HELEN CARLA SANTANA AMORIM

PLANT AFFECTED LEAD SPECIATION AND AVAILABILITY IN A SOIL CONTAMINATED BY LEAD SMELTER

Dissertação apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Solos e Nutrição de Plantas, para obtenção do título de *Magister Scientiae*.

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BIOGRAFIA

Helen Carla Santana Amorim, filha de Carlos Henriques Ribeiro de Amorim e Elazir Rosa de Santana Amorim, nasceu em 26 de fevereiro de 1991 em Abre Campo, Minas Gerais.

Cursou o ensino médio no Colégio de Aplicação da Universidade Federal de Viçosa (COLUNI), concluindo o mesmo em dezembro de 2009.

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Em março de 2016, ingressou no Programa de Mestrado em Solos e Nutrição de Plantas da Universidade Federal de Viçosa, submetendo-se à defesa em fevereiro de 2018.

SUMÁRIO

ABSTRACT

AMORIM, Helen Carla Santana, M.Sc., Universidade Federal de Viçosa, February, 2018. **Plant affected lead speciation and availability in a soil contaminated by lead smelter.** Advisor: Maurício Paulo Ferreira Fontes. Co-advisors: Ivo Ribeiro da Silva and Leonardus Vergütz.

Soil contamination by lead (Pb) represents a relevant problem for public and environmental health worldwide. Pb chemical species determine its mobility in soil, its availability to plants, and therefore, its potential toxicity in the environment. Understanding the factors that affect Pb speciation in soil-plant system is vital to develop effective remediation techniques for contaminated sites. Thus, the main goal of this study was to assess Pb speciation in a soil contaminated by lead smelter particulate material and to relate it to availability to different plant species. Soil samples from a Typic Hapludert (0-5 and 5-15 cm) were collected in Santo Amaro, BA, Brazil, prepared to obtain the fine earth fraction (< 2 mm), had their physical, chemical and mineralogical properties determined. Total Pb concentration and Pb fractions in soil were determined. Due to their different total Pb concentrations, soil samples were named HPb and LPb, respectively. Soil samples were stored in plastic pots and used in a glasshouse experiment. It was arranged a randomized block design in a 2 x factorial scheme (3 + 1), corresponding to the two soil depths (HPb and LPb) and to the three plant species: eucalyptus (*Eucalyptus urophylla x Eucalyptus grandis*), brachiaria (*Brachiaria brizantha* cv. Marandu), mustard (*Brassica juncea* L.), and one control treatment without plant, with six replicates. After three months, plants were collected, separated in roots and shoots, and had the plant dry mass and the total Pb content in plant tissue determined. Soil samples adhered to the roots of the plant species composed the rhizosphere soil; soil samples of non-planted pots were considered bulk soil. Soil samples had the total Pb concentration and fractions again determined. Pb speciation was assessed by synchrotron-based x-ray absorption spectroscopy (XAS) only in bulk and rhizosphere soil samples from the 0-5 cm depth (HPb). Differences in Pb total concentration in soil, Pb fractions in soil, Pb content in plant material, and dry mass were tested using ANOVA followed by the Tukey test ($p<0.05$). XANES spectra were normalized, corrected, and the proportions of Pb species in soil samples were obtained

through linear combination fitting (LCF). The cultivation of eucalyptus caused an increase in the total Pb concentration in rhizosphere soil from both soil depths investigated. The SEP demonstrated the predominance of Pb bound to Fe- and Mn-(hydr)oxides (Pb-Red) for rhizosphere and bulk soil samples. The cultivation of mustard and eucalyptus caused a decrease in the proportion of Pb-Red for both soil samples (HPb and LPb), respectively, indicating a possible dissolution of these compounds in soil. In terms of chemical speciation, bulk soil demonstrated the predomince of Pb-kaolinite species, followed by Pb-goethite and Pb-ferrihydrite. In rhizosphere soils, it was observed a decrease in the proportion of Pb-kaolinite species, the absence of Pb bound to Fe-(hydr)oxides species, and the presence of Pb acetate. This suggests that, under rhizosphere conditions, a possible dissolution of Pb bound to Fe-(hydr)oxides species, coupled to the complexation of Pb by low molecular weight organic acids may be occurring. The cultivation of mustard favored the lowest proportion of Pb-acetate in rhizosphere soil, and a higher Pb content in roots, indicating its potential as Pb accumulator specie. The cultivation of brachiaria favored the highest proportion of Pb-acetate in rhizosphere soil, and a lower Pb content in roots, indicating that the cultivation of this plant specie favors the accumulation of more soluble Pb species in soil. Eucalyptus accumulated the highest Pb stock in plant tissue, indicating its potential in phytoremediation studies. Our work confirms that the presence of plants and their associated rhizospheres alter Pb chemical speciation in soils. The presence of Pb soluble species in soil depends on the plant specie cultivated, and not necessarily represents a higher Pb uptake by plants.

RESUMO

AMORIM, Helen Carla Santana. M.Sc., Universidade Federal de Viçosa, fevereiro de 2018. **Especiação afetada por plantas e disponibilidade de chumbo em um solo contaminado por metalúrgica de chumbo**. Orientador: Maurício Paulo Ferreira Fontes. Coorientadores: Ivo Ribeiro da Silva e Leonardus Vergütz.

A contaminação dos solos por chumbo (Pb) representa um problema relevante para a saúde pública e ambiental mundialmente. As espécies químicas de Pb determinam sua mobilidade no solo, sua disponibilidade para as plantas, e portanto, sua toxicidade potencial no ambiente. Compreender os fatores que afetam a especiação de Pb no sistema solo-planta é fundamental para o desenvolvimento de técnicas de remediação efetivas para sítios contaminados. Assim, o objetivo deste estudo foi avaliar a especiação química de Pb em um solo contaminado por material particulado de metalurgia e relacioná-la a sua biodisponibilidade para diferentes espécies de plantas. Amostras de um Vertissolo (0-5 e 5-15 cm) foram coletadas em Santo Amaro, BA, Brasil, preparadas para a obtenção da fração < 2 mm, e caracterizadas física, química e mineralogicamente. Foram determinados os teores totais e as frações de Pb no solo. Em função dos diferentes teores de Pb no solo, as amostras de 0-5 e 5-15 cm foram nomeadas HPb e LPb, respectivamente. As amostras de solo foram acondicionadas em vasos para a condução do experimento em casa de vegetação. O experimento foi disposto em um delineamento em blocos casualizados, em um esquema fatorial 2 x (3+1), correspondente às duas profundidades (HPb e LPb) e às três espécies de plantas: eucalipto (*Eucalyptus urophylla x Eucalyptus grandis*), braquiária (*Brachiaria brizantha* cv. Marandu), mostarda (*Brassica juncea* L.), e um controle sem planta, com seis repetições. Após três meses de cultivo, as plantas foram coletadas, separadas em raiz e parte aérea, e tiveram a massa de matéria seca e os teores totais de Pb no material vegetal determinados. Amostras de solo aderidas às raízes das plantas constituíram o solo rizosférico; considerou-se como solo não rizosférico o solo dos vasos sem planta. Novamente, determinou-se os teores totais e as frações de Pb no solo. A especiação de Pb foi determinada através de espectroscopia de absorção de raios-X (XAS) baseada em luz síncrotron apenas nas amostras de solo rizosférico e não rizosférico da profundidade

0-5 cm (HPb). Os dados de massa de matéria seca, os teores totais de Pb nas amostras de solo, nas frações, e no material vegetal foram submetidos a ANOVA e comparados pelo teste de Tukey ($p < 0.05$). Os espectros obtidos através da XAS foram normalizados, corrigidos, e as proporções entre as espécies químicas de Pb nas amostras de solo foram obtidas através de *linear combination fitting* (LCF). O cultivo de eucalipto proporcionou um aumento nos teores totais de Pb no solo rizosférico nas duas profundidades de solo avaliadas. A extração sequencial mostrou o predomínio de formas de Pb ligadas a (hidr)óxidos de Fe e Mn (Pb-Red) nos solos cultivados e não cultivado. O cultivo de mostarda e eucalipto promoveu uma redução na proporção de Pb-Red em ambas as camadas de solo (HPb e LPb), respectivamente, indicando uma possível dissolução destes compostos no solo. Em termos de especiação química, o solo não cultivado apresentou o predomínio de formas de Pb-caulinita, seguido por Pb-goethita e Pb-ferridrita. Nos solos cultivados, observou-se diminuição da proporção de formas de Pb-caulinita, a ausência de formas de Pb associadas a (hidr)óxidos de Fe e a presença de Pb-acetato. Isto sugere que, em condições de rizosfera, uma possível dissolução de compostos de Pb ligados a (hidr)óxidos de Fe, seguida pela complexação de Pb por ácidos orgânicos de baixo peso molecular possa estar ocorrendo. O cultivo de mostarda proporcionou a menor proporção de acetato de Pb no solo e uma maior concentração de Pb nas raízes, indicando o potencial dessa espécie como acumuladora de Pb. O cultivo de braquiária proporcionou a maior proporção de acetato de Pb no solo e uma menor concentração de Pb nas raízes, indicando que o cultivo dessa espécie favorece a acumulação de formas mais solúveis de Pb no solo. O eucalipto acumulou o maior estoque de Pb em material vegetal, indicando o potencial dessa espécie para estudos de fitorremediação. Nosso trabalho confirma que a presença das plantas e suas rizosferas associadas alteram a especiação de Pb no solo. A presença de formas solúveis de Pb no solo é dependente da espécie de planta cultivada, e não necessariamente implica em uma maior absorção pelas plantas.

INTRODUCTION

Lead (Pb) pollution became one of the most concerning problems to ecosystem and human health in the last decades. Pb mining and smelting activities have led to heavy contamination of soil and water (Shahid *et al*., 2014; Cai *et al*., 2015; Li *et al*., 2015), especially in developing countries (Li *et al*., 2014; Yabe *et al*., 2015) where environmental regulations are not very strict or, sometimes, absent. In Brazil, one of the most polluted sites is located in Santo Amaro, Bahia (Anjos, 2003; Andrade Lima & Bernardez, 2017). This city hosted for 33 years (1960-1993) a Pb smelter that improperly managed the Pb slag and emitted high amounts of particulate material, damaging agricultural activities and causing serious diseases to the population. Several studies have been conducted to assess total Pb concentrations in these soils. Although this information is crucial to the development of environmental guidelines, it is limited, since it does not show how the metal is bound to the soil constituents. By identifying Pb-bearing forms, the fate of the contaminant in the environment can be predicted with greater accuracy, this its potential toxicity to living organisms. Therefore, knowledge on Pb forms in soil is vital to assess the hazard of Pb pollution in soils, and to develop proper remediation technologies.

Pb in soils exists in several forms, such as: dissolved in soil solution, exchangeable in organic and inorganic components, structural components of mineral lattices, and insoluble precipitated components (Alloway, 1990; Kabata-Pendias, 2011). Its bioavailability is controlled by soil properties, including mineralogy (Fontes *et al*., 2000; Fontes & Santos, 2010), organic matter content and quality (Duan *et al*., 2014; McBride, 2016), pH, redox (Eh) conditions, and cation exchange capacity (CEC) (Vega

et al., 2010; Mao *et al*., 2014). There is also growing evidence that the presence of plants and their associated rhizospheres may alter Pb bioavailability (Hashimoto *et al*., 2011; Yang *et al*., 2012). Root induced changes in pH and Eh as well as root exudates and rhizodeposits may alter Pb species in rhizosphere soil. In addition, metals can be released from exchangeable sites due to complex formation of metal-organic chelates with organic acids in root exudates (Shahid *et al*., 2012). This may be dependent on rhizosphere biochemistry and plant species involved, but research on this topic is still scarce. Thus, it is important that we understand the transformations of Pb species that occur in the rhizosphere zone and how they relate to Pb bioavailability.

Traditionally, Pb bioavailability in soils is evaluated through indirect methods of determination such as sequential extraction procedures (SEP) (Rauret *et al*., 1999; Zimmermann & Weindorf, 2010; Sungur *et al*., 2014). These procedures consist on applying sequentially, in the same soil sample, extractants with lower, medium and higher extraction power, which allows separating the metal in more and less labile forms. Despite of its widespread use, these procedures only provide operationally defined forms of metals, they are not chemically specific, and may produce artifacts, such as dissolution of a non-target phase, precipitation and readsorption into soil constituents (Calmano *et al*., 2001). Moreover, indirect approaches do not provide information on the actual relationship, at a molecular level, between the contaminant and a certain phase (Manceau *et al*., 2002; Nevidomskaya *et al*., 2016). Therefore, to quantify Pb species in soils, and to understand the behavior of the contaminant in soil, is highly desirable to apply a more selective and non-destructive technique.

X-Ray Absorption Near Edge Structure (XANES) spectroscopy has emerged as the best available tool to assess Pb chemical species in soils and sediments (Manceau *et al*., 1996; Barret *et al*., 2010, Duan *et al*., 2014; Luo *et al*., 2016). Synchrotron-based XANES has high resolution, low detection limits, elemental selectivity, and it does not require previous preparation of the sample. It is the only technique that would allow us to obtain detailed information on Pb oxidation state and Pb coordination number in a complex matrix such as soil samples. These specific electronic and structural properties are fundamental to understand the transformations that occur on Pb species in contaminated soils when cultivated with different plant species. By assessing Pb chemical species in rhizosphere soil, the mechanisms involved in metal sorption, complexation and uptake by plants can be better elucidated. Such detailed information is vital for management and rehabilitation of Pb-contaminated soils.

Finally, to completely understand Pb bioavailability in the metal-soil-plant system, it is necessary to assess the plants' response to Pb toxicity. Pb accumulation induces severe destructive effects in plants. It damages plant growth, root elongation, seedling development, chlorophyll production, and cell division (Gupta *et al*., 2009, 2010; Maestri *et al*., 2010). The extent of these effects depends on the plant's stage of development, compartments evaluated, and tolerance mechanisms. Such mechanisms include Pb complexation on cell walls (Jiang & Liu, 2010), protein induced detoxification and excretion (Meyers *et al*., 2008), and activation of antioxidant enzymes (Singh *et al*., 2010; Gupta *et al*., 2010). The efficiency of these detoxification mechanisms defines the tolerance or the sensitivity of different plant species to Pb toxicity. Pb uptake by plants is also affected by Pb concentration and speciation in soils.

Although much is known on how Pb concentration in soils affects plants growth and productivity, the relationship between Pb speciation in soils and its accumulation in plants remains unexplored. To obtain a whole understanding on Pb bioavailability in the environment is highly desirable to couple specific information on Pb forms in soils to plants' growth.

The main objective of this study was to understand how different plant species affect Pb speciation and availability in a soil contaminated by lead smelter. Our specific objectives were i) assess Pb speciation in rhizosphere and bulk soils and ii) relate Pb speciation in soils to its availability to different plant species. We tested the hypothesis that Pb speciation in rhizosphere soils is plant specie-dependent. A XANES analysis was applied to provide a qualitative and quantitative estimate of Pb species in bulk and rhizosphere soils. A modified BCR sequential extraction procedure was used to support the spectroscopic analysis. Pb bioavailability to plant species will be assessed by determining plant dry mass and Pb content in plant tissue. Three different plant species were examined: eucalyptus, fast growing specie with high economic value; brachiaria, plant specie cultivated in the lead smelter area; and mustard, that is a Pb hyperaccumulator plant specie.

MATERIALS AND METHODS

Soil Sampling

The study site is located northwest of the urban area of Santo Amaro, Bahia, Brazil, near to an inactive lead smelter (12° 32' 25"S, 38° 43' 44" W). According to the Koppen classification (Koppen & Geiger, 1936), climate in this area is predominantly Af, tropical with average rainfall of at least 60 mm in every month. Average annual temperature is 25.4 ºC (average maximum temperature of 31 ºC and average minimum temperature of 21.9 ºC), and average annual rainfall is 1540 mm, with the rain season concentrated between April and June (Embrapa, 2000; Anjos, 2003). Geologically, this area consists majorly of dark grey and green shales, fine and clayey sandstones, and limestones (Brasil, 1981). The soil was classified as a Typic Hapludert (Vertisol) according to the U.S. Soil Taxonomy (Soil Survey Staff, 2010) due to its shrinking and swelling properties (presence of cracks in dry seasons), dark color, high base saturation, clayey texture and pH > 5.0.

Briefly, surface soil samples (0-5 and 5-15 cm depth) were collected inside the area of influence of the lead smelter, under secondary vegetation, at the upper portion of the landscape. The sampling site was carefully chosen in order to represent only soils contaminated by the atmospheric emissions of the lead smelter in the past, and to avoid contamination with the lead slag heap, that was located at the lowest portion of the landscape. Approximately 120 kg of soil material (60 kg of soil from 0-5 cm depth and 60 kg of soil from 5-15 cm) were manually collected and after collection, the soil samples were kept on plastic bags and transported to the laboratory within two days.

Soil Samples Characterization

Soil samples were air-dried, ground to obtain the fine earth fraction (< 2mm) and homogenized. These samples were used for the glasshouse experiment and for the laboratory analyses. Physical and chemical analyses were performed following the methodology recommended by Embrapa (1997). Soil texture analysis was preceded by chemical dispersion of the fine earth fraction using 1 mol L^{-1} sodium hydroxide (NaOH) under continuous stirring at 50 rpm during 16 hours. After complete dispersion, the samples were wet-sieved through a 53-μm mesh screen to separate the sand from the silt and clay fractions. The fraction <53 μm was used for quantifying clay and silt content by using the pipette method, by applying the Stoke's law. The fractions obtained were dried at 105 °C during 48 hours and weighed.

Soil samples were characterized for pH in a 1:2.5 soil: solution (H_2O) suspension; Ca^{2+}, Mg^{2+} and Al^{3+} were extracted with 1 mol L^{-1} potassium chloride (KCl); H + Al were extracted using 0.5 mol L^{-1} calcium acetate ($(C_2H_3O_2)_2Ca$) adjusted to pH 7.0; P and K+ were extracted using 0.05 mol L^{-1} hydrochloric acid (HCl) + 0.0125 mol L^{-1} sulfuric acid (H_2SO_4) (Mehlich-1), and quantified using colorimetry and flame photometry, respectively. Effective cation exchange capacity (ECEC), cation exchange capacity (CEC), percentage of base saturation (BS) and percentage of aluminum saturation (AS) were calculated according to Embrapa (1997). The soil organic matter (SOM) content was determined using 0.5 mol^{-1} potassium dichromate ($K_2Cr_2O_7$) + concentrated H_2SO_4, followed by titration of the excess of dichromate with 0.05 mol L^{-1} ammonium ferrous sulfate ($(NH_4)_2(SO4)_2.6H_2O$), and using diphenylamine ($(C_6H_5)_2NH$) as an indicator (Walkley-Black method).

For the mineralogical analysis, soil samples were submitted to oxidation of organic matter (Embrapa et al., 1997) and dispersion (Almeida *et al*., 2013). 5 g of the fine earth fraction were treated with 20 mL of 6% v/v sodium hypochlorite (NaOCl) adjusted to pH 9.5 in a water- bath during 15 minutes at 75°C to remove organic matter. The residue was centrifuged at 1500 rpm for 5 minutes, the supernatant was discarded and the residue was washed with deionized water. This procedure was repeated three times in total, until a clear solution was obtained. Finished the oxidation of organic matter, the samples were dispersed into 2.5 mL of 1 mol L^{-1} sodium hexametaphosphate ($(NaPO_3)_6$) buffered with sodium carbonate (Na_2CO_3) and 25 mL of deionized water into a 50 mL centrifuge tube during 16 hours under continuous stirring at 50 rpm.

After dispersion, the samples were wet-sieved through a 53 μm mesh screen to separate the sand- from the silt- and clay-sized fractions. The clay fraction was separated using a siphon after the sedimentation of the silt fraction according to the Stoke's Law (Embrapa, 1997). After the complete separation of the silt and clay fractions, the material was dried at 45 °C and the mineralogical composition was qualitatively assessed by X-ray diffraction (XRD) using finely powdered material (<149 μm). Non oriented samples were prepared with sand and silt samples. Oriented samples were prepared with clays in natural state (natural clays) and clays that had the iron oxides removed by using dithionite-citrate buffered with sodium carbonate (iron-free clays). The XRD analyses were conducted using Co-Ka radiation (λ= 0.178896 nm) at 40 kV and 40 mA from a multifunctional diffractometer (Panalytical X'Pert Pro PW 3040/60). XRD patterns were obtained between 4 and 70 °2θ a scan rate of 0.0167 °2θ s^{-1} in the sand and silt samples, and between 4 and 50 °2θ at a scan rate of 0.0167 °2θ s^{-1}

in the natural and iron-free clays. In order to identify the 2:1 minerals, iron-free clays were saturated with 0.5 mol^{-1} magnesium chloride ($MgCl_2$), glycerol: ethanol (1:1), and 1 mol^{-1} KCl at 25°C, 350°C and 550°C. XRD patterns in these samples were obtained between 4 and 40 °2θ at a scan rate of 0.0167 °2θ s^{-1}.

Samples had the total concentration of Si, Al and Fe determined after sulfuric acid digestion (Embrapa, 1997). One gram of the fine earth fraction was weighed in digestion tubes and treated with 20 ml of concentrated sulfuric acid (H_2SO_4) diluted in deionized water (1:1) during 1 hour at 180°C at the digestion block. After cooling, the digestion tubes were rinsed with approximately 20 ml of deionized water, the digest was filtered with slow filter papers (Whatman no. 42) in volumetric flasks, and had the volume completed to 250 ml with deionized water. Al and Fe were determined in the filtrate through Atomic Absorption Spectrometry (AAS). The residue of the filtration was treated with 4 ml of 40% (v/v) NaOH, kept at the heating block at 220°C, and left boiling during 2 minutes. After cooling, the basic-digested residue was transferred to volumetric flasks, added 10 ml of 6 mol^{-1} HCl, and had the volume completed to 200 ml with deionized water. This extract was used to determine Si by Atomic Absorption Spectrometry (AAS).

Clay samples were submitted to selective dissolution of poorly crystalized Al- and Fe-(hydr)oxides using ammonium oxalate (AO) following McKeague & Day (1966) and crystalline Al- and Fe-(hydr)oxides using dithionite-citrate-bicarbonate (DC) following Mehra & Jackson (1960). The procedure to extract the amorphous Al- and Fe-(hydr)oxides consisted on treating 0.2 g of clay with 10 mL of 0.2 mol L^{-1} AO pH 3.0 in the dark. The samples were kept in the dark by centrifuge tubes covered with

aluminum foils, and were shaken for 2 hours at 120 rpm. After the extraction procedure, the samples were centrifuged at 3000 rpm, the supernatant was collected and stored into centrifuge tubes to quantify the amount of Al and Fe released during the treatment. The extraction of crystalline Al- and Fe-(hydr)oxides consisted on treating 0.2 g of clay with 10 mL of 0.2 mol L^{-1} DC in centrifuge tubes, which were kept under water bath for 30 minutes at 50°C. Afterwards, the samples were centrifuged at 2000 rpm during 20 minutes, the supernatant collected and stored. The extraction procedure using DC was repeated three times. The amounts of Al and Fe extracted by AO and DC were determined by Atomic Absorption Spectrometry (AAS).

Total Pb concentration in soil

In order to assess the total concentration of Pb, soil samples were submitted to acid digestion following the method USEPA 3052 from the United States Environmental Protection Agency (USEPA, 1996). 0.5 g of finely powdered material was weighed, transferred to Teflon vessels, treated with 9 ml of 68% (v/v) nitric acid (HNO_3) + 3 ml of 40% (v/v) hydrofluoric acid (HF), and pre-digested for 30 min with vessels loosely capped. The vessels were sealed and submitted to microwave irradiation (Multiwave 3000 Anton Paar). The system was programmed to rise to 180 ± 5°C and 16 atm of pressure in approximately 5.5 min, and to remain at 180 ± 5°C for 9.5 min, reaching 15 min of digestion period. After cooling, the digest was filtered through slow filter papers (Whatman no. 42) in volumetric flasks, and had the volume completed to 50 ml with deionized water. The amount of Pb extracted was determined by Atomic Absorption Spectrometry (AAS).

Sequential Extraction Procedure (SEP)

A modified three step SEP was employed to partition Pb in the soil samples, following the method developed by the European Community Bureau of Reference (*Bureau Communautaire de Référence*, BCR) (Ure *et al.*, 1993). To perform the procedure, 1 g of soil was treated with 40 ml of 0,11 mol L^{-1} acetic acid (CH_3COOH) in centrifuge tubes (50 ml), and kept under mechanical agitation for 16 h at 40 rpm using an end-over-end shaker. After this step, the samples were centrifuged at 3000 rpm for 25 min and the supernatant collected, acidified with 2% (v/v) HNO_3, and stored in centrifuge tubes (15 ml) to quantify the amount of Pb released during the extraction. Then, the residue was washed by adding 20 ml of deionized water, shaken for 15 min and centrifuged for 20 min at 3000 rpm. The supernatant was discarded carefully to avoid losses of the solid residue. The fraction collected in this step of the procedure was named Pb-Exc, which stands for the exchangeable Pb forms (water soluble and bound to carbonates).

The second step consisted on treating the residue from the previous step with 40 ml of 0,5 mol L^{-1} hydroxylammonium chloride ($NH_2OH.HCl$) + 2,0 mol L^{-1} HNO3 in centrifuge tubes, and keeping it under mechanical agitation for 16 h at 40 rpm. After that, the samples were centrifuged at 3000 rpm for 25 min and the supernatant collected, acidified with 2% (v/v) HNO_3, and stored in centrifuge tubes to quantify the amount of Pb released during the extraction. Then, the residue was washed by adding 20 ml of deionized water, shaken for 15 min and centrifuged for 20 min at 3000 rpm. The supernatant was discarded carefully to avoid losses of the solid residue. The fraction

collected in this step of the procedure was named Pb-Red, which stands for the reducible Pb forms (bound to amorphous Fe- and Mn- (hydr)oxides).

The third step consisted on treating the residue from the previous step with 10 ml 8,8 mol L^{-1} hydrogen peroxide (H_2O_2), followed by digestion at room temperature for 1 h with occasional manual shaking with the vessels loosely capped. Then, the samples were placed in a water-bath at 85°C for 1 h digestion, and the vessels were uncapped so that the volume was reduced to less than 3 ml. After cooling, the samples were treated with 40 ml of 1,0 mol L^{-1} ammonium acetate (CH_3COONH_4) and kept under mechanical agitation for 16 h at 40 rpm. Then, the samples were centrifuged at 3000 rpm for 25 min and the supernatant collected, acidified with 2% (v/v) HNO_3, and stored in centrifuge tubes to quantify the amount of Pb released during the extraction. The residue was washed by adding 20 ml of deionized water, shaken for 15 min and centrifuged for 20 min at 3000 rpm. The supernatant was discarded carefully to avoid losses of the solid residue. The fraction collected in this step of the procedure was named Pb-SOM, which stands for the Pb forms bound to organic matter.

The amount of Pb extracted in each step was determined by Atomic Absorption Spectrometry (AAS). The Pb forms not recovered in the previous steps were considered to be occluded forms of Pb, or structural Pb forms in Fe- and Al-(hydr)oxides, insoluble in the applied reagents. To determine this fraction, we subtracted the amount of Pb extracted in each step (sum of step 1 + step 2 + step 3) from the total amount of Pb obtained in the USEPA 3052 digestion, which is a total decomposition method for siliceous and other complexes matrices. Therefore, this fraction was named residual (Pb-Res).

Table 1. Procedures and reagents used in each step of the sequential extraction procedure following the BCR method.

Fraction	Extractable Fraction	Reagents	Volume mL	Temperature °C	Extraction Method
Pb-Exc	Water soluble and bound to carbonates	0,11 mol L^{-1} acetic acid (CH_3COOH)	40	Room temp. (22 ± 2)	Mechanical agitation for 16 h
Pb-Red	Bound to amorphous Fe- and Mn-(hydr)oxides	0,5 mol L^{-1} hydroxylammonium chloride ($NH_2OH.HCl$) + 2,0 mol L^{-1} HNO_3	40	Room temp. (22 ± 2)	Mechanical agitation for 16 h
Pb-Mos	Bound to organic matter	8,8 mol L^{-1} hydrogen peroxide (H_2O_2)	10	Room temp. (22 ± 2)	Digestion for 1 h with occasional manual shaking
			10	85 ± 2	Digestion for 1 h in water-bath
		1,0 mol L^{-1} ammonium acetate (CH_3COONH_4)	40	Room temp. (22 ± 2)	Mechanical agitation for 16 h
Pb-Res	Structural Fe- and Al-(hydr)oxides				Pb total concentration - ∑(S1+S2+S3)

Glasshouse Experiment

After physical and chemical characterization, the Pb contaminated soil samples were used in a glasshouse experiment. The experiment was conducted in a randomized block design, in a factorial scheme with two factors with three levels each (2x(1+3)). The first factor corresponded to the effect of different Pb concentrations in soil: 0-5 cm depth is the higher Pb concentration (HPb soil) and 5-15 cm is the lower Pb concentration (LPb soil). The second factor corresponded to the plant species effect.

Three plant species were tested in this experiment: eucalyptus (*Eucalyptus urophylla* x *Eucalyptus grandis*), a grass specie (*Brachiaria brizantha cv. Marandu*) and Indian mustard (*Brassica juncea* L) (all commercially available). A treatment without plant (control) was used as the control group. Six repetitions of each treatment were assembled, totalizing 48 experimental units.

Plastic pots (Ø 14 cm; 3 kg capacity) were internally lined with polythene bags and filled with approximately 2.5 kg of soil. Each pot received seedlings of eucalyptus, or approximately five seeds of grass or mustard, except the control treatment, and 300 mg dm^{-3} of phosphorus (P) as monoammonium phosphate ($NH_4H_2PO_4$). After two weeks, the soil pots cultivated with grass and mustard were thinned out, remaining only three plants/pot. Plants were grown in the glasshouse for 14 weeks; the temperature in the glasshouse ranged from 18 to 35°C during the experimental period. After 14 weeks, the experiment was disassembled. Firstly, shoots were harvested, carefully washed using a three step washing procedure (tap water, 0.1 mol L^{-1} HCl then deionized water) and dried at 65°C for 48 hours. Afterwards, the shoot dry matter yield was recorded, the samples were ground (< 1 mm) and kept in airtight plastic bags until further analysis.

Secondly, the soil pots were dismantled in order to collect the rhizosphere soil samples. Due to the clayey texture of the soil, a hand-shaking operation did not work well to separate bulk soil (soil falling from the roots) from rhizosphere soil (soil that remained adhered to the roots). Since the whole pot was taken by the plants' roots, it was not possible to separate rhizosphere from bulk soil. Therefore, a small soil sample collected close to the roots of each plant was considered the rhizosphere soil, and the soil sample collected at the control treatment with no plant pot was considered the bulk

soil. The soil samples were freeze-dried and kept in plastic vessels until further analysis. After soil sampling, roots were harvested, washed following the three step washing procedure discussed above, and dried at 65°C for 48 hours. The roots dry mass was recorded, the samples were ground and kept in airtight plastic bags until further analysis.

Total Pb content and Pb stocks in plant material

The dry plant material (shoots and roots) were submitted to acid digestion following the method performed by Oliva *et al*. (2003). 0.5 g of the dried material was transferred to digestion tubes, treated with 5 ml of concentrated HNO_3, and left digesting overnight. Afterwards, the digestion tubes were placed in a heating block; the temperature was slowly raised until 120°C then kept until the color of the extract became clear. The digestion tubes were removed from the heating block and left cooling. Then, 2 ml of H_2O_2 were added to each digestion tube and submitted again to heat until 120°C. When necessary, a few drops of H_2O_2 were added until the extract became clear. The samples were kept under heating until the volume was reduced to approximately 2 ml. After cooling, the extracts were filtered through quantitative filter papers (Whatman no. 40) in volumetric flasks and had the volume completed to 15 ml with deionized water. The amount of Pb in the extract was determined by Atomic Absorption Spectrometry (AAS).

Additionally, Pb stocks in plant material were calculated by multiplying the total Pb content (mg kg-1 of dry matter) obtained through the acid digestion by the dry mass (g) of each plant specie.

XANES Spectroscopy

X-ray absorption near-edge structure (XANES) analysis was used to determine Pb speciation in soil samples following the glasshouse experiment. The experiment was carried out using the XAFS2 beamline of the Brazilian Synchrotron Light Laboratory (LNLS), with an electron beam energy of 1.37 GeV. A double crystal monochromator of Si (111) was applied to obtain an energy resolution of $1.7\times10{-}4$ $\Delta E/E$ at 7 keV, and a Rh-coated toroidal bendable mirror was used in a system of focusing mirrors to get an ultimate beam size of 450 x 250 μm^2.

Due to the challenges of conducting XANES analysis in soil samples with low Pb concentrations, and the limited beamtime available, only the HPb soil samples were used for the XANES measurements. Rhizosphere and bulk soil samples were ground, and the powder was pressed into pellets with 10 mm in diameter and 1 mm thick. The reference compounds were selected based on previous characterization of the soil samples and previous studies (Hashimoto *et al*., 2010; Luo *et al*., 2016) Nine reference compounds were included: PbO, PbO_2, $PbCO_3$, $Pb(CH_3COO)_2$, $Pb_5(PO_4)_3Cl$, Pb sorbed to goethite, kaolinite, illite, and montmorillonite. The detailed procedure for preparation of the reference compounds can be found on Table 2. Spectra for Pb sorbed to ferrihydrite were provided by Dr. Alain Manceau (Institut des Sciences de la Terre, Grenoble).

Table 2. Procedures and reagents used in the synthesis of the XANES spectroscopy reference compounds.

Reference compound	Description
PbO	Sigma Aldrich PA reagent, finely powdered
PbO2	Sigma Aldrich PA reagent, finely powdered
PbCO3	Sigma Aldrich PA reagent, finely powdered
$Pb(CH_3COO)_4$	Sigma Aldrich PA reagent, finely powdered
Pb-kaolinite	A suspension was prepared with 0.5 g of kaolinite (China Clay 46E0995) and 20 ml of PbNO3 0.01 M. The suspension was stirred during 24 h at 50 rpm, had the pH adjusted to 6.0, and afterwards was dried at 40°C for 24 hours.
Pb-illite	A suspension was prepared with 0.5 g of illite (Green Shale Rochester NY USA 46E0315) and 20 ml of PbNO3 0.01 M. The suspension was stirred during 24 h at 50 rpm, had the pH adjusted to 6.0, and afterwards was dried at 40°C for 24 hours.
Pb-montmorillonite	A suspension was prepared with 0.5 g of montmorillonite (Bentonite 46E0435) and 20 ml of PbNO3 0.01 M. The suspension was stirred during 24 h at 50 rpm, had the pH adjusted to 6.0, and afterwards was dried at 40°C for 24 hours.
Pb-ferrihydrite	Pb sorbed to ferrihydrite, 1.5% concentrated, pH 6.5.

The XANES data collection was performed under ambient conditions across the Pb L_{III} absorption edge at 13035 eV. Spectra from most of the reference compounds were collected in transmission mode, with an energy range of 12800 to 13900 eV, step size of 0.5 eV and acquisition time of 1 s at the absorption edge. Spectra from soil

samples and the Pb-goethite reference compound were collected in fluorescence mode, with an energy range of 12800 to 13900 eV, step size of 0.5 eV, and acquisition time of 3 s at the absorption edge. Measurements of XANES spectra of all samples were repeated at least three times per sample to obtain high reproducibility and smooth spectra.

Statistical analysis

As discussed above, the glasshouse experiment was assembled in a randomized block design, in a factorial scheme 2(1+3) corresponding to the two Pb concentrations and the three plant species. However, the statistical analyses were performed separately in HPb and LPb soil samples, since comparing Pb concentrations that were previously known to be different due to the nature of the contamination (deposition of particulate material) did not have practical meaning. Thus, Pb total concentrations in bulk and rhizosphere soils, Pb fractions obtained in the SEP, Pb content in plant material, and shoot and root dry mass were tested using ANOVA followed by the Tukey test ($p<0.05$). All analyses were performed using the SISVAR 5.6 software (Ferreira, 2011). The XANES raw spectra averaging, normalization, and least-squares linear combination fitting (LCF) were performed using the Athena software (Ravel & Newville, 2005). The fits were performed initially with the 10 reference compounds, following a sequential elimination of the reference compounds that did not fit to the soil sample. A fit with all possible combinations between the 10 reference compounds was also performed. The best possible combination of reference compounds were defined as the one with the lowest fit residual value (R-factor value) yielded.

RESULTS

Soil Samples Characterization

Original soil samples (HPb and LPb) have similar textures. HPb soil is composed by 70,9% clay, 25.5% silt, and 3.5% sand, and LPb soil is composed by 69.7% clay, 26.7% silt, and 3.7% sand (Table 1). Both soil samples are classified as clayey soil samples.

Table 1. Soil texture from two soil layers of a Typic Hapludert used in the glasshouse experiment (HPb: high Pb concentration; LPb: low Pb concentration)

Sample	Sand*	Silt**	Clay**	Texture Class
	……g kg^{-1} soil...……			
HPb soil	35	255	709	Clay
LPb soil	37	267	697	Clay

*Separated through wet-sieving with a 53-µm mesh screen; **Quantified through the pipette method, by applying the Stoke's law.

Both soil samples have neutral to alkaline pH (pH_{H20} > 6.0); high base saturation (> 90%), high CEC (> 40 $cmol_c$ dm^{-3}), and high organic matter content (> 4 dag kg^{-1}) (Table 2).

Table 2. Soil chemical properties from two soil layers of a Typic Hapludert used in the glasshouse experiment (HPb: high Pb concentration; LPb: low Pb concentration)

Sample	pH_{H2O} (1:2.5)	P*	K*	Ca^{2+}**	Mg^{2+}**	Al^{3+}**	H +Al¤
		mg dm^{-3}		……..cmol$_c$ dm^{-3} ……			
HPb soil	6.34	6.2	257	34.47	8.05	0.00	1.4
LPb soil	6.45	7.1	170	41.71	9.51	0.00	2.9

*Extracted by Mehlich-1; **Extracted by 1 mol L^{-1} KCl; ¤Extracted by 0.5 mol L^{-1} Calcium acetate pH 7.0.

Sample	SB§	ECEC↓	CEC¶	BS#	AS†	OM∆
	……..……...cmol$_c$ dm^{-3}……..……			%		dag kg^{-1}
HPb soil	43.18	43.18	44.58	96.9	0.0	6.26
LPb soil	51.66	51.66	54.56	94.7	0.0	4.28

§SB=(Ca^{2+}+Mg^{2+}+K^{+}): Sum of bases; ↓ECEC=SB+Al^{3+}: Effective cation exchange capacity; ¶CEC=SB+(H+Al): Cation exchange capacity at pH 7.0; #BS=((SB/CEC)x100): Base saturation; †AS=((Al^{3+}/ECEC)x100): Aluminum saturation; ∆OM= (C.Org x 1,724) Walkley-Black.

Mineralogical analyses indicate in the HPb soil samples the presence of mica, quartz and feldspar in sand fraction (Figure 1); montmorillonite, illite, quartz, and feldspar in the silt fraction (Figure 2); and montmorillonite, illite, and kaolinite in the clay fraction (Figures 3 and 4).

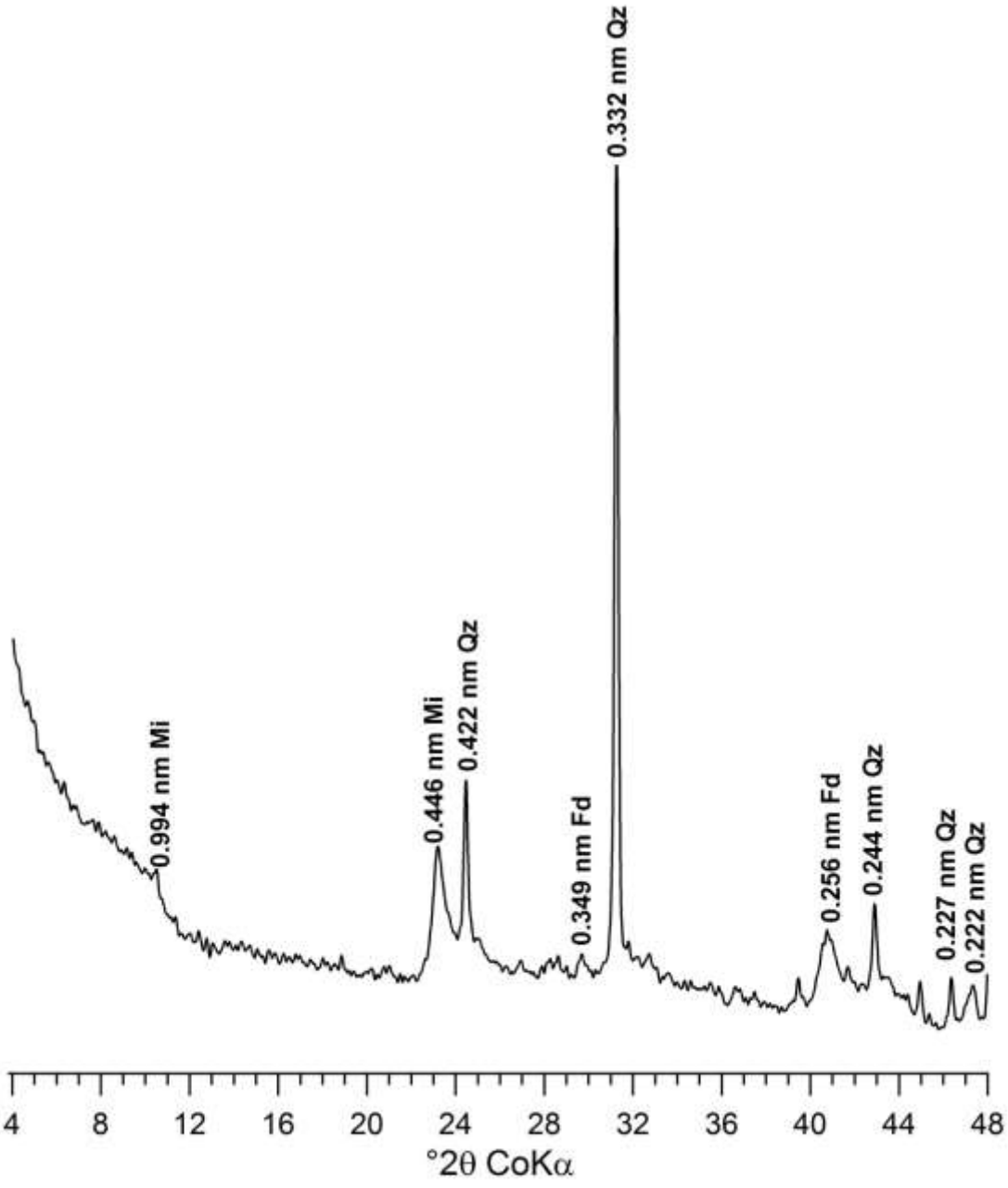

Figure 1. X-ray diffraction pattern from the sand fraction of the HPb soil. Mi: mica; Qz: quartz; Fd: feldspar.

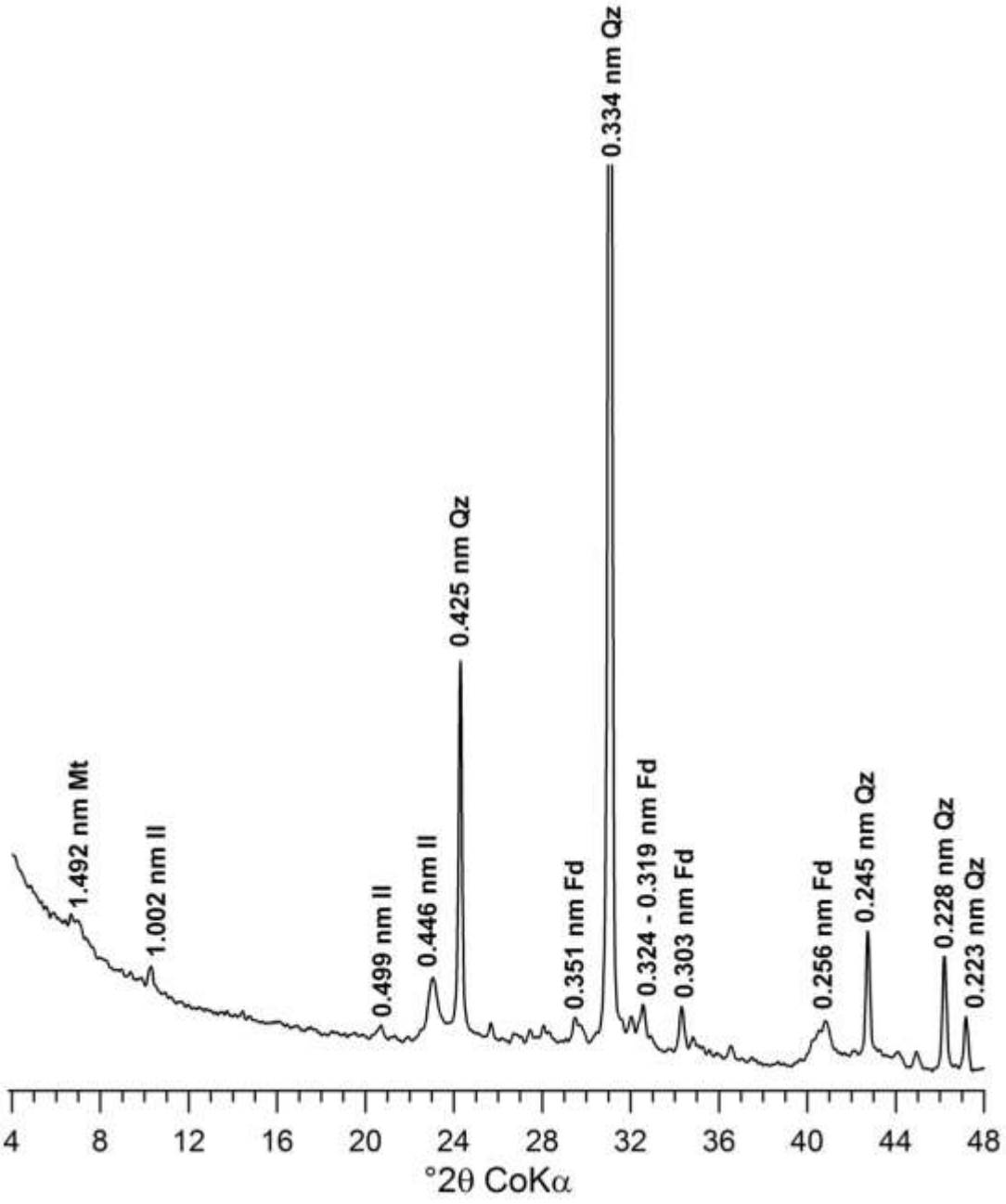

Figure 2. X-ray diffraction pattern from the silt fraction of the HPb soil. Mt: montmorillonite; Il: illite; Qz: quartz; Fd: feldspar.

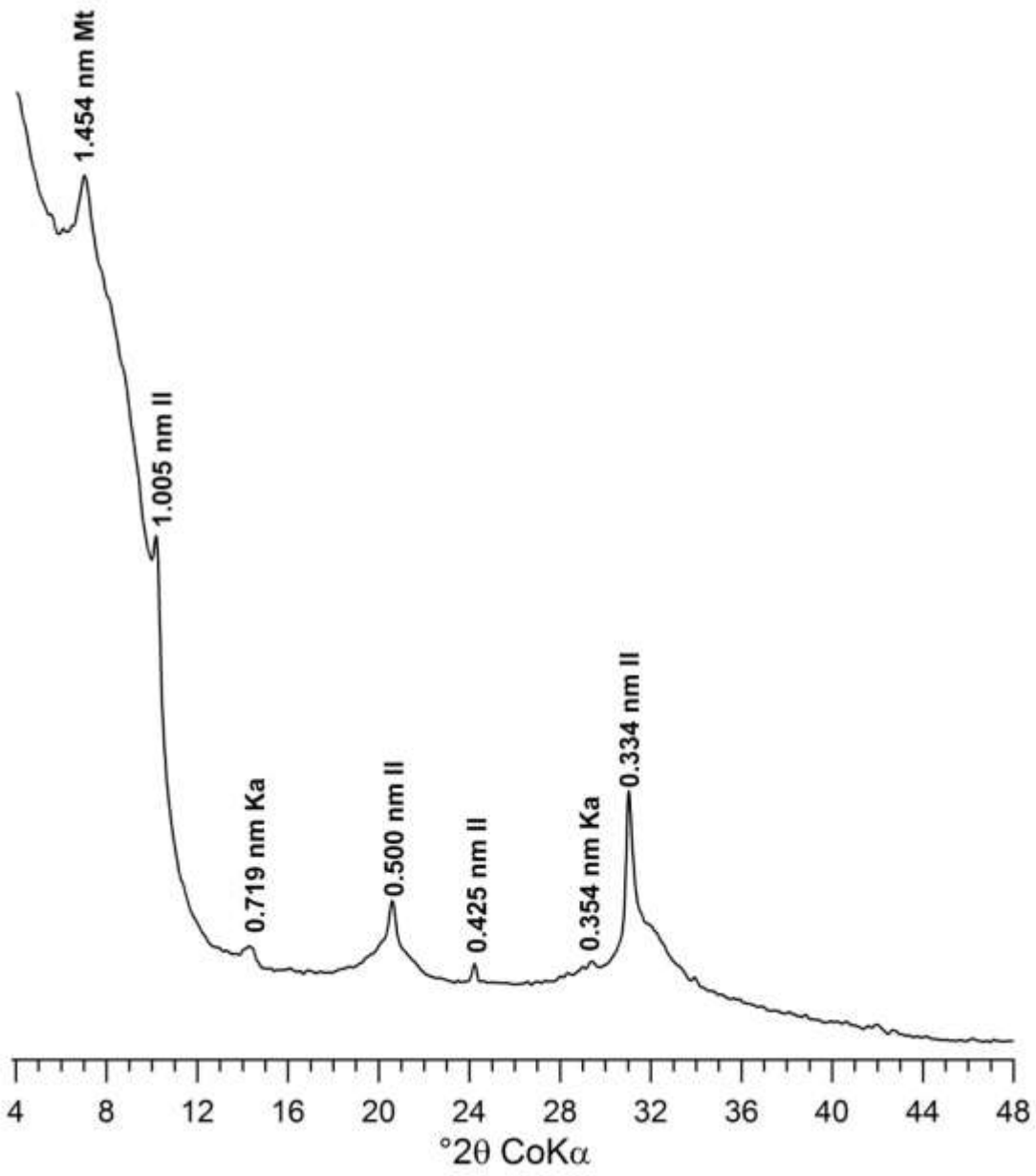

Figure 3. X-ray diffraction pattern from the non-treated clay fraction (natural clay) of the HPb soil. Mt: montmorillonite; Il: illite; Ka: kaolinite.

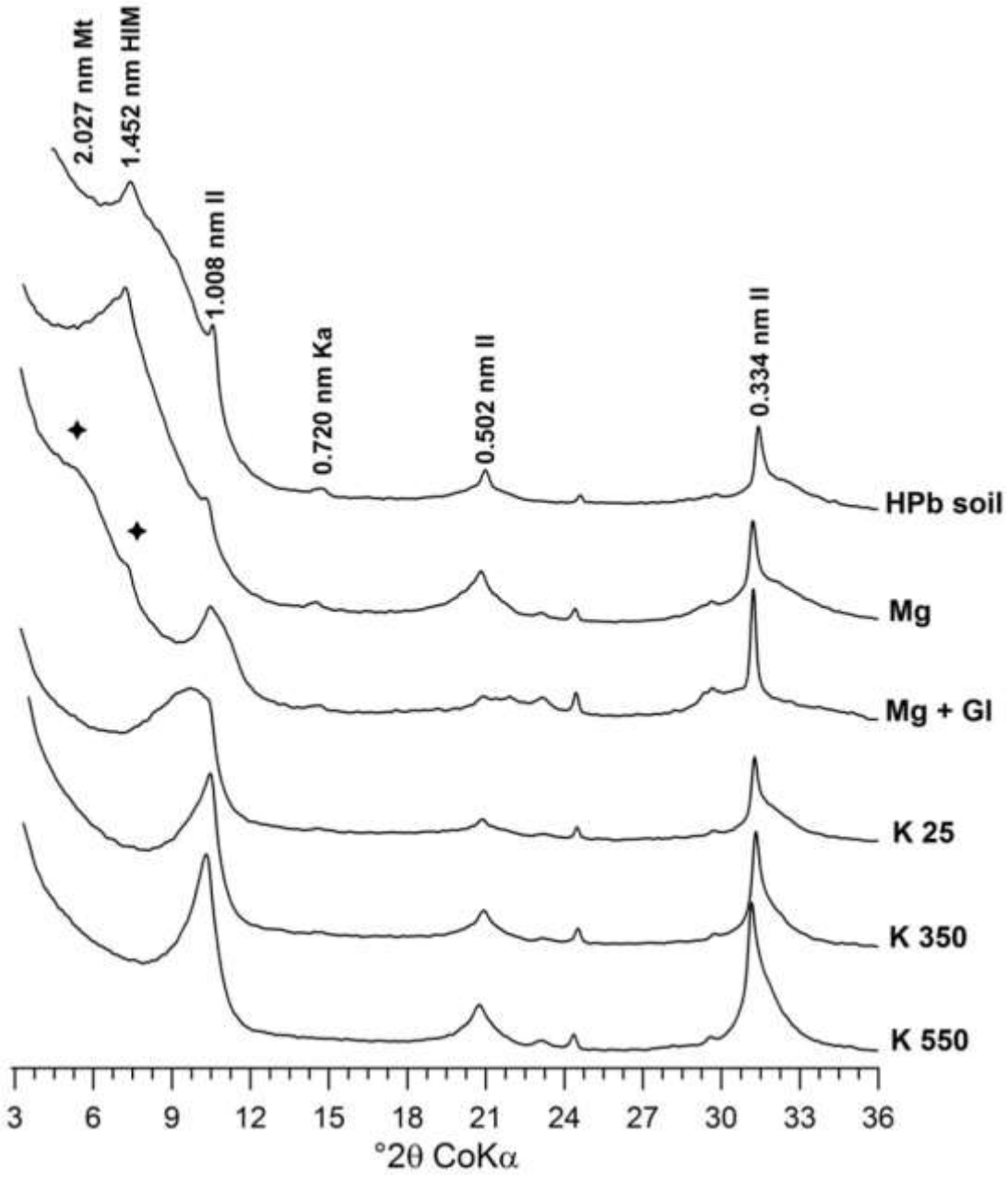

Figure 4. X-ray diffraction patterns from the HPb soil iron-free clays treated with $MgCl_2$ (Mg); glycerol: ethanol 1:1 (Mg + Gl); and KCl at 25°C (K 25), 350°C (K 350) and 550°C (K 550). Mt: montmorillonite; HIM: Hydroxy-interlayered montmorillonite; Il: illite; Ka: kaolinite. ♦ HIM peak shift.

In the LPb soil samples, mineralogical analyses indicate the presence of mica, quartz and feldspar in the sand fraction (Figure 5); montmorillonite, illite, kaolinite, quartz, and feldspar in the silt fraction (Figure 6); and montmorillonite, illite, kaolinite in the clay fraction (Figures 7 and 8).

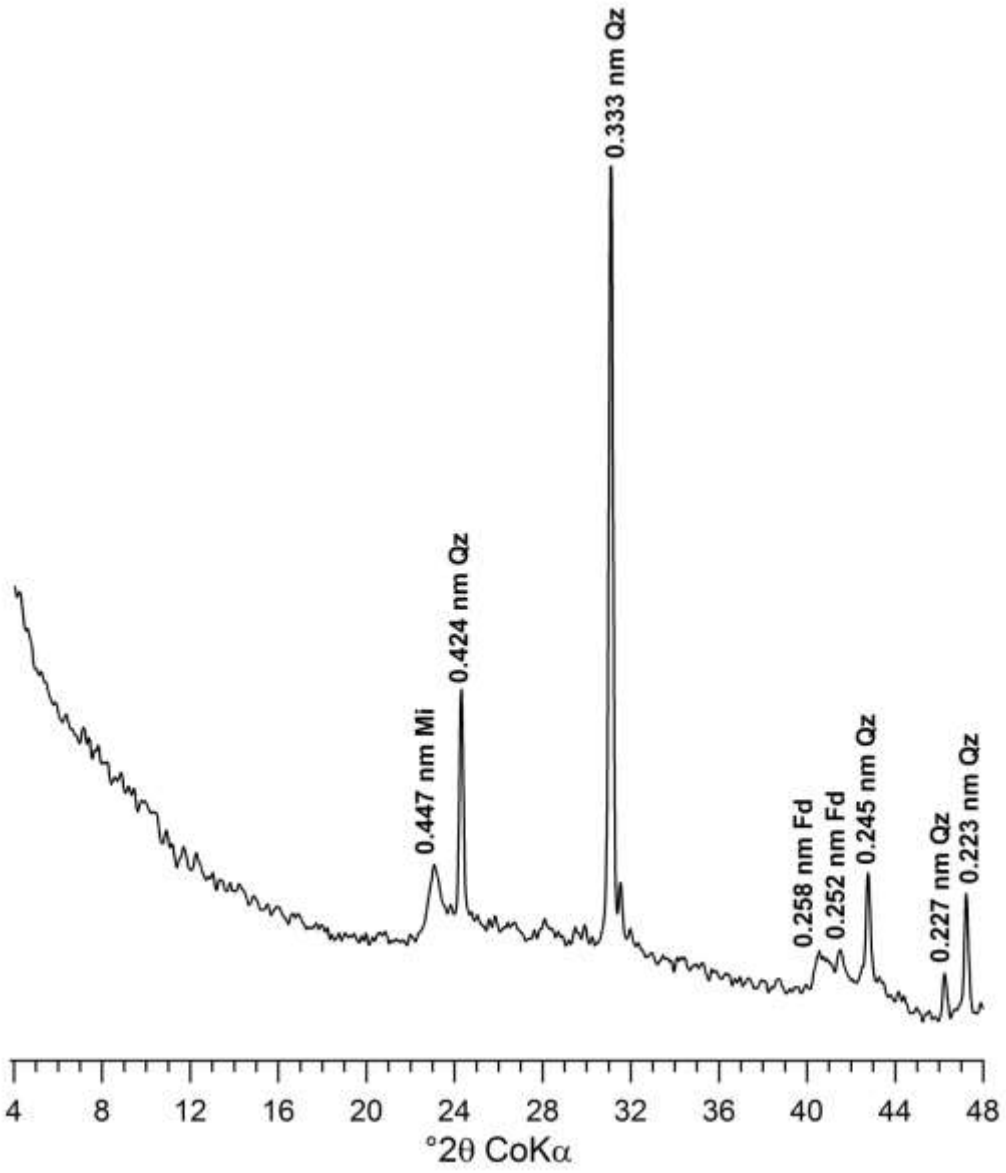

Figure 5. X-ray diffraction pattern from the sand fraction of the LPb soil. Mi: mica; Qz: quartz; Fd: feldspar.

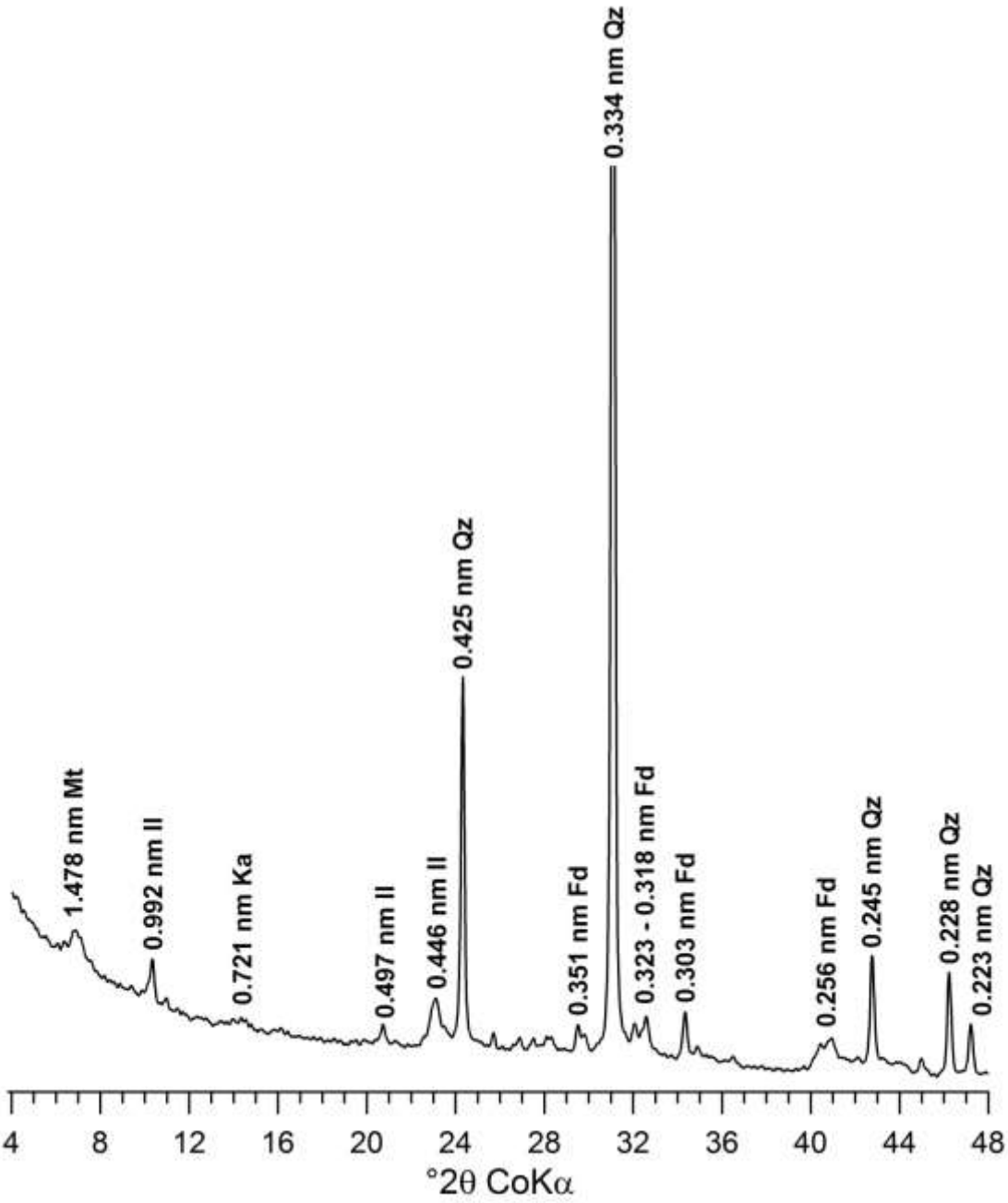

Figure 6. X-ray diffraction pattern from the silt fraction of the LPb soil. Mt: montmorillonite; Il: illite; Ka: kaolinite; Qz: quartz; Fd: feldspar.

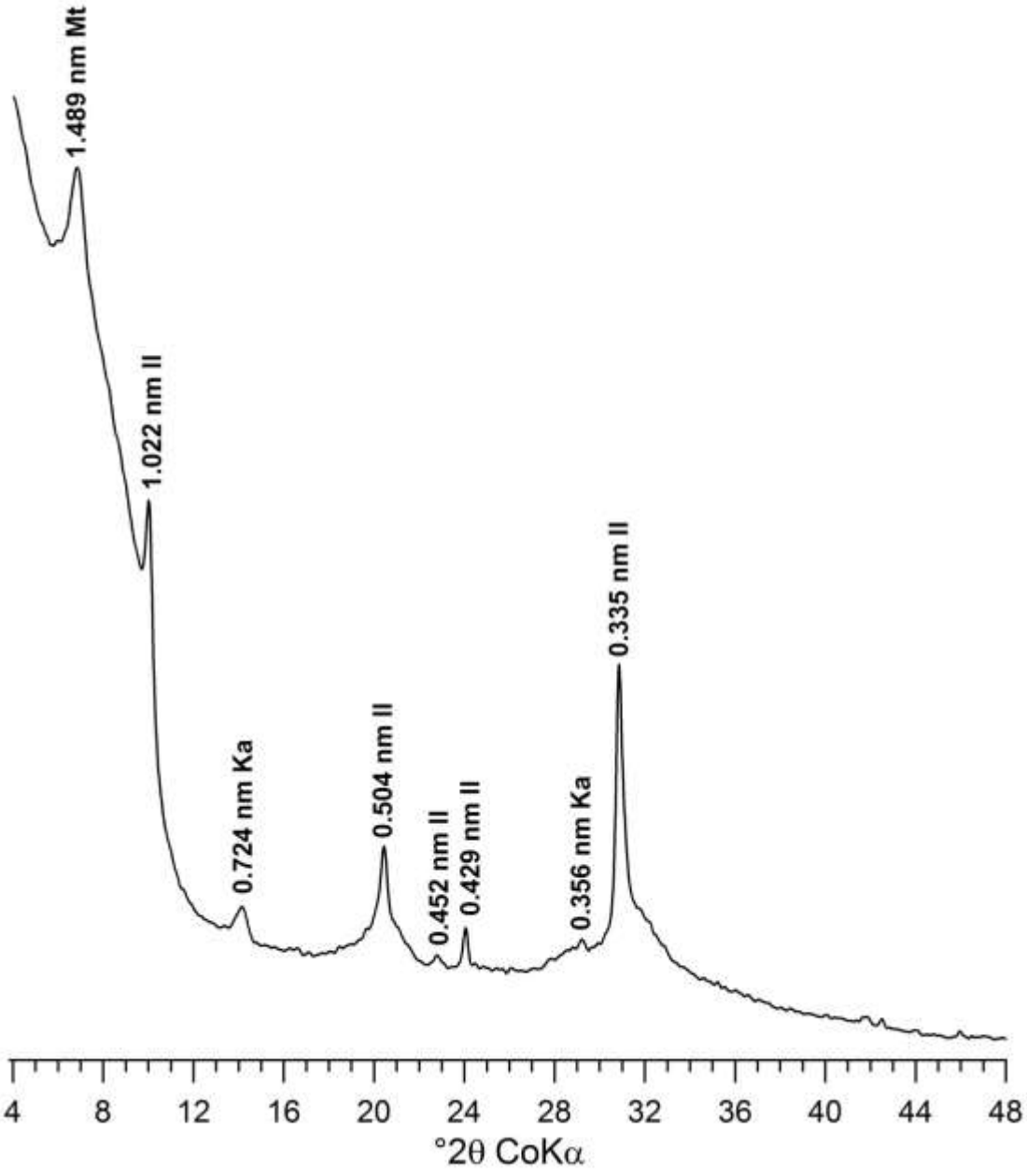

Figure 7. X-ray diffraction pattern from the non-treated clay fraction (natural clay) of the LPb soil. Mt: montmorillonite; Il: illite; Ka: kaolinite.

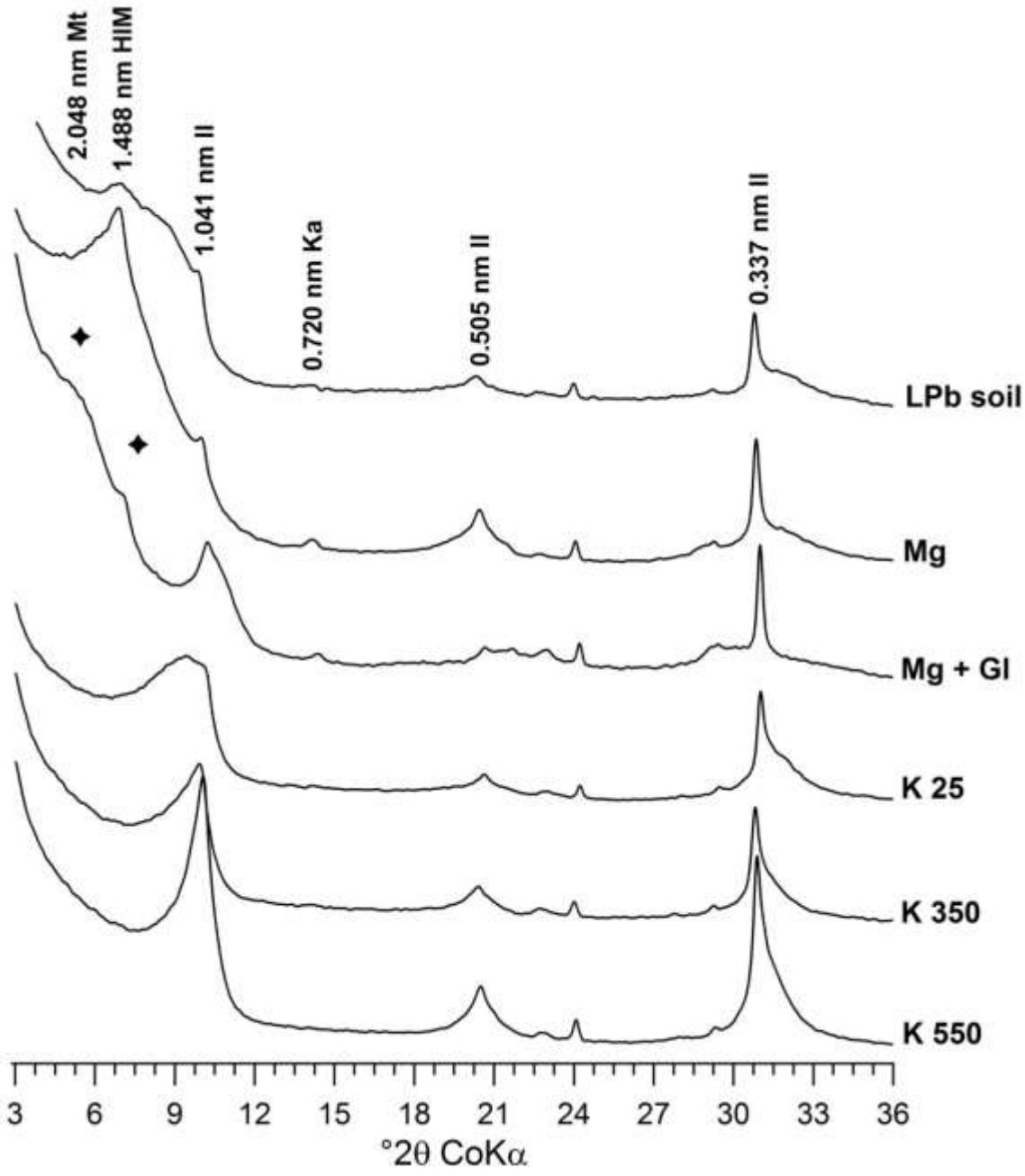

Figure 8. X-ray diffraction patterns from the LPb soil iron-free clays treated with $MgCl_2$ (Mg); glycerol: ethanol 1:1 (Mg + Gl); and KCl at 25°C (K 25), 350°C (K 350) and 550°C (K 550). Mt: montmorillonite; HIM: Hydroxy-interlayered montmorillonite; Il: illite; Ka: kaolinite. ♦ HIM peak shift.

Total Si, Al, Fe, and Mn concentrations are similar in both soil samples. Total Si concentration is higher in the LPb soil sample (349 g kg^{-1} soil). Total Al, Fe, and Mn concentrations are slightly higher in the LPb soil sample (148, 78, and 1.8 g kg^{-1}, respectively) (Table 3).

Table 3. Total concentration of Si, Al, Fe, and Mn from two soil layers of a Typic Hapludert used in the glasshouse experiment (HPb: high Pb concentration; LPb: low Pb concentration)

Soil Sample	SiO_2*	Al_2O_3**	Fe_2O_3**	MnO**	Ki¤
	…….....g kg^{-1} soil.....				
HPb soil	319	140	71	1.6	3.87
LPb soil	349	148	78	1.8	4.01

*Determined by sulfuric digestion H_2SO_4 (1:1), followed by basic digestion 40% NaOH (v/v); **Determined by sulfuric digestion H_2SO_4 (1:1); ¤Weathering index= $1.7x(SiO_2/Al_2O_3)$.

For both soil samples, content of crystalline Al- and Fe-(hydr)oxides in the clay fraction is higher than the content of amorphous Al- and Fe-(hydr)oxides (Table 4).

Table 4. Content of crystalline and amorphous Al- and Fe-(hydr)oxides from the clay fraction of two soil layers of a Typic Hapludert used in the glasshouse experiment (HPb: high Pb concentration; LPb: low Pb concentration)

Soil Sample	Al_{DC}*	Fe_{DC}*	Al_{AO}**	Fe_{AO}**
	…..……..…g kg^{-1} soil…….……			
HPb soil	10.1	12.3	7.9	4.3
LPb soil	10.4	12.9	8.5	3.9

*Extracted by 0.2 mol L^{-1} dithionite-citrate-bicarbonate; **Extracted by 0.2 mol L^{-1} ammonium oxalate pH 3.0 in the dark.

As expected, total Pb concentration in the HPb soil is higher than in the LPb soil (Table 5). For both soil samples, Pb fractions decrease in the following order: Pb-Red (bound to amorphous Fe- and Mn- (hydr)oxides) > Pb-Res (residual) > Pb-MOS (bound to organic matter) > Pb-Exc (water soluble, exchangeable and bound to carbonates) (Table 5). In the HPb soil sample, Pb-Exc accounts for 2.7% of the total Pb content in soil, Pb-Red for 67.4%, Pb-MOS for 7.8%, and Pb-Res for 22.1%. In the LPb soil, Pb-Exc accounts for 2.8% of the total Pb content in soil, Pb-Red for 72%, Pb-MOS for 8.4%, and Pb-Res for 16.8% (Figure 9).

Table 5. Total Pb content and Pb fractions from two soil layers of a Typic Hapludert used in the glasshouse experiment (HPb: high Pb concentration; LPb: low Pb concentration)

Soil Sample	**Pb total***	**Pb-Exc****	**Pb-Red*****	**Pb-SOM[§]**	**Pb-Res[¤]**
		…… mg kg^{-1} soil ……			
HPb soil	2121.74	57.08	1428.97	165.73	469.96
LPb soil	1312.68	37.30	945.18	110.52	219.68

*Determined by acid digestion 68% (v/v) HNO_3 + 40% (v/v) HF; **Pb-Exc: Water soluble fraction, exchangeable and bound to carbonates; ***Pb-Red: Fraction bound to amorphous Fe- and Mn- (hydr)oxides; [§]Pb-SOM: Fraction bound to organic matter; [¤]Pb-Res: Residual fraction.

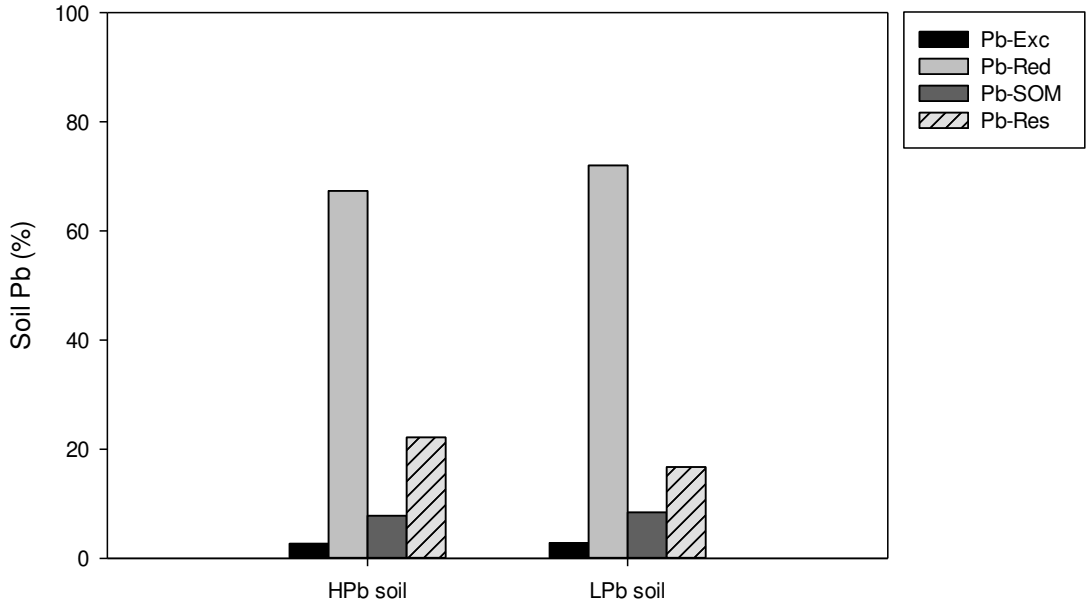

Figure 9. Soil Pb fractions (%) in the HPb and LPb soil samples determined by the BCR sequential extraction procedure. Pb-Exc: Water soluble fraction, exchangeable and bound to carbonates; Pb-Red: Fraction bound to amorphous Fe- and Mn- (hydr)oxides; Pb-SOM: Fraction bound to organic matter; Pb-Res: Residual fraction.

Total Pb concentration in soil

For both soil samples (HPb and LPb soil), only the cultivation of eucalyptus affected significantly the Pb total concentration in soil (Figure 10). An increased total Pb concentration was observed in eucalyptus rhizosphere soil for both HPb and LPb soil samples. No significant differences in total Pb concentrations were observed in brachiaria and mustard rhizosphere soils when compared to bulk soil.

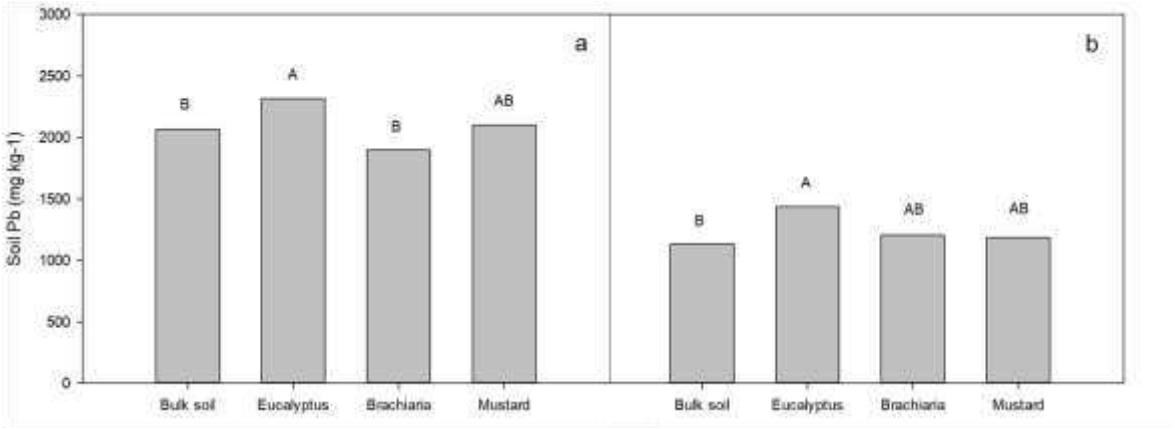

Figure 10. Total Pb concentration (mg kg-1) in the HPb (a) and LPb (b) soil samples. Eucalyptus, Brachiaria and Mustard: rhizosphere soil from the respective plant species. Means followed by the same case letter do not differ by the Tukey test ($p<0.05$).

Pb fractions in soil

The cultivation of the three plant species altered Pb fractions in HPb soil samples (Figure 11). Compared to the bulk soil, the soil sample cultivated with eucalyptus showed an increased residual fraction (Pb-Res); a decrease in the Pb fraction bound to amorphous Fe- and Mn-(hydr)oxides (Pb-Red) was observed in the soil sample cultivated with brachiaria; and an increased Pb-Res fraction and a decreased Pb-

Red fraction were observed in the soil sample cultivated with mustard. The cultivation of the three plant species did not affect the exchangeable (Pb-Exc) and the soil organic matter bound (Pb-SOM) fractions compared to the bulk soil. For both rhizosphere and bulk soil samples, the distribution of Pb fractions decreased in the following order: Pb-Red > Pb-Res > Pb-MOS = Pb-Exc. In terms of relative percentages (Table 6), most of the significant alterations mentioned above became non-significant; only the cultivation of mustard affected the Pb fractions in soil. It showed an increased Pb-Res fraction and a decreased Pb-Red fraction compared to the bulk soil.

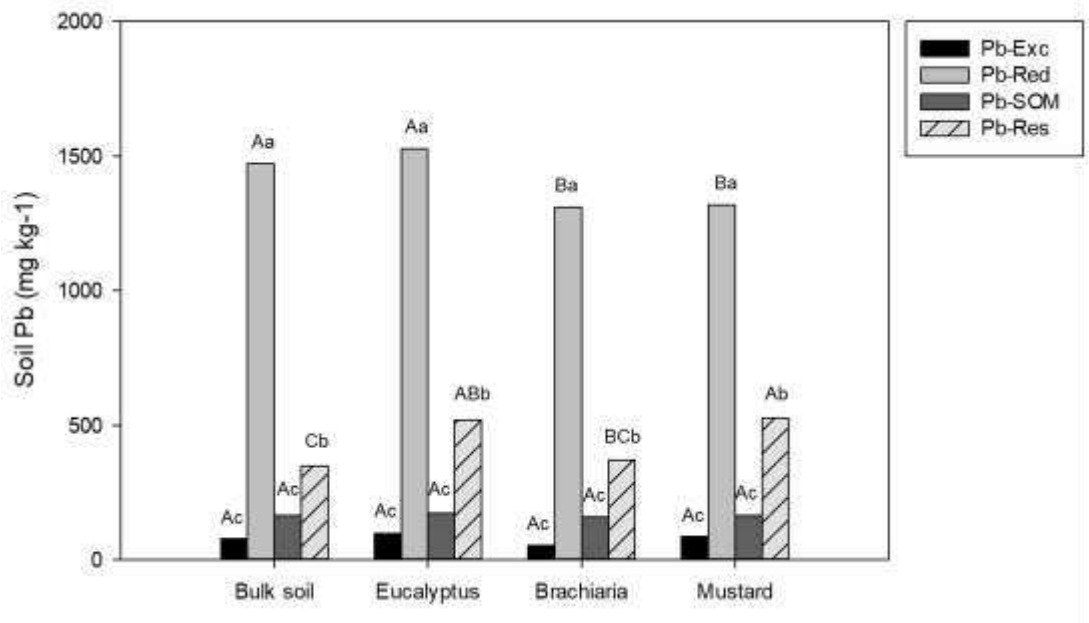

Figure 11. Pb fractions (mg kg^{-1}) in the HPb soil determined by the BCR sequential extraction procedure. Eucalyptus, Brachiaria and Mustard: rhizosphere soil from the respective plant species. Pb-Exc: Water soluble fraction, exchangeable and bound to carbonates; Pb-Red: Fraction bound to amorphous Fe- and Mn- (hydr)oxides; Pb-SOM: Fraction bound to organic matter; Pb-Res: Residual fraction. Means followed by the same uppercase letter do not differ in Pb concentration (%) between treatments by the Tukey test ($p<0.05$). Means followed by the same lowercase letter do not differ in Pb concentration (%) between fractions by the Tukey test ($p<0.05$).

Table 6. Relative abundance of Pb fractions (%) in the HPb soil determined by the the BCR sequential extraction procedure.

	Bulk soil	Eucalyptus	Brachiaria	Mustard
Pb-Exc	3.8 Ac	4.2 Ac	2.8 Ac	4.2 Ac
Pb-Red	71.4 Aa	65.9 Aba	69.1 ABa	62.8 Ba
Pb-SOM	7.9 Ac	7.5 Ac	8.5 Ac	7.9 Ac
Pb-Res	16.9 Bb	22.4 ABb	19.6 ABb	25.1 Ab

Means followed by the same uppercase letter do not differ in Pb concentration (%) between treatments by the Tukey test ($p<0.05$). Means followed by the same lowercase letter do not differ in Pb concentration (%) between fractions by the Tukey test ($p<0.05$).

The cultivation of different plant species also affected Pb fractions in the LPb soil samples. When cultivated with eucalyptus, soil samples presented higher Pb-Red and Pb-Res fractions compared to the bulk soil. The soil samples cultivated with brachiaria also showed an increased Pb-Red fraction when compared to the bulk soil. The cultivation of mustard did not affect soil Pb fractions when compared to the bulk soil. The distribution of Pb fractions in soil was also affected by the cultivation of different plant species. For bulk soil, the distribution of soil Pb fractions decreased in the following order: Pb-Red > Pb-SOM ≥ Pb-Res ≥ Pb-Exc. When cultivated with eucalyptus, Pb fractions decreased as follows: Pb-Red > Pb-Res > Pb-SOM > Pb-Exc; and when cultivated with brachiaria, it decreased in the following order: Pb-Red > Pb-SOM > Pb-Res = Pb-Exc. The cultivation of mustard did not alter the distribution of soil Pb fractions when compared to the bulk soil.

In terms of relative percentages, only the cultivation of eucalyptus affected the Pb fractions in soil (Table 7). It showed a decreased Pb-Red fraction and an increased Pb-Res fraction compared to the bulk soil. The distribution of soil Pb fractions in

eucalyptus rhizosphere soil presented a slight change, decreasing as follows: Pb-Red > Pb-Res > Pb-SOM = Pb-Exc.

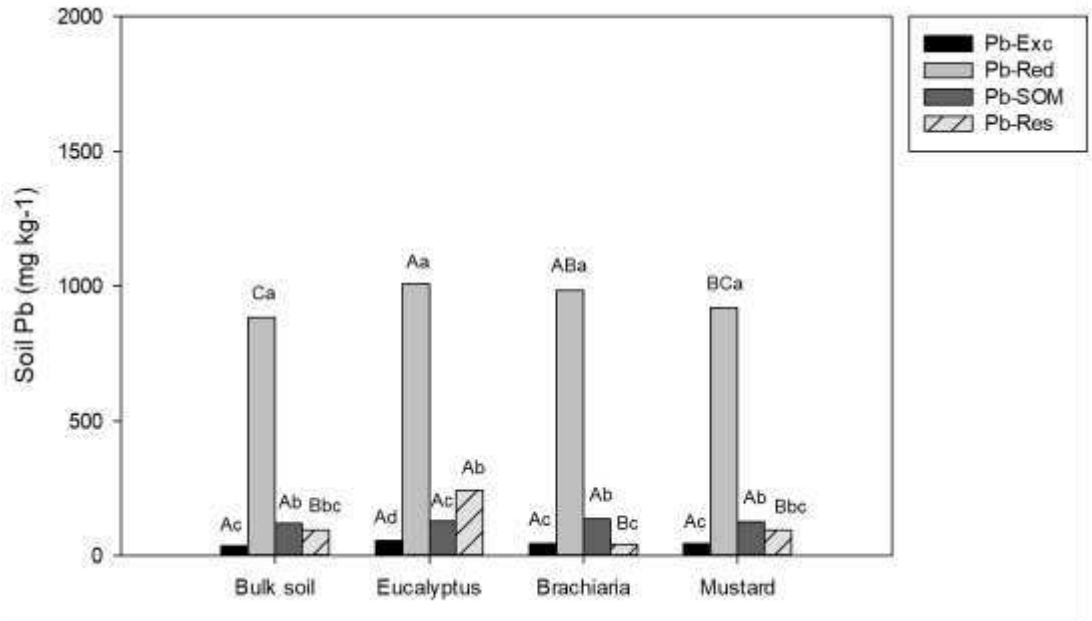

Figure 12. Pb fractions (mg kg^{-1}) in the LPb soil determined by the BCR sequential extraction procedure. Eucalyptus, Brachiaria and Mustard: rhizosphere soil from the respective plant species. Pb-Exc: Water soluble fraction, exchangeable and bound to carbonates; Pb-Red: Fraction bound to amorphous Fe- and Mn- (hydr)oxides; Pb-SOM: Fraction bound to organic matter; Pb-Res: Residual fraction. Means followed by the same uppercase letter do not differ in Pb concentration (%) between treatments by the Tukey test ($p<0.05$). Means followed by the same lowercase letter do not differ in Pb concentration (%) between fractions by the Tukey test ($p<0.05$).

Table 7. Relative abundance of Pb fractions (%) in the LPb soil determined by the the BCR sequential extraction procedure.

	Bulk soil	**Eucalyptus**	**Brachiaria**	**Mustard**
Pb-Exc	3.3 Ac	3.9 Ac	3.7 Ac	3.7 Ac
Pb-Red	77.7 Aa	70.3 Ba	81.4 Aa	77.6 Aa
Pb-SOM	10.6 Ab	8.9 Ac	11.3 Ab	10.6 Ab
F-Res	8.4 Bbc	16.9 Ab	3.6 Bc	8.1 Bbc

Means followed by the same uppercase letter do not differ in Pb concentration (%) between treatments by the Tukey test ($p<0.05$). Means followed by the same lowercase letter do not differ in Pb concentration (%) between fractions by the Tukey test ($p<0.05$).

Pb speciation in soil

The cultivation of the three plant species altered the Pb speciation of the HPb soil sample. According to the LCF of the XANES spectra (Table 8), Pb was bound to ferrydrite (14.23%), goethite (26.61%), and kaolinite (59.16%) in the bulk soil sample. After cultivation, Pb in rhizosphere soil was found as PbO_2, Pb bound to kaolinite, Pb bound to montmorillonite, and PbCOOH. The Pb speciation in eucalyptus and mustard rhizosphere soils were similar: 11.97 and 11.24% of Pb as PbO_2, respectively; 56.18 and 53.69% of Pb bound to kaolinite, respectively; 7.13 and 7.9% of Pb bound to montmorillonite, respectively; and 24.71 and 27.16% of PbCOOH, respectively. The cultivation of brachiaria led to a similar abundance of Pb as PbO_2 (11.65%); however, it led to a lower percentage of Pb bound to kaolinite (32.85%), and higher percentages of Pb bound to montmorillonite (14.2%) and PbCOOH (41.3%). The XANES spectra of the soil samples and a comparison with the reference compounds are shown in figure 13.

Table 8. Relative abundance of Pb species (%) in the HPb bulk and rhizosphere soil samples determined by linear combination fitting (LCF) of the XANES spectra.

	Bulk soil	**Eucalyptus rhiz. soil**	**Brachiaria rhiz. soil**	**Mustard rhiz. soil**
PbO	-	-	-	-
PbO_2	-	11.97	11.65	11.24
$PbCO_3$	-	-	-	-
Pb-ferrihydrite	14.23	-	-	-
Pb-goethite	26.61	-	-	-
Pb-kaolinite	59.16	56.18	32.85	53.69
Pb-montmorillonite	-	7.13	14.2	7.9
Pb-illite	-	-	-	-
Pyromorphite	-	-	-	-
$(CH_3COO)_2Pb$	-	24.71	41.3	27.16
R-factor	5.58E-08	9.31E-05	0.0001365	0.0001061

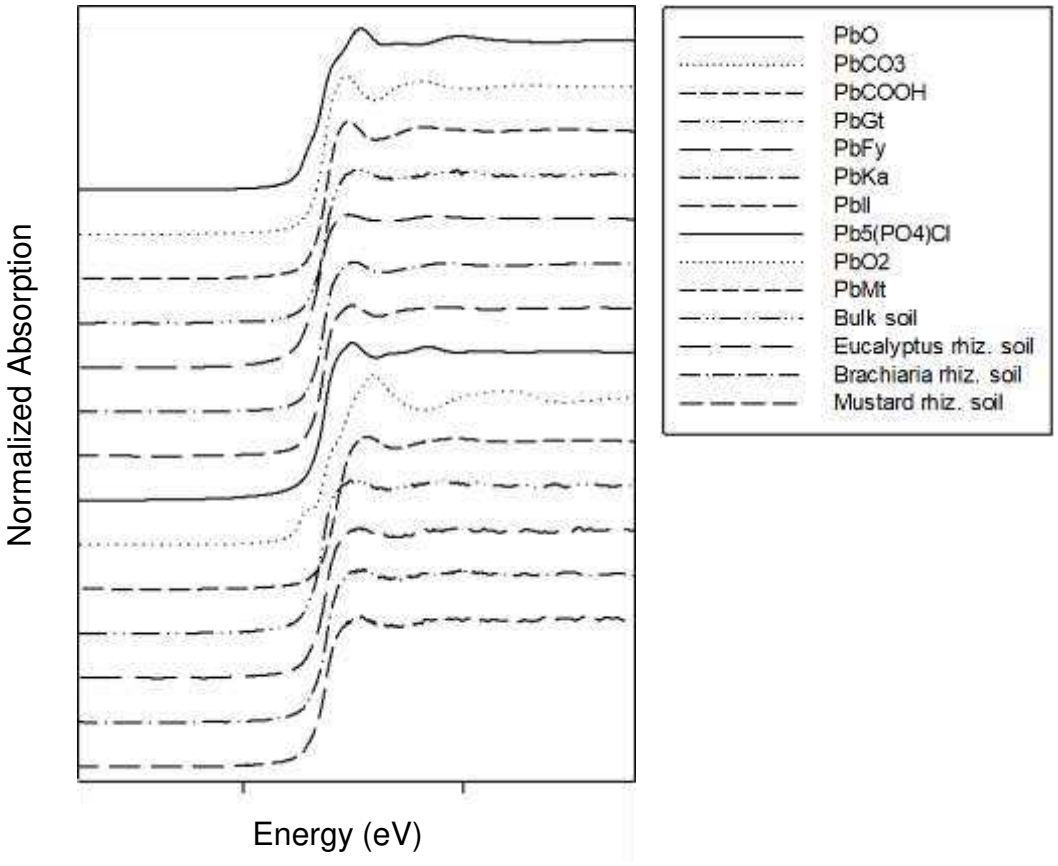

Figure 13. Pb L3 edge XANES spectra of the reference compounds and soil samples.

Total Pb content in plant tissue

For both HPb and LPb soils, the highest Pb accumulation occurred in the roots of all cultivated plant species (Figure 14). In the HPb soil (Figure 14a), mustard was the plant specie with the highest Pb accumulation (mg kg^{-1} of dry matter) in the roots, followed by eucalyptus and brachiaria. No significant differences were found in the Pb accumulation in shoot of the three plant species when grown in the HP soil.

In the LPb soil, mustard had the highest Pb accumulation in root, followed by eucalyptus and brachiaria, which did not differ in terms of Pb accumulation (Figure 14b). Mustard also presented the highest Pb accumulation in shoot, followed by eucalyptus and brachiaria, which did not differ in Pb accumulation.

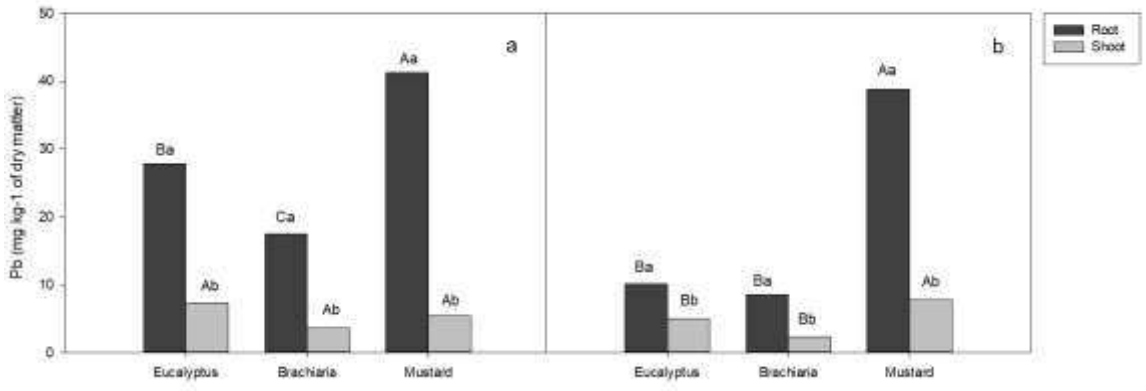

Figure 14. Pb content (mg kg^{-1} of dry matter) in plant tissue of plants grown in the HPb soil (a) and LPb soil (b). Means followed by the same uppercase letter do not differ in Pb content between plant species by the Tukey test ($p<0.05$). Means followed by the same lowercase letter do not differ in Pb content between plant compartments by the Tukey test ($p<0.05$).

Plant dry mass

Shoot dry mass (g) was higher than root dry mass (g) for all plant species cultivated in both HPb and LPb soils (Figure 15). Exception was brachiaria when cultivated in the LPb soil, which obtained similar shoot and root dry mass yield (Figure 15b). Brachiaria had the highest root dry mass when cultivated in both HPb and LPb soils, followed by eucalyptus and mustard. For the HPb soil, eucalyptus had the highest shoot dry mass, followed by brachiaria and mustard (Figure 15a). For the LPb soil, eucalyptus and brachiaria had similarly high shoot dry mass yield, followed by mustard (Figure 15b).

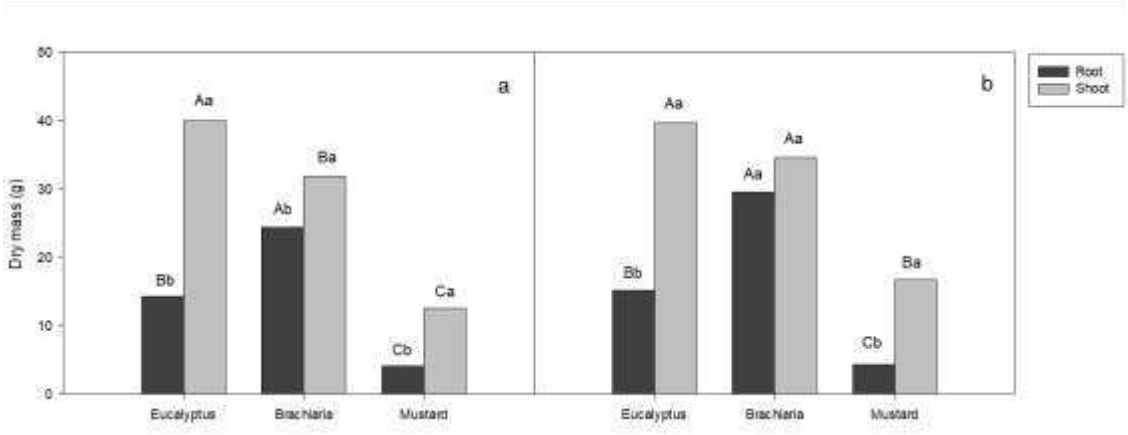

Figure 15. Root and shoot dry mass (g) of plants grown in the HPb soil (a) and LPb soil (b). Means followed by the same uppercase letter do not differ in dry mass between plant species by the Tukey test ($p<0.05$). Means followed by the same lowercase letter do not differ in dry mass between plant compartments by the Tukey test ($p<0.05$).

Pb stocks in plant tissue

For plant species cultivated in the HPb soil, Pb stock was higher in roots than in shoots (Figure 16a). Eucalyptus showed the highest Pb stock in roots and shoots, followed by brachiaria and mustard, which did not differ in Pb stock in shoot. When cultivated in the LPb soil, Brachiaria showed a higher Pb stock in roots than in shoots; eucalyptus and mustard did not present significant differences in Pb stock between roots and shoots (Figure 16b). Brachiaria had the highest Pb stock in roots, followed by eucalyptus and mustard. For the shoot compartment, Pb stock in eucalyptus was higher than that of brachiaria, but it did not differ from the Pb stock in mustard (Figure 16b).

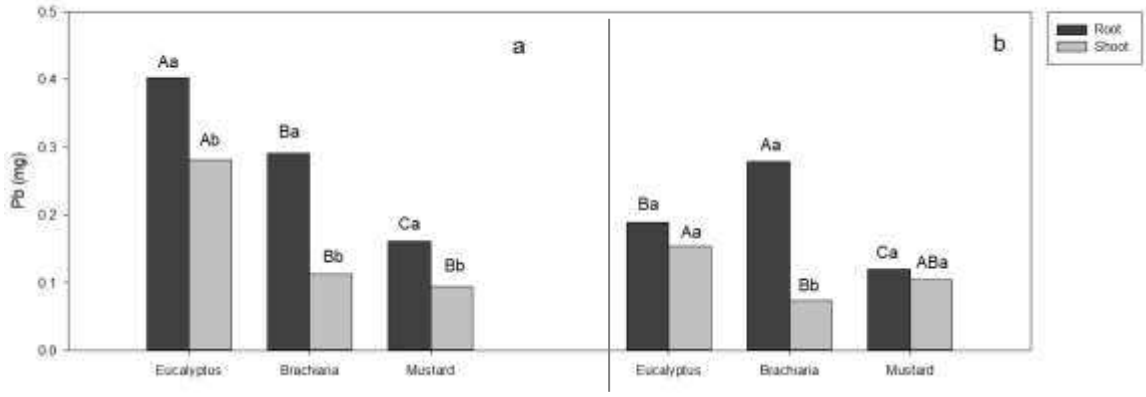

Figure 16. Pb stocks (mg) in plant tissue of plants grown in the HPb soil (a) and LPb soil (b). Means followed by the same uppercase letter do not differ in Pb stocks between plant species by the Tukey test ($p<0.05$). Means followed by the same lowercase letter do not differ in Pb stocks between plant compartments by the Tukey test ($p<0.05$).

DISCUSSION

Total Pb concentration in original, bulk, and rhizosphere soils

We conducted a pot experiment with three different plant species and assessed the total Pb concentration in the original, bulk and rhizosphere soils. As expected, in the original soil samples, Pb concentration was higher in the surface layer and decreased with depth, which is aligned with previous studies (Cecchi *et al*., 2008; Souza, 2014). The higher Pb concentration in the surface layer of the original soil is primarily explained by the own route of the contamination, which occurred through deposition of particulate material emitted by the lead smelter chimneys during 37 years of operation (Anjos, 2003; Rabelo, 2010). Secondly, the strong binding between Pb and SOM have favored its accumulation in the surface layer, where the organic matter content was higher than that in the deeper layer (Table 2).

Pb is known to be a roughly immobile cation in soils, and its strong association with SOM may limit its uptake by plants. The amount of Pb extracted by plants is generally much lower than the total Pb content in soils (Wilson & Cline, 1966; Davies, 1995), and it may not lead to significant differences between initial and final Pb concentrations in soils. This might explain our results, since no difference was observed between total Pb concentration in bulk soil and soil samples cultivated with brachiaria and mustard. On the other hand, soil samples cultivated with eucalyptus showed a higher total Pb concentration compared to bulk soil. The increased total Pb concentration in eucalyptus rhizosphere soil might indicate a possible tolerance mechanism. When grown in highly contaminated sites, plants defend against Pb

entrance in their tissue mainly by Pb complexation in root surface (Jiang & Liu, 2010). Therefore, total Pb concentration in the root zone may be higher than total Pb concentration in bulk soil.

Rhizosphere effects on Pb fractionation and speciation in soils

In the current work, we applied a sequential extraction procedure following the BCR method to investigate Pb lability in soils under bulk and rhizosphere conditions, after a three month glasshouse experiment. For both HPb and LPb soil samples, we found that Pb is majorly bound to amorphous (reducible by hydroxylamine) Fe- and Mn-(hydr)oxides (Figures 11 and 12), despite of the cultivation of the plant species, ranging from 62.8 to 71.4% of total soil Pb in HPb soil sample and from 70.3 to 81.4% of total soil Pb in LPb soil (Tables 6 and 7). Fe- and Mn-(hydr)oxides have long been recognized by their vital role in controlling the fate of heavy metals in soils (McKenzie, 1980; Schwertmann & Taylor, 1989; Manceau *et al*., 1992) due to their very reactive –OH functional groups exposed on the particle surface. In soils with high pH, the deprotonation of these functional groups increases the electrostatic interaction between Pb^{2+} and the negatively charged surface (McBride, 1994), increasing the adsorption capacity of these oxides even when present in low proportions in soils.

Despite of being operationally defined, the fact that most of the soil Pb in the studied site was bound to amorphous Fe- and Mn-(oxides) has an important implication. In our study, the Pb-Red fraction was extracted using hydroxylamine, which is a strong reductive agent. Therefore, this fraction recovers the Pb forms bound to Fe- and Mn-(hydr)oxides that are unstable under reductive conditions (Tessier *et al*., 1979). This

suggests that most of the soil Pb of the studied site is unstable under reductive conditions, such as a rising water table, organic matter accumulation, or increased biological activity. Reductive conditions are also verified in the rhizosphere of plants (Rudolph-Mohr *et al*., 2017). This might explain the reduced Pb-Red fraction observed in mustard rhizosphere soil in HPb soil sample (Table 6) and in eucalyptus rhizosphere soil in LPb soil sample (Table 7). Under acidic and anaerobic conditions, the reduction of oxidized forms of Fe (Fe^{3+} to Fe^{2+}) and Mn (Mn^{3+} and $^{4+}$ to Mn^{2+}) takes place (Kabata-Pendias, 2011), increasing the dissolution of Fe- and Mn-(hydr)oxides in soils and, therefore, promoting the release of metals associated with them.

The three other Pb fractions obtained in the SEP (exchangeable, oxidable and residual) ranged from 28.6 to 37.2% of the total soil Pb in the HPb soil (Table 6), and from 18.6 to 29.7% of the total soil Pb in the LPb soil (Table 7). The residual fraction, that corresponds to Pb bound to silicates and occluded in crystalline Fe- and Al-(hydr)oxides, was the second most abundant Pb fraction in soil. It represents the most stable Pb forms in soils, since they are part of the crystalline structure of the mineral and, therefore, hardly released under normal environmental conditions (Tessier *et al*., 1979). The oxidable fraction extracted in this procedure comprised roughly 8% and 10% of the total soil Pb in HPb and LPb soil samples, respectively, and represents the Pb forms stabilized by soil organic matter. Along with Fe- and Mn-(hydr)oxides, organic matter is also known to be a major sink from trace metals in soils, due to its strong complexing capacity for metallic contaminants. However, our results show that the oxides played a much greater role in retaining Pb in soils than organic matter, and, therefore, in controlling its fate in the environment. The exchangeable fraction

accounted for less than 4% of the total soil Pb in both HPb and LPb soil samples, which means that only a small portion of the total soil Pb is readily available in the environment for plant uptake or leaching.

Our results from the XANES spectroscopy show in a very accurate way that the presence of plants and their associated rhizospheres altered Pb-bearing forms in soils. The LCF of the XANES spectra indicated that Pb was mostly associated with kaolinite, followed by goethite and ferrydrite in the bulk soil (Table 8). To our knowledge, this is the first time that Pb bound to kaolinite appeared as a major fraction in soils contaminated by lead smelter emissions. Kaolinite is a 1:1 aluminosilicate mineral, very common in Vertisols, with negative charge arising from broken edges on the clay crystal and isomorphic substitution. Although it is the least reactive clay (Kabata-Pendias, 2011), its pH dependency could enhance or reduce the adsorption of heavy metals according to the soil pH. The formation of outer- and inner- sphere surface complexes in kaolinite is also pH dependent, and may alter its stability in the environment (Gu & Evans, 2008). In addition, the occurrence of Fe- and Al-(hydr)oxides coatings on kaolinite surface can increase its surface affinity and surface area, and therefore, improve its adsorptive capacity (Fontes, 1992; Khan & Khan, 2015).

The presence of Fe-(hydr)oxides in the original soil samples was already shown by the bulk chemical analysis (Tables 3 and 4), by the sequential extraction procedure (Figure 11 and Table 6), and then confirmed in the bulk soil by the LCF of the XANES spectra (Table 8). As discussed above, Fe-(hydr)oxides are very common constituents of tropical soils, known by their strong ability to bind trace elements, and therefore,

with great potential to be used to ameliorate metal-polluted sites. According to Kabata-Pendias (2011), goethite is apparently the most frequently occurring Fe-mineral in soils involved in sorption processes, including absorption on external surfaces, and fixation inside the mineral particles. Several authors have also reported the great adsorption capacity of amorphous Fe-(hydr)oxides to different heavy metals (Cheney *et al*., 2004; Pett-Ridge *et al*., 2007). The stability of heavy metals bound to Fe-compounds in soils depends on the processes that control Fe-compounds solubility in soils. These processes include: hydrolysis, formation of complexed species, and redox behavior of Fe. Lindsay (1979) demonstrated that Fe solubility increases with pH decrease and reduction of Fe^{3+} to Fe^{2+}. Thus, environmental conditions that increase Fe solubility is also expected to increase Pb solubility when bound to Fe-(hydr)oxides.

In our study, such conditions were imposed by the presence of the plant species and their associated rhizospheres. When cultivated with eucalyptus, brachiaria and mustard, we noticed a slight decrease in the abundance of Pb-kaolinite, and the absence of the Pb bound to ferrihydrite and goethite. The alterations occurred in Pb speciation in the rhizosphere soils are probably due to changes in pH, redox conditions, and due to the increased microbial activity in the rhizosphere region. We did not observe a strong acidification in the rhizosphere soils as compared to the bulk soil, except for the soil cultivated with brachiaria (Supplementary Table 2); therefore, the transformations occurred in Pb speciation in rhizosphere soils might be majorly due to the dissolution of Fe-(hydr)oxides under reductive conditions (Schwertmann, 1991). We hypothesized that the rhizosphere conditions were reductive enough to dissolve the Fe-(hydr)oxides

and the possible Fe-(hydr)oxides coatings on kaolinite, and to promote the release of Pb to soil solution.

Simultaneously to the dissolution of Fe-(hydr)oxides, we noticed the appearance of lead dioxide (PbO_2) and Pb acetate ($(CH_3COO)_2Pb$) species in all rhizosphere soils. The former compound is a tetravalent Pb specie, very soluble (log K° 49.68), and therefore, unstable under environmental conditions. The latter compound is a divalent specie bound to a carboxylic COOH group (Manceau *et al*., 1996). Previous studies also reported the presence of acetate ligands complexing heavy metals in rhizosphere soil (Bluskov *et al*., 2006); however, the evidence of Pb bound to acetate in rhizosphere soils is previously unreported, and may be a unique aspect for Pb speciation in soils. We believe that Pb-acetate may represent Pb bound to low molecular weight organic acids (LMWOAs) exuded by the plants' roots. LMWOAs are able to form soluble complexes and chelates with metal ions, and modify the mobility of heavy metals in soils (Chen *et al*., 2003; Shakoor *et al.*, 2014; Khan *et al.*, 2016). Under Pb stress conditions, plants release LMWOAs at higher rates due to the oxidative stress (Quartacci *et al*., 2006; Evangelou *et al*., 2007), which increases Pb desorption from soil components and Pb compounds dissolution (Wu *et al*., 2003; Qin *et al*., 2004).

In our study, the LMWOAs produced by the plants' roots may have contributed to the dissolution of Pb species bound to Fe-(hydr)oxides and may have caused a slight desorption of Pb bound to kaolinite. Thus, the free Pb^{2+} ions in soil solution were complexed by the LMWOAs, which explains the presence of the Pb-acetate specie in the rhizosphere soils. Part of the free Pb^{2+} ions was adsorbed onto montmorillonite surface, due to its high surface area and CEC. The higher proportion of Pb-acetate and

the lower proportion of the Pb-kaolinite in the rhizosphere soil of brachiaria (Table 8) were probably due to its more extensive root mass (Figure 15a). This possibly contributed to higher LMWOAs production, which boosted their effects on Pb complexation and dissolution of Pb compounds. Several studies support our findings in terms of dissolution of Pb mineral phases by LMWOAs followed by Pb complexation (Debela *et al*, 2013; Li *et al*, 2013); however, future research may be needed to quantify and characterize the LMWOAs present in our rhizosphere soils.

Pb bioavailability as affected by Pb species in soils

In the present work, we evaluated Pb bioavailability by assessing Pb content in plant tissue (mg kg^{-1} of dry matter) in different plant species. Along with the parameters evaluated in the soil samples (Pb total concentration, Pb fractionation and Pb speciation), that parameter would allow us to understand if the alterations occurred in rhizosphere soils would affect Pb bioavailability and its accumulation in plant tissue. In terms of bulk chemical analysis (total soil Pb and Pb fractions), the cultivation of eucalyptus promoted an increase in total soil Pb for both soil samples, and a decrease in the Pb-Red fraction in the LPb soil. These alterations were not followed by a higher Pb content (mg kg^{-1} of dry matter) in plant roots and shoots (Figure 14), which indicates that the cultivation of eucalyptus in contaminated sites contributes to a higher Pb accumulation in soils. On the other hand, the cultivation of mustard promoted a decrease in the Pb-Red fraction in the HPb soil, and that was followed by higher Pb accumulation in plant tissue (Figure 14a). This indicates that the cultivation of mustard contributes to dissolution of poorly crystallized Pb forms, and the plants are able to take

up the free Pb^{2+} ions released. Therefore, mustard may represent a potential specie to be used in phytoremediation of contaminated sites.

The potential of mustard as an accumulator plant specie was supported by our XANES spectroscopy results. It was shown that the lowest proportion of Pb-acetate was observed in the rhizosphere soils of eucalyptus and mustard, which may indicate a lower exudation of LMWOAs and following Pb complexation, or possibly a higher Pb uptake by these plant species. Despite of the similarity in Pb speciation for both rhizosphere soils, the highest Pb content in plant tissue was observed in mustard roots (Figure 14a). Therefore, the solubilization of Pb by the LMWOAs in the rhizosphere soil of mustard was followed by an increased Pb accumulation in plant tissue, which indicates the potential use of mustard as an accumulator plant specie. Plants from the *Brassicaceae* family are known by their ability to accumulate heavy metals in plant tissue, especially roots (Bluskov *et al*., 2006; John *et al*., 2009; Shakoor *et al*., 2014), where Pb interacts with cell walls and it is complexed by pectin carboxyl groups (Meyers *et al*., 2008). Due to this efficient detoxification mechanism, mustard is highly tolerant to metal-induced stress, being very useful in phytoremediation of heavily polluted soils.

The cultivation of brachiaria yielded the highest proportion of Pb acetate in rhizosphere soil, and that was not coupled to a higher Pb accumulation in plant tissue (Figure 14a). Contrarily, brachiaria was the plant specie that presented the lowest Pb content in root tissue. This suggests that the Pb solubilization in soil was higher than the plant uptake, which indicates a lower efficiency of brachiaria in accumulating Pb in plant tissue. As discussed above, the highest proportion of Pb-acetate in brachiaria

rhizosphere soil may reflect greater exudation of LMWOAs, that may facilitate Pb desorption from soil components and dissolution of Pb compounds. Moreover, Pb ions complexed by LMWOAs are easily released into the soil solution, due to the increased biodegradability of LMWOAs in soils (Romkens *et al*., 2002). From this point of view, the cultivation of brachiaria in this specific site is not recommended, since it seems to favor the accumulation of unstable Pb species in soils. However, to be conclusive about the cultivation of brachiaria in this site, a proper characterization of the LMWOAs present in rhizosphere soils is required, as well as constant monitoring of the area in terms of Pb leaching.

Eucalyptus and mustard presented similar abundance of Pb-acetate in rhizosphere soils; however, eucalyptus accumulated the highest Pb stock (mg) compared to the other plant species (Figure16a), which is mainly due to its increased dry mass yield (Figure 15a). Our results suggest that biomass production should be take into account in phytoremediation studies, as well as Pb accumulation ability of each plant specie, since it favors high Pb extraction from soils. Several authors have demonstrated the potential of eucalyptus to grow in highly contaminated sites (Pyatt, 2001) and accumulate Pb (Peng *et al*., 2012). In our study, eucalyptus presented low solubilization of Pb compounds in rhizosphere soil and higher total Pb uptake, which is highly desirable from an environmental view. Moreover, the cultivation of eucalyptus may prevent Pb to enter into human food chain. From an economic perspective, the production of wood from a currently marginal area would be very profitable. Therefore, the cultivation of eucalyptus may represent an alternative use for the contaminated site investigated in this work.

CONCLUSIONS

1. The cultivation of eucalyptus caused an increase in total Pb concentration in soils, which may reflect a tolerance mechanism of Pb stabilization.

2. SEP results shown that Pb is majorly bound to Fe- and Mn-(hydr)oxides in both soil depths, indicating that most of the Pb in soils is unstable under reductive conditions.

3. XANES spectroscopy shown that Pb is majorly found as Pb-kaolinite, followed by Pb-goethite and Pb-ferrihydrite in bulk soil.

4. Rhizosphere soils of the three plant species shown a slight decrease in the abundance of Pb-kaolinite, and the absence of Pb-goethite and Pb-ferrihydrite, which might be due to the dissolution of Fe-(hydr)oxides under reductive conditions.

5. Coupled to the absence of Fe-(hydr)oxides, the presence of Pb-acetate was noticed in the rhizosphere soils of the three plant species, suggesting that complexation of Pb by low molecular weight organic acids may be occurring.

6. Mustard presented the highest Pb content in roots, and its cultivation caused a lower dissolution of Pb compounds in soil, which indicates the potential of mustard as accumulator plant specie.

7. Brachiaria presented the lowest Pb content in roots, and its cultivation caused the highest dissolution of Pb compounds in soil, which indicates the lower efficiency of brachiaria in accumulating Pb.

8. Eucalyptus presented the highest Pb stock in plant tissue, and a lower dissolution of Pb compounds in soil, suggesting that its cultivation may represent an alternative use for the contaminated site

9. The presence of plants and their associated rhizosphere altered Pb chemical speciation in soils.

10. The presence of Pb soluble species in soil depends on the plant specie cultivated, and not necessarily represents a higher Pb uptake by plants.

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SUPPLEMENTARY MATERIAL

Supplementary Table 1. Recovery rates of Pb for the reagents used in each step of the sequential extraction procedure following the BCR method.

	Recovery rates (%)		
Certified Sample	Exchangeable Fraction	Reducible Fraction	Oxidable Fraction
BCR 701	94	106	100

Supplementary Table 2. Soil pH in bulk and rhizosphere soils.

Soil Sample	Bulk soil	Eucalyptus rhiz. soil	Brachiaria rhiz. soil	Mustard rhiz. soil
HPb soil	5.98ab	6.09ab	5.87b	6.15a
LPb soil	6.13a	6.40a	6.42a	6.19a

Soil pH determined in H2O (1: 2.5). Means followed by the same case letter in the same row do not differ by the Tukey test ($p<0.05$).

Supplementary Table 3. ANOVA results from total soil Pb concentration (mg kg^{-1}) in rhizosphere (eucalyptus, brachiaria and mustard) and bulk soil samples.

Soil Sample	SV	DF	p value
HPb soil	Treatment	3	0.0016
	Block	5	0.3917
	Residue	15	-
	CV (%)	6.96	
LPb soil	Treatment	3	0.0195
	Block	5	0.9776
	Residue	15	-
	CV (%)	12.50	

SV: source of variation; DF: number of degrees of freedom.

Supplementary Table 4. ANOVA results from soil Pb fractions (mg kg^{-1}) in rhizosphere (eucalyptus, brachiaria and mustard) and bulk soil samples.

Soil Sample	SV	DF	p value
HPb soil	Treatment	3	0.0057
	Fraction	3	0.0000
	Treatment*Fraction	9	0.0071
	Block	5	1.0000
	Residue	75	-
	CV (%)	19.02	
LPb soil	Treatment	3	0.0000
	Fraction	3	0.0000
	Treatment*Fraction	9	0.0000
	Block	5	1.0000
	Residue	75	-
	CV (%)	15.02	

SV: source of variation; DF: number of degrees of freedom.

Supplementary Table 5. ANOVA results from HPb soil Pb fractions (mg kg^{-1}) in rhizosphere (eucalyptus, brachiaria and mustard) and bulk soil samples, unfolded by treatment and fraction.

Soil Sample	SV	DF	p value
HPb soil/Treatment	Pb-Exc	3	0.8892
	Pb-Red	3	0.0002
	Pb-MOS	3	0.9968
	Pb-Red	3	0.0019
	Residue	75	-
HPb soil/Fraction	Bulk soil	3	0.0000

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(continuation)

	Eucalyptus	3	0.0000
	Brachiaria	3	0.0000
	Mustard	3	0.0000
	Residue	75	-

SV: source of variation; DF: number of degrees of freedom.

Supplementary Table 6. ANOVA results from LPb soil Pb fractions (mg kg^{-1}) in rhizosphere (eucalyptus, brachiaria and mustard) and bulk soil samples, unfolded by treatment and fraction.

Soil Sample	**SV**	**DF**	**p value**
LPb soil/Treatment	Pb-Exc	3	0.9218
	Pb-Red	3	0.0000
	Pb-MOS	3	0.9498
	Pb-Red	3	0.0000
	Residue	75	-
LPb soil/Fraction	Bulk soil	3	0.0000
	Eucalyptus	3	0.0000
	Brachiaria	3	0.0000
	Mustard	3	0.0000
	Residue	75	-

SV: source of variation; DF: number of degrees of freedom.

Supplementary Table 7. ANOVA results from soil Pb fractions (%) in rhizosphere (eucalyptus, brachiaria and mustard) and bulk soil samples.

Soil Sample	SV	DF	p value
HPb soil	Treatment	3	1.0000
	Fraction	3	0.0000
	Treatment*Fraction	9	0.0160
	Block	5	1.0000
	Residue	75	-
	CV (%)	18.77	
LPb soil	Treatment	3	1.0000
	Fraction	3	0.0000
	Treatment*Fraction	9	0.0000
	Block	5	1.0000
	Residue	75	-
	CV (%)	15.02	

SV: source of variation; DF: number of degrees of freedom.

Supplementary Table 8. ANOVA results from HPb soil Pb fractions (%) in rhizosphere (eucalyptus, brachiaria and mustard) and bulk soil samples, unfolded by treatment and fraction.

Soil Sample	SV	DF	p value
HPb soil/Treatment	Pb-Exc	3	0.9550
	Pb-Red	3	0.0131
	Pb-MOS	3	0.9881
	Pb-Red	3	0.0208
	Residue	75	-
HPb soil/Fraction	Bulk soil	3	0.0000

(continues)

(continuation)

	Eucalyptus	3	0.0000
	Brachiaria	3	0.0000
	Mustard	3	0.0000
	Residue	75	-

SV: source of variation; DF: number of degrees of freedom.

Supplementary Table 9. ANOVA results from LPb soil Pb fractions (%) in rhizosphere (eucalyptus, brachiaria and mustard) and bulk soil samples, unfolded by treatment and fraction.

Soil Sample	**SV**	**DF**	**p value**
LPb soil/Treatment	Pb-Exc	3	0.9927
	Pb-Red	3	0.0000
	Pb-MOS	3	0.7189
	Pb-Red	3	0.0000
	Residue	75	-
LPb soil/Fraction	Bulk soil	3	0.0000
	Eucalyptus	3	0.0000
	Brachiaria	3	0.0000
	Mustard	3	0.0000
	Residue	75	-

SV: source of variation; DF: number of degrees of freedom.

Supplementary Table 10. ANOVA results from total Pb content in plant tissue (mg kg^{-1}) of plants cultivated in HPb and LPb soil samples.

Soil Sample	**SV**	**DF**	**p value**
HPb soil	Treatment	2	0.0001
	Section	1	0.0000

(continues)

(continuation)

	Treatment*Section	2	0.0004
	Block	2	0.2341
	Residue	10	-
	CV (%)	18.42	
LPb soil	Treatment	2	0.0000
	Section	1	0.0000
	Treatment*Section	2	0.0000
	Block	2	0.1100
	Residue	10	
	CV (%)	9.82	

SV: source of variation; DF: number of degrees of freedom.

Supplementary Table 11. ANOVA results from total Pb content in plant tissue (mg kg^{-1}) of plants cultivated in HPb soil samples, unfolded by treatment and plant section.

Soil Sample	**SV**	**DF**	**p value**
HPb soil/Section	Eucalyptus	1	0.0000
	Brachiaria	1	0.0000
	Mustard	1	0.0003
	Residue	10	-
HPb soil/Treatment	Root	2	0.0000
	Shoot	2	0.3748
	Residue	10	-

SV: source of variation; DF: number of degrees of freedom.

Supplementary Table 12. ANOVA results from total Pb content in plant tissue (mg kg^{-1}) of plants cultivated in LPb soil samples, unfolded by treatment and plant section.

Soil Sample	SV	DF	p value
LPb soil/Section	Eucalyptus	1	0.0004
	Brachiaria	1	0.0001
	Mustard	1	0.0000
	Residue	10	-
LPb soil/Treatment	Root	2	0.0000
	Shoot	2	0.0008
	Residue	10	-

SV: source of variation; DF: number of degrees of freedom.

Supplementary Table 13. ANOVA results from plant dry matter (g) of plants cultivated in HPb and LPb soil samples.

Soil Sample	SV	DF	p value
HPb soil	Treatment	2	0.0000
	Section	1	0.0000
	Treatment*Section	2	0.0000
	Block	5	0.0929
	Residue	25	-
	CV (%)	17.39	
LPb soil	Treatment	2	0.0000
	Section	1	0.0000
	Treatment*Section	2	0.0000
	Block	5	0.2024
	Residue	25	-
	CV (%)	18.47	

SV: source of variation; DF: number of degrees of freedom.

Supplementary Table 14. ANOVA results from plant dry matter (g) of plants cultivated in HPb soil samples, unfolded by treatment and plant section.

Soil Sample	SV	DF	p value
HPb soil/Section	Eucalyptus	1	0.0000
	Brachiaria	1	0.0018
	Mustard	1	0.0005
	Residue	25	-
HPb soil/Treatment	Root	2	0.0000
	Shoot	2	0.0000
	Residue	25	-

SV: source of variation; DF: number of degrees of freedom.

Supplementary Table 15. ANOVA results from plant dry matter (g) of plants cultivated in LPb soil samples, unfolded by treatment and plant section.

Soil Sample	SV	DF	p value
LPb soil/Section	Eucalyptus	1	0.0000
	Brachiaria	1	0.0545
	Mustard	1	0.0000
	Residue	25	-
LPb soil/Treatment	Root	2	0.0000
	Shoot	2	0.0000
	Residue	25	-

SV: source of variation; DF: number of degrees of freedom.

Supplementary Table 16. ANOVA results from total Pb stocks in plant tissue (mg) of plants cultivated in HPb and LPb soil samples.

Soil Sample	SV	DF	p value
HPb soil	Treatment	2	0.0000
	Section	1	0.0000
	Treatment*Section	2	0.0050
	Block	2	0.6633
	Residue	10	-
	CV (%)	9.87	
LPb soil	Treatment	2	0.0071
	Section	1	0.0001
	Treatment*Section	2	0.0005
	Block	2	0.4590
	Residue	10	-
	CV (%)	19.83	

SV: source of variation; DF: number of degrees of freedom.

Supplementary Table 17. ANOVA results from total Pb stocks in plant tissue (mg) of plants cultivated in HPb soil samples, unfolded by treatment and plant section.

Soil Sample	SV	DF	p value
HPb soil/Section	Eucalyptus	1	0.0000
	Brachiaria	1	0.0000
	Mustard	1	0.0040
	Residue	10	-
HPb soil/Treatment	Root	2	0.0000
	Shoot	2	0.0000
	Residue	10	-

SV: source of variation; DF: number of degrees of freedom.

Supplementary Table 18. ANOVA results from total Pb stocks in plant tissue (mg) of plants cultivated in LPb soil samples, unfolded by treatment and plant section.

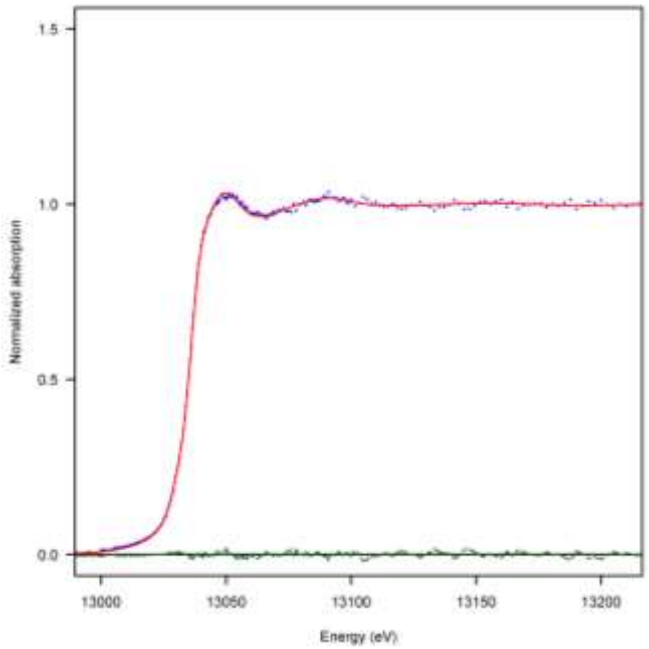
Soil Sample	SV	DF	p value
LPb soil/Section	Eucalyptus	1	0.1802
	Brachiaria	1	0.0000
	Mustard	1	0.5434
	Residue	10	-
LPb soil/Treatment	Root	2	0.0003
	Shoot	2	0.0276
	Residue	10	-

SV: source of variation; DF: number of degrees of freedom.

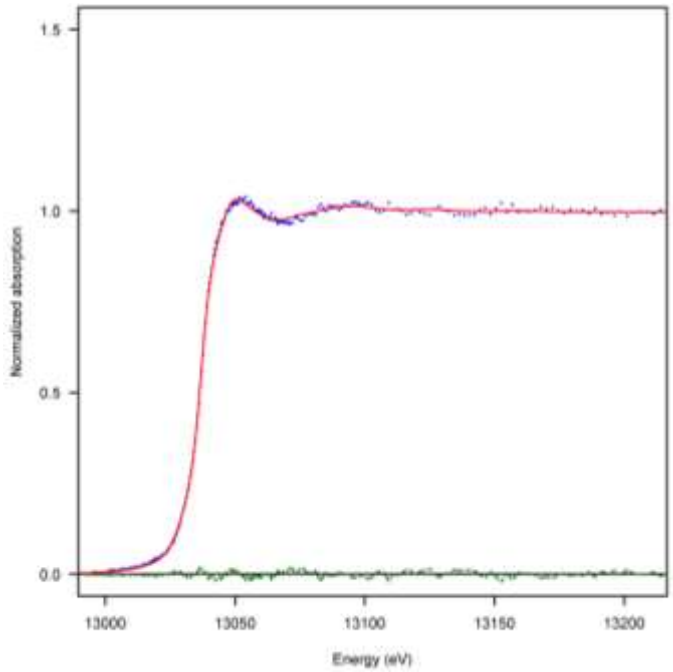
Supplementary Table 19. ANOVA results from pH values in rhizosphere (eucalyptus, brachiaria and mustard) and bulk soil samples.

Soil Sample	SV	DF	p value
HPb soil	Treatment	3	0.0220
	Block	2	0.5519
	Residue	6	-
	CV (%)	1.32	
LPb soil	Treatment	3	0.1058
	Block	2	0.2781
	Residue	6	-
	CV (%)	2.30	

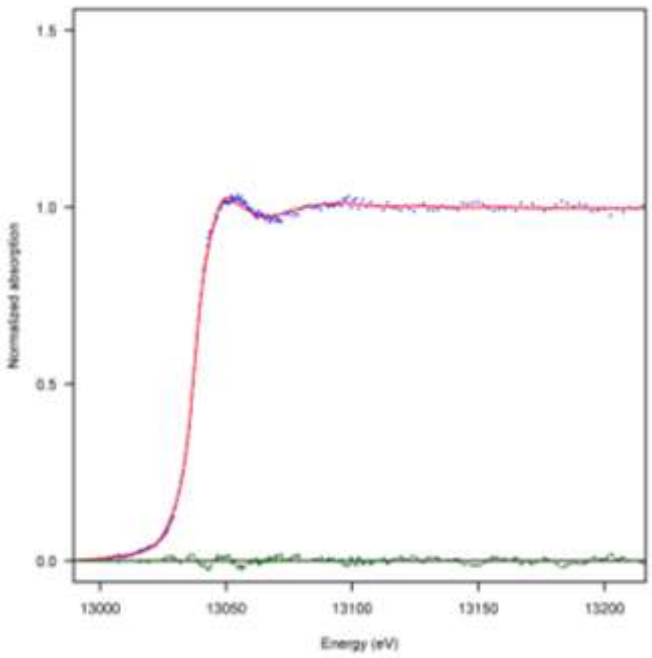
SV: source of variation; DF: number of degrees of freedom.



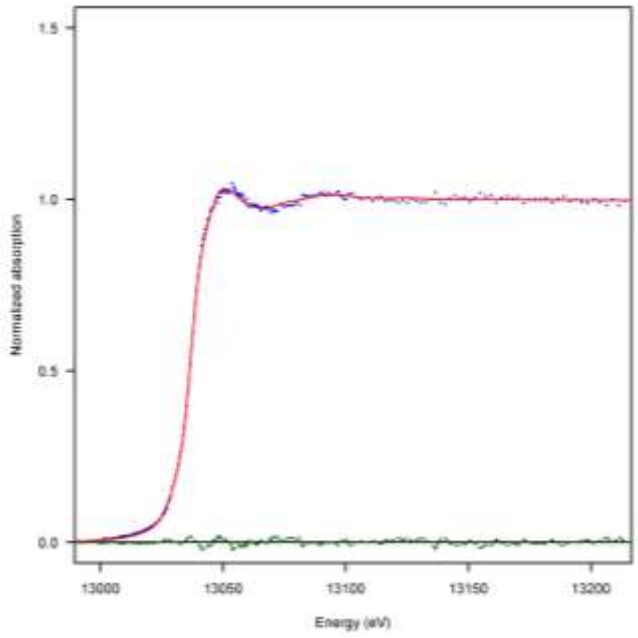
Supplementary Figure 1. XANES spectrum of *bulk* soil and spectrum of the best linear combination fitting.



Supplementary Figure 2. XANES spectrum of eucalyptus rhizosphere soil and spectrum of the best linear combination fitting.



Supplementary Figure 3. XANES spectrum of brachiaria rhizosphere soil and spectrum of the best linear combination fitting.



Supplementary Figure 4. XANES spectrum of mustard rhizosphere soil and spectrum of the best linear combination fitting.