Effect of biochar amendment on mobility and plant uptake of Zn, Pb and Cd in contaminated soil

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Abstract

This study aimed to assess the effect of rice straw biochar application as a soil amendment on the mobility, availability, speciation and plant uptake of Zn, Pb and Cd in contaminated soil. A pot experiment with maize (Zea mays L.) was conducted using different rates 0, 1, 2, and 5% (w/w) of rice straw biochar. The soil pore water properties; pH, EC, and DOC concentration, the dissolved metal concentrations in soil pore water as well as plant metals uptake were determined at the end of the experiment. The BCR sequential extraction procedure was adopted to determine the effect of biochar on speciation and partitioning of the studied metals.

Results showed that the application of biochar is significantly increased the plant shoots biomass by 94.5% with 5% biochar rates compared to untreated soil. Similarly, the soil pore water properties pH, EC, and DOC concentration were also increased with biochar addition compared to untreated soil. The dissolved metal concentrations were decreased in soil pore water with the increasing of biochar rates by 92%, 81.5%, and 90% for Zn, Pb and Cd, respectively at 5% biochar rate. In the same trend, the plant metals uptake reduced significantly with the increasing of biochar dose. Compared to untreated soil, the BCR sequential extraction showed that the biochar addition induced the transformation of the exchangeable metal fractions to oxidizable and residual fractions. These results confirmed the ability of rice straw biochar to immobilize the studied metals and therefore reducing their bioavailability and their uptake by plant.

Keywords: Biochar, heavy metals availability, plant metal uptake, BCR sequential extraction.

1. Introduction

In the past few decades, the contamination of soils by heavy metals became a serious issue around the world due to their seriously threatening the eco-system [1]. The anthropogenic activities, such as mining and smelting activities, chemical fertilizers, wastewater irrigation and sewage sludge are considered as the most important sources of heavy metals that introduced to soils [2]. Depending on their biological role in eco-system, the heavy metals were classified into two categories; essential metals such as Cu, Zn, Fe and Mn, and non-essential metals such as Hg, Pb, and Cd. These metals occurred naturally in low concentration, the increasing of their concentrations above the threshold limits will poses a serious threat to humans and animals through the accumulation of these metals in food chain [3,4]. The adverse effect of the heavy metals become from the fact that unlike organic matter, these metals are non-degradable, non-destroyable contaminants and do not stabilized in the contaminated sites, but can move to the surrounding environment (soil or ground water) via wind erosion or leaching [5].

It is well known that the total metals content dose not refers to their toxicity and accessibility to plants, whereas the metal phytoavailability refers to the available metal content which considered available or can be absorb by plants. As a result, the remediation of the contaminated soils from heavy metals has become an important objective to rehabilitate the contaminated soils for safe food production. Despite the conventional remediation techniques such as soil washing, dig, dump and solidification and stabilization have been used for a long time, these methods in fact have some limitations: very expensive, disruptive to soil and not a practicable methods [6].
The alternative technique for remediation and rehabilitation of contaminated soils is the phytoremediation or also called "green remediation" which defined as the use of plants to remediate the heavy metals contaminated soils.

Biochar is an organic porous carbon-rich material resulting from the burning of agricultural biomass under oxygen-limited conditions. Due to its functional, sorptive, low density, higher pH and CEC characteristics, recent studies have demonstrated its effectiveness role in remediation of the metal contaminated soils by reducing the heavy metal concentrations in both soil solution and plant organs [7][8]. In addition, the biochar has the ability to increase the cation exchange capacity of the soil and metals by forming stable complexes with the cationic metals [9]. In fact, although many studies have been focused on the effect of biochar on heavy metals immobilization as well as its availability [10][11][12]): further studies are also required to investigate and understand its potential role into a wide range of polluted soils with different physico-chemical properties.

To our knowledge there are a little studies have been investigated on the effect of biochar on the soil pore water (SPW): therefore, the aim of this study was to evaluate the: (i) effect of biochar application on soil pore water properties: pH, EC, and dissolved organic carbon (DOC) concentration. (ii) efficiency of biochar to immobilize Zn, Pb and Cd and therefore reducing their availability and uptake by maize (Zea mays L.) grown in spiked contaminated soil. (iii) effect of biochar application on speciation and re-distribution of the studied heavy metals.

2. Materials and methods

2.1 Soil sampling and analysis

The top 0-20 cm depth soil samples (control soil) were collected with a stainless steel spade from a garden soil located at Jadidat Al- Shat (Diyala – Iraq) which never cultivated or treated before with sewage sludge, wastewater or animal manure. Soil samples were transferred to laboratory of analytical chemistry (University of Technology) with clean polyethylene bags, dried at room temperature for 72h, homogenized, sieved through 2mm sieve.

The physico-chemical properties of the studied soil were characterized in laboratory depending on standardized procedures (AFNOR 1999 and ISO 1999). The method of loss of weight on ignition (LOI) was applied to determine organic matter. The calcium carbonate was determined by titration method [13]. Cation exchange capacity (CEC) was measured according to Aran et al. [14] by using 0.05N cobalt–hexamine method. Total metal concentrations were determined according to Zhang et al. [15] by using aqua regia/hydrofluoric acid digestion, and then determined by flame atomic absorption spectrometer (Perkin Elmer Analyst 400, USA). pH and EC were measured with distilled water (1:2.5 w/v) according to (NF ISO 10390 (2005)) using a combined pH–EC meter (WTW, ProfiLine 1970i; Germany) calibrated with standard solutions of pH 4, 7 and 10. The main soil properties are presented in Table 1.

| Table 1: Main physico-chemical characteristics of the studied soil. | pH | 7.14 ± 0.1 |
|---|---|---|
| Electric conductivity (µs cm⁻¹) | 113 ± 7 |
| Cation Exchange Capacity (cmol⁺kg⁻¹) | 9.4 ± 0.41 |
| Organic matter (%) | 8.3 ± 0.23 |
| CaCO₃ (%) | 23.4 ± 0.11 |
| Total metal concentration (mg.kg⁻¹) : Zn | 9.43 ± 0.62 |
| Pb | 4.87 ± 0.21 |
| Cd | 0.87 ± 0.02 |
2.2 Soil samples preparation and biochar
The selected soil was spiked with 600 mg kg\(^{-1}\) of Zn as ZnCO\(_3\), 500 mg kg\(^{-1}\) of Pb as PbCO\(_3\), and 100 mg kg\(^{-1}\) of Cd as CdSO\(_4\). The spiked soils were watered twice a week for one month to equilibrate. The soil samples were treated with 0%, 1%, 2% and 5% (w/w) of biochar (named as RS0%, RS1%, RS2%, RS5%): and placed in plastic pots, watered daily with deionized water to reach 80% of water holding capacity. They were left for one month to equilibrate. The biochar used in the current study was produced by continuous slow pyrolysis of rice straw at 500°C for 3h, cooled to room temperature, ground then sieved through 2mm sieve. The main physico-chemical properties of the rice straw biochar were presented in Table 2.

Table 2: Main physico-chemical characteristics of the rice straw biochar

| Characteristic                               | Value |
|----------------------------------------------|-------|
| pH (H\(_2\)O)                                | 9.96  |
| Electric conductivity (\(\mu\)s cm\(^{-1}\)) | 172   |
| Cation Exchange Capacity (cmol kg\(^{-1}\))  | 44.6  |
| Total Nitrogen (g kg\(^{-1}\))              | 17.4  |
| Total Carbon (g kg\(^{-1}\))                | 493   |
| Total Hydrogen (g kg\(^{-1}\))              | 17.4  |
| Total phosphorus (g kg\(^{-1}\))            | 2.8   |
| Total organic carbon (%)                    | 63.7  |
| Surface area (BET) (m\(^2\).g\(^{-1}\))     | 36.2  |
| Ash (%)                                     | 41.3  |
| Surface alkalinity (cmol.kg\(^{-1}\))       | 151   |
| Total metal concentration (mg.kg\(^{-1}\))  |
| Zn                                          | 104   |
| Pb                                          | 4.54  |
| Cd                                          | 0.02  |
| Cu                                          | 1.65  |

2.3 Pot experiment and analysis
Maize (Zea mays L.) seeds were sown in plastic pots filled with 3kg of prepared soils; the pot experiment was conducted in five replicates for control soil and for each amendment ratio. After two weeks, it thinned to two plants in each pot. The plant grew in a laboratory growth chamber under controlled conditions (20 – 25°C, 16h day/8h night, 500 \(\mu\)Em\(^{-2}\).s\(^{-1}\) of light intensity, and 65 – 80 % of water holding capacity).

After six weeks of growth, the plants were harvested, rinsed thoroughly with distill water, separated to roots, and shoots. The plant tissues were dried at 70°C in laboratory oven for 72h, powdered by a laboratory grinder, and then digested with a mixture of HNO\(_3\):HCl (1:3v/v). The metal concentrations in the digested materials were determined by flame atomic absorption spectrophotometer.

2.4 Soil pore water sampling and analysis
Rhizon soil moisture samplers (Rhizosphere Research Products, Wageningen, The Netherlands) were used to collect the soil pore water (SPW) in the current study. One rhizon was inserted into each pot at an angle of 45°, the pots were covered with parafilm to avoid the losses of water by evaporation. The SPW was collected twice, at the beginning and at the end of the experiment.

The collected SPW were taken directly to measure the pH and EC with (pH–EC meter (WTW, ProfiLine 1970i; Germany): the dissolved organic carbon (DOC) measured using an automatic carbon
analyzer (Shimadzu© TOC 5000A). Flame atomic absorption spectrometer was used to determine the dissolved metal concentrations in SPW.

2.5 Sequential extraction procedure
The 4-steps BCR sequential extraction procedure [16] was adopted to determine the distribution of the studied metals with and without amendments. This method involves four steps that related to four fractions, in the first step of this method of extraction which represents the acid-soluble fraction, 1g of dried soil was treated with 20mL of 0.1M of acetic acid, the second step which represents the reducible fraction (bound to Mn and Fe oxides) was performed using 20mL of 0.5M of NH$_2$OH.HCl (pH=2). To determine the oxidizable fraction (bound to organic matter): 8.8M of 30% of hydrogen peroxide (H$_2$O$_2$, pH=2-3) followed by 1M ammonium acetate (pH= 2) was used. In the fourth step, a solution of aqua regia has been used to determine the residual fraction.

For all of steps, the soil samples were agitated with the reagents in 50-mL polyethylene centrifuge tubes using end-over-end shaking, centrifuged and the resulting supernatants were filtered with a 0.45µ filter paper, the metal concentrations in each fraction were determined by flame atomic absorption spectrometer.

2.6 Statistical analysis
The obtained results were analyzed with SPSS statistical package (version 21.0, USA). The reported values that represent the means of five replications are expressed with their standard error (±SE) and were compared with one way ANOVA and LSD test. Data were considered significant at P < 0.05. The effect of each biochar rates (0%, 1%, 2% and 5%) on studied soils was studied.

3. Results and discussion

3.1 Effect of biochar application on plant dry biomass
The dry weight of shoots and roots of maize (Zea mays L.) at the end of the experiment is presented in Fig.1. The maize biomass is significantly increased ($p < 0.05$) as biochar dose increased relative to untreated soil, which considerably varied with the variation of biochar dose. The highest maize biomass was noted at the application of 5% biochar followed by 2% and the lowest value was observed with 1% biochar compared to untreated soil.

Our results showed that the application of rice straw biochar increased plant shoots by 94.5%, 50.2% and 23% at 5%, 2% and 1% biochar rates, respectively. The results of enhancement of plant yield after biochar addition is consistent with that reported by many researchers [17,18]. The increased of maize biomass as a function of biochar increasing can be attributed to the effects of biochar on soil properties such as pH, improving of soil nutrients such as N, P, K, water retention and its effect on cation exchange capacity [19,20]. Compared to untreated soil, the increased of plant growth might be also attributed to the decreasing of heavy metals bioavailability after biochar addition by adsorbing heavy metals and therefore reduce metals plant uptake and phytotoxicity that induce the plant development [21], whilst in untreated soil, the lower soil pH, the non-improvement nutrients as well as the increased of metal bioavailability might cause phytotoxicity and resulting in low plant yield.

3.2 Effect of biochar application on soil pore water properties
The rice straw biochar application was affected the physico-chemical properties of the SPW, the effects of biochar addition on pH, EC, and DOC concentration were presented in Table 3. At the end of the experiment, it can be seen that SPW pH was increased significantly ($p < 0.05$) with the increasing of biochar dose, pH values were increased by approximately 2 units when amended with 5% biochar, 1.3 units with 2% biochar and by 0.8 units with 1% biochar addition compared to untreated soil.
Figure 1: Effect of biochar on plant dry biomass grown in contaminated spiked soil, values are mean ± SE (n=5): letters represented the differences between untreated soil and biochar treated soil.

| Biochar dose | pH   | aEC(µs.cm⁻¹) | bDOC(mg L⁻¹) |
|--------------|------|--------------|--------------|
| RS 0%        | 6.13 | 113          | 8.23         |
| RS 1%        | 6.91 | 204          | 14.55        |
| RS 2%        | 7.46 | 432          | 18.13        |
| RS 5%        | 8.61 | 718          | 21.26        |

aEC: Electrical conductivity  
bDOC: Dissolved organic carbon

Our results are in agreement with other researcher studies, Lebrun [12] noted that pinewood biochar addition increased SPW pH by more than 2 units, Beesley [22] also found that SPW pH was increased when the soil was treated with various types of amendments (biochar and alperujo compost). In addition, Jones [23] confirmed these findings by reported that the addition of 1-3% (w/w) biochar was enhanced pH value in Cu contaminated soil. The increased of pH value after biochar application is attributed to: i) the alkaline nature of biochar content, ii) the pyrolysis process converts some of ions such as Mg²⁺, Ca²⁺, and K⁺ that already existed in some compounds content to hydroxides or to oxides, and because of their incorporation into char and contact with water, the pH will increased as a result of dissociation and OH⁻ release.

The SPW pH of the untreated biochar soil was also increased but still much lower compared to biochar treated soil, it was increased by 0.3 units compared to initial SPW pH value. This increasing in SPW pH value can be attributed to the uptake of anions by plant in different rates to maintain the electrical neutrality within root-soil interface and to balance the excess of the negative charges taken by plant roots [24,25] or might be as a result to the changes in Ca²⁺ concentration [26].

The same trend was also observed for EC value, the SPW EC was increased as a biochar dose increase (Table 3). The highest EC value was noted with the application of 5% biochar, it increased by six times compared to untreated soil, followed by four times with 2% biochar, and the lowest value was
obtained with 1% biochar addition. The increasing of EC as a result of biochar addition can be correlated to the pH increases that enhance the dissolution of the soil nutrients and salts or through the accretion of the ash [27]. Our results were in agreement with that of Lomaglio [28] who reported that 2% and 5% biochar dose stimulated both of pH and EC in soil pore water in polluted mine technosols. The current study confirmed that the application of the different dose of rice straw biochar increased significantly ($p < 0.05$) the SPW DOC concentration compared to untreated soil (Table 3). The greatest DOC concentration was observed with 5% biochar addition, it increased by 2 times compared to untreated soil, the lowest SPW DOC was noted with 1% biochar dose.

In the literature, Cao [29] and Lin [30] are well noted that the organic amendments as biochar are capable to increase DOC concentration in soil. In contrast to these conclusions, Karami [31]; Jones [32] and Clemente [33] reported that no significant changes were observed on DOC concentration after biochar application in their field or pot studies. The increased of DOC concentration after biochar addition can be attributed to the increase of microbial activity in biochar treated soil. In addition, Kalbitz [34] noted that the increased of pH value after biochar addition will affect the organic matter functional groups (carboxyl and hydroxyl groups) which leads to increase the negative charge and reduce the DOC sorption to soil matrix. In the untreated soil, an increasing in DOC concentration was also observed but it remained lower than biochar treated soil, this can be explained as a result of root exudates as well as the solubilization of the soil organic matter caused by microbial activity [35][36].

### 3.3 Effect of biochar application on dissolved metal concentrations

The dissolved Zn, Pb, and Cd concentrations in SPW are shown in Fig. 2. The initial concentrations of the studied metals in SPW before biochar treatment were 462µg.L$^{-1}$ for Zn, 281µg.L$^{-1}$ for Pb and 158µg.L$^{-1}$ for Cd. As can be seen in Fig. 2, the application of rice straw biochar showed a significant effect on the reduction of dissolved Zn, Pb, and Cd concentrations in SPW. At the end of the experiment, the decreasing of SPW metal concentrations was related to the biochar rates, the highest reduction was noted with the application of 5% biochar which reduces the dissolved metal concentrations by 92%, 81.5%, and 90% for Zn, Pb, and Cd respectively.

Our results were in agreement with that of Lu [37] who reported that rice straw biochar showed a strong metals adsorption which leads to reduce metals pore water concentrations. Huang [38] also reported that rice straw ash application was significantly decreased the available Cu in SPW of a rice paddy contaminated soils. In the same manner, Yang [39], confirmed these results by noted that the addition of rice straw biochar reduced the SPW metal concentrations as a function of biochar dose application.

It is also well reported that the decrease of dissolved metal concentration in SPW is related to the higher pH value as well as the total carbon of the used biochar [40,41] which plays an important role on the heavy metals retention by increasing the capacity of soils for metal sorption and therefore influence the metal solubility [42]. The [5] confirmed these findings by reported that the application of biochar reduced the extractable concentrations of Zn, Pb, and Cd with DTPA as a result of pH increasing that induce the metal precipitation, and therefore reduce heavy metals solubility.

In general, the reducing of the SPW metal concentration was improved by biochar treatment that related to biochar physico-chemical properties such as functional groups, higher pH value as well as microporous biochar structure that induce the biochar capacity for adsorbing heavy metals [43]. Several mechanisms concerning the ability of biochar to immobilize heavy metals have been reported [44,45,46], these mechanisms included: formation of stable complexes between metals and biochar functional groups (COOH and OH): formation of biochar-metals complexes via ion exchange process, and formation of biochar-metals inner sphere complexes.

### 3.4 Effect of biochar application on the accumulation of heavy metals in plant tissues

The changes in the studied metal concentrations in plant tissues as affected by various biochar rates are presented in Fig. 3. As can be seen in Fig. 3, the highest metal concentrations reduction was noted with the higher biochar rate (5%): whilst the lowest reduction was noted with the lower biochar rate (1%). Compared to untreated soil, the addition of biochar at different rates was significantly decreased ($p < 0.05$) the SPW metal concentrations compared to untreated soil, this can be explained as a result of root exudates as well as the solubilization of the soil organic matter caused by microbial activity [35][36].
0.05) Zn concentration in plant shoots by 64.5%, 24%, and 11% at 5%, 2% and 1% biochar rates respectively. The Zn concentration was also reduced in plant roots as a result of biochar application by 65%, 32%, and 29% with 5%, 2% and 1% biochar rates respectively.

Similarly, the biochar addition decreased ($p < 0.05$) the Pb concentration in both plant organs by 39%, 34%, and 13% in shoots, and by 44%, 31%, and 14% in roots at 5%, 2% and 1% biochar rates respectively. The same trend was also observed with Cd concentration in plant tissues. The Cd concentration reduced ($p < 0.05$) by 82%, 42%, and 23% for plant shoots, and by 83%, 66%, and 31% for plant roots at 5%, 2% and 1% biochar rates, respectively. These results indicating a good efficiency of biochar in reducing the metals plant uptake from the soil even at a low biochar rate.

Our results were in agreement with that of Zheng [47] who reported that the application of 5% straw biochar resulted in great reduction in Pb and Cu concentrations in rice plant shoots. Similarly, Sizmur [48] also noted that a significant decrease ($p < 0.05$) in Zn, Pb and Cd concentrations in Sedum plumbizincicola X. tissues was also observed after biochar treatments. In the same pattern, Namgay [49] also reported that the concentration of Cd, Pb and Zn were significantly reduced in maize (Zea mays L.) as a result of wood biochar application to soil.

It is well reported that the reduction of the plant metal concentrations is corresponded to the decreasing of extractable metals concentration measured by various chemical extractants such as DTPA, CaCl$_2$, and NH$_4$NO$_3$ or to their dissolved concentrations reduction in soil pore water. This reduction can be attributed to the both of biochar adsorption capacity as well as the dilution effect that related to the increasing of plant yield [42].

Our results were in consistent with these studies; the application of rice straw biochar reduced the dissolved studied metals bioavailability in soil pore water as well as in acid soluble metal fraction (BCR sequential extraction (section 3.5)) by transforming the readily available metal fractions to unavailable residual metal fractions [50]. This process will reduce the heavy metals mobility and their bioavailability and therefore reduce their concentrations in plant tissues by reducing the heavy metals translocation.
from plant roots to plant shoots. Zhang [51] and Chen [52] reported that the application of wheat straw biochar, oil mallee plants biochar and wheat chaff biochar were decreased the translocation of Cd from root to shoot in rice and Juncus subsecundus plants.

The large biochar surface area induces the heavy metals affinity to form stable complexes onto its surface functional groups either by adsorption process or by ion exchange process [53,9], in addition, the mineral biochar components such as phosphate and carbonate will contribute in co-precipitation of heavy metals in form of phosphate or carbonate that reduce the metal mobility [42,54]. In the one hand, the reducing of studied metal concentrations in plant tissues can be also related to the higher Si concentration in rice straw biochar, this was improved by Li [55] who reported that the used of Si rich amendments such as Na₂SiO₃·9H₂O was reduced the plant metal uptake as a result of the metal bioavailability decreasing.

In the current study, based on what was mentioned above, the reduction of the studied metal concentrations in plant tissues after rice straw application could be related to (i) adsorption of Zn, Pb, and Cd on biochar surface, (ii) precipitation of these metals as CdCO₃ or Pb₅(PO₄)₃OH as a result of pH increased, and (iii) the high Si content in rice straw biochar played an important role in reducing the metal plant uptake [49,56,57,58,59]. Therefore, the biochar application in contaminated soils helps to reduce metals bioavailability and therefore reduce its toxicity for plants.

3.5 Effect of biochar application on heavy metals speciation

The results of the metal speciation that obtained from the BCR sequential extraction procedure are presented in Fig. 4. For control soil, it can be seen that the reducible fraction was predominant for both Zn (36%) and Pb (47%) of the total content, followed by the exchangeable fraction which represented about 28% for Zn and 22% for Pb of the total content. Cd was distributed mainly in exchangeable fraction which represented 43% of the total content, followed by reducible fraction which accounting 39% of the total content. For all the studied heavy metals the bound to organic matter and residual fractions were lesser.

After the biochar application, the distribution of the studied metals changed obviously, the Zn and Pb reducible fraction decreased by 61% and 68% respectively with 5% biochar rate, whereas, their exchangeable fractions were reduced by 39% and 32%, respectively. Likewise, Cd exchangeable fraction was reduced by 76% with 5% biochar rate. In the same manner; the Cd reducible fraction was also decreased by 46% of total amount. In contrast, the oxidizable and residual metal fractions were increased after the application of rice straw biochar.

Our results are in the same line with Jiang [60] who reported that the application of the biochar was significantly reduced the acid soluble fraction of Pb and Cd. It’s also in agreement with that obtained by Zhu [61], who noticed that the wine lees-derived biochar application transformed the exchangeable Pb and Cd to more stable fractions (oxidizable and residual fractions). Similarly, to our results, Jiang and Xu [62] found that the biochar addition was significantly increased the oxidizable fraction that represent the metals bound to organic matter fraction which indicates that biochar application induces the heavy metals stabilization or immobilization in contaminated treated soils.

**Figure 4:** Effect of biochar on Zn, Pb, and Cd speciation (F1: Exchangeable fraction, F2: Reducible fraction, F3: Oxidizable fraction and F4: Residual fraction).
Therefore, the rice straw biochar had the capacity to reduce the exchangeable heavy metal contents and induce the transformation of the exchangeable metal fraction to oxidizable and residual fractions which leads finally to decrease the heavy metals mobility in the studied contaminated soil.

The heavy metals in soil existing in different fractions, their mobility, availability and their toxicity are depends on these fractions. According to the BCR sequential extraction procedure, these fractions were classified as: (i) acid soluble fraction (exchangeable); (ii) reducible, (iii) oxidizable, and (iv) residual fraction [63]. The exchangeable fraction is considered as the more available fraction that represents the amount of the metals released to the soils when the conditions is acidic and directly absorbed by living organisms, this fraction is the more toxic fraction for the environment. The reducible fraction represents the metal content that bound to manganese and iron hydroxides which released under reducing conditions, these oxides are considered as a sinks for heavy metals in the environment and are unstable. The oxidizable fraction represents the amount of heavy metals that bound to organic matters; these metals can be release to the environment under oxidative conditions, it consider as a slow release fraction compared to the exchangeable fraction since it bound to the solid matrix. The residual fraction represents the amount of the heavy metals that bound to the minerals crystalline structures and hardly absorb by living organisms, it considers as the more difficult to extract [64][65].

Yang [66] noticed that the reduction of the exchangeable studied metals accompanied by an increase in oxidizable and residual fractions can be attributed to the effect of biochar on soil physico-chemical properties which therefore change the soil metal speciation. Similarly, Ahmad [59] and Xu [67] also reported that the transformation of the exchangeable heavy metals fraction to the oxidizable and residual fractions is related to the increasing in soil pH as well as the organic matters after biochar application, these results were confirmed by Mohamed [68] who reported that the high exchangeable heavy metals content is related to the low pH value in the moderate acidic contaminated soil.

The decreasing in the exchangeable heavy metals content can be attributed to the formation of metal silicate and metal carbonate as a result of the increasing of SiO$_3^{2-}$ and CO$_3^{2-}$ in soil pore water after biochar addition [69]. In addition, Rinklebe [70] reported that the reduction of the exchangeable heavy metals content can be attributed to the complex formation between heavy metals and the biochar hydroxyl functional groups. The capacity of biochar to improve the aeration structure of the treated soils, increasing the microorganisms metabolism as well as the soil aggregates formation were helped also to induce the transformation of the exchangeable heavy metals fraction to the oxidizable and residual fractions [71]. The higher pyrolysis temperature as well as the feedstock composition of the rice straw biochar compared to the other types makes it as an effective biochar to reduce the heavy metals solubility [72,73].

4. Conclusion
Our results showed that the application of rice straw biochar has the potential to reduce the Zn, Pb and Cd mobility and their availability in contaminated spiked soil. Compared to untreated soil, the addition of biochar increased plant dry biomass; a significant change was observed in the soil pore water properties including an increase in pH, EC, and DOC concentration after biochar addition. The concentrations of the dissolved studied metals decreased significantly in soil pore water with the increasing of biochar rates especially with 5%.

In the same manner, the biochar application decreased significantly the studied metal concentrations in plant tissues, thereby reducing the metals acid soluble fractions and induces the transformation of the studied metals to more stable oxidizable and residual fractions. Therefore, the addition of biochar can be used to enhance the immobilization of the studied metals in contaminated soil and therefore reduce their availability and improve plant growth.

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