Evaluation of Mobility, Bioavailability and Toxicity of Pb and Cd in Contaminated Soil Using TCLP, BCR and Earthworms

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Abstract: The objective of the present study was to investigate the reduction of mobility, availability and toxicity found in soil contaminated with lead (Pb) and cadmium (Cd) from Santo Amaro Municipality, Bahia, Brazil using two combined methods, commonly tested separately according to the literature: metal mobilization with phosphates and phytoextraction. The strategy applied was the treatment with two sources of phosphates (separately and mixed) followed by phytoremediation with vetiver grass (*Vetiveria zizanioides* (L.)). The treatments applied (in triplicates) were: T1—potassium dihydrogen phosphate (KH$_2$PO$_4$); T2—reactive natural phosphate fertilizer (NRP) and; T3—a mixture 1:1 of KH$_2$PO$_4$ and...
NRP. After this step, untreated and treated soils were planted with vetiver grass. The extraction procedures and assays applied to contaminated soil before and after the treatments included metal mobility test (TCLP); sequential extraction with BCR method; toxicity assays with *Eisenia andrei*. The soil-to-plant transfer factors (TF) for Pb and Cd were estimated in all cases. All treatments with phosphates followed by phytoremediation reduced the mobility and availability of Pb and Cd, being KH₂PO₄ (T1) plus phytoremediation the most effective one. Soil toxicity however, remained high after all treatments.

**Keywords:** contaminated soil; toxic metals; remediation; phosphate; phytoremediation; vetiver grass; availability; TCLP; BCR; *Eisenia andrei*

1. Introduction

The worldwide environmental problem caused by soil and sediments contamination has stimulated scientific investigations to develop new technologies and materials for the removal and/or reduction of toxic metal concentrations to acceptable levels. Among the contaminants, lead (Pb) and cadmium (Cd) are of great concern due to the high toxicity they pose to the environment and humans, which is aggravated by the fact that metals concentrate in tissues with magnification in the food web [1]. Several studies show high correlation between exposure to contaminated soils and concentration of metals in the blood [2–4].

Since metals are not degraded, one strategy applied for remediation of soils contaminated with metals is *in situ* immobilization achieved when one metal is transformed into a more stable geochemical form, which reduces its bioavailability. Several investigations have been conducted with the purpose of clarifying the mechanisms responsible for immobilization of metals using, for instance, natural and synthetic phosphates [5–9].

Phosphate fertilizers are a source of phosphorus (P) available in the market and, therefore, easily obtained in large amounts at relatively low cost when compared to mineral phosphates and phosphate salts [10]. Phosphate fertilizers such as superphosphate simple [11] diammonium phosphate [12], triple superphosphate [10,13], calcium magnesium phosphate [13] and molten phosphate [14] reduce solubility, leaching, transportation and therefore, bioavailability.

Another strategy is to remove the metals from the soil and several plant species have shown capacity to extract and accumulate metals in roots or aerial parts [15–17]. Among the investigated species, the vetiver grass (*Vetiveria zizanioides* (L.)) has been investigated in different parts of the world to phytoremediate soils contaminated by organic compounds and metals [18,19].

Although the application of phosphates and phytoremediation are both techniques that can achieve different levels of success, there is no record in the literature of any application of both strategies simultaneously or in sequence, probably because they are based on opposite strategies: immobilization by phosphate application *vs.* extraction-phystoaccumulation by the use of plants. Even though, one hypothesis waiting to be tested is the possibility or obtaining a complementary effect and remediation enhancement by using both techniques: the plants would extract those metals that phosphate has not immobilized.
Therefore, the objective of the present study was to assess the effect of two different sources of phosphate on metals immobilization in a soil contaminated with Pb and Cd, followed by the extraction of the same metals by vetiver grass (*Vetiveria zizanioides* (L.)), as a complementary technique for soil remediation. The study also assessed the toxicity posed by the contaminated soil before and after treatment by single and combined techniques.

2. Experimental Setup

2.1. Treatment of Soil Contaminated with Pb and Cd Using Phosphates and Vetiver Grass

The soil samples were collected at 12°32’24.7″ latitude South and 38°43’40.9″ longitude West in Santo Amaro Municipality, state of Bahia in Brazil, where soil contamination by Pb and Cd has been reported due to decades of operation of a factory that produced lead ingots. The contaminated site and impacts on the human health have been previously reported [20]. For the purpose of the present study, soil samples from two well-known contaminated hotspots within the site were collected with the sole purpose of conducting laboratory studies with the focus on remediation using phosphates combined with vetiver grass. The samples obtained from 20 cm-depth once they arrived in the laboratory, they were dried at 40 °C, sieved (335 μm) and homogenized. The 335-μm sieve was chosen due to the high clay content found in vertisol and high colloidal activity (see Results, Table 1), which makes sieving in smaller net inappropriate for column tests and root development. Each column formed by PVC rings with 25 cm high, 6.5 cm in diameter received approximately 1 kg of soil. The experiment included in total, 12 columns (representing three treatments and one control in triplicates). Treatment T1 consisted of potassium dihydrogen phosphate; treatment T2 consisted of reactive rock phosphate fertilizer; treatment T3 consisted of a 1:1 (molar ratio) mixture of both phosphates used in T1 and T2. The same 6:1 P:metal molar ratio was applied in all treatments, according to preliminary tests in the laboratory with Brazilian soils with very high clay content and colloidal activity [21]. The soil treated with phosphates were homogenized in a mixer and transferred to the columns built up in triplicates. Contaminated soil with no treatment (T0) was used as positive control. After 120 days of treatment, which has previously been considered a time period enough to promote sorption of metals by phosphates [22,23], pots with soils from all treatments were planted with vetiver grass *Vetiveria zizanioides* (L.) in 12 pots. After 90 days, the plants were taken for analysis.

| Texture of the Fine Soil (g·kg⁻¹) | pH (Water) | Organic Carbon (g·kg⁻¹) | Assimilable P (mg·kg⁻¹) | Metal Concentration (mg·kg⁻¹) |
|-----------------------------------|-----------|------------------------|-------------------------|-------------------------------|
| Sand                              | 8.0       | 11                     | 1                       | Pb 3196                      |
| Silt                              |           |                        |                         |                               |
| Clay                              |           |                        |                         | Cd 33                         |

| Sorptive complex (cmol⁺·kg⁻¹) | CEC (cmol⁻·kg⁻¹) | N (g·kg⁻¹) | Detection Limit-DL (µg·L⁻¹) |
|-------------------------------|-----------------|-----------|-----------------------------|
| Ca²⁺                          | 34              |           | Pb 60                      |
| Mg²⁺                          | 11              | 2.6       |                               |
| K⁺                             | 0.36            |           |                               |
| Na⁺                            | 0.28            |           |                               |
| P                                | 46              |           |                               |
| Cd                              | 3               |           |                               |

Table 1. Physical and chemical characteristics of the soil.
2.2. Soil and Plant Biomass Characterization

2.2.1. Physical and Chemical Characterization of Soil Samples

The methodologies recommended by EMBRAPA-Solos [24] were used to characterize the soil samples regarding texture, organic matter content, water retention capacity, cationic exchange capacity (CEC) and pH. The methodology applied for Pb and Cd quantification was based on USEPA 3051A [25].

2.2.2. Metal Extraction from Plant Tissues Using Nitro-Peroxide Method

Entire plants collected from the pots were washed with distillate water, dried at 60 °C during 24 h. Then, roots and aerial part were shredded separately in a Model 11 Basic automatic shredder (IKA do Brasil, Sao Paulo, Brazil).

Triplicates of 0.5 g from each anatomic part were placed in quartz tubes where 5 mL of HNO₃ (70% ultra-pure, Vetec Quimica Fina Ltd., Rio de Janeiro, Brazil) and 2 mL of H₂O₂ (30%–32% supra-pure Vetec Química Fina Ltd.) were added.

The microwave received a program for digestion of plant tissue, with temperature reaching 180 °C and pressure of 27 atm in 5.5 min, remaining in this temperature during 9.5 min and then, declining in 15 min. After reaching room temperature, the extract was centrifuged (Model 206-R, FANEM, Sao Paulo, Brazil) and the volume completed to 30 mL with distillate water followed by filtration [24].

The reference material SRM1515 Apple Leaves (NIST, Gaithersburg, MD, USA) was used to compare metal recovery with certified values. The recovery rates (above 75%) obtained for Pb and Cd were considered satisfactory.

2.3. Mobility, Availability and Toxicity of Soil

2.3.1. Toxicity Characteristic Leaching Procedure (TCLP)

Soil samples (2.5 g) in triplicates were sieved and reduced to 1 mm in size and then, placed in 100 mL tubes with extractor fluid. The fluid was formed by 5.7 mL of glacial acetic acid (CH₃COOH) added with distilled water to complete 1 L with final pH 2.88 ± 0.05. The tubes were placed in a mechanic shaker (pendulous shaking table Model TE 240-Tecnal, Sao Paulo, Brazil) at 30 ± 2 h in room temperature [26]. After this shaking period, the samples were filtrated and the concentrations of Pb and Cd were determined by an ICP-OES (OPTIMA 3000, Perkin Elmer, MA, USA).

2.3.2. Calculation of the Soil-Plant Transfer Factor (TF) for Pb and Cd

The soil-plant transfer factor (TF) is defined as the ratio between the total concentration of a certain contaminant in the plant tissue and the total concentration of the same contaminant in the soil. This relation depends not only on the total concentration of metal in the soil but also on the chemical species, type of soil and plant species. Therefore, a great variability in TFs has been found. The transfer of each metal (Pb or Cd) from soil to plant was estimated according to Intawongse and Dean [27]:

\[
FT = \frac{C_p}{C_s}
\]
\[ FT = \frac{C_{pr}}{C_{a}} \]

where: \( C_{pr} \) = metal concentration in the root dry biomass; \( C_{a} \) = metal concentration in the aerial dry biomass; \( C_{s} \) = metal concentration in the soil (dry weight).

2.3.3. Chemical Fractionation of Soil Samples According to the BCR Method

The BCR sequential method is recommended to determine the fractionation or distribution of metals in soils, or in other words, to determine metal concentrations in different fractions. Four different steps in the BCR method split the metals in the following fractions: water-soluble, exchangeable and linked to carbonate (E1); linked to iron and manganese oxides (E2); linked to organic fraction (E3); and residual (E4). In order to apply the BCR method, soil (1 g, in triplicate) was mixed with different extracting solutions, according to the procedure described in the literature [28]. For each step, the samples were washed to remove residues and filtered in paper filter (medium pores). For that, they were agitated during 15 min with Milli-Q water (20 mL) and centrifuged during 20 min at 3000 rpm and the supernatants were discarded [28].

A certified reference material (CRM BCR 701) was subjected to the BCR protocol and the Pb and Cd recovery observed was higher than 75% in all steps, indicating satisfactory recovery of the fractionation process.

2.3.4. Toxicity Assays with the Bioindicator *Eisenia andrei*

The toxicity of the contaminated soil was assessed before and after treatments with phosphates and phytoremediation using the bioindicator *Eisenia andrei*. The objective of this assay was to evaluate if the treatments applied to the contaminated soil were sufficient to eliminate or reduce the acute toxicity (lethality) and chronic toxicity (reproduction rate and biomass loss) caused by the presence of toxic metals Pb and Cd in the soil.

For all experimental units (four replicates for each treatment), the worms were previously selected according to the sexual development with weight between 300 to 600 mg, washed in tap water and weighted [29]. Each 500 mL beaker containing 200 g of soil, received 10 adults, according to the ISO 11268-1 protocol [30]. For each treatment, 200 individuals in total were exposed. After the 7th day of exposure, for the lethality assays, the dead organisms were counted and removed from the beakers and the survivors were kept until the 14th day. For the chronic assay (reproduction), the norm applied was the ISO 11268-2 [31]. All assays were developed under controlled environment with temperature of 20 ± 2 °C, photoperiod light: dark 12:12 h. Every week the beakers were weighed for checking and correction of the soil moisture content, food and counting of eggs and juveniles. This procedure took about five weeks.

2.3.5. Experimental Design and Statistical Analyses

The experimental design using columns and pots was entirely random with triplicates. The software SAEG [32] was used for variance analysis (ANOVA). Tukey or Scott Knott tests were applied for mean comparison and data grouping [33]. The significance level assumed was \( p < 0.05 \).
2.3.6. Analytical Procedure for Metal Analyses

The metals Pb and Cd in soil and plant biomass were quantified with a Perkin Elmer Optima 3000 ICP-OES Spectrometer. The standards were Perkin Elmer Pure IV, lot 9-81YPY1, 1,000 mg·L\(^{-1}\) of Cd and Pb. The calibration curve was 0.5, 1.0, 2.0, 5.0 and 10 mg·L\(^{-1}\), with 15 L·min\(^{-1}\) flux from an argon plasma, auxiliary flux of 0.8 L·min\(^{-1}\) argon and nebulizer flux of 0.5 L·min\(^{-1}\). The sample flow was 2.0 mL·min\(^{-1}\) with power of 1,500 Watts being 214,438 nm for Cd and 220,353 nm for Pb (detection limits DL of 3 and 60 µg·L\(^{-1}\) respectively).

3. Results and Discussion

3.1. Soil Characterization

The physical and chemical analyses showed that the soil had a clayey texture with high cationic exchange capacity (CEC) related to the high colloidal activity, as expected for a vertisol (Table 1). Some properties including pH, organic matter, type of clay, surface charge among others are responsible for controlling the behaviour of the contaminants in soils [34].

3.2. Mobility, Availability and Toxicity of Soil Samples

3.2.1. Toxicity Characteristic Leaching Procedure (TCLP)

The results obtained with the TCLP after phytoremediation showed that all treatments were effective in reducing the mobility of Pb and Cd (Table 2).

| Treatments            | Mean (± SD) Pb | Mean (± SD) Cd |
|-----------------------|----------------|----------------|
| T0—Control           | 181 (±11.0)    | 9.8 (±7.3)     |
| T1—KH\(_2\)PO\(_4\)  | 26 (±1.4)      | 6.4 (±4.4)     |
| T2—NRP               | 164 (±5.9)     | 9.4 (±2.9)     |
| T3—KH\(_2\)PO\(_4\) + NRP | 48 (±2.7)  | 6.9 (±2.9)     |

Notes: Different letters (\(^a\), \(^b\), \(^c\), \(^d\)) within the same column mean differences statistically significant among treatments \((p < 0.05)\). T1 was the treatment that resulted in less release of Pb and Cd in the extraction solution, but even in this case the solution had concentrations above the threshold limits established by USEPA [22], which is 5 mg·L\(^{-1}\) and 1 mg·L\(^{-1}\), respectively. T3 was the treatment that released less after T1, followed by T2 and finally T0 (the positive control) which as expected, was the one that released more Pb and Cd. The treatments T1, T3 and T2 promoted a reduction in the release of Pb of 86%, 74% and 10% respectively compared to the control T0. Regarding Cd, the treatments T1, T3 and T2 promoted a reduction 40%, 30% and 8%, respectively. The concentrations in the extraction solution in all cases were found to be above the threshold limits.
3.2.2. Pb and Cd Transfer Factor from Soil to Plant

According to Intawongse and Dean [23] the transfer factors (TF) are considered low when they are within the range of 0.0–0.9 for Pb and 0.0–2.7 for Cd. The TF found for Pb in the aerial biomass, regardless the treatments applied were all very low (Table 3). For both Pb and for Cd the TF values were higher in the root biomass; however, the TF for Cd in both root and aerial biomasses were approximately 10 times higher than the TF for Pb. These results suggest that the Cd availability is higher than the Pb availability in the studied soil even after treatment with phosphates. According to Alloway [35], Cd has a tendency for being more mobile in soils and therefore, more available for plants than many other metals, including Pb. According to Magna et al. [36], after these metals are transferred to the plant, they accumulate mostly in the roots, which are the first anatomic part affected by soil contamination.

The pre-treatment with phosphates (which reduced the mobility and availability of the metals), does not explain alone the low TF values observed, since the positive control (T0) had showed also a low TF (Table 3). One cannot ruled out the fact that this is a vertisol, with very high clay content and high cation exchange capacity (CEC), making it to act as natural barriers against the contaminant dispersion [37] affecting the absorption by plants [36]. Since some transfer of metals from soil to plant biomass, mostly to the root biomass was observed even after treatment with phosphates, a complementary removal of metals from soil by vetiver grass is occurring. A complete assessment of a combined treatment would require successive planting periods and an increasing reduction of the TF should be expected. According to the results, the treatment T2 was the one that allowed more metal transfer from soil to plant biomass (Table 3), which is agreement with the fact this treatment promoted less phosphate-metal sorption in the soil.

Table 3. Metal concentrations and soil-plant transfer factors (TFs) calculated for Pb and Cd after soil treatments with different phosphates.

| Treatments | Pb and Cd Transfer Factor from Soil to Plant (mg·kg⁻¹ (Dry Weight)) | Transfer Factor (TF) |
|------------|-------------------------------------------------------------------|----------------------|
|            | Soil                  | Aerial               | Root   | TF (Total) | TF (Aerial) | TF (Root) |
|            | Pb  | Cd  | Pb  | Cd  | Pb  | Cd  | Pb  | Cd  | Pb  | Cd  | Pb  | Cd  |
| T0         | 4233 | 44  | 10  | 1.8 | 158 | 15  | 0.040 | 0.386 | 0.002 | 0.345 | 0.037 | 0.041 |
| T1         | 4001 | 44  | 8   | 1.8 | 60  | 14  | 0.017 | 0.359 | 0.002 | 0.320 | 0.015 | 0.039 |
| T2         | 3943 | 46  | 17  | 2.8 | 224 | 33  | 0.061 | 0.790 | 0.004 | 0.071 | 0.057 | 0.719 |
| T3         | 3838 | 41  | 14  | 2.7 | 156 | 23  | 0.044 | 0.613 | 0.004 | 0.060 | 0.041 | 0.554 |

3.2.3. Chemical Fractionation with Soil Samples According to the BCR Method

Figure 1a shows the results obtained with the sequential extraction after phosphate treatment and phytoremediation with vetiver grass (*Vetiveria zizanioides* (L.)) with the average values transformed into percentage. T1 followed by T3 was the treatment that resulted in the highest reduction of Pb concentration obtained with step E1. Additionally, T3 was the treatment that increased the Pb concentrations extracted with steps E3 and E4. The typical behaviour of Pb in contaminated soils is one of high retention, low mobility and therefore, low bioavailability [35].
In Figure 1b one can observe different steps of the sequential extraction for Cd. Treatments T1 and T3 were those that reduced more the concentrations of Cd in E1, followed by treatment T2. For the treatments T1 and T2, no significant changes occurred during steps E3 and E4 if compared with T0. T3 was the treatment that increased the concentrations of Cd in the steps of less availability (E3 and E4).

In this study, Cd was more labile than Pb. Saheen [38] mentioned that Pb seems to suffer more easily complexation with functional groups in the surface of the soil particles and in the internal sphere when compared to Cd, and this would be the reason Pb is less labile than Cd.

3.2.4. Toxicity Assay with the Bioindicator *Eisenia andrei*

The results of the toxicity assay with *Eisenia andrei* based on the observations after 7 and 14 days of exposure are found in Table 4. The treatments T0 and T1 showed the same mortality rate (7%); the same occurred for the treatments T2 and T3 (2%). Treatment T1 however, was the one that was the most efficient in immobilizing the metals in the soil. Therefore, the higher toxicity observed for T1 (as high as the toxicity posed by T0) is likely to be associated to the type of phosphate used for metal immobilization (KH₂PO₄). The worms in treatment T1 might respond to the effect of the treatment itself, which could attenuate with time. Therefore, to assess eventual toxicity posed by KH₂PO₄ a sensibility test with this phosphate is required. It is important to highlight that the soil pH observed in different treatments (including T0-control) varied from 7.0 up to 7.9, suggesting that pH had no relevance on the final toxicity.

| Treatment                  | Lethality (%) |
|----------------------------|---------------|
|                            | 7 Days | 14 Days |
| T0—Control                 | 7      | 7       |
| T1—KH₂PO₄                  | 7      | 7       |
| T2—NRP                     | 2      | 2       |
| T3—KH₂PO₄ + NRP            | 2      | 2       |

Table 4. Acute toxicity test with *E. andrei* and soil contaminated with Pb and Cd after treatment with phosphates and phytoremediation with *V. zizanioides* (L.).
Figure 2 shows biomass loss of *E. andrei* (in % compared to the initial weight) when the worms were exposed to the contaminated soil with no treatment (control-T0) and after treatments with different sources of phosphate (T1, T2, T3) followed by plantation with vetiver grass. The treatments that included NRP (T2 and T3) were those that caused less biomass loss, particularly during the first weeks (Figure 2). At the 5th week however, almost no difference in biomass loss was observed among treatments. The interpretation of the results took into account the fact that since phosphate in NRP (T2) is less soluble than in KH$_2$PO$_4$ (T1), more metals were bioavailable during the first weeks in the soil treated with NRP, allowing removal by the vetiver grass, resulting in fewer metals in the soil.

**Figure 2.** Biomass loss (in %) of *E. andrei* after exposure to Pb and Cd contaminated soil and treated soil with different sources of phosphate (T0-control, T1, T2, T3). Different letters within each week mean differences statistically significant ($p < 0.05$). Bars mean standard deviations (SD).

Regarding the reproduction test, there was no significant difference among treatments; in other words, low reproduction was observed in untreated soil (control-T0) as well as in treated soils (T1, T2 and T3) with low production of eggs by the exposed worms. In 5 weeks, few eggs and no juvenile forms were found. Spurgeon *et al.* [39] investigated the effects on worms due to exposure to different concentrations of Cd, Zn, Cu and Pb and concluded that egg production was more affected than survival for all metals, mostly Cd and Cu.

### 4. Conclusions

When vertisoil contaminated with Pb and Cd was treated with two different types of phosphates followed by planting with vetiver grass (*Vetiveria zizanioides* (L.)), the mobility and the availability of both metals was significantly reduced with all treatments. The treatment with KH$_2$PO$_4$ (T1) was the most effective in reducing availability followed by the mixture of KH$_2$PO$_4$ + natural reactive phosphate-NRP (T3) and finally, natural reactive phosphate alone (T2). The treatments were more efficient in immobilizing Pb than Cd. Regardless the reduction in mobility combined with transfer
from soil to plant of metals still bioavailable, after all treatments with phosphate, the soil remained toxic to *E. andrei*.

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**Author Contributions**

Maria Luiza F. M. Kede was the main responsible for the experimental work and drafted the manuscript revised by all authors; Fabio V. Correia, Paulo F. Conceição Conceição and Sidney F. Salles Junior were responsible for the planning and development of the toxicity assays; Marcia Marques, Josino C. Moreira and Daniel V. Pérez had the original idea for the study and carried out the design with all co-authors; additionally, Daniel V. Pérez was responsible for the statistical analyses and M. Marques for the final version of the manuscript.

**Conflicts of Interest**

The authors declare no conflict of interest.

**References**

1. El Razek, A.A.A. The mobility and speciation of lead and cadmium in Bahr El Baqar region, Egypt. *J. Environ. Chem. Eng.* 2014, 2, 685–691.
2. Ren, H.M.; Wang, J.D.; Zhang, X.L. Assessment of soil lead exposure in children in Shenyang, China. *Environ. Pollut.* 2006, 144, 327–335.
3. Oulhote, Y.; le Tertre, A.; Etchevers, A.; le Bot, B.; Lucas, J.P.; Mandin, C.; le Strat, Y.; Lanphear, B.; Glorennec, P. Implications of different residential lead standards on children’s blood lead levels in France: Predictions based on a national cross-sectional survey. *Int. J. Hyg. Environ. Health* 2013, 216, 743–750.
4. Taylor, M.P.; Camenzuli, D.; Kristensen, L.J.; Forbes, M.; Zahran, S. Environmental lead exposure risks associated with children’s outdoor playgrounds. *Environ. Pollut.* 2014, 178, 447–454.
5. Cao, R.X.; Ma, L.Q.; Chen, M.; Singh, S.P.; Harris, W.G. Impacts of phosphate amendments on lead biogeochemistry at a contaminated Site. *Environ. Sci. Technol.* 2002, 24, 5296–5304.
6. Zupancic, M.; Bukovec, N.; Milacic, R.; Scancar, J. Comparison of various phosphate stabilization agents for the immobilization of Ni and Zn in sewage sludge. *Water Air Soil Pollut.* 2004, 156, 57–69.
7. Sneddon, I.R.; Orueetxebarria, M.; Hodson, M.E.; Schofield, P.F.; Valsami-Jones, E. Field trial using bone meal amendments to remediate mine waste derived soil contaminated with zinc, lead and cadmium. *Appl. Geochem.* 2006, 23, 2414–2424.
8. Giammar, D.E.; Xie, L.; Pasteris, J.D. Immobilization of lead with nanocrystalline carbonated apatite present in fish bone. *Environ. Eng. Sci.* 2008, 25, 725–735.
9. Munksgaard, N.C.; Lottermoser, B.G.; Blake, K. Prolonged testing of metal mobility in mining-impacted soils amended with phosphate fertilisers. *Water Air Soil Pollut.* 2012, 223, 2237–2255.

10. Thawornchaisit, U.; Polprasert, C. Evaluation of phosphate fertilizers for the stabilization of cadmium in highly contaminated soils. *J. Hazard. Mater.* 2009, 165, 1109–1113.

11. Wang, B.; Xie, Z.; Chen, J.; Jiang, J.; Su, Q. Effects of field application of phosphate fertilizers on the availability and uptake of lead, zinc and cadmium by cabbage (*Brassica chinensis* L.) in a mining tailing contaminated soil. *J. Environ. Sci.* 2008, 20, 1109–1117.

12. Basta, N.T.; McGowen, S.L. Evaluation of chemical immobilization treatments for reducing heavy metal transport in a smelter-contaminated soil. *Environ. Pollut.* 2004, 127, 73–82.

13. Chen, S.; Sun, T.; Sun, L.; Zhou, Q.; Chao, L. Influences of phosphate nutritional level on the phytoavailability and speciation distribution of cadmium and lead in soil. *J. Environ. Sci.* 2006, 6, 1247–1253.

14. Hong, C.O.; Chung, D.Y.; Lee, D.K.; Kim, P.J. Comparison of phosphate materials for immobilizing cadmium in soil. *Arch. Environ. Contam. Toxicol.* 2010, 58, 268–274.

15. Göhre, V.; Paszkowski, U. Contribution of the arbuscular mycorrhizal symbiosis to heavy metal phytoremediation. *Planta* 2006, 223, 1115–1122.

16. Mun, H.W.; Hoe, A.L.; Koo, L.D. Assessment of Pb uptake, translocation and immobilization in kenaf (*Hibiscus cannabinus* L.) for phytoremediation of sand tailings. *J. Environ. Sci.* 2008, 20, 1341–1347.

17. Shelmerdine, P.A.; Black, C.R.; McGrath, S.P.; Young, S.D. Modelling phytoremediation by the hyperaccumulating fern, *Pteris vittata* of soils historically contaminated with arsenic. *Environ. Pollut.* 2009, 157, 1589–1596.

18. Pang, J.; Chan, G.S.Y.; Zhang, J.; Liang, J.; Wong, M.H. Physiological aspects of vetiver grass for rehabilitation in abandoned metalliferous mine wastes. *Chemosphere* 2003, 52, 1559–1570.

19. Chen, Y.; Shena, Z.; Li, X. The use of vetiver grass (*Vetiveria zizanioides*) in the phytoremediation of soils contaminated with heavy metals. *Appl. Geochem.* 2004, 19, 1553–1565.

20. De Andrade Lima, L.R.P.; Bernardez, L.A. Characterization of the lead smelter slag in Santo Amaro, Bahia, Brazil. *J. Hazard. Mater.* 2011, 189, 692–699.

21. Kede, M.L.F.M.; Moreira, J.C.; Mavropoulos, E.; Rossi, A.M.; Bertolino, L.C.; Pérez, D.V.; Rocha, N.C.C. Study of lead behaviour in Brazilian latosols treated with phosphates: Contributions to the remediation of contaminated sites. *Química Nova* 2008, 3, 579–584.

22. Melamed, R.; Cao, X.; Chen, M.; Ma, L.Q. Field assessment of lead immobilization in a contaminated soil after phosphate application. *Sci. Total Environ.* 2003, 305, 117–127.

23. Cao, X.; Wahbi, A.; Ma, L.; Li, B.; Yang, Y. Immobilization of Zn, Cu, and Pb in contaminated soils using phosphate rock and phosphoric acid. *J. Hazard. Mater.* 2009, 164, 555–564.

24. EMBRAPA-Solos; Nacional Centre of Soil Research. *Manual de Métodos de Análise do Solo*; Embrapa: Rio de Janeiro, Brazil, 2011. (In Portuguese)
25. USEPA. Method 3051A Microwave Assisted Acid Digestion of Sediments, Sludges, Soils, and Oils. Available online: http://www.epa.gov/osw/hazard/testmethods/sw846/pdfs/3051a.pdf (accessed on 30 March 2011).
26. USEPA. Method 1311 Toxicity Characteristic Leaching Procedure (TCLP). Available online: http://www.epa.gov/osw/hazard/testmethods/sw846/pdfs/1311.pdf (accessed on 20 November 2004).
27. Intawongse, M.; Dean, J.R. Uptake of heavy metals by vegetable plants grown on contaminated soil and their bioavailability in the human gastrointestinal tract. Food Addit. Contam. 2006, 23, 36–48.
28. Pueyo, M.; Mateu, J.; Rigol, A.; Vidal, M.; López-Sánchez, J.F.; Rauret, G. Use of the modified BCR three-step sequential extraction procedure for the study of trace element dynamics in contaminated soils. Environ. Pollut. 2008, 152, 330–341.
29. OECD. Guideline for Testing of Chemicals: Earthworm’s Acute Toxicity Test. No. 207, 4 April 1984. Available online: http://www.oecd.org/chemicalsafety/risk-assessment/1948293.pdf (accessed on 29 October 2014).
30. International Organization for Standardization. ISO 11268-1:1993 Soil Quality. Effects of Pollutants on Earthworms Part 1: Determination of Acute Toxicity Using Artificial Soil Substrate. Geneva, Switzerland, 1993.
31. International Organization for Standardization. ISO 11268-2:1993 Soil Quality. Effects of Pollutants on Earthworms Part 2: Determination of Effects on Reproduction. Geneva, Switzerland, 1993.
32. Sistema Para Análise Estatística e Genética (SAEG). V 9, Viçosa; Universidade Federal de Viçosa: Viçosa, State of Minas Gerais, Brazil, 2005. (In Portuguese)
33. Fernandez, G.C.J. Residual analysis and data transformations—Important tools in statistical analysis. Hortscience 1992, 27, 297–300.
34. Naidu, R.; Bolan, N.S. Contaminant chemistry in soils: Key concepts and bioavailability. In Chemical Bioavailability in Terrestrial Environmental; Naidu, R., Ed.; Developments in Soil Science: Oxford, UK, 2008; Volume 32, pp. 9–37.
35. Alloway, B.J. Soil processes and the behavior of metals. In Heavy Metals in Soils; Alloway, B.J., Ed.; Blackie Academic & Professional: London, UK, 1995; pp. 11–37.
36. Magna, G.A.M.; Machado, S.L.; Portella, R.B.; de Carvalho, M.F. Chumbo e cádmio detectados em alimentos vegetais e gramineas no município de Santo Amaro-Bahia. Química Nova 2013, 7, 989–997.
37. Sánchez-Jiménez, N.; Gismera, M.J.; Sevilla, M.T.; Cuevas, J.; Rodríguez-Rastrero, M.; Procopio, J.R. Clayey materials as geologic barrier in urban landfills: Comprehensive study of the interaction of selected quarry materials with heavy metals. Appl. Clay Sci. 2012, 56, 23–29.
38. Shaheen, S.M. Sorption and liability of cadmium and lead in different soils from Egypt and Greece. Geoderma 2009, 153, 61–68.
39. Spurgeon, D.J.; Hopkin, S.P.; Jones, D.T. Effects of cadmium, copper, lead and zinc on growth, reproduction and survival of the earthworms *Eisenia fetida* (Savigny): Assessing the environmental impact of point-source metal contamination in terrestrial ecosystems. *Environ. Pollut.* **1994**, *84*, 120–130.

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