

# Comparison of Pb stabilization in a contaminated calcareous soil by application of vermicompost and sheep manure and their biochars produced at two temperatures

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## ABSTRACT

Soil contamination with potentially toxic elements (PTEs) is increasing due to human activities including the widespread use of chemical fertilizers, sludge and industrial wastewater in agriculture. In situ stabilization of soil PTEs using organic amendments is a cost effective and efficacious method. A laboratory experiment was conducted to investigate the effect of two organic materials (sheep manure (SM) and vermicompost (VC)) and their biochars (SMB and VCB) produced at two pyrolysis temperatures (300 and 500 °C) at 2 wt. % on Pb immobilization in a contaminated calcareous soil. A sequential extraction procedure, mobility factor and release kinetics were used for evaluating efficiency of the amendments. Addition of the biochars resulted in a significant decrease in exchangeable (10.4–19.6%) and carbonate (3.8–10.5%) forms of soil Pb, while Pb bound to amorphous Fe oxides (4.3–52.9%) was increased. The application of VCB produced at 500 °C resulted in the greatest mobility factor reduction (11%), likely due to its characteristics such as high pH (10.5), high P (0.39%) and ash content (67.7%). Two first-order reactions model best fitted the Pb release data extracted by EDTA, indicating Pb release took place from two sections of the soil with different adsorption and solubility properties. The lowest amount of Pb extracted in Q<sub>1</sub> and Q<sub>2</sub> phases were observed in VCB (115.2 mg kg<sup>-1</sup>) and SMB (105.5 mg kg<sup>-1</sup>) produced at 500 °C treatments, respectively. Generally, the effect of biochars produced at higher temperature (500 °C) on the soil Pb stabilization was greater than those produced at lower temperature (300 °C), while the feedstocks (SM and VC) had no significant effect in stabilizing soil Pb.

## 1. Introduction

There are many reports of soil contamination with lead (Pb), especially in areas close to mines and battery factories (de Andrade Lima and Bernardez, 2011; Ogundiran and Osibanjo, 2009). High concentrations of soil Pb can disrupt the physiological activity of plants by altering soil microbial nutrient cycles (Zeng et al., 2006). Furthermore, high levels of soil Pb and its entry into the human food cycle can have a negative impact on the nervous system, reproduction, metabolism and behavior of humans (Ogundiran et al., 2015).

Several innovative ways have been designed to remediate contaminated sites with potentially toxic elements (PTEs), but most of them are very expensive, time-consuming and costly (Derakhshan-Nejad and Jung, 2017; Derakhshan-Nejad et al., 2017). One of the promising ways to remediate soils polluted with PTEs is in situ stabilization (Sengupta, 2007). Simplicity, speed, high public acceptance,

low cost and coverage of a wide range of inorganic contaminants are the advantages of in situ stabilization procedure (Martin and Ruby, 2004). During the stabilization process, PTEs in the soil are removed from the soil solution through adsorption, complexation and sedimentation reactions, and ultimately, are prevented from plant uptake or leaching into groundwater (Bolan and Duraisamy, 2003).

Recently, biochars have been widely used as amendments for the stabilization of PTEs in the soil (Boostani et al., 2018a). Biochar is a carbon-rich material produced by pyrolysis (300–700 °C) of organic matter (wood chips, plant debris and organic manure) under limited oxygen conditions (Klinar, 2016). Due to high surface area, oxygen functional group (hydroxyl, carboxylic and phenol) content, and porous structure, biochars can be applied as effective and environmentally friendly adsorbent material for immobilizing PTEs in aqueous, soil and sedimentary environments (Yang et al., 2016). The efficacy of biochars in stabilizing PTEs in the soil depends on the porosity, chemical

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composition, pyrolysis temperature, duration of pyrolysis and the type of raw material (Lahori et al., 2017).

Determination of PTE fractions in the soil solid phase and the PTE release kinetics can be used to evaluate the stabilization efficiency of soil amendments (Boostani et al., 2018a; Jalali and Tabar, 2013). Derakhshan-Nejad and Jung (2017) studied the effect of application of maple tree leaves and rice husk biochars (produced at 550 °C) at different levels (0.5, 1 and 2 wt. %) in a PTE contaminated soil and reported that the use of biochars caused a significant reduction in the leaching of Cd, Pb, Cu and Zn from soil profiles. In addition, the uptake of Cd, Cu, Pb and Zn by plants decreased by 66–79%, 13–19%, 87–86% and 37–36%, resp., in biochar treatments. They indicated that the main mechanism of biochars in decreasing the availability of soil PTEs was the increase of PTEs in organic and carbonate forms. Meier et al. (2017) reported that the addition of poultry manure biochar to a polluted soil significantly reduced the concentration of exchangeable Cu, while significantly increasing the concentration of residual and organic Cu fractions. Dume et al. (2016) showed that the concentration of soluble and exchangeable Pb was significantly reduced, while the concentration of Pb bound to carbonates, Fe and Mn oxides, organic materials, and minerals was increased by application of coffee bran biochar (produced at 500 °C) at 15 ton ha<sup>-1</sup> in a contaminated soil for 90 days.

Given the fact that the type of raw materials and pyrolysis temperature are the most important factors in determining biochar characteristics (Tang et al., 2013), it was hypothesized that biochars produced at different pyrolysis temperatures from sheep manure and vermicompost could have different effects on soil Pb stabilization. Vermicomposting is currently considered a practical and economic method of dealing with urban organic wastes, turning it into fine, peat-like composts (Sharma and Garg, 2018). Sheep manures are also widely available, N-rich organic materials. Therefore, the purpose of this study was to investigate the effect of two organic materials (sheep manure and vermicompost) and their biochars produced at two different temperatures (300 and 500 °C) applied at a mixing ratio of 2 wt. % on the release kinetics and chemical forms of soil Pb in a Pb-contaminated calcareous soil. The efficacy of the different treatments in stabilization of soil Pb was compared via the two first-order reactions model and mobility factor. This is the first study to examine the effect of the vermicomposts and their biochars on Pb-stabilization in a calcareous soil.

## 2. Materials and methods

### 2.1. Biochar production and characterization

The sheep manure and vermicompost samples were obtained from local sources and then dried at 60 °C for 24 h, crushed and then passed through a 2 mm sieve. The biochars were produced using a slow-pyrolysis method under limited oxygen conditions (Sadaf et al., 2017). This involved taking 80 g of each raw material and placing it on a 250 mm glass sheet, covering it with thick aluminum sheets and tightening with a wire to create limited oxygen conditions. Pyrolysis of samples was carried out in an electric furnace at 300 °C and 500 °C for 4 h. The rate increase of the furnace temperature was about 10 °C per minute. After allowing the biochars to cool overnight, they were passed through a 0.5 mm sieve. The raw materials and biochars were characterized by

the following standard laboratory methods. The pH and electrical conductivity (EC) were determined in a suspension of 1:10 solid to distilled water, and the percentage of total carbon (C), hydrogen (H) and nitrogen (N) were measured by the CHN Analyzer (Thermo-Finnigan Flash EA 1112 Series). A dry combustion method and dissolution of resulting ash in 2N HCl was used to extract total phosphorous (P) and Pb (Ding et al., 2016). In the obtained extract, P concentration was determined colorimetrically (Page et al., 1982) and Pb concentration by atomic absorption spectroscopy (AAS) (PG990, PG Instruments Ltd. UK). Fourier-transform infrared (FTIR) spectroscopy (Shimadzu DR-8001) was used to identify functional groups. The percentage of ash was determined by a dry combustion method at 900 °C for 12 h (Boostani et al., 2018b).

### 2.2. Soil sampling and Pb-treatment

A composite surface (0–30 cm) sample of a calcareous soil from southern part of Iran was collected. After air drying and passing through a 2 mm sieve, some of the chemical and physical properties of the soil were measured by standard laboratory methods (Page et al., 1982). Soil samples (100 g) were placed in plastic containers. To each of the samples, 50 ml of aqueous solution containing Pb prepared from distilled water and 4.8 M lead nitrate salt (Pb(NO<sub>3</sub>)<sub>2</sub>) was added and mixed completely, so that the Pb concentration in the soil was 500 mg kg<sup>-1</sup>. After drying at room temperature, the samples were wetted to field capacity using distilled water, and then allowed to dry out at room temperature again. Successive wetting and drying of the Pb-treated soil samples was carried out five times, to achieve equilibrium and simulate field conditions (Boostani et al., 2018a).

### 2.3. Experimental design and soil incubation test

An experiment was conducted in a completely randomized design with seven treatments and three replications. Applied treatments included: sheep manure (SM), sheep manure biochar produced at 300 °C (SMB300), sheep manure biochar produced at 500 °C (SMB500), vermicompost (VC), vermicompost biochar produced at 300 °C (VCB300) and vermicompost biochar produced at 500 °C (VCB500) each applied at 2 wt. % and a control (CL) treatment (without the use of amendment). Lead-contaminated soil samples (100 g) were mixed with different treatments according to the experimental design. During the incubation period (70 days at room temperature), the moisture of the soil samples was maintained about field capacity with distilled water by daily weighing. After the incubation period, soil samples were air dried, gently crushed and then passed through a 2 mm sieve for chemical analysis. Statistical analysis of data and correlation coefficients were performed using SPSS 15.0 software. Comparison of means was done using Duncan test at 5% level.

### 2.4. Sequential extraction procedure and mobility factor

Soil Pb was fractionated by a sequential extraction procedure of Singh et al. (1988). The description of this method is given in Table 1. This procedure separates soil Pb into seven different forms including water soluble and exchangeable (WsEx), carbonate (Car), bound to

**Table 1**  
Sequential extraction procedure of Singh et al. (1988).

Chemical forms of Zn	abbreviation	Time of shaking (h)	Used extractant	Specific gravity (g.cm <sup>-3</sup> )
Exchangeable and soluble	WsEx-Pb	2	1 M Mg(NO <sub>3</sub> ) <sub>2</sub>	1.10
carbonatic	Car-Pb	5	1 M NaOAc (pH = 5)	1.04
organic	OM-Pb	0.5	0.7 M NaOCl (pH = 8.5)	1.00
Mn oxide	MnOx-Pb	0.5	0.1 M NH <sub>2</sub> OH, HCl (pH = 2, HNO <sub>3</sub> )	1.00
Amorphous Fe oxides	AFex-Pb	0.5	0.25 M NH <sub>2</sub> OH, HCl + 0.25 M HCl	1.01
Crystalline Fe oxides	CFex-Pb	0.5	0.2 M (NH <sub>4</sub> ) <sub>2</sub> C <sub>2</sub> O <sub>4</sub> + 0.2 M H <sub>2</sub> C <sub>2</sub> O <sub>4</sub> + 0.1 M C <sub>6</sub> H <sub>8</sub> O <sub>6</sub>	1.02

organic matter (OM), bound to manganese oxides (MnOx), bound to amorphous iron oxides (AFeOx), bound to crystalline iron oxides (CFeOx) and residual (Res). Lead content in Res form was calculated by the difference between the total amount of soil Pb and other extractable chemical forms (Kamali et al., 2011). Total Pb concentration in soil was also determined in the extract obtained from 2 g soil which was digested for one night with 12.5 ml of nitric acid 4 normal at 80 °C (Sposito et al., 1982). The Pb concentration in extracts was measured by AAS. It should be noted that Pb standards are prepared in solutions that are similar in composition and concentration to each of the sequential extracts.

The mobility factor of soil Pb was calculated using the Salbu and Krekling (1998) method.

$$\text{Mobility factor} = \frac{\text{Exchangable} + \text{Carbonate}}{\text{Sum of fractions}} \times 100$$

### 2.5. Two first-order reactions kinetic model

The Pb release kinetics study was performed using 0.01M EDTA solution with pH = 7 (Dang et al., 1994). This entailed adding 25 ml of EDTA solution to 5 g of treated Pb-contaminated (incubated for 70 days) soil sample and then shaking by a shaker incubator for 0.16, 0.5, 1, 2, 6, 12, 24 and 48 h at a temperature of  $22 \pm 2$  °C. After that, soil samples were immediately centrifuged at 4000 rpm for 5 min and filtered with Whatman filter paper 42. The Pb concentration in the extracted solution was measured by AAS. Then, Pb release data were fitted with a two first-order reactions kinetics model provided by Santos et al. (2010). This model divides soil Pb into three fractions ( $Q_1$ ,  $Q_2$ , and  $Q_3$ ):

$$q = Q_1 (1 - e^{-k_1 t}) + Q_2 (1 - e^{-k_2 t}), Q_3 = q_t - (Q_1 + Q_2)$$

Where  $q$  ( $\text{mg kg}^{-1}$ ) indicates the amount of Pb released at time  $t$ ,  $Q_1$  ( $\text{mg kg}^{-1}$ ), the labile Pb content or easy extractable fraction in relation to the release rate coefficient of  $k_1$ ,  $Q_2$  ( $\text{mg kg}^{-1}$ ), the less labile Pb or lesser extractable fraction in relation to the release rate coefficient of  $k_2$ , and  $Q_3$  ( $\text{mg kg}^{-1}$ ) also represents a non-extractable fraction which obtained from the difference between total Pb and sum of  $Q_1$  and  $Q_2$  forms. Suitability of this model was evaluated by standard error of estimate (SE) and coefficient of determination ( $r^2$ ). The parameters of this kinetic model were obtained through nonlinear fit of the release data by software Sigma plot 12.0.

## 3. Results and discussions

### 3.1. Properties of the soil and amendments

Some of the physical and chemical properties of the studied soil are shown in Table 2. The soil textural class was loamy. The soil was calcareous, non-saline and slightly alkaline reaction in nature. It also had low organic carbon (OC) content. The soil is classified as coarse-loamy, carbonatic, hyperthermic Typic Haplustepts according to the USDA Soil Taxonomy (Soil Survey Staff, 2014). The amount of soil available P was  $15 \text{ mg kg}^{-1}$  and the magnitude of cation exchange capacity (CEC) was

**Table 2**  
Selected physicochemical properties of soil sample before Pb treatment.

Property	Value	Property	Value
Soil texture	Loamy	Available-P ( $\text{mg kg}^{-1}$ )	15.0
pH	7.5	CEC ( $\text{cmol}(+) \text{ kg}^{-1}$ )	17.0
EC ( $\text{dS m}^{-1}$ )	1.0	CCE (%)	50.0
OC (%)	0.6	Total Pb ( $\text{mg kg}^{-1}$ )	bd

CEC = cation exchange capacity; EC = electrical conductivity; CCE = calcium carbonate equivalence; OC = organic carbon; bd = below detection.

**Table 3**  
Some chemical characteristics of applied amendments.

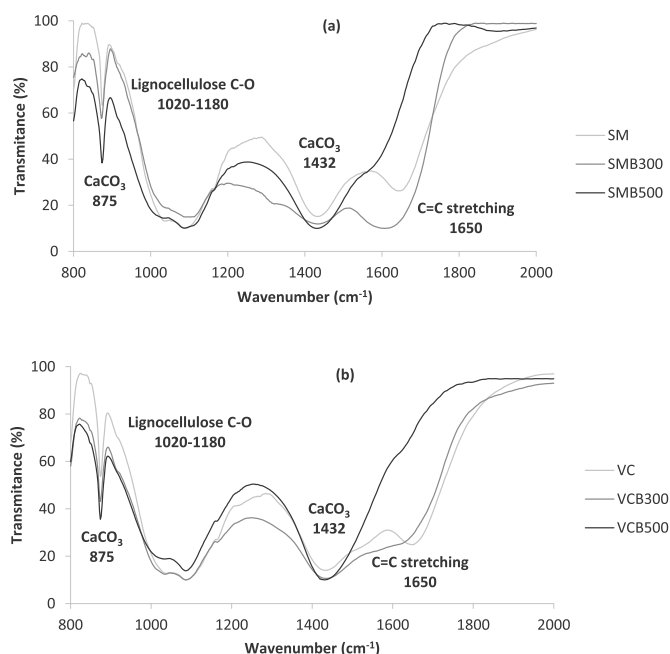
Property	SM	SMB300	SMB500	VC	VCB300	VCB500
Electrical conductivity (1:10) ( $\text{dS m}^{-1}$ )	3.94	7.22	9.24	1.34	2.01	3.15
pH (1:10)	8.04	10.25	10.40	8.09	9.78	10.50
Lead ( $\text{mg kg}^{-1}$ )	nd	nd	nd	nd	nd	nd
Phosphorous (%)	0.16	0.28	0.35	0.18	0.32	0.39
Carbon (%)	29.94	42.23	52.74	24.41	40.00	51.00
Hydrogen (%)	3.36	2.56	1.19	2.73	1.79	0.48
Nitrogen (%)	3.67	3.35	2.84	2.18	2.20	1.25
Ash content (%)	43.57	53.07	59.56	53.95	56.57	67.22
H:C mole ratio	1.35	0.73	0.27	1.34	0.53	0.11

SM = sheep manure; SMB300 = sheep manure biochar produced at 300 °C; SMB500 = sheep manure biochar produced at 500 °C; VC = vermicompost; VCB300 = vermicompost biochar produced at 300 °C; VCB500 = vermicompost biochar produced at 500 °C.

$17 \text{ cmol}(+) \text{ kg}^{-1}$ . Furthermore, the concentration of total Pb was lower than the detection limit by atomic absorption spectroscopy.

Characteristics of the applied amendments are given in Table 3. The EC values of the amendments increased as a result of pyrolysis and with increase in the pyrolysis temperature from 300 °C to 500 °C. The VC and its biochars contained less water soluble salinity than those of the SM and its biochars. The release of mineral elements such as K, Ca, Mg and P due to pyrolysis can increase the salinity of organic materials (Wang et al., 2017). In general, the ash content in the VC and its biochars were much higher than those of SM and its biochars, and increased with the increase of pyrolysis temperature. The pH values of amendments were also raised by pyrolysis and increasing of pyrolysis temperature, so that pH increased from around 8 in the raw materials to over 10 in the biochars produced at 500 °C. This pH increase could be attributed to the loss of surface acidic functional groups and increase in the ash percentage due to pyrolysis (Reeves et al., 2007). The P concentration in the VC and its biochars was slightly higher than that of the SM and its biochars, and P content increased with increasing pyrolysis temperature. The Pb concentration of the amendments was below the detection limit by atomic absorption. The C content was also increased due to pyrolysis and increasing pyrolysis temperature, while the H and N contents were decreased. This is also reflected by the substantial decrease in the H:C mole ratio of the amendments, a lower value indicates a greater degree of aromatic condensation and carbonization (Krull et al., 2009). Therefore the VCB500 was the most carbonized of the amendments. Claoston et al. also reported reduction of N content and an increase of basic cation content of rice rolls biochar with increasing pyrolysis temperature.

The FTIR results at the range of 800–2000  $\text{cm}^{-1}$  wavelength showed that the identified peaks for all the organic amendments were similar but, in term of peak intensity were different (Fig. 1). The most prominent peaks detected in the organic amendments were C-O bound associated with lignocellulose in the range of 1020–11180  $\text{cm}^{-1}$  (Keiluweit et al., 2010), calcium carbonate in peaks of 875 and 1432  $\text{cm}^{-1}$  (Bruckman and Wriessnig, 2013; Chen et al., 2008) and a C=C dual tensile bond at a peak of 1650  $\text{cm}^{-1}$  associated with lignin (Asim et al., 2015). As shown in the FTIR spectra, with an increase in the pyrolysis temperature, the peaks intensity of 875 and 1432  $\text{cm}^{-1}$  were increased, indicating a higher content of calcium carbonate. The higher pH values of the biochars produced at higher pyrolysis temperatures confirm this observation (Table 3). Also, the increase of pyrolysis temperature led to the substantial reduction of the lignin type C=C bonds (1650  $\text{cm}^{-1}$ ) biochars produced at 500 °C, which could also indicate enhanced formation of fused aromatic rings (like graphite) which are not visible in IR spectra (Francioso et al., 2011). This is also confirmed by the substantially lower H:C ratios of the biochars produced at 500 °C (Table 3).



**Fig. 1.** FTIR spectra of (a) fresh sheep manure (SM) and SM biochars produced at 300 °C (SMB300) and 500 °C (SMB500), and (b) fresh vermicompost (VC) and VC biochars produced at 300 °C (VCB300) and 500 °C (VCB500), in the wavelength range of 800–2000  $\text{cm}^{-1}$ .

### 3.2. Pb chemical fractions and mobility factor

The results of data variance analysis showed that the effect of SM, VC and their biochars on the soil Pb concentration in all the chemical forms were significant (Table 4).

The WsEx form of Pb significantly decreased with application of all the amendments compared to control treatment, and the greatest reduction (19.6%) was observed in the SM treatment (Fig. 2). The effect of all the biochar treatments on the reduction of WsEx form was the same as statistically. The effect of applied treatments on the Car fraction were different, so that the use of SM and VC caused a significant increase, while the application of VCB300, VCB500 and SMB300 caused a significant decrease in the Car form of Pb in the soil by 3.8, 10.5 and 4.3%, respectively. The soil Pb concentration in the OM form was not affected by application of biochars produced at 500 °C compared to the control, while the application of raw materials (SM and VC) and their biochars generated at 300 °C caused a significant increase in this form in the soil. The highest increase OM Pb form was attributed to the SM treatment (37.1%) in comparison to the control soil. This is likely due to the high organic C content of the raw materials and biochars produced at 300 °C compared to the more strongly pyrolyzed biochars produced at 500 °C, as reflected by the FTIR spectra (Fig. 1) and H:C ratios (Table 3) of the amendments.

**Table 4**

The Results of variance analysis of effect of sheep manure, vermicompost and their biochars on Pb chemical forms in Pb-contaminated calcareous soil.

Chemical forms	DF	Mean squares	F-value
WsEx	6	23.25	6.93**
Car	6	333.62	102.39**
OM	6	5.95	24.69**
MnOx	6	6.10	110.04**
AFex	6	1987.33	333.44**
CFex	6	14.784	23.72**
Res	6	1431.06	66.78**

\*\* , \* are significant at 1 and 5% probability level, respectively and ns, not significant.

The use of all the amendments significantly reduced the soil Pb concentration in MnOx form compared to the control, so that the SMB300 had the highest reduction of 39.0%. The soil Pb concentration in the MnOx fraction was in the order of: CL > SM = VCB500 > VC > SMB500 > VCB300 > SMB300. The application of SM and VCB300 treatments resulted in a significant decrease in the AFeOx form compared to the control soil, while the concentration of soil Pb in this fraction was considerably increased with the use of biochars produced at 500 °C. The application of VC and SMB300 treatments did not significantly change the concentration of Soil Pb in the AFeOx form. The concentration of soil Pb in the CFex form, with the use of biochars produced at 300 °C, was not significantly different from that of control, but the application of SM, VC and their biochars generated at 500 °C reduced the Pb concentration in this fraction, so that the highest reduction was observed in the SMB500 by 40.2%. The application of SM and VC did not affect the soil Pb concentration in Res form, whereas biochars produced at 500 °C resulted in a significant reduction, while biochars produced at 300 °C resulted in a significant increase of the Res fraction. Generally, the biochars reduced the concentration of Pb forms with more bioavailability (WsEx and Car forms) and increased the concentration of Pb forms with less bioavailability (AFex and Res), and VCBs were more effective than SMBs.

The addition of all the amendments to the soil in this study increased soil pH compared to control (from 0.46 to 0.82 units), so that the least and the highest increase was related to the SM and VCB500 treatments (data not shown). There was a significant and negative correlation between soil pH in different treatments with WsEx ( $r = -0.57^{**}$ ) and MnOx ( $r = -0.78^{**}$ ) forms. Also, the AFeOx fraction had a significant and positive correlation ( $r = +0.54^{*}$ ) with the soil pH. This suggests that the increase in soil pH as affected by application of the biochars can be one of the most important factors in the soil Pb stabilization and its conversion into chemical fractions with low bioavailability. Dume et al. (2016) showed that the concentration of WsEx form of soil Pb was significantly reduced and the concentration of soil Pb in the fractions of Car, FeMnOx, OM, and Res was increased by application of coffee bran biochar (prepared 500 °C for 3 h) at 15  $\text{ton ha}^{-1}$  in a contaminated soil for 90 days. Hamzenezad Taghliabad and Sepehr (2018), with addition of different levels of grape-pruning-residue biochar (0, 2, 5 and 10 wt. %) prepared at 500 °C to a contaminated calcareous soil for 8 weeks showed that the soil Pb concentration in the WsEx and Car forms reduced by 42% and 62%, and that the FeMnOx, OM and Res fractions increased by 33, 36 and 11%, respectively. They stated that an increase in the concentration of soil Pb in the oxide forms could be due to the formation of  $\text{Pb}(\text{OH})_2$ , which is more stable at soil pH above 8 (Lindsay, 1979).

The relative dynamic index of soil PTEs is determined using a mobility factor (MF) calculated by data obtained from a sequential extraction procedure. In determining this factor, among the chemical forms of PTEs, Car and WsEx fractions are identified as dynamic and high-risk forms (Saffari et al., 2015). A high MF value is indicative of high metal mobility and bioavailability (Kabala and Singh, 2001). Mean comparison of the MF values in the different treatments (Table 5) showed that the application of VC caused a significant increase in the Pb mobility compared to the control soil, while there was no significant difference between SM and control treatment. All of the biochar treatments significantly reduced the amount of soil Pb mobility compared to the control treatment. The maximum relative reduction was associated with the application of VCB500 by 11%, which is probably due to its high pH value (10.50), high P concentration (0.39%) and high ash percentage (67.72%) (Table 3) compared to the other biochar treatments. Furthermore, there was a negative and significant correlation between P concentration in the produced biochars and MF values ( $r = -0.83^{**}$ ). This could promote the formation of precipitates with Pb. Therefore, the applied biochars had an important role in the soil Pb stabilization. A significant and negative correlation ( $r = -0.51^{*}$ ) was obtained between MF and soil pH. This likely indicates that the





**Fig. 2.** The effect of organic material and biochar treatments on soil Pb fractions (mg Pb kg<sup>-1</sup> soil) (CL = control; SM = sheep manure; SMB300 = sheep manure biochar produced at 300 °C; SMB500 = sheep manure biochar produced at 500 °C; VC = vermicompost; VCB300 = vermicompost biochar produced at 300 °C; VCB500 = vermicompost biochar produced at 500 °C).

**Table 5**  
Effect of sheep manure, vermicompost and their biochars application on the mobility factor of Pb in a Pb-polluted calcareous soil.

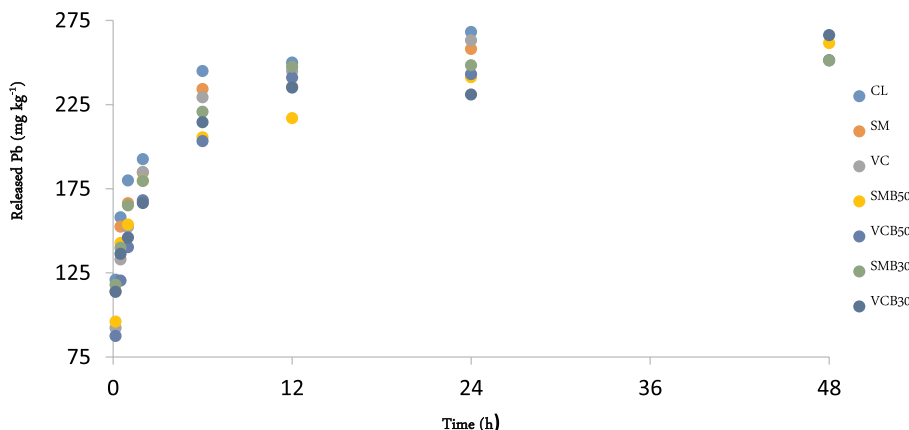
	CL	SM	VC	SMB500	VCB500	SMB300	VCB300
Mobility factor (%)	39.3 bc	40.0 b	42.0 a	38.6 c	35.0 e	37.0 d	36.6 d

\* Numbers followed by same letters in each rows in each section are not significantly (P < 0.05) different.  
CL = control; SM = sheep manure; SMB300 = sheep manure biochar produced at 300 °C; SMB500 = sheep manure biochar produced at 500 °C; VC = vermicompost; VCB300 = vermicompost biochar produced at 300 °C; VCB500 = vermicompost biochar produced at 500 °C.

interaction between Pb<sup>2+</sup> and surface functional groups is increased with increasing the soil pH as influenced by application of the amendments and consequently, Pb is bounded to the soil particles with higher strength (Park et al., 2011). The increase of soil organic C as affected by the addition of organic amendments can increase the adsorption and complexity of the elements specifically through activated surface functional groups (Kunhikrishnan et al., 2013). Hamzenejad Taghliabad and Sepehr (2018), with addition of grape-pruning-residue biochar (10 wt. %) to a polluted calcareous soil concluded that the MF values for the Cd, Pb, Cu and Zn were reduced by 47, 62, 70 And 49%, respectively. Boostani et al. (2018a) also reported that the application of five different types of biochars at 3 wt. % in a contaminated calcareous soil for 90 days significantly reduced the MF of soil Cd compared to the control and the highest decrease was obtained in sheep manure biochar treatment.

3.3. Pb release and two first-order reactions kinetic model

The pattern of Pb release from the soil during the period of 0.16–48 h as cumulative (Fig. 3) for all the applied treatments were similar, so that



**Fig. 3.** Cumulative release of Pb by EDTA solution from a contaminated calcareous soil as influenced by application of different amendments (CL = control; SM = sheep manure; SMB300 = sheep manure biochar produced at 300 °C; SMB500 = sheep manure biochar produced at 500 °C; VC = vermicompost; VCB300 = vermicompost biochar produced at 300 °C; VCB500 = vermicompost biochar produced at 500 °C).

the amount of Pb release during the first 6 h was fast (first phase) and then continued with slower rate (second phase). The biphasic desorption of PTEs from soil has also been reported by other researchers (Boostani et al., 2018a; Tabar and Jalali, 2013). In general, Pb released during the first phase consists of a water-soluble and exchangeable forms which are easily extracted by EDTA (Jones and Turki, 1997). Whereas, the second slow-release phase is associated with Pb complexes that require more time to detach, or Pb fractions attached to the soil particles with high adsorption energies (Kandpal et al., 2005). The amount of Pb release by EDTA after 48 h in different treatments was in the order of: VC (287.7 mg kg<sup>-1</sup>) > SM (282.3 mg kg<sup>-1</sup>) > CL (281.8 mg kg<sup>-1</sup>) > VCB300 (266.2 mg kg<sup>-1</sup>) > SMB500 (261.5 mg kg<sup>-1</sup>) > SMB300 (251.3 mg kg<sup>-1</sup>) > VCB500 (251.1 mg kg<sup>-1</sup>).

A two first-order reactions kinetic model was fitted to the Pb release data in different treatments, and its parameters are given in Table 6. Given the values of r<sup>2</sup> and SE, this model described well the Pb release kinetics from the soil in different treatments. A good description of PTEs release kinetics from the soil by a two-phase reaction kinetic model has also been reported by other researchers (Saffari et al., 2015; Tabar and Jalali, 2013). This model allows that the amount and rate of Pb release

**Table 6**

The parameters of two first-order reactions model in different applied treatments in soil.

	Q <sub>1</sub> (mg kg <sup>-1</sup> )	k <sub>1</sub> (h <sup>-1</sup> )	Q <sub>2</sub> (mg kg <sup>-1</sup> )	k <sub>2</sub> (h <sup>-1</sup> )	Q <sub>3</sub> (mg kg <sup>-1</sup> )	R <sup>2</sup>	SE
CL	154.08	8.43	118.48	0.20	227.43	0.98	9.36
SM	153.00	7.70	118.24	0.14	228.78	0.97	12.50
VC	136.00	5.96	139.23	0.16	224.77	0.98	12.04
SMB500	153.02	5.48	105.47	0.09	241.51	0.98	9.11
VCB500	115.18	7.43	132.97	0.20	251.85	0.99	6.60
SMB300	131.90	11.61	118.34	0.25	249.75	0.99	5.10
VCB300	125.12	12.60	124.14	0.19	250.73	0.97	12.30

CL = control; SM = sheep manure; SMB300 = sheep manure biochar produced at 300 °C; SMB500 = sheep manure biochar produced at 500 °C; VC = vermicompost; VCB300 = vermicompost biochar produced at 300 °C; VCB500 = vermicompost biochar produced at 500 °C.

**Table 7**Effect of sheep manure, vermicompost and their biochars application on changes of Q<sub>1</sub>/Q<sub>3</sub> and Q<sub>2</sub>/Q<sub>3</sub> ratios of Pb in soil.

Ratios	CL	SM	VC	SMB500	VCB500	SMB300	VCB300
Q <sub>1</sub> /Q <sub>3</sub>	0.677 a	0.668 a	0.605 b	0.633 b	0.457 d	0.528 c	0.500 c
Q <sub>2</sub> /Q <sub>3</sub>	0.527b	0.517 b	0.619 a	0.436 e	0.521 b	0.474 d	0.495 c

\* Numbers followed by same letters in each rows in each section are not significantly ( $P < 0.05$ ) different.

CL = control; SM = sheep manure; SMB300 = sheep manure biochar produced at 300 °C; SMB500 = sheep manure biochar produced at 500 °C; VC = vermicompost; VCB300 = vermicompost biochar produced at 300 °C; VCB500 = vermicompost biochar produced at 500 °C.

**Table 8**

The Pearson correlation coefficient between Pb chemical forms and constants of two first-order reactions kinetic equation.

	WsEx	Car	OM	MnOx	AFeOx	CFeOx	Res	Q <sub>1</sub>	Q <sub>2</sub>	Q <sub>3</sub>
WsEx	1									
Car	0.01 <sup>ns</sup>	1								
OM	-0.42 <sup>ns</sup>	0.33 <sup>ns</sup>	1							
MnOx	0.60 <sup>**</sup>	0.41 <sup>ns</sup>	-0.53 <sup>*</sup>	1						
AFeOx	0.08 <sup>ns</sup>	-0.33 <sup>ns</sup>	-0.57 <sup>**</sup>	-0.09 <sup>ns</sup>	1					
CFeOx	0.04 <sup>ns</sup>	-0.38 <sup>ns</sup>	0.03 <sup>ns</sup>	0.12 <sup>ns</sup>	-0.65 <sup>**</sup>	1				
Res	-0.25 <sup>ns</sup>	-0.60 <sup>ns</sup>	0.49 <sup>*</sup>	-0.03 <sup>ns</sup>	-0.90 <sup>**</sup>	0.80 <sup>**</sup>	1			
Q <sub>1</sub>	0.30 <sup>ns</sup>	0.65 <sup>**</sup>	0.06 <sup>ns</sup>	0.39 <sup>ns</sup>	0.04 <sup>ns</sup>	-0.02 <sup>ns</sup>	-0.36 <sup>ns</sup>	1		
Q <sub>2</sub>	-0.13 <sup>ns</sup>	0.01 <sup>ns</sup>	0.13 <sup>ns</sup>	-0.07 <sup>ns</sup>	-0.38 <sup>ns</sup>	-0.07 <sup>ns</sup>	0.46 <sup>*</sup>	-0.63 <sup>**</sup>	1	
Q <sub>3</sub>	-0.30 <sup>ns</sup>	-0.85 <sup>**</sup>	0.03 <sup>ns</sup>	-0.50 <sup>*</sup>	0.35 <sup>ns</sup>	0.25 <sup>ns</sup>	0.04 <sup>ns</sup>	-0.70 <sup>**</sup>	-0.10 <sup>ns</sup>	1

\*\* , \* are significant at 1 and 5% probability level, respectively and ns, not significant.

to be calculable in two fast (Q<sub>1</sub>) and slow (Q<sub>2</sub>) release phases (Tabar and Jalali, 2013). The fitting of this model to the release data showed two different stages of Pb extraction (slope and flat): the first step is the quick and easy extraction of Pb (Q<sub>1</sub>) and the second is the extracted Pb with slower rate. The amount of Pb-extracted relative to the total amount of soil Pb in the fast (Q<sub>1</sub>) and slow phases (Q<sub>2</sub>) were 23–28% and 25–31% in different treatments, respectively. The lowest values of Pb extracted in Q<sub>1</sub> (115.18 mg kg<sup>-1</sup>) and Q<sub>2</sub> (105.47 mg kg<sup>-1</sup>) phases were observed in the VCB500 and SMB500 treatments, respectively. Biochars due to their high porosity, high active surface functional groups, high specific surface, and high cation exchange capacity, can effectively immobilize PTEs through adsorption, ion exchange, surface and sedimentation complexes, and then reduce their extractability (Bian et al., 2014). Generally, the values of Pb release rate coefficient related to the first phase of extraction (k<sub>1</sub>) in applied treatments were considerably higher than the release rate coefficient related to the second extraction phase (k<sub>2</sub>). This result is in agreement with the findings of Jalali and Tabar (2013) and Saffari et al. (2015). The EDTA solution by two different processes can extract heavy elements from soil: a) complexation of metal cations by breaking down weak bounds between elements and soil particles (rapid release); b) slow solving of part of the soil structure and release of metals bounded to oxides and soil organic matter (slow release) (Sposito, 2004; Tsang et al., 2007).

Among the Q<sub>1</sub>, Q<sub>2</sub>, Q<sub>3</sub> parameters and Q<sub>1</sub>/Q<sub>2</sub>, Q<sub>2</sub>/Q<sub>3</sub> and Q<sub>1</sub>/Q<sub>3</sub>

ratios, only the ratios of Q<sub>1</sub>/Q<sub>3</sub> ( $r = 0.81^{**}$ ) and Q<sub>2</sub>/Q<sub>3</sub> ( $r = 0.44^{*}$ ) had a significant and positive correlation with the Pb mobility factor (MF) in the soil. Therefore, these particular ratios were used to evaluate the effect of applied treatments on the soil Pb stabilization. The results of mean comparison of data showed that the use of all organic treatments (except SM) significantly decreased the Q<sub>1</sub>/Q<sub>3</sub> ratio compared to control and the highest reduction was observed in the VCB500 treatment by 32.5% (Table 7). The amount of Q<sub>2</sub>/Q<sub>3</sub> ratio was significantly increased by application of VC treatment while the use of SMB500, SMB300 and VCB300 treatments significantly decreased this ratio compared to control (Table 7). The highest reduction of the Q<sub>2</sub>/Q<sub>3</sub> ratio was observed in the SMB500 by 17.3%. From the above findings, it is deduced that with application of the VCB500 in the soil, soil Pb is more reduced from the Q<sub>1</sub> phase and added to the stable phase (Q<sub>3</sub>), while soil Pb is more decreased from the Q<sub>2</sub> phase as affected by addition of SMB500 to the soil.

The phases of soil PTEs (Q<sub>1</sub>, Q<sub>2</sub> and Q<sub>3</sub>) obtained from the two first-order reactions kinetic model are associated with their chemical fractions in soil (Saffari et al., 2015). The results of Pearson correlation coefficients between parameters of the kinetic model and Pb chemical forms showed that the Q<sub>1</sub> parameter had a positive and significant correlation with the Car form, while the Q<sub>2</sub> parameter positively and significantly correlated with the Res fraction (Table 8). The Q<sub>3</sub> parameter also had a negative and significant correlation with the Car and

MnOx forms (Table 8). There was also a significant correlation between some of the Pb forms in the soil (Table 8), which indicates the close relationship among the soil Pb chemical fractions and the ability of them to convert to one another.

#### 4. Conclusions

The effect of two kinds of organic materials (VC and SM) and their biochars produced at 300 and 500 °C at 2 wt. % on the Pb stabilization were investigated in a contaminated calcareous soil. The results showed that the concentration of soil Pb in the WsEx and Car forms were reduced and the concentration of soil Pb bounded to AFeOx fraction was considerably increased by addition of the biochars. The increase of soil pH as affected by application of the amendments was one of the most important factors contributing to the stabilization of soil Pb, according to the correlation study. The maximum reduction of MF was attributed to the addition of VCB500 by 11%, which is probably due to its high pH value, high P and ash content compared to the other biochar treatments. The two first-order reactions kinetic model fitted the Pb release data extracted by EDTA very well. This indicated that Pb release could take place from two separate parts of the soil with different adsorption and solubility properties. The lowest amount of Pb extracted in the Q<sub>1</sub> and Q<sub>2</sub> phases were observed in the VCB500 and SMB500 treatments. The ratios of the Q<sub>1</sub>/Q<sub>3</sub> and Q<sub>2</sub>/Q<sub>3</sub> had a significant and positive correlation with the mobility factor and the highest reduction in the Q<sub>1</sub>/Q<sub>3</sub> and Q<sub>2</sub>/Q<sub>3</sub> ratios in comparison with the control soil were obtained in the VCB500 and SMB500 treatments. The effect of biochars produced at higher temperature (500 °C) on the soil Pb stabilization were greater than those produced at lower temperature (300 °C), and the raw materials (SM and VC) had no significant effect in stabilizing the soil Pb.

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#### Appendix A. Supplementary data

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#### References

- Asim, M., Abdan, K., Jawaid, M., Nasir, M., Dashtizadeh, Z., Ishak, M., Hoque, M.E., 2015. A review on pineapple leaves fibre and its composites. *Int. J. Polym. Sci.* 2015.
- Bian, R., Joseph, S., Cui, L., Pan, G., Li, L., Liu, X., Zhang, A., Rutledge, H., Wong, S., Chia, C., 2014. A three-year experiment confirms continuous immobilization of cadmium and lead in contaminated paddy field with biochar amendment. *J. Hazard Mater.* 272, 121–128.
- Bolan, N.S., Duraisamy, V., 2003. Role of inorganic and organic soil amendments on immobilization and phytoavailability of heavy metals: a review involving specific case studies. *Soil Res.* 41, 533–555.
- Boostani, H., Hardie, A., Najafi-Ghiri, M., Khalili, D., 2018a. Investigation of cadmium immobilization in a contaminated calcareous soil as influenced by biochars and natural zeolite application. *Int. J. Environ. Sci. Technol.* 15, 2433–2446.
- Boostani, H.R., Najafi-Ghiri, M., Hardie, A.G., 2018b. Single and competitive adsorption isotherms of some heavy metals onto a light textured calcareous soil amended with agricultural wastes-biochars. *Arch. Agron Soil Sci.* 1–14.
- Bruckman, V.J., Wriessnig, K., 2013. Improved soil carbonate determination by FT-IR and X-ray analysis. *Environ. Chem. Lett.* 11, 65–70.
- Chen, Z., Ma, W., Han, M., 2008. Biosorption of nickel and copper onto treated alga (*Undaria pinnatifida*): application of isotherm and kinetic models. *J. Hazard Mater.* 155, 327–333.
- Dang, Y., Dalal, R., Edwards, D., Tiller, K., 1994. Kinetics of zinc desorption from Vertisols. *Soil Sci. Soc. Am. J.* 58, 1392–1399.
- de Andrade Lima, L., Bernardez, L., 2011. Characterization of the lead smelter slag in Santo amaro, Bahia, Brazil. *J. Hazard Mater.* 189, 692–699.
- Derakhshan-Nejad, Z., Jung, M.C., 2017. The effects of biochar and inorganic amendments on soil remediation in the presence of hyperaccumulator plant. *Int. J. Energy Environ. Eng.* 8, 317–329.
- Derakhshan-Nejad, Z., Jung, M.C., Kim, K.-H., 2017. Remediation of soils contaminated with heavy metals with an emphasis on immobilization technology. *Environ. Geochem. Health* 1–27.
- Ding, Z., Hu, X., Wan, Y., Wang, S., Gao, B., 2016. Removal of lead, copper, cadmium, zinc, and nickel from aqueous solutions by alkali-modified biochar: Batch and column tests. *J. Ind. Eng. Chem.* 33, 239–245.
- Dume, B., Mosissa, T., Nebiyu, A., 2016. Effect of biochar on soil properties and lead (Pb) availability in a military camp in South West Ethiopia. *Afr. J. Environ. Sci. Technol.* 10, 77–85.
- Francioso, O., Sanchez-Cortes, S., Bonora, S., Roldán, M.L., Certini, G., 2011. Structural characterization of charcoal size-fractions from a burnt Pinus pinea forest by FT-IR, Raman and surface-enhanced Raman spectroscopies. *J. Mol. Struct.* 994, 155–162.
- Hamzenejad Taghliadab, R., Sepehr, E., 2018. Heavy metals immobilization in contaminated soil by grape-pruning-residue biochar. *Arch. Agron Soil Sci.* 64, 1041–1052.
- Jalali, M., Tabar, S.S., 2013. Kinetic extractions of nickel and lead from some contaminated calcareous soils. *Soil Sediment Contam.: Int. J.* 22, 56–71.
- Jones, B., Turki, A., 1997. Distribution and speciation of heavy metals in surficial sediments from the Tees Estuary, north-east England. *Mar. Pollut. Bull.* 34, 768–779.
- Kabala, C., Singh, B.R., 2001. Fractionation and mobility of copper, lead, and zinc in soil profiles in the vicinity of a copper smelter. *J. Environ. Qual.* 30, 485–492.
- Kamali, S., Ronaghi, A., Karimian, N., 2011. Soil zinc transformations as affected by applied zinc and organic materials. *Commun. Soil Sci. Plant Anal.* 42, 1038–1049.
- Kandpal, G., Srivastava, P., Ram, B., 2005. Kinetics of desorption of heavy metals from polluted soils: influence of soil type and metal source. *Water Air Soil Pollut.* 161, 353–363.
- Keilueit, M., Nico, P.S., Johnson, M.G., Kleber, M., 2010. Dynamic molecular structure of plant biomass-derived black carbon (biochar). *Environ. Sci. Technol.* 44, 1247–1253.
- Klinar, D., 2016. Universal model of slow pyrolysis technology producing biochar and heat from standard biomass needed for the techno-economic assessment. *Bioresour. Technol.* 206, 112–120.
- Krull, E.S., Baldock, J.A., Skjemstad, J.O., Smernik, R.J., 2009. Characteristics of biochar: organo-chemical properties. *Biochar Environ. Manage. Sci. Technol.* 53.
- Kunhikrishnan, A., Bolan, N.S., Naidu, R., Kim, W.-I., 2013. Recycled water sources influence the bioavailability of copper to earthworms. *J. Hazard Mater.* 261, 784–792.
- Lahori, A.H., Zhanyu, G., ZHANG, Z., Ronghua, L., Mahar, A., Awasthi, M.K., Feng, S., Sial, T.A., Kumbhar, F., Ping, W., 2017. Use of biochar as an amendment for remediation of heavy metal-contaminated soils: prospects and challenges. *Pedosphere* 27, 991–1014.
- Lindsay, W.L., 1979. *Chemical Equilibria in Soils*. John Wiley and Sons Ltd.
- Martin, T.A., Ruby, M.V., 2004. Review of in situ remediation technologies for lead, zinc, and cadmium in soil. *Remed. J.: J. Environ. Cleanup Costs Technol. Tech.* 14, 35–53.
- Meier, S., Curaqueo, G., Khan, N., Bolan, N., Cea, M., Eugenia, G.M., Cornejo, P., Ok, Y.S., Borie, F., 2017. Chicken-manure-derived biochar reduced bioavailability of copper in a contaminated soil. *J. Soils Sediments* 17, 741–750.
- Nejad, Z.D., Jung, M.C., 2017. The effects of biochar and inorganic amendments on soil remediation in the presence of hyperaccumulator plant. *Int. J. Energy Environ. Eng.* 8, 317–329.
- Ogundiran, M., Osibanjo, O., 2009. Mobility and speciation of heavy metals in soils impacted by hazardous waste. *Chem. Speciat. Bioavailab.* 21, 59–69.
- Ogundiran, M.B., Lawal, O.O., Adejumo, S.A., 2015. Stabilisation of Pb in Pb smelting slag-contaminated soil by compost-modified biochars and their effects on maize plant growth. *J. Environ. Protect.* 6, 771.
- Page, A., Miller, R., Keeney, D., 1982. *Methods of Soil Analysis. Part 2. Chemical and Microbiological Methods*. Am. Soc. Agronomy/Soil Sci. Soc. Am., Madison, WI, pp. 323–336.
- Park, J.H., Choppala, G.K., Bolan, N.S., Chung, J.W., Chuasavathi, T., 2011. Biochar reduces the bioavailability and phytotoxicity of heavy metals. *Plant Soil* 348, 439.
- Reeves, J.B., McCarty, G.W., Rutherford, D.W., Wershaw, R.L., 2007. Near infrared spectroscopic examination of charred pine wood, bark, cellulose and lignin: implications for the quantitative determination of charcoal in soils. *J. Near Infrared Spectrosc.* 15, 307–315.
- Sadaf, J., Shah, G.A., Shahzad, K., Ali, N., Shahid, M., Ali, S., Hussain, R.A., Ahmed, Z.I., Traore, B., Ismail, I.M., 2017. Improvements in wheat productivity and soil quality can accomplish by co-application of biochars and chemical fertilizers. *Sci. Total Environ.* 607, 715–724.
- Saffari, M., Karimian, N., Ronaghi, A., Yasrebi, J., Ghasemi-Fasaie, R., 2015. Stabilization of nickel in a contaminated calcareous soil amended with low-cost amendments. *J. Soil Sci. Plant Nutr.* 15, 896–913.
- Salbu, B., Krekling, T., 1998. Characterisation of radioactive particles in the environment. *Analyst* 123, 843–850.
- Santos, S., Costa, C.A., Duarte, A.C., Scherer, H.W., Schneider, R.J., Esteves, V.I., Santos, E.B., 2010. Influence of different organic amendments on the potential availability of metals from soil: a study on metal fractionation and extraction kinetics by EDTA. *Chemosphere* 78, 389–396.
- Sengupta, A., 2007. *Solidification and Stabilization of Contaminated Soil*.
- Sharma, K., Garg, V.K., 2018. Vermicomposting: A green technology for organic waste management. In: Agarwal, R., Kumar, R., Sukumaran, R. (Eds.), *Waste to Wealth. Energy, Environment, and Sustainability*. Springer, Singapore, pp. 199–235.
- Singh, J., Karwasra, S., Singh, M., 1988. Distribution and forms of copper, iron, manganese, and zinc in calcareous soils of India. *Soil Sci.* 146, 359–366.

- Soil Survey Staff, 2014. Keys to Soil Taxonomy. Department of Agriculture: Natural Resources Conservation Service.
- Sposito, G., 2004. The Surface Chemistry of Natural Particles. Oxford University Press on Demand.
- Sposito, G., Lund, L., Chang, A., 1982. Trace metal chemistry in arid-zone field soils amended with sewage sludge: I. Fractionation of Ni, Cu, Zn, Cd, and Pb in solid phases 1. *Soil Sci. Soc. Am. J.* 46, 260–264.
- Tabar, S.S., Jalali, M., 2013. Kinetics of Cd release from some contaminated calcareous soils. *Nat. Resour. Res.* 22, 37–44.
- Tang, J., Zhu, W., Kookana, R., Katayama, A., 2013. Characteristics of biochar and its application in remediation of contaminated soil. *J. Biosci. Bioeng.* 116, 653–659.
- Tsang, D.C., Zhang, W., Lo, I.M., 2007. Copper extraction effectiveness and soil dissolution issues of EDTA-flushing of artificially contaminated soils. *Chemosphere* 68, 234–243.
- Wang, Z., Chen, L., Sun, F., Luo, X., Wang, H., Liu, G., Xu, Z., Jiang, Z., Pan, B., Zheng, H., 2017. Effects of adding biochar on the properties and nitrogen bioavailability of an acidic soil. *Eur. J. Soil Sci.* 68, 559–572.
- Yang, X., Liu, J., McGrouther, K., Huang, H., Lu, K., Guo, X., He, L., Lin, X., Che, L., Ye, Z., 2016. Effect of biochar on the extractability of heavy metals (Cd, Cu, Pb, and Zn) and enzyme activity in soil. *Environ. Sci. Pollut. Control Ser.* 23, 974–984.
- Zeng, L.-S., Liao, M., Chen, C.-L., Huang, C.-Y., 2006. Effects of lead contamination on soil microbial activity and rice physiological indices in soil–Pb–rice (*Oryza sativa* L.) system. *Chemosphere* 65, 567–574.