exchange capacity (CEC), zero charge point (ZCP), and surface proteolytic equilibria K_A and K_B constants after modification by acid, whereas a reduction by of 12% occurred in electric conductivity (EC) for acid-modified biochar compared to unmodified biochar (Table S2). From the variation in the number of moles of protons exchanged per gram of solid as a function of pH (Fig. S1), it can be deduced that basic and acidic sites are present, the latter involved in the complexation of metal cations. From the values of the protolysis constants obtained (Table S2), the constant K_A can be assigned to substituted phenolic groups, while the constant K_B could be identified with the presence of pyronic groups. Acidic sites (type A) are involved in the adsorption process of metal ions (Table S2).

The FTIR spectra of unmodified (B) biochar and TA-modified (TA-B) biochar are illustrated in Fig. 1. The TA-modified biochar was characterized by the highest band intensities for the organic functional groups with a band of –OH stretching (3352 cm^{-1}), C=O stretching of carboxyl (1700 cm^{-1}), aromatic C=O or C=C ring stretching

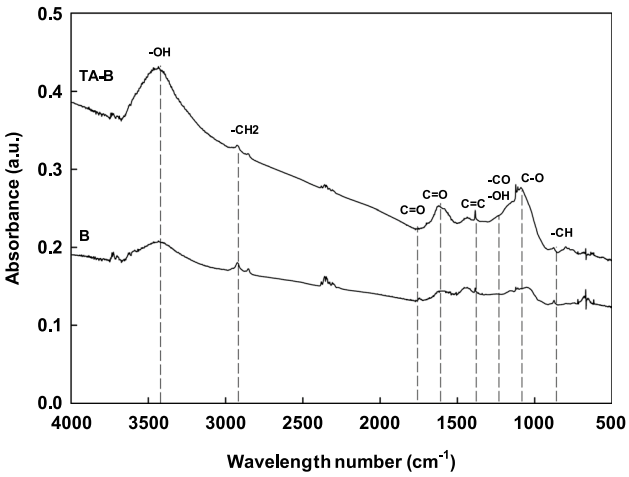

Fig. 1 FT-IR spectra for unmodified (B) and tannic acid-modified (TA-B) biochar

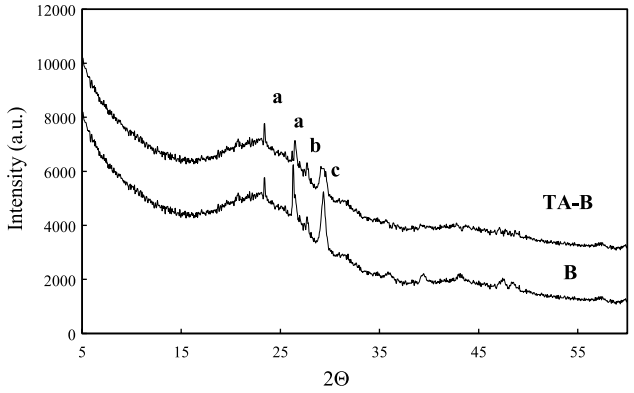

Fig. 2 XRD spectra of unmodified (B) and tannic acid-modified (TA-B) biochar (**a** SiO_2, **b** KCl, **c** K_2SO_4)

(1653 cm^{-1}), and C–O stretching (1031 and 1098 cm^{-1}) whereas the aliphatic CH_2 (2919 cm^{-1}) and Si–O–Si stretching (798 and 1031 cm^{-1}) were decreased.

XRD spectra for unmodified (B) and tannic acid-modified (TA-B) biochar are shown in Fig. 2. The band observed in the 2Θ 18–28° range is indicative of the organic matter. Potassium Chloride (KCl), silicon dioxide (SiO_2) and potassium sulphate (K_2SO_4) were also identified in all biosorbents. Adsorption isotherms that describe the adsorption capacity of biochar for Cd and Pb elements (Q_E in function of equilibria concentrations C_E of Pb(II) or Cd(II)) are shown in Fig. 3. The experimental data agree with the Langmuir model (Langmuir 1918):

$$Q_E = Q_0 \frac{K_L C_E}{1 + K_L C_E}$$

where Q_0 (mg g^{-1}) represents the maximum quantity of metal ions adsorbed per gram of biochar, while K_L (dm^3 mg^{-1}) is the Langmuir constant whose value is linked to the affinity between adsorbent and adsorbate. The isotherm models reveal that chemically treating biochar with tannic acid significantly enhanced its capacity to adsorb Cd, while substantially reducing its effectiveness for Pb. To investigate the potential competition between Cd and Pb for binding sites, a sorption experiment was conducted using a combination of Cd and Pb ions on TA-modified biochar. The results indicate that the sorption isotherm of individual ions remain unaffected when both ions are combined (Fig. S2). It appears that the treatment with tannic acid substantially modified the biochar inducing changings both in physical and chemical properties that might explain the changings in sorption properties observed for both ions. Since the isotherm models for Cd and Pb ions of the modified biochar does not change if evaluated in single or double components, it is reasonable to say that different mechanisms are involved in adsorption by biochar for Cd and Pb. It is possible to stay

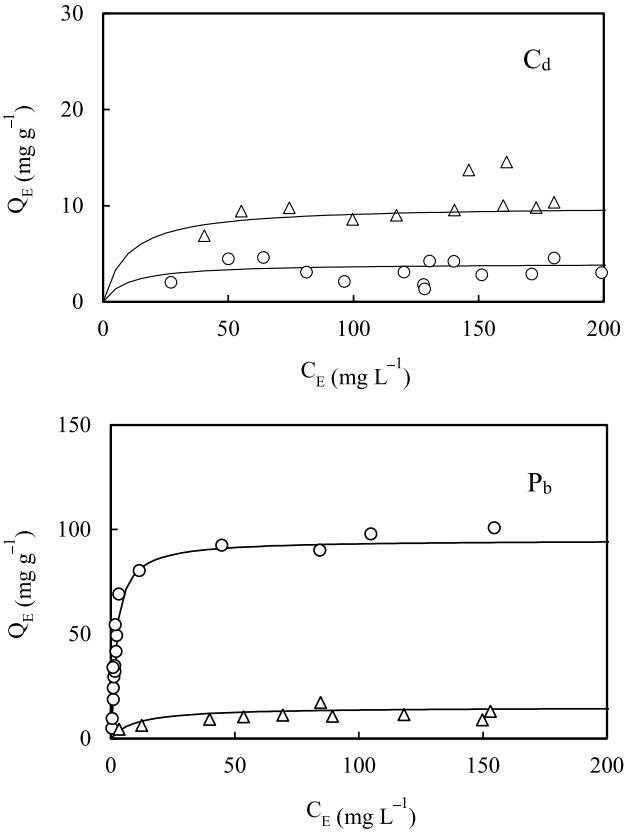

Fig. 3 Adsorption capacity (Q_E, mg g^{-1}) of cadmium (Cd) and lead (Pb) ions onto B (circle) and TA-B (triangle) at equilibrium concentration (C_E, mg dm^{-3}) in 0.1 M of $NaClO_4$, at 25 °C

that the increased capability of TA-B substrate to absorb Cd was due to enhanced complexation capability with oxygen-containing functional groups. In this study, an increase in band intensities for the oxygen-containing functional groups, such as –OH stretching, C=O stretching of carboxyl, aromatic C=O, and C–O stretching was observed, confirming our hypothesis. These modifications are common in treated biochar with both inorganic (Chen et al. 2019) and organic acids (Lonappan et al. 2020) and are known to improve the effectiveness of biochar in sequestering Cd (Xiong et al. 2024), so making it less available to plants. However, tannic acid treatment improves the biochar's ability to adsorb Cd but not Pb, so partially confirming our hypothesis. It is reasonable to stay that this occurs as consequence of reduced total pore volume that limits the physical sorption of Pb ions, a larger ion in size than Cd ion. Physical sorption by biochar occurs as Pb ions are attracted to and captured by the available sites within the biochar pores (Chatterjee and Abraham 2019). The physical alterations observed in the present study to treated biochar, in particular the reduction in the specific surface area (S_{BET}) and total pore volume (V_p), are in contrast with those reported in other studies which by using acids to modify biochar find an increase in those

physical properties (Ravindiran et al. 2024). Since functional groups also are involved in the adsorption of Pb by biochar, it is also likely to suppose that tannic acid may have altered the binding sites, such as Si–O-Si sites (798 and 1031 cm^{-1}) (Han et al. 2017) making them ineffective in Pb absorption, as evidenced by the isotherm model.

Effect of TA-modified biochar on soil characteristics

According to the two-way ANOVA, the C_{tot} and N_{tot} content were significantly influenced by the time ($P < 0.05$), by treatment (at least $P < 0.05$), as well as by the interactions ($P < 0.05$) between treatment and time (Table S3). In particular, C_{tot} content was higher in B and TA-B at T0 and T5 and it was the highest in T5 (Table S4). The N_{tot} content was higher in B and TA-B at T5, and it was higher at T5 in B and TA-B (Table S4). Total soil Cd and Pb content (ranging from 10–12 and 100–111 mg kg^{-1} soil d.w., respectively for Cd and Pb) and the relative bioavailable fractions (ranging from 0.09–0.11 and 12–13 mg kg^{-1} soil d.w., respectively for Cd and Pb), determined at T5, did not differ among treatments.

All the soil biological characteristics were significantly influenced ($P < 0.001$) by the time (T0-T5) and, except the qCO_2 (at least $P < 0.05$), by the treatment as well as by the interactions (at least $P < 0.01$) between treatment and time (Table S4, Fig. 4), except for urease activity. In particular, the microbial biomass was higher in CTRL and B at T2 and in TA-B in T0, T1 and T2 than at the other times (Fig. 4). Within the same time, the microbial biomass was higher in CTRL and TA-B than B at T0 and was higher in TA-B than in CTRL and B at the T1 (Fig. 4). The basal respiration was higher in CTRL at T0, T1 and T4, in B at T0, T2 and T4 and in TA-B in T2 than at the other times (Fig. 4). Within the same time, the basal respiration was higher in CTRL and TA-B than B at T0 and T1 and was higher in TA-B than in CTRL and B at the T2 (Fig. 3). The qCO_2 was higher in CTRL and B at T4, in TA-B in T0, T2 and T4 than at the other times (Fig. 4). Within the same time, the qCO_2 was higher in CTRL than in B and TA-B at T4 and was higher in TA-B than in CTRL and B at the T2 (Fig. 4). The β-glu was higher in CTRL, B and TA-b at T0, T1, T3 and T5 than at the other times (Fig. 4). Within the same time, the β-glu was higher in CTRL than in B and TA-B at T0 and was higher in TA-B than in CTRL and B at the T1 (Fig. 3). The Ure was higher in CTRL, B and TA-B at T1 and T5 than at the other times (Fig. 4). Within the same time, the Ure was higher in CTRL and TA-B than in B at T3 (Fig. 4).

The results obtained in the present study indicate a positive impact of biochar application on soil C and N content as well as on microbial biomass and activities, especially the in beginning of the application. In particular, the application of biochar, regardless the type (biochar or tannic modified) increased the C and N content, whereas the

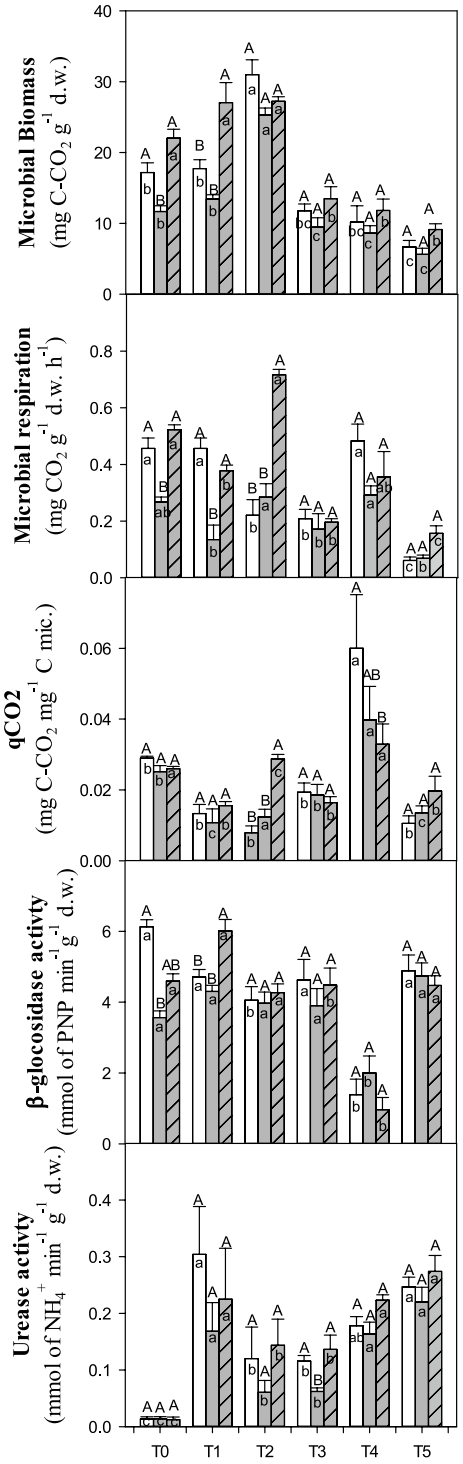

Fig. 4 Microbial biomass, microbial respiration, qCO_2, β-glucosidase and urease activities measured at different time (T0-T5) in soil untreated (CTRL, white bar), treated with biochar (B, gray bar) and with tannic acid-modified biochar (TA-B, dashed gray bar). Data are means ($n = 4$) ± SE. Different capital and small letters indicate significant differences among the treatments at each time and among different times in each treatment, respectively

tannic acid modified (TA-B) biochar mainly influenced the soil microbial biomass and activities. It is well known that biochar application improves soil properties, particularly physical properties, nutrient content, microbial composition and increase crop production. However, the impact of biochar on C and N content are controversial, as the wide C/N ratio of biochar may negatively affect the soil N availability or accelerate the soil organic carbon mineralization through a greater soil microbial activity (Dai et al. 2020). In the present study, the microbial biomass and activities were favoured by biochar application, but the content of C increased suggesting that the added C was not mineralized. Conversely, the biochar application seemed to make N less available for biological transformations, as suggested by the increase of its concentrations only in amended soil. Biochar application in the soil could limit nutrients availability, as biochar acts as an absorbent material for both organic and inorganic fertilizers (Lehmann et al. 2009).

The observed positive impact of TA-modified biochar on microbial biomass could be due to the micropores created by the biochar that may protect the bacteria, improving their habitat (Azeem et al. 2016). In addition, the high microbial biomass after the application of fresh biochar, could be due to the supply of small amounts of organic substrates, that might prompt a trigger response to enhance microbial biomass (Guenet et al. 2010).

Biochar, being a source of organic material, changed soil physical and chemical properties, also impacting soil enzyme activities of β-glucosidase catalyses the hydrolysis of maltose and cellobiose and is widely distributed in the soil (Utobo and Tewari 2015), while urease is involved in the hydrolysis of urea and represents a key regulator of the nitrogen cycle in the soil. For these reasons, both β-glucosidase and Urease activities are widely used as soil quality indicators. In the present study, the enzyme activities were improved by the application of TA-modified biochar, as biochar brings new carbon and nitrogen in soil, influencing the enzyme activities related to C and N cycles (Khadem and Raiesi 2017). The positive impact of TA-modified biochar was particularly evident on urease activity, as it has been demonstrated that adding biochar to soil increases the activities of enzymes related to N utilization (Bailey et al. 2011). Besides to constitute a source of organic material, biochar, once added to the soil, results in increased surface carboxyl groups, with a great ability to absorb cations (Cheng et al. 2008). The sorption capability of biochar may positively impact enzymes (and consequently their activity) by sorbing toxic cations that usually inhibit their activities (Huang et al. 2023). In fact, in the present study, microbial biomass and activities are mainly favoured by TA-modified biochar, probably due to the high amount of functional chemical groups capable to retain Cd whereas Pb is more uptakes by plants, so making these metals less available for soil microorganisms.

Cd and Pb uptake and their toxic effects on plant

The growth of lettuce plants on polluted soil amended with either unmodified biochar (B) or chemically modified biochar (TA-B) did not differ from that of plants grown on unamended soil (CTRL), except for the number of leaves, which was lower than in CTRL plants (Table 1). Plants grown on soil amended with TA-B exhibited a reduced Cd content compared to those grown on unamended soil or soil amended with unmodified biochar (Table 2). On the contrary, Pb was accumulated more in TA-B than in CTRL

Table 1 Biometrical determinations in control (CTRL) plants and in plants grown on soil amended with unmodified (B) and modified (TA-B) biochar

Treatment	Plant (g f.w. pl^{-1})	Shoot (g f.w. pl^{-1})	Root (g f.w. pl^{-1})	Leaves (g f.w. pl^{-1})	Stem (g f.w. pl^{-1})	Leaf area (cm^2)	n° green leaves	n° senescent leaves
CTRL	185 ± 15^a	169 ± 15^a	16 ± 1^a	156 ± 14^a	13.0 ± 2^a	2883 ± 256^a	57.0 ± 2^a	4.0 ± 1^a
B	191 ± 2^a	171 ± 2^a	20 ± 2^a	161 ± 2^a	10.0 ± 1^a	3333 ± 58^a	46.0 ± 1^b	4.0 ± 1^a
TA-B	177 ± 11^a	160 ± 10^a	17 ± 2^a	149 ± 9^a	11.0 ± 1^a	2899 ± 127^a	42.0 ± 3^b	4.0 ± 1^a

Data are means ($n = 4$) ± SE. Different letters denote statistically significant differences among treatments. $p < 0.05$

Table 2 Cd and Pb content in roots and leaves in control (CTRL) plants and in plants grown on soil amended with unmodified (B) and tannic acid-modified (TA-B) biochar. Data are means ($n = 4$) ± SE

Treatments	Cd			Pb		
	Root	Leaves	Total	Root	Leaves	Total
CTRL	14.0 ± 1.0^a	6.1 ± 0.4^a	20.1 ± 1.4^a	29 ± 2^a	3.4 ± 0.8^a	32.6 ± 1.6^a
B	12.0 ± 1.0^a	6.1 ± 0.5^a	18.5 ± 0.5^a	49 ± 7^b	2.0 ± 0.3^b	51.2 ± 7.1^b
TA-B	7.0 ± 0.9^b	6.2 ± 0.3^a	13.0 ± 1.2^b	41 ± 7^b	4.9 ± 1.0^c	45.6 ± 6.7^b

Different letters denote statistically significant differences among treatments. $p < 0.05$

plants, and this occurred also for B plants. However, in TA-B plants Pb content was higher in leaves while in B plants it was higher in roots (Table 2).

Plants grown on amended soil with modified biochar (TA-B) showed lowest values of maximum net photosynthetic rate (A_{Nmax}), photosynthetic pigments (Chl and Car) and photochemical quenching (qP) (Table 3). No significant difference in non-photochemical quenching (NPQ) among treatments was detected. The highest A_{Nmax}, as well as qP index was measured in plants grown on amended soil with unmodified biochar (B).

The growth on soil amended with biochar determined an increase in bioactive compounds such as anthocyanins and flavonoids which was more marked in TA-B plants (Table 3); on the contrary, total polyphenols dropped in plants grown on soil amended with biochar. A significant increase in the antioxidant capacity as well as in H_2O_2 content occurred in TA-B plants, whereas it was reduced in B plants as compared to CTRL (Table 3). The soluble proteins content also increased in TA-B compared to CTRL and B plants.

The analysis of correlations among leaf characteristics is reported in Table S4. Leaf Pb content resulted negatively correlated with A_{Nmax}, Q_{pp}, Chl and Car content, and qP, whereas it was positively correlated to NPQ, flavonoids content, and antioxidant capacity (Table S5). A_{Nmax} was positively correlated to Chl and Car content, and qP, but it was negatively correlated to flavonoids and soluble proteins content and antioxidant capacity. Both photosynthetic pigments (Chl and Car) were negatively correlated to leaf H_2O_2 content, as well as to flavonoids, soluble proteins, and antioxidant capacity. Moreover, they were positively correlated to qP and negatively to NPQ. Interesting it is the positive correlation found between soluble proteins and flavonoids, anthocyanins, and antioxidant capacity. No significant correlation between leaf Cd content and leaf physiological and biochemical parameters was found.

It appears that the treatment of biochar with tannic acid limits the plant uptake for Cd but not for Pb which is mainly translocated in leaves. Recently, Shahzad et al. (2023) found that the acidified biochar, by using H_2SO_4 as acidifying agent, was efficient in reducing root and shoot Pb content in *M. piperita* plants, resulting in an increasing shoot and root weight, shoot and root length, and shoot and root N, P, and K contents. Similar results have been found also by Hamzah et al. (2022) when used humic acid-coated biochar.

The biochar treatment with tannic acid modified the physical–chemical properties of amendment therefore reducing the biochar effectiveness to absorb lead and limit its uptake by plants. Consequently, Pb accumulated in the leaves of TA-B plants in amounts higher than in the other treatments with negative effects on photosynthesis. On the contrary, the untreated biochar (B) enhanced photosynthesis—as previously reported by other studies—resulting

Table 3 Maximum net photosynthetic rate (A_{Nmax}), chlorophylls (Chl), carotenoids (Car), anthocyanins (Antho), flavonoids (Flav), total polyphenols (TP), antioxidant capacity (FRAP), soluble proteins (SP), hydrogen peroxide (H_2O_2), photochemical quenching (qP), and non-photochemical quenching (NPQ) in control (CTRL) plants and in plants grown on soil amended with unmodified (B) and modified (TA-B) biochar

Treatment	A_{Nmax} (μmol m^{-2} s^{-1})	Chl (μg cm^{-2})	Car (μg cm^{-2})	Anthocyanins (μmol g^{-1} FW)	Flavonoids (mg CAT eq g^{-1} FW)	Total polyphenols (mg GA eq g^{-1} FW)	FRAP (μ mol Trolox eq g^{-1} FW)	Soluble proteins (mg eq BSA g^{-1} FW)	H_2O_2 (μ mol g^{-1} FW)	qP (a.u.)	NPQ (a.u.)
CTRL	16.6 ± 0.1^a	49 ± 2^a	8.5 ± 0.4^a	0.50 ± 0.04^a	12.0 ± 0.1^a	0.60 ± 0.01^a	0.80 ± 0.04^a	0.35 ± 0.02^a	0.11 ± 0.01^a	0.37 ± 0.00^a	1.87 ± 0.01^a
B	18.8 ± 0.7^b	48 ± 2^a	8.5 ± 0.3^a	0.60 ± 0.01^b	10.9 ± 0.4^a	0.20 ± 0.04^b	0.60 ± 0.04^b	0.27 ± 0.02^a	0.10 ± 0.00^a	0.44 ± 0.02^b	1.81 ± 0.04^a
TA-B	15.5 ± 0.7^c	36 ± 2^b	6.7 ± 0.2^b	0.90 ± 0.01^c	19.3 ± 0.3^b	0.30 ± 0.05^b	1.20 ± 0.04^c	0.65 ± 0.04^b	0.18 ± 0.02^b	0.30 ± 0.03^c	2.0 ± 0.01^a

Data are means ($n = 4$) ± SE. Different letters denote statistically significant differences among treatments. $p < 0.05$

in improved photochemical capacity of photosystem II, as indicated by the highest qP values. Recently, Ikkonen and Kaznina (2022) demonstrated that Pb concentration of 4 mg kg^{-1} in shoot biomass, comparable to values found in our study for the leaves, exerted a negative effect on photosynthesis in lettuce plants. Considering that plants grown on soil amended with TA-biochar also showed a reduced content in photosynthetic pigments, it is likely that the Pb translocation in leaves may have altered the synthesis of Chl and Car (Abdelhameed et al. 2024), contributing to the reduction of A_{Nmax}. Carotenoids, in addition to light harvesting, are also involved in the thermal dissipation of the excess of absorbed light acting as photoprotective mechanisms of photosynthetic apparatus. Indeed, they are involved in the quenching of Chl triplet and scavenging of oxygen singlet and other reactive oxygen species (ROS) (Pospíšil 2012). It is noteworthy that the reduction of carotenoid pool under limiting CO_2 assimilation conditions, as found in the present study, limits the thermal dissipation processes, and favours the reactive oxygen species synthesis, such as H_2O_2, which increased of 65% in TA-B plants compared to the other treatments. ROS overproduction can negatively affect the photosynthetic apparatus if the antioxidant defences fail to keep ROS concentrations at levels below the dangerous threshold. Although the reported results demonstrated that the soluble antioxidants, such as anthocyanins and flavonoid, and the antioxidant capacity (FRAP) significantly increased in TA-B plants, it is supposed that the level of antioxidant system was inadequate to keep ROS below dangerous levels, thus compromising the photosynthetic apparatus functionality and, in turn, the photosynthetic rates. Generally, Pb accumulation also determines a decrease in soluble proteins (Xia et al. 2019); conversely, in the present study an increase in soluble protein content was found which could be the result of the induction of stress proteins including antioxidant enzymes (Lamhamdi et al. 2013) or the result of phytochelatins production for favouring Pb detoxification (Schat et al. 2002).

In this research, it has been also found that Pb accumulates in the roots to a greater extent than shoots confirming that only a small part of absorbed Pb is translocated to aerial parts of plants (Ikkonen and Kaznina 2022). It is hypothesized that root endoderm barrier may have limited the transport of Pb from roots to leaves (Sharma and Dubey 2005). However, soil contamination did not affect the plant biomass. The low effect of lead and cadmium on plant biomass found in this study indicates that the plants were not strongly stressed—nevertheless Cd is present in a greater extent than the legal limits—likely because the fraction available for these metals was low.

Conclusion

The treatment with tannic acid altered the physical–chemical characteristics of biochar improving the biochar capability to absorb Cd and worsening that for Pb, partially confirming our hypothesis. Biochar application improved the C concentration in soil, regardless the kind; by contrast the tannic acid modified biochar exerted a positive impact on microbial biomass and activities. Plants grown with tannic acid modified biochar accumulated more Pb in the leaves than the untreated biochar, inhibiting the photosynthetic pigment production and in turn the photosynthetic capacity. This issue caused an increase in H_2O_2 content and an enhancement in the antioxidant compounds as response to the Pb accumulation stress. The results found in this study demonstrate that the treatment of biochar with tannic acid is a suitable for reclamation of polluted soils by Cd but not by Pb because tannic acid treatment enhances the oxygen-containing functional groups but reduce the biochar porosity, likely an effective sorption mechanism for Pb by biochar. Future studies should focus on understanding the reasons behind the reduction in porosity induced by tannic acid treatment.

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Author contributions Luca Vitale conceptualized and designed the study. Gaetano De Tommaso, Fulvio De Paola, Giuseppina Roviello, Elena Chianese, Claudio Ferone, Mauro Iuliano carried out the soil chemical and plant analysis, biochar modification and characterization, and conducted the data statistical analysis and interpretation. Lucia Santorufo, Giuseppe Maglione, Luca Vitale, Maria Riccardi and Giulia Maisto carried out the soil biological analysis and conducted the statistical analysis and interpretation. Giulia Costanzo and Carmen Arena carried out the biochemical analysis on plant samples and conducted the statistical analysis and interpretation. Luca Vitale and Carmen Arena performed physiological measurements on plants. Lucia Santorufo and Luca Vitale wrote the draft of manuscript. Franca Polimeno supervised the statistical analysis. All authors revised the final version and approved manuscript.

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Data availability The data will be available on request by corresponding author.

Declarations

Conflict of interest Authors have no conflicts of interest to declare. All co-authors have seen and agree with the contents of this manuscript and there is no financial interest to report.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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