

# Lead acetate ecotoxicity in tropical soils

**Rômulo CS Alexandrino**

Fundação Estadual do Meio Ambiente - FEAM

**Francielle Lima** (✉ [frandislim@gmail.com](mailto:frandislim@gmail.com))

UFLA: Universidade Federal de Lavras <https://orcid.org/0000-0003-0186-7004>

**Gabriel C Martins**

Instituto Tecnológico Vale

**Tiago Natal-da-Luz**

Universidade de Coimbra

**José Paulo Sousa**

Universidade de Coimbra

**Luiz Roberto Guimarães Guilherme**

UFLA: Universidade Federal de Lavras

**João José Marques**

UFLA: Universidade Federal de Lavras

---

## Research Article

**Keywords:** toxicity, soil invertebrates, crops, phytotoxicity, lead

**Posted Date:** March 24th, 2021

**DOI:** <https://doi.org/10.21203/rs.3.rs-338895/v1>

**License:**   This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

---

# Abstract

Lead acetate (AcPb) is an important raw material used in chemical industries worldwide. The potential toxicity of AcPb is generally attributed to the presence of Pb. However, the effect of AcPb on the environment as a whole is still poorly known. This study aimed to evaluate AcPb toxicity on three standard species of soil invertebrates and two plant species using ecotoxicology tests. Three tropical soils (Oxisol, Inceptisol, and Tropical Artificial Soil (TAS)) were contaminated with different concentrations of AcPb and one dose of K-acetate (positive control). These soils were used in tests with *Eisenia andrei* (earthworm), *Folsomia candida* (springtail), *Enchytraeus crypticus* (enchytraeid), *Zea mays* (maize), and *Phaseolus vulgaris* (common bean). Dose-response curves obtained in the laboratory tests were used to estimate the EC<sub>50</sub> values for each species. Among invertebrates, the highest sensitivity to AcPb was observed for *E. crypticus* in the TAS (EC<sub>50</sub> = 29.8 mg AcPb kg<sup>-1</sup>), whereas for *E. andrei* and *F. candida* the highest sensitivity was observed in the Oxisol (EC<sub>50</sub> = 141.9 and 1835 mg AcPb kg<sup>-1</sup>, respectively). *Folsomia candida* was the least sensitive invertebrate species to AcPb in all soils. Among plant species, *Z. mays* was less sensitive (EC<sub>50</sub> = 1527.5 mg AcPb kg<sup>-1</sup>) than *P. vulgaris* (EC<sub>50</sub> = 560.5 mg AcPb kg<sup>-1</sup>) in the Oxisol. The present study evidenced that the toxicity of AcPb should not be attributed uniquely to the presence of Pb, as the treatment containing uniquely Ac provoked the same toxicity as the highest dose of AcPb.

## Highlights

- Lead acetate significantly affected all standard species tested
- *Enchytraeus crypticus* was the most sensitive species in all tested soils
- *Zea mays* was less sensitive to AcPb than *Phaseolus vulgaris*
- K-acetate equivalent to the highest AcPb dose was as toxic as the highest AcPb dose

## 1. Introduction

Soil pollution is a current worldwide challenge that has direct and/or indirect impacts on human health, food security, environmental quality, provision of ecosystem services, and the economy (FAO 2018). Among the main types of pollutants to the environment are heavy metals, such as lead (Pb), which stands out due to its potential harm and persistence in the environment (Wani et al. 2015; Frank et al. 2019; Verma et al. 2020).

Lead is an old and well-known public health hazard and is still responsible for poisoning hundreds of millions of children worldwide (Rees and Fuller 2020). The widespread number of sites contaminated by Pb is probably due to its historical and current broad use and human activities, for example, as a raw material in lead-acid batteries, some paints, glazes, leaded glass and ceramics, as well as mining, smelting, refining, and recycling of lead (Who 2019; Rees and Fuller 2020). When these activities and products/wastes are not properly managed, there is an increased risk of environmental contamination with severe lasting implications.

The solubility of Pb compounds can be quite different, with lead acetate, lead chloride, and lead nitrate being among the most soluble Pb salts (NTP 2016). These soluble Pb compounds have many industrial uses, e.g., lead acetate (AcPb) is used as a color additive in hair dyes; as a mordant in cotton dyes; in lead coating of metals; as a drier in paints; varnishes and pigment inks; and in medicines, such as astringents (NTP 2016; Pohanish 2017). Besides, AcPb has been used in the production of highly efficient perovskite solar cells (Li et al. 2018, 2020) and on lead-acetate test paper, a product designed to detect sulfur in different materials (Selwyn 2017). All the uses mentioned above make it a potential source of soil contamination when proper management is not assigned.

Although lead is an element that has been widely studied (Brown et al. 2016; Rehman et al. 2017; Entwistle et al. 2019; Alexandrino et al. 2020), the knowledge on the disposal and detailed information on the exposure to materials containing AcPb for soil organisms is still limited. Moreover, the majority of the studies conducted to date to evaluate the toxicity of Pb to plants, invertebrates, and soil microorganisms have used nitrates and chlorides as a source of Pb (Sobolev and Begonia 2008; Xu et al. 2009; Smolders et al. 2015; Chandrasekhar and Ray 2019; Zhang et al. 2019a; Dai et al. 2020). To the best of our knowledge, most studies using AcPb as a Pb source have been devoted to assessing its toxicity in animals (e.g. Ibrahim et al. 2012 and Haouas et al. 2014). The few studies using AcPb in soils have been focused mainly on the toxicity of Pb (Päivöke 2002; Liao et al. 2007; Zeng et al. 2007; Cândido et al. 2020). Consequently, data on AcPb toxicity to soil organisms are lacking, a useful piece of information on the management of areas contaminated by this Pb source.

Studies that investigated the toxicity of AcPb and evidenced its potential harm in soils include those of Liao et al. (2007), Zeng et al. (2007), and Cândido et al. (2020). For example, Liao et al. (2007) showed that AcPb (concentrations  $> 500 \text{ mg Pb kg}^{-1}$ ) caused a significant decline in soil microbial biomass. Zeng et al. (2007) reported that concentrations up to  $900 \text{ mg Pb kg}^{-1}$  as AcPb cause damage to rice plants, enzyme activity, and soil microbial biomass, and Cândido et al. (2020) found Pb concentrations of 2760 and 1788  $\text{mg Pb kg}^{-1}$  (as AcPb) that decrease the shoot dry matter production by 50% in sorghum and soybean, respectively. The wide range of effective concentrations presented for these studies shows that Pb toxicity can be quite different, considering the soil, test organisms, and endpoints evaluated.

It is widely known that distinct soils can induce dissimilar toxicities for the same concentration of a specific contaminant due to its interaction with soil attributes (Kabata-Pendias and Szteke 2015). Also, the exposure mode and own protection and detoxification strategies of each species also contribute to diversify the toxicity values, which reinforces the need to obtain toxicity data for different soils, organisms, and endpoints.

Whereas there is a lack of knowledge on the toxicity of AcPb, mainly due to the stress caused by contamination of high concentrations of AcPb compared with acetate for plants and soil invertebrates, it becomes important to clarify the toxic effects of AcPb on these organisms. Ecotoxicology studies are needed to fill this gap and improve the understanding of AcPb effects on the environment, mainly for tropical soils, which may have physical and chemical attributes quite different from those found in temperate regions and standard substrates. Thus, this study aimed to evaluate AcPb and acetate toxicity in tropical soils through bioassays including plant species and soil invertebrates. It is expected that these results may clarify some aspects of AcPb toxicity, and the first step in this direction has been made in the tropical region but studies in other regions of the globe are needed.

## 2. Materials And Methods

### 2.1 Test Soils

In all experiments, two natural soils from areas of native vegetation with minimal anthropogenic interference of State of Minas Gerais, Brazil, were used: an Oxisol (Typic Hapludox) ( $21^{\circ}17'10.3'' \text{ S}$  and  $44^{\circ}47'45.5'' \text{ W}$ ) and an Inceptisol (Typic Dystrudept) ( $21^{\circ}13'48.3'' \text{ S}$  and  $44^{\circ}59'11.6'' \text{ W}$ ) (Soil Survey Staff 2014). Both soils were collected from the top 20-cm layer, air-dried, and sieved to 2 mm. These soils were selected because Oxisols and Inceptisols are relevant soil classes in tropical regions, covering approximately 20 and 9% of the total area of South America, respectively (Gardi et al. 2015).

In tests with soil invertebrates, in addition to the natural soils, a Tropical Artificial Soil (TAS; Garcia et al. 2004) composed of a mixture of 75% fine sand, 20% kaolinite clay, and 5% coconut fiber was used.

The physical and chemical properties of the natural soils are presented in Table 1. Briefly, soil pH was determined using a 1 mol L<sup>-1</sup> KCl solution (1:5, w:v; ISO 2005), cation exchange capacity (CEC) was calculated by the sum of exchangeable cations and potential acidity according to Teixeira et al. (2017), the particle-size analysis was performed by the pipette method (Day 1965), water holding capacity was measured according to ISO 11269-2 (ISO 2005), and organic matter content was determined by the potassium dichromate (K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>) method (Walkley and Black 1934). The soil pH and water holding capacity of TAS are 5 and 49%, respectively.

Table 1  
Physical and chemical properties of the Oxisol, Inceptisol, and Tropical Artificial Soil (TAS) used in laboratory experiments

Attributes	Oxisol	Inceptisol	TAS
pH (KCl)	4.3	4.9	5.0
Cation exchange capacity at pH 7 (cmol <sub>c</sub> dm <sup>-3</sup> )	5.0	6.1	2.3
Water holding capacity (%)	40.0	59.0	49.0
Organic matter (%)	1.6	2.9	5.0
Texture	23.0	46.0	20.0 <sup>1</sup>
Clay (%)			
Silt (%)	3.0	19.0	
Sand (%)	74.0	35.0	75.0 <sup>1</sup>
Texture class	Sandy clay loam	Clay	
<sup>1</sup> Value determined by weighing.			

## 2.2 Higher plant growth test

To provide conditions for the development of plants, thirty five days before sowing, the pH of natural soils was adjusted to approximately 6.0 and the base saturation was increased to 50%, by the application of CaCO<sub>3</sub> and MgCO<sub>3</sub> in a 3:1 molar ratio, as recommended by Alvarez and Ribeiro (1999). Fifteen days before sowing, the soils were fertilized considering the critical levels of nutrients (Alvarez and Ribeiro 1999) and minimum levels of fertilization for plants in pots (Malavolta 1980). Both procedures were performed to avoid reductions in plant growth due to the lack of nutrients in the test substrate, which could mask toxic effects provoked by the presence of the test substance in the soil. A nutrient solution composed of 200 mg P kg<sup>-1</sup>, 5 mg Zn kg<sup>-1</sup>, 1.5 mg Cu kg<sup>-1</sup>, 1 mg B kg<sup>-1</sup>, and 3 mg Mn kg<sup>-1</sup> prepared using analytical-grade ammonium dihydrogen phosphate, Zn-sulfate, boric acid, and Mn-sulfate was mixed in the soils. In both incubation periods and throughout the higher plant growth test, the soil moisture was kept at 60% of the water holding capacity.

The fertilized soils were air-dried immediately before receiving treatments. The same concentrations of AcPb and AcK were tested in both soils, comprising a gradient of increasing concentrations of AcPb composed of 0, 78, 157, 314, 628, 1256, 2512, 5024 mg Pb(CH<sub>3</sub>COO)<sub>2</sub>·3H<sub>2</sub>O kg<sup>-1</sup> and a Pb-free acetate control (AcK; 3200 mg CH<sub>3</sub>CO<sub>2</sub>K kg<sup>-1</sup>). The

AcPb concentrations were defined following a multiplying factor of two and were prepared by the addition of different volumes of a stock solution of  $91.5 \text{ g AcPb L}^{-1}$  prepared in distilled water. The acetate concentration in the AcK control was used to have a treatment free of Pb but with an acetate concentration equivalent to the acetate concentration present in the  $5024 \text{ mg AcPb kg}^{-1}$  treatment (the highest AcPb concentration tested). The AcK control was used in the tests to evaluate the influence of the acetate itself in the final toxicity.

Higher plant growth tests using *Zea mays* (maize) and *Phaseolus vulgaris* cv. Carioquinha (common bean) were performed following ISO 11269-2 (ISO 2005). These species were selected due to their relevance as staple crops. Each replicate corresponded to one pot (110 mm height, 120 mm diameter) containing 600 g of soil (dry weight equivalent; DW). Ten seeds were sown in each replicate up to 24 hours after the soil spiking and the correction of soil moisture to 60% of its water holding capacity.

Four replicates were used per treatment and plant species. Plant tests were carried out in a growth chamber at  $25 \pm 2^\circ\text{C}$  with a photoperiod of 16:8 hours (light: dark), using a light intensity of  $8.000 \pm 2.000 \text{ lux}$  in the light periods. Soil moisture was reestablished by capillarity (each test pot was connected to an individual container filled with distilled water through a rope).

The test started after the emergence of 50% of the seeds in the control replicates. In all treatments, surplus plants were trimmed to leave only five plants per pot. During the experiment, the test replicates were randomly distributed within the growth chamber. The N and K nutrients were applied 7th and 14th days after starting the test on the soil surface, with a total of  $300 \text{ mg N kg}^{-1}$  applied as urea and  $150 \text{ mg K kg}^{-1}$  as a nutrient solution of analytical-grade KCl. After 21 days of the beginning of the test, plants were harvested at the stem base in all replicates. Then, the harvested plants were dried at  $75^\circ\text{C}$  for 72 h and weighted to determine the shoot dry matter (SDM).

## 2.3 Ecotoxicological tests with soil invertebrates

Laboratory reproduction tests were performed using the three soils (Oxisol, Inceptisol, and TAS) without pH adjustment (Table 1) and the springtails *Folsomia candida*, the earthworms *Eisenia andrei*, and the enchytraeids, *Enchytraeus crypticus* as test organisms and following the ISO guidelines 11267 (1999), 11268-2 (1998) and 16387 (2003), respectively.

The organisms used in the tests were grown in the laboratory, under a temperature of  $25 \pm 2^\circ\text{C}$  and a photoperiod of 16:8 hours light: dark. Springtails were kept in plastic boxes (11 cm diameter and 4 cm height) containing a mixture of plaster of Paris and activated charcoal in a ratio of 11:1 (w:w), being fed weekly with dry granulated yeast. Earthworms were kept in plastic boxes (36 cm length, 22 cm width, and 11 cm height) with a substrate containing a mixture of horse dung (previously defaunated through two freeze-thawing cycles of 48h at  $-20^\circ\text{C}$  followed by 48h at  $25^\circ\text{C}$ ) and peat (in a proportion of 1:1, w:w), and moisture was maintained between 40 and 60% of the water holding capacity of the mixture. Earthworms were fed twice a month with one spoon of horse dung. Enchytraeids were kept on Petri dishes (9 cm diameter and 1 cm height) filled with agar as described by Cesar et al. (2015b) and were fed weekly with finely ground autoclave-sterilized oat.

Natural soils were defaunated by two freeze-thawing cycles (48 hours at  $-20^\circ\text{C}$  followed by 48 hours at  $25^\circ\text{C}$ ), after which, their microbial community was restored by inoculation of 100 mL of elutriates per kg of fresh soil. The elutriates were obtained by stirring fresh and non-defaunated soil samples with water in a ratio proportion of 1:10 (w:v) for 30 minutes. After this procedure, both soils were stored for 10 days at room temperature in the dark before being spiked with AcPb or AcK in the laboratory for testing.

In each laboratory test, the invertebrate species were exposed to a concentration gradient composed of the following increasing concentrations of AcPb: 0, 314, 628, 1256, 2512, 5024, 10048, 20095 mg AcPb kg<sup>-1</sup> soil dry weight and a Pb-free acetate control with 12800 mg of CH<sub>3</sub>CO<sub>2</sub>K kg<sup>-1</sup>. The concentrations selected for the reproduction tests were based on data from the available literature concerning the toxicity of Pb to *Eisenia fetida* (Neuhauser et al. 1985; Spurgeon et al. 1994; Davies et al. 2002, 2003), *Folsomia candida* (Sandifer and Hopkin 1997; Jie et al. 2009) and *Enchytraeus albidus* (Lock and Janssen 2003) using different lead salts as a source of contamination. For each treatment, soil aliquots were spiked through the addition of different volumes of a stock solution of 130.6 g AcPb L<sup>-1</sup> or 135.2 g AcK L<sup>-1</sup> (prepared with water) and water to obtain the desired concentration and soil moisture of 50% of its maximum water holding capacity. Soil spiking was performed immediately before the beginning of the laboratory tests. Tests with invertebrate species were performed at 25 ± 2°C and under a photoperiod of 16:8 hours (light: dark).

In the reproduction tests with *F. candida*, ten synchronized organisms 10–12 days old were used per replicate in a total of five replicates per treatment. Each replicate consisted of cylindrical plastic containers (7 cm diameter and 6 cm height) with 30 g of soil (fresh weight). During the experiment, the test organisms were fed by adding ~ 2 mg of dry granulated yeast in the test container at the beginning of the test and after 14 days of exposure. Once a week, the vessels were opened to allow aeration and to restore water losses by the addition of few drops of distilled water. After 28 days the test was finished and the content of each vessel was transferred to a larger container and filled with water. Drops of blue ink were added and the soil was gently stirred in the bottom of the vessels. Then, the water surface was photographed, and the number of juveniles and living adults was determined using the software ImageJ. Soil moisture and pH were measured at the beginning of the experiment in all treatments. An additional replicate without organisms was prepared per treatment for soil pH and moisture determinations at the end of the experiment.

In the reproduction tests with *E. andrei*, four replicates were prepared per treatment. Each replicate consisted of one plastic pot (11 cm diameter and 12 cm height) containing 500 g of soil (dry weight). Ten earthworms with fully developed clitellum, more than two months old, previously rinsed, and 386 ± 74 mg of individual weight (average ± standard deviation; *n* = 1080) were introduced in each replicate. Each test container was covered by a transparent lid with small holes to allow aeration. These covers were used to reduce water losses by evaporation and prevent the organisms from escaping. Fifteen grams of horse dung, previously defaunated, were added to each pot as food at the beginning of the experiment and at the 14th and 28th days of the test. On the 28th day, living adults were removed, counted, and weighted to determine the number of survivals and the percentage of initial biomass. On the 56th day of the experiment, the test ended and the experimental units were placed into a water bath at 50–60 °C to force the juveniles to raise in the soil surface, allowing to determine the number of juveniles in each test container.

In the reproduction tests with *E. crypticus*, replicates containing ten individuals of similar size and developed clitellum were used for each treatment. Four replicates were prepared per treatment, except for control (0 mg AcPb kg<sup>-1</sup>) in which eight replicates were used. Each replicate consisted of cylindrical glass vessels (6 cm diameter and 9 cm height) with 20 g of soil (dry weight equivalent). Finely ground oats, 2 mg per replicate, were provided as food at the 0, 7th, 14th, and 21st days of the test. Once a week, test containers were opened to allow aeration, and water losses were restored whenever weight losses were higher than 2%. After a test period of 28 days, the organisms were killed by adding a few milliliters of an 80% ethanol solution in the replicates and stained with a few drops of a Rose Bengal solution (1% in ethanol). After 12h, the soil was rinsed in a 0.25 mm-sieve, enchytraeids were transferred to a Petri dish and the total number of organisms was determined using a binocular magnifying glass (Chelinho et al. 2014). The number of adults could not be determined as the size of juveniles did not allow us to distinguish the surviving adults from some juveniles at the end of the test. As for Collembola reproduction tests, soil pH and moisture were

determined at the beginning of the test and an additional replicate without organisms was prepared per each treatment for soil pH and moisture determinations at the end of the experiment.

## 2.4 Chemical analyses

A composite sample was collected per treatment to determine Pb concentration, immediately after soil spiking with AcPb and AcK in all tests. Lead extraction was performed according to the USEPA 3051A method (USEPA 2007). Lead was determined by air-acetylene flame atomic absorption spectrophotometry, with detection and quantification limits of the method of 0.3 and 0.9 mg L<sup>-1</sup>, respectively (Penha et al. 2017). The reference material BCR 142 R - light sandy soil (Community Bureau of Reference, Brussels) was used to verify the accuracy of Pb measurements. The recovery of Pb ranged between 90 and 110% of the reference material.

## 2.5 Statistical analyses

The shoot dry matter (SDM) of plants, the percentage of initial biomass of surviving earthworms, and the reproductive output of earthworms, collembolans, and enchytraeids were statistically analyzed by one-way ANOVAs followed by Dunnett's post hoc test to test for significance of the difference between the control and the AcPb contaminated soils and AcK control in each test. When the ANOVA assumptions of normality (Kolmogorov–Smirnov test for  $p > 0.05$ ) and homoscedasticity (Bartlett test for  $p > 0.05$ ) were violated, Kruskal-Wallis ANOVA by Ranks (Kruskal and Wallis 1952) followed by a multiple comparison test was used.

The effective concentrations - EC<sub>20</sub> and EC<sub>50</sub> values - and the respective 95% confidence intervals were calculated to estimate the concentrations that produce 20 and 50% change in the response (i.e., effects) on SDM in plants and reproduction of earthworms, collembolans, and enchytraeids. These values were estimated through non-linear regressions, using an exponential, Gompertz, or Logistic model (EC 2007). The model selected was the one that presented the highest determination coefficient ( $R^2$ ) and the smallest 95% confidence interval. Non-linear regressions followed the method Levenberg-Marquardt and the assumptions of non-linear regressions were checked by the analysis of the normality of the residuals via Q-Q plots.

One-way ANOVAs and non-linear regressions were performed using the Statistica 7.0 software (Statsoft 2004). Significant differences between EC<sub>50</sub> values of different test soils for the same species or between EC<sub>50</sub> values of different species in the same soil were determined using a generalized likelihood ratio test.

## 3. Results

Lead concentrations in the soils for the control treatment (without the addition of Pb) were 4.1, 9.9, and 7.6 mg kg<sup>-1</sup> in the Oxisol, Inceptisol, and TAS, respectively. These concentrations for natural soils (Oxisol and Inceptisol) are within the range of values considered normal for Pb *background* in the area (Guevara et al. 2018). The concentrations of Pb in the AcPb treatments in the Oxisol, Inceptisol, and TAS represented the added Pb concentration (Table 2 and Table 3). Thus, chemical measurements confirmed that the test organisms (plants and soil invertebrates) were exposed to a gradient of increasing Pb concentrations. Since the actual concentrations showed a percentage of nominal concentrations close to 100%, the effective concentrations (EC<sub>50</sub> and EC<sub>20</sub>) were estimated based on the nominal concentrations of AcPb.

Table 2

Lead acetate (AcPb) and lead (Pb) nominal concentrations and Pb actual concentrations (mean  $\pm$  standard deviation;  $n = 3$ ; expressed in  $\text{mg kg}^{-1}$ ) in treatments of Oxisol and Inceptisol (and respective percentages of Pb nominal concentrations for each soil) used in the laboratory higher plant growth tests

Treatment	AcPb nominal	Pb nominal	Oxisol		Inceptisol	
			Pb actual	% of Pb nominal	Pb actual	% of Pb nominal
C0	0	0	$4.1 \pm 0.3$	-	$9.9 \pm 0.4$	-
C1	78	50	$49 \pm 2.5$	98	$55 \pm 1.5$	110
C2	157	100	$119 \pm 1.7$	119	$92 \pm 2.5$	92
C3	314	200	$198 \pm 2.6$	99	$188 \pm 4.0$	94
C4	628	400	$412 \pm 1.5$	103	$400 \pm 3.1$	100
C5	1256	800	$796 \pm 1.0$	99.5	$798 \pm 2.1$	99.8
C6	2512	1600	$1599 \pm 1.0$	99.9	$1591 \pm 3.0$	99.4
C7	5024	3200	$3225 \pm 0.6$	100.8	$3187 \pm 4.5$	99.6
AcK	5320 <sup>1</sup>	0	$6.4 \pm 0.2$	-	$8.1 \pm 0.4$	-

<sup>1</sup> Potassium acetate nominal concentration (in  $\text{mg kg}^{-1}$ )

Table 3

Lead acetate (AcPb) and lead (Pb) nominal concentrations and Pb actual concentrations (mean  $\pm$  standard deviation;  $n = 3$ ; expressed in  $\text{mg kg}^{-1}$ ) in treatments of Oxisol, Inceptisol and tropical artificial soil (TAS; and respective percentages of Pb nominal concentrations for each soil) used in the laboratory reproduction tests with soil invertebrates

Treatment	AcPb nominal	Pb nominal	Oxisol		Inceptisol		TAS	
			Pb actual	% of Pb nominal	Pb actual	% of Pb nominal	Pb actual	% of Pb nominal
C0	0	0	4.1 $\pm$ 0.2	-	9.9 $\pm$ 0.3	-	7.6 $\pm$ 0.3	-
C1	314	200	193 $\pm$ 3.1	96.5	191 $\pm$ 2.1	95.5	186 $\pm$ 2.6	93
C2	628	400	395 $\pm$ 1.5	98.8	397 $\pm$ 2.6	99.3	392 $\pm$ 2.1	98
C3	1256	800	789 $\pm$ 2.1	98.6	798 $\pm$ 3.5	99.8	788 $\pm$ 4.4	98.5
C4	2512	1600	1598 $\pm$ 3.0	99.9	1593 $\pm$ 3.1	99.6	1596 $\pm$ 2.1	99.8
C5	5024	3200	3200 $\pm$ 1.0	100	3198 $\pm$ 1.5	99.9	3197 $\pm$ 4.2	99.9
C6	10048	6400	6400 $\pm$ 1.7	100	6404 $\pm$ 2.0	100.1	6437 $\pm$ 3.8	100.6
C7	20095	12800	12800 $\pm$ 1.2	100	12790 $\pm$ 5.6	99.9	12807 $\pm$ 3.6	100.1
AcK	12121 <sup>1</sup>	0	5.1 $\pm$ 0.3	-	9.1 $\pm$ 0.3	-	10.3 $\pm$ 0.3	-

<sup>1</sup> Potassium acetate nominal concentration (in  $\text{mg kg}^{-1}$ )

The AcK treatments were toxic to all test species (plant, springtails, enchytraeids, and earthworms), considering the Oxisol and Inceptisol for SDM (Fig. 1) and Oxisol, Inceptisol, and TAS for the number of juveniles, and the initial percentage of biomass (Figs. 2 and 3). As expected, Pb chemical measurements in the Oxisol, Inceptisol, and TAS showed that AcK treatments had Pb concentrations ( $\text{mg kg}^{-1}$ ) similar to that of control treatments without application of Pb, for the tests with plants (Oxisol – 6.4  $\pm$  0.2 and Inceptisol – 8.1  $\pm$  0.4) and soil invertebrates (Oxisol – 5.1  $\pm$  0.3, Inceptisol – 9.1  $\pm$  0.3 and TAS – 10.3  $\pm$  0.3).

In the higher plant growth tests, all validity criteria defined in the abovementioned protocols were met. The percentage of emergence in the control treatment was 80 and 81% for *Z. mays* and 81 and 83% for *P. vulgaris* in the Oxisol and Inceptisol, respectively. For *P. vulgaris*, SDM was significantly affected in both natural soils (Oxisol and Inceptisol) by concentrations higher than or equal to 157  $\text{mg AcPb kg}^{-1}$  and in AcK treatment (Fig. 1a). In the Inceptisol, concentrations greater than or equal to 157  $\text{mg AcPb kg}^{-1}$  significantly affected *Z. mays* SDM. The same was observed for AcK at 3200  $\text{mg kg}^{-1}$  (Fig. 1b). It was observed a significant effect on SDM when *Z. mays* plants were grown in the Oxisol with concentrations higher than or equal to 628  $\text{mg AcPb kg}^{-1}$  (628, 1256, 2512 e 5024  $\text{mg kg}^{-1}$ ),

except for the 78 mg AcPb kg<sup>-1</sup>, which also had a significant effect. A significant decrease was also observed in the AcK treatment (Fig. 1b).

In the laboratory tests with soil invertebrates, all validity criteria were met. In the control treatment, the percentage of adult survival of *E. andrei* and *F. candida* were 98, 100, 100% and 84, 82, 82% in the Oxisol, the Inceptisol, and the TAS, respectively. The mean of juveniles (and associated coefficient of variation) produced in control treatment in the Oxisol, the Inceptisol, and the TAS were, respectively, 41 (20%), 61 (30%), and 44 (10%) for *E. andrei*; 412 (24%), 713 (28%), and 775(29%) for *F. candida*; 573 (37%), 1228 (17%), and 1204 (15%) for *E. crypticus*.

Effects on *F. candida* reproduction were observed in concentrations higher than or equal to 2512 mg kg<sup>-1</sup> in natural soils, and higher than or equal to 5024 mg AcPb kg<sup>-1</sup> in the TAS soil (Fig. 2a). The exception was the 314 mg kg<sup>-1</sup> concentration in the Inceptisol in which significant effects were observed (Fig. 2a). However, this decrease was not consistent with the reproductive pattern observed over the concentration gradient used in the test. Living adults of *F. candida* were not observed in concentrations higher than or equal to 2512 mg AcPb kg<sup>-1</sup> in both the Oxisol and the Inceptisol, and in the TAS in concentrations higher than 10048 mg AcPb kg<sup>-1</sup> (Fig. 2a).

For *E. crypticus* and *E. andrei*, effects on reproduction were observed in concentrations greater than or equal to 314 mg AcPb kg<sup>-1</sup> (Fig. 2b and 2c).

Effects of AcPb on *E. andrei* initial biomass were observed only for the highest AcPb (20095 mg AcPb kg<sup>-1</sup>) concentration in the Oxisol (Fig. 3).

The EC<sub>50</sub> values for AcPb calculated for the five test species are presented in Table 4. The monocotyledonous *Z. mays* showed a sensitivity significantly lower than that of the dicotyledonous *P. vulgaris* in the Oxisol. In the Inceptisol, the average EC<sub>50</sub> values for *P. vulgaris* were also lower; however, the confidence interval does not confirm the difference between the EC<sub>50</sub> values of *Z. mays* and *P. vulgaris*. For the three species of soil invertebrates, in general, the sensitivity decreased in the following order: *E. crypticus* > *E. andrei* > *F. candida*.

Table 4

EC<sub>50</sub> and EC<sub>20</sub> values (and respective 95% confidence intervals) for the effects on growth of *Z. mays* and *P. vulgaris* and reproduction of *E. andrei*, *F. candida*, and *E. crypticus* exposed to an Oxisol, an Inceptisol, and a Tropical Artificial Soil (TAS) spiked with increasing concentrations of Pb acetate (AcPb). Values are expressed in mg of AcPb kg<sup>-1</sup> of soil

Species	Soil	EC <sub>20</sub>	EC <sub>50</sub>
		—— mg kg <sup>-1</sup> ——	
<i>E. andrei</i>	Oxisol	68.8 (5–132)	141.9 (48–235)
	Inceptisol	NV <sup>1</sup>	177.4 (113–245)
	TAS	73.6 (10–138)	370.5 (209–534)
<i>F. candida</i>	Oxisol	NV	1835 (36–3633)
	Inceptisol	NV	3606 (1129–6089)
	TAS	2050.3 (651–3451)	4601.5 (2879–6325)
<i>E. crypticus</i>	Oxisol	NV	52.3 (30–75)
	Inceptisol	NV	72.2 (35–109)
	TAS	NV	29.8 (22–38)
<i>Z. mays</i>	Oxisol	1078.5 (568–1587)	1527.5 (893–2162)
	Inceptisol	NV	1229.3 (490–1967)
<i>P. vulgaris</i>	Oxisol	NV	560.5 (251–871)
	Inceptisol	100.5 (9–191)	802.2 (413–1192)

<sup>1</sup> NV – Data not validated by non-linear regression models ( $p < 0.05$ ).

## 4. Discussion

The toxicity of AcPb changed according to soil type and composition. An increased soil pH can contribute to a reduced concentration of Pb in soil solution, owing to the lower solubility of AcPb (Harter 1983; Alloway 2013). Conversely, the reduction of soil pH following the application of AcPb in the natural tropical soils can be explained by the strong affinity between  $\text{Pb}^{2+}$  and Fe oxides, namely hematite (Pierangeli et al. 2001a, b), on which the specific adsorption of  $\text{Pb}^{2+}$  may release  $\text{H}^+$  and cause soil acidification. The influence of pH on Pb toxicity and availability was reported by other authors (Bur et al. 2012; Ardestani et al. 2014; Romero-Freire et al. 2015). As the TAS is not rich in Fe oxides, it was not observed pH reduction following the AcPb addition. Thus, the attributes of natural soils provided greater sensitivity to soil invertebrates exposed to AcPb. Besides pH (Pierangeli et al. 2001a), Pb availability in soils is affected by specific adsorption to soil solid phases (Pierangeli et al. 2001b), precipitation of poorly soluble compounds, and formation of relatively stable complexes and chelates with soil organic matter. Lead sorption is also dependent on the distribution of soil particle-size fractions, owing to their varying reactivity and specific surface area (Romero-Freire et al. 2015; Zhang et al. 2019b). This can explain the increased toxicity observed in the Oxisol compared with the Inceptisol, as the latter has increased clay and organic matter contents relatively to the Oxisol, and these attributes are related to greater Pb retention capacity.

According to Zhang et al. (2019b), soil pH is the main predictive factor for Pb toxicity to enchytraeid reproduction. In that study, *E. crypticus* individuals were exposed for 21 days to the natural standard soils Lufa 2.1, Lufa 2.2, Lufa 2.3, Lufa 2.4, and Lufa 5 M (Speyer, Germany) and a grassland soil, all contaminated with  $\text{Pb}(\text{NO}_3)_2$  (Zhang et al. 2019b). The  $\text{EC}_{50}$  values for *E. crypticus* reproduction ranged between 81.4 and 1,008 mg of  $\text{Pb kg}^{-1}$ . The lowest value was observed for the Lufa 2.1 soil (OM = 1.3%; CEC = 2.2  $\text{cmol}_c \text{ kg}^{-1}$ ; pH = 4.8) and the highest value for the LUFA 5M soil (OM = 2.6%; CEC = 10.1  $\text{cmol}_c \text{ kg}^{-1}$ ; pH = 6.9). The conclusion of Zhang et al. (2019b) was attributed after finding similar  $\text{EC}_{50}$  values in soils with different properties (CEC, OM content, and clay content), but with approximate values of pH (LUFA 2.4, LUFA 5 M, and grassland soil), indicating that pH was the main factor describing the toxicity of Pb on enchytraeid reproduction. Along with pH, CEC and Ca concentration in porewater were identified as the factors determining  $\text{EC}_{50}$  and  $\text{EC}_{10}$  based on total Pb concentration (Zhang et al. 2019b). The three soils used in the present study presented pH around 6.0 after the application of carbonates and the  $\text{EC}_{50}$  values observed in the natural soils (Oxisol 33.3 mg  $\text{Pb kg}^{-1}$  and Inceptisol 46 mg  $\text{Pb kg}^{-1}$ ) for *E. crypticus* reproduction were lower for the Oxisol (OM = 1.6%; CEC = 5.0  $\text{cmol}_c \text{ kg}^{-1}$ ) than for the Inceptisol (OM = 2.9%; CEC = 6.1  $\text{cmol}_c \text{ kg}^{-1}$ ), indicating the difference of toxicity related to differences in OM content. Literature data support the greater effect of OM on  $\text{EC}_{50}$  values (Figure S1).

The organic matter content in the TAS (5.0%) may have contributed to diminishing the toxic effects of AcPb on soil organisms. However, this was not observed for *E. crypticus*, with an  $\text{EC}_{50}$  value of 19 mg  $\text{Pb kg}^{-1}$  being the lowest average  $\text{EC}_{50}$  value for the species (plants and soil invertebrates). The different  $\text{EC}_{50}$  values for *E. crypticus* reproduction in both natural soils (Oxisol and Inceptisol) may be also related to  $\text{Ca}^{2+}$  concentrations in each soil. It is known that  $\text{Ca}^{2+}$  outruns  $\text{Pb}^{2+}$  for specific absorption sites in living organisms (Zhang et al. 2019b). This may have contributed to the reduced toxicity of AcPb in the Inceptisol, as it presented a higher  $\text{EC}_{50}$  value and higher  $\text{Ca}^{2+}$  concentration compared with the Oxisol.

The survey of  $\text{EC}_{50}$  data (Table 5) in the literature for Pb shows how soil chemistry (pH, OM, and Ca), Pb salt used, invertebrate, and plant species influence Pb toxicity (Cheyins et al. 2012; Smolders et al. 2015). In a study with springtails (*F. candida*) exposed to an artificial soil (10% Sphagnum peat, 20% kaolinite clay, and 70% quartz sand)

contaminated with  $\text{Pb}(\text{NO}_3)_2$ , Menta et al. (2006) did not observe effects from concentrations lower than  $500 \text{ mg Pb kg}^{-1}$ , and decreased reproduction (16%) was only observed at the  $1000 \text{ mg kg}^{-1}$  concentration. These results are consistent with the present study since significant decreases in reproduction were not observed in AcPb concentrations lower than  $1256 \text{ mg kg}^{-1}$  ( $800 \text{ mg Pb kg}^{-1} \text{ kg}^{-1}$ ), except for the dose  $314 \text{ mg kg}^{-1}$  of AcPb ( $200 \text{ mg Pb kg}^{-1}$ ) in the Inceptisol. The salt used as a source of contamination may itself influence the performance of the assays. For instance,  $\text{Pb}(\text{NO}_3)_2$  is more toxic to *F. candida* than  $\text{PbCl}_2$  (Fountain and Hopkin 2005) for the same Pb amount. Thus, results from different sources of contamination may not be directly comparable. Nevertheless, the  $\text{EC}_{50}$  values for *F. candida* reproduction in this study, using AcPb, are consistent with the ones reported generically for Pb ( $580$  to  $3160 \text{ mg kg}^{-1}$  at  $20 \text{ }^\circ\text{C}$  - ISO 11267:1999; Fountain and Hopkin 2005). The toxicity of AcPb varies among soil invertebrates due to different routes of exposure to the contaminant. Both species *E. andrei* and *E. crypticus* have soft bodies and are directly exposed through their derma, which favors the absorption of contaminants present in the soil solution (Cesar et al. 2015a). *F. candida* has an exoskeleton and, unlike the soft-bodied species, it absorbs the contaminant with water through specialized organs (Peijnenburg et al. 2012). These different exposure pathways may have contributed to the increased sensitivity in the soft-bodied organisms (*E. andrei* and *E. crypticus*) compared with the hard-bodied ones (*F. candida*) (Fig. 2). Generally, *F. candida* organisms have a subcylindrical or spherical body, very fragile, with a cuticle coating their exoskeleton. The exoskeleton molts can eliminate substances accumulated on the body surface, such as metals (Peijnenburg et al. 2012). Besides the dermal contact with the contaminant, the ingestion of contaminated soil organic matter should be also considered as another exposure pathway (Briones 2018).

Table 5

EC<sub>50</sub> values for the effects on growth of plants and reproduction of soil invertebrates. Values are expressed in mg of Pb kg<sup>-1</sup> of soil

Site	Soils	Soil properties				Species	EC50 mg kg <sup>-1</sup>	Pb source	Reference
		pH	CEC	OM	OC				
			cmolc kg <sup>-1</sup>	%	%				
Brazil	Oxisol	6.1	5.0	1.6	-	<i>E. andrei</i>	90	Pb acetate	Present study
	Inceptisol	5.7	6.1	2.9	-		113		
	TAS <sup>1</sup>	6.0	-	5.0	-		236		
Spain	Calcic Luvisol	7.4	14.3	-	1.2	<i>E. fetida</i>	480	Pb chloride	Smolders et al. (2015)
United Kingdom	Dystric Luvisol	6.1	26.5	-	4.3		2400		
		6.1	26.5	-	4.3		4530		
Belgium	Haplic Luvisol	6.2	8.4	-	1.0		1710		
Netherlands	Lufa 2.1 <sup>2</sup>	4.9	2.2	1.3	-	<i>E. crypticus</i>	81	Pb nitrate	Zhang et al. (2019b)
	Lufa 2.2	5.7	7.6	3.7	-		238		
	Lufa 2.3	5.4	4.0	1.4	-		205		
	Lufa 2.4	6.9	20.1	5.4	-		948		
	Lufa 5M	7.0	10.1	2.6	-		1008		
	Grassland soil	6.9	20.0	12.8	-		991		
Brazil	Oxisol	6.1	5.0	1.6	-	<i>E. crypticus</i>	33.3	Pb acetate	Present study
	Inceptisol	5.7	6.1	2.9	-		46		
	TAS	6.0	-	5.0	-		19		
	Oxisol	6.1	5.0	1.6	-	<i>F. candida</i>	1169		
	Inceptisol	5.7	6.1	2.9	-		2297		
	TAS	6.0	-	5.0	-		2931		

<sup>1</sup> TAS - tropical artificial soil.

<sup>2</sup> LUFA - Landwirtschaftliche Untersuchungs- und Forschungsanstalt, Speyer, Germany.

<sup>3</sup> CEC - effective cation exchange capacity at soil pH.

Site	Soils	Soil properties				Species	EC50 mg kg <sup>-1</sup>	Pb source	Reference
		pH	CEC	OM	OC				
			cmolc kg <sup>-1</sup>	%	%				
Spain	Calcic Luvisol	7.4	14.3	-	1.2	<i>F. candida</i>	712	Pb chloride	Smolders et al. (2015)
China	-	6.5	20.1	-	1.6	<i>F. candida</i>	2361	Pb chloride	Xu et al. (2009)
China	Forest soil	5.1	15.2	1.21	-	<i>F. candida</i>	1244	Pb nitrate	Dai et al. (2020)
Brazil	Oxisol	6.1	5.0	1.6	-	<i>Z. mays</i>	973	Pb acetate	Present study
	Inceptisol	5.7	6.1	2.9	-		783		
	Oxisol	6.1	5.0	1.6	-	<i>P. vulgaris</i>	357		
	Inceptisol	5.7	6.1	2.9	-		511		
	Rhodic Acrudox	5.7	11.7	4.0	-	<i>S. bicolor</i> L.	2359	Pb acetate	Cândido et al. (2020b)
	Typic Hapludox	6.3	6.1	2.1	-		2760		
	Typic Hapludox	6.3	6.1	2.1	-	<i>G. max</i> L.	1788		
EUA	Udic Argiustolls	4.8	4.1	-	0.4	<i>L. perenne</i> L.	785	Pb nitrate	Anderson and Basta (2009)
	Typic Hapludults	5.5	4.1	-	0.7		961		
	Udertic Paleustolls	6.3	14.2	-	1.4		856		
	Aridic Argiustolls	7.8	27.9	-	0.7		2693		
	Typic Endoaquolls	6.1	25.7	-	2.4		4191		
Spain	Calcic Luvisol	7.4	14.3	-	1.2	<i>L. esculentum</i>	2900	Pb chloride	Smolders et al. (2015)
United Kingdom	Dystric Luvisol	6.1	26.5	-	4.3		6140		
Belgium	Haplic Luvisol	6.2	8.4	-	1.0		1240		

<sup>1</sup> TAS - tropical artificial soil.

<sup>2</sup> LUFA - Landwirtschaftliche Untersuchungs- und Forschungsanstalt, Speyer, Germany.

<sup>3</sup> CEC - effective cation exchange capacity at soil pH.

Site	Soils	Soil properties				Species	EC50 mg kg <sup>-1</sup>	Pb source	Reference
		pH	CEC	OM	OC				
			cmolc kg <sup>-1</sup>	%	%				
Spain	Arable land	7.4	14.7 <sup>3</sup>	-	1.4	<i>L. esculentum</i>	6000	Pb chloride	Cheyns et al. (2012)
United Kingdom	Grassland	6.5	27.1 <sup>3</sup>	-	3.1		6500		
Belgium	Arable land	6.7	8.7 <sup>3</sup>	-	1.0		2200		
Denmark		5.7	4.2 <sup>3</sup>	-	1.5		2700		
Denmark	Grassland	5.2	7.6 <sup>3</sup>	-	2.1		1600		
Netherlands		4.7	41.7 <sup>3</sup>	-	31.0		5400		
Belgium	Arable land	6.7	8.7 <sup>3</sup>	-	1.0	<i>H. vulgare</i>	4900		
Denmark	Grassland	5.2	7.6 <sup>3</sup>	-	2.1		1900		
Netherlands		4.7	41.7 <sup>3</sup>	-	31.0		8300		
Spain	Calcic Luvisol	7.4	14.3	-	1.2	<i>H. vulgare</i>	2380	Pb chloride	Smolders et al. (2015)
	Dystric Luvisol	6.1	26.5	-	4.3		6750		
	Haplic Luvisol	6.2	8.4	-	1.0		1710		
Australia	Tenosol	7.0	20.9	-	3.0	<i>C. sativa</i> L.	4200	Pb nitrate	Kader et al. (2016)
		7.8	7.4	-	5.5		3840		

<sup>1</sup> TAS - tropical artificial soil.

<sup>2</sup> LUFA - Landwirtschaftliche Untersuchungs- und Forschungsanstalt, Speyer, Germany.

<sup>3</sup> CEC - effective cation exchange capacity at soil pH.

Site	Soils	Soil properties				Species	EC50 mg kg <sup>-1</sup>	Pb source	Reference
		pH	CEC	OM	OC				
			cmolc kg <sup>-1</sup>	%	%				
		6.3	24.1	-	8.4		6250		
		8.7	5.8	-	1.8		3490		
	Ferrosol	5.2	5.3	-	3.5		2240		
		5.3	11.7	-	5.0		4380		
	Vertosol	8.1	24.2	-	1.1		5240		
	Kurosol	5.1	7.3	-	1.5		5590		
	Dermosol	7.4	29.1	-	3.9		5570		
	Calcarosol	8.1	19.3	-	3.5		2560		
Spain	Leptic Cambisol (eutric)	6.7	9.9	-	0.6	<i>L. sativa</i>	3479	Pb nitrate	Romero- Freire et al. (2015)
Spain	Leptic Regosol (eutric)	7.2	25.9	-	8.2		6240		
Spain	Leptic Regosol (distic)	5.9	3.8	-	0.5		1303		
Spain	Cutanic Luvisol (chromic)	7.0	15.5	-	0.7		1765		
<sup>1</sup> TAS - tropical artificial soil.									
<sup>2</sup> LUFA - Landwirtschaftliche Untersuchungs- und Forschungsanstalt, Speyer, Germany.									
<sup>3</sup> CEC - effective cation exchange capacity at soil pH.									

Shoot dry matter (SDM) is the most relevant variable in toxicity tests with plants (ISO 2005). It was observed that *P. vulgaris* SDM was the most sensitive to AcPb exposure in the Oxisol (Table 4 and S2). It is known that the toxic effects of Pb may be due to its action in vital processes in plants, such as photosynthesis inhibition and nutrient absorption. Also, Pb may impair water balance, hormone status, and membrane permeability and structure (Sharma and Dubey 2005). Furthermore, several factors influence the absorption of metals such as Pb by plants, leading to different toxicity effects among plant species. According to Chlopecka (1994), the absorption of metals by plants is not only affected by contaminant concentration and form, or by soil physical and chemical attributes, but also by the characteristics of the tested plant species, nutrition, and growth stage. As most of these factors were standardized in the toxicity assays, the main differences are due to traits related to Pb absorption and translocation in soils (root system) and due to the attributes of the tested soils. However, it is worth remembering that little is known about the effects of acetate (in different concentrations) on the absorption of Pb in different plant species.

The higher EC<sub>50</sub> values for *Zea mays* in the Oxisol (Table 4 and S2) can be explained by the increased tolerance to Pb concentrations in soil presented by the species (Gupta et al. 2013). Some plant species, such as *Brassica pekinensis* and *Pelargonium*, present defense mechanisms when exposed to Pb, with internal pathways for detoxification, including selective absorption, excretion, complexation by specific ligands, and compartmentalization (Pourrut et al. 2011). Some species, including *Z. mays*, tolerate Pb by complexation and inactivation, with Pb deposits in cell walls and vacuoles (Wierzbicka and Antosiewicz 1993). On the other hand, plant species with increased sensitivity, such as *Brassica napus* and *Phaseolus vulgaris*, have some metabolic routes blocked by Pb (Gupta et al. 2013).

Exposure to Pb reduces the capacity of nutrient absorption in plants such as *Zea mays*, *Oryza sativa*, *Brassica oleracea*, *Raphanus sativus*, and *Medicago sativa* (Pourrut et al. 2011). Divalent cations, including Zn<sup>2+</sup>, Mn<sup>2+</sup>, Mg<sup>2+</sup>, Ca<sup>2+</sup>, and Fe<sup>2+</sup>, are some of the nutrients that can have their uptake reduced by Pb exposure. However, factors associated with the reduction in nutrient absorption are not fully understood (Sharma e Dubey 2005; Pourrut et al. 2011). In the literature, there are reports on the several adverse effects of Pb on plant tissue at the subcellular level, namely chloroplasts disorganization, cell wall damage, and presence of osmiophilic bodies in stems and leaves harvested at the physiological maturity stage (Ferreiroa et al. 2017). Although these parameters were not evaluated in this study, they may have caused the observed phytotoxic effects of AcPb.

Contrasting EC<sub>50</sub> values were found in different soils contaminated with Pb-nitrate and cultivated with *Lolium perenne* L. (ryegrass; Anderson and Basta 2009). The authors reported EC<sub>50</sub> values between 795 and 4191 mg Pb kg<sup>-1</sup> and attributed these differences within soils to clay, Fe oxides, and organic matter contents. The phytotoxic limit for total Pb in soil, i.e., Pb present in all soil fractions, is highly variable, ranging from 100 to 400 mg Pb kg<sup>-1</sup> (Kabata-Pendias and Pendias 2004). In most cases, Pb bioavailability is low, which explains the relatively high total concentrations required to induce toxicity. The variation within toxicity limits reflects differences in sorption-desorption in soils, absorption processes in the root-soil solution interface, and the sensitivity of varying species.

The acetate concentration used in the tested soils had a significant saline/toxic effect on soil invertebrates and plant species, which was proved by the AcK treatments. According to its safety data sheet (Carl Roth 2016), AcK salinity may cause toxic effects on organisms, such as mice, rabbits, fish, and algae. On the other hand, the presence of acetate as a source of organic C may exert a stimulant effect on soil invertebrates (Briones 2018) and may even be beneficial to *Lens culinaris* (lentil) plants under copper stress, with a test in 0.3 and 3.0 mmol L<sup>-1</sup> Cu concentrations and addition of 10 mmol L<sup>-1</sup> Na acetate (Hossain et al. 2020). Possibly, at lower concentrations, acetate will also present beneficial effects on Pb stress, and in this study the acetate concentration tested was only related to higher concentrations (acetate) that were added with Pb, 20095 and 3200 mg kg<sup>-1</sup> for invertebrates and plants, respectively. Acetate can also improve drought tolerance in plants (Kim et al. 2017), as shown with acetate concentrations between 20 and 30 mmol L<sup>-1</sup> in the soil causing enhanced drought tolerance in both monocots and dicots, such as rice, wheat, maize, and rapeseed plants, being presented as a basic and simple biochemical compound, which connects fundamental metabolism, epigenetic regulation, and hormone signaling. The absence of the benefits of acetate (AcK treatment) in this study can be explained mainly by the high acetate levels added in this treatment.

Considering the effects of AcPb in soils, invertebrates, and plant species, the composition and disposal of products containing AcPb should be carefully evaluated. Disposal of waste containing AcPb may represent a source of contamination. The evaluation of Pb concentrations in plant tissue and invertebrates was not considered in the present study, but Pb uptake by plants and soil invertebrates likely happened, taking into consideration their reduced production, reproduction, and survival.

## 5. Conclusions

Lead acetate concentrations tested in the soils showed toxicity to at least one of the tested species (*E. crypticus*, *E. andrei*, *F. candida*, *P. vulgaris*, and *Z. mays*).

The acetate concentrations of 3200 and 20095 mg kg<sup>-1</sup> showed toxicity for plants and soil invertebrates, respectively. The effect of various acetate concentrations on the soil-plant system needs to be better understood.

The lower organic matter content associated with smaller CEC in the Oxisol with increasing AcPb concentrations is a more harmful environment to the studied species comparatively to the Inceptisol and the TAS.

## Declarations

### Acknowledgements

The authors would like to thank the National Council of Technology and Scientific Development (CNPq), the Coordination for the Improvement of Higher Education Personnel (CAPES), and the Foundation for Research Support of the State of Minas Gerais (FAPEMIG) for financial support for this study. Authors also acknowledge the Environment Foundation of Minas Gerais (FEAM) and Universidade Federal de Lavras (UFLA) via cooperation term FEAM –UFLA n° 209101050013, as well as Universidade de Coimbra for the infrastructure and professionals involved in this study. Lastly, the authors thank Juliano Fernandes Mota and Paulo Roger for helping with field and laboratory activities.

**Funding:** This study was funded by FAPEMIG, The State of Minas Gerais Research Foundation (grant number APQ 1084/15).

**Conflicts of interest/Competing interests:** The authors declare that they have no competing interests.

**Availability of data and materials:** All data generated or analysed during this study are included in this published article and its supplementary information files.

**Code availability:** Not applicable.

**Authors' contributions:** All authors contributed to the conceptualization, investigation, data analysis, and writing of the manuscript. Alexandrino, R.C.S.: Conceptualization; Data curation; Methodology; Formal analysis; Writing - original draft and Writing - review & editing. Lima, F.R.D.: Writing - original draft and Writing - review & editing. Martins, G.C.: Writing - original draft and Writing - review & editing. Natal-da-Luz, T.: Supervision; Writing - original draft and Writing - review & editing. Sousa, J.P.: Supervision; Writing - original draft and Writing - review & editing. Luiz R. G. Guilherme: Funding acquisition and Writing - review & editing. João J. G. S. M. Marques: Conceptualization; Funding acquisition; Supervision; Writing - review & editing and Methodology.

**Animal Research (Ethics):** Not applicable.

**Consent to participate (Ethics):** Not applicable.

**Consent for publication (Ethics):** Not applicable.

**Plant Reproducibility:** Not applicable.

**Clinical Trials Registration:** Not applicable.

## References

- Alexandrino RCS, Marques JJGSM, Silva SHG, Simão FR (2020) The importance of the assessment of natural concentration of Pb in soils of the State of Minas Gerais. *Res Soc Dev* 9: 8. <https://doi.org/10.33448/rsd-v9i8.5022>
- Alloway B (2013) *Heavy Metals in Soils: Trace Metals and Metalloids in Soils and their Bioavailability*. Springer Netherlands, Dordrecht. <https://doi.org/10.1007/978-94-007-4470-7>
- Alvarez VH, Ribeiro AC (1999) Calagem. In: Ribeiro AC, Guimarães PTG, Alvarez VH (eds) *Recomendações para o uso de corretivos e fertilizantes em Minas Gerais: 5ª aproximação*. Comissão de Fertilidade do Solo do Estado de Minas Gerais, Viçosa, pp 43–60 (in Portuguese)
- Anderson RH, Basta NT (2009) Application of ridge regression to quantify marginal effects of collinear soil properties on phytotoxicity of arsenic, cadmium, lead, and zinc. *Environ Toxicol Chem* 28:1018-1027. <https://doi:10.1897/08-062.1>
- Ardestani MM., van Straalen NM, van Gestel CAM (2014) Uptake and elimination kinetics of metals in soil invertebrates: A review. *Environ. Pollut* 193:277–295. <https://doi.org/10.1016/j.envpol.2014.06.026>
- Bartlett MS (1937) Properties of sufficiency and statistical tests. *Proc R Soc A* 160:268-282. <https://doi.org/10.1098/rspa.1937.0109>
- Briones MJ (2018) The serendipitous value of soil fauna in ecosystem functioning: the unexplained explained. *Front Environ Sci* 149:1-11. <https://doi.org/10.3389/fenvs.2018.00149>
- Brown SL, Chaney RL, Hettiarachchi, GM (2016) Lead in Urban Soils: A Real or Perceived Concern for Urban Agriculture? *J Environ Qual* 45:26-36. <https://doi.org/10.2134/jeq2015.07.0376>
- Bur T, Crouau Y, Bianco A, Gandois L, Probst A (2012) Toxicity of Pb and of Pb/Cd combination on the springtail *Folsomia candida* in natural soils: Reproduction, growth and bioaccumulation as indicators. *Sci Total Environ* 414:187–197. <https://doi.org/10.1016/j.scitotenv.2011.10.029>
- Cândido GS, Martins GC, Vasques ICF, Lima FRD, Pereira P, Engelhardt MM, Reis RHCL, Marques JJ (2020) Toxic effects of lead in plants grown in Brazilian soils. *Ecotoxicology* 29:305-313. <https://doi.org/10.1007/s10646-020-02174-8>
- Carl Roth (2016) *Ficha de Dados de Segurança - Acetato de Potássio*. Department Health, Safety and Environment. Karlsruhe, Alemanha. [https://www.carlroth.com/downloads/sdb/pt/4/SDB\\_4986\\_PT\\_PT.pdf](https://www.carlroth.com/downloads/sdb/pt/4/SDB_4986_PT_PT.pdf). Accessed 20 December 2018
- Castro-Ferreira MP, Roelofs D, van Gestel CAM, Verweij RA, Soares AMVM, Amorim MJB (2012) *Enchytraeus crypticus* as model species in soil ecotoxicology. *Chemosphere* 87:1222-1227. <https://doi.org/10.1016/j.chemosphere.2012.01.021>
- Cecchi M, Dumat C, Alric A, Felix-Faure B, Pradere P, Guiresse M (2008) Multimetal contamination of a calcic cambisol by fallout from a lead-recycling plant. *Geoderma* 144:1-2. <https://doi.org/10.1016/j.geoderma.2007.11.023>

- Cesar R, Rodrigues AP, Bidone E, Castilhos Z, Polivanov H, Campos T (2015a) Proposta de um índice de risco ecológico para disposição sustentável de sedimentos de dragagem em Latossolos e Chernossolos. *Geociências* 34:275-285
- Cesar R, Natal-da-Luz T, Silva F, Bidone E, Castilhos Z, Polivanov H, Sousa JP (2015b) Ecotoxicological assessment of a dredged sediment using bioassays with three species of soil invertebrates. *Ecotoxicology* 24:414-423. <https://doi.org/10.1007/s10646-014-1390-8>
- Chandrasekhar C, Ray JG (2019) Lead accumulation, growth responses and biochemical changes of three plant species exposed to soil amended with different concentrations of lead nitrate. *Ecotox Environ Safe* 171:26-36. <https://doi.org/10.1016/j.ecoenv.2018.12.058>
- Chelinho S, Domene X, Campana P, Andrés P, Römbke J, Sousa JP (2014) Toxicity of phenmedipham and carbendazim to *Enchytraeus crypticus* and *Eisenia andrei* (Oligochaeta) in Mediterranean soils. *J Soils Sediments* 14:584-599. <https://doi.org/10.1007/s11368-013-0818-8>
- Cheyns K, Peeters S, Delcourt D, Smolders E (2012) Lead phytotoxicity in soils and nutrient solutions is related to lead induced phosphorus deficiency. *Environ Pollut* 164:242-247. <https://doi.org/10.1016/j.envpol.2012.01.027>
- Chlopeck AA (1994) Forms of Cd, Cu, Pb, and Zn in soil and their uptake by cereal crops when applied jointly as carbonates. *Water Air Soil Pollut* 87:297-309. <https://doi.org/10.1007/BF00696843>
- Dai W, Holmstrup M, Slotsbo S, Ke X, Li Z, Gao M, Wu L (2020) Compartmentation and effects of lead (Pb) in the collembolan, *Folsomia candida*. *Environ Sci Pollut Res* 27:43638-43645. <https://doi.org/10.1007/s11356-020-10300-6>
- Day PR (1965) Particle fractionation and particle-size analysis. In: Black CA (ed) *Methods of soil analysis: physical and mineralogical properties including statistics of measurement and sampling, part 1*. American Society of Agronomy, Madison, pp 545-567. <https://doi.org/10.2134/agronmonogr9.1.c43>
- Dunnett CW (1955) A multiple comparison procedure for comparing several treatments with a control. *J Am Stat Assoc* 50:272, 1096-1121. <http://dx.doi.org/10.1080/01621459.1955.10501294>
- Entwistle JA, Amaibi PM, Dean JR, Deary ME, Medock D, Morton J, Rodushkin I, Bramwell L (2019) An apple a day? Assessing gardeners' lead exposure in urban agriculture sites to improve the derivation of soil assessment criteria. *Environ Int* 122:130-141. <http://dx.doi.org/10.1016/j.envint.2018.10.054>
- Food and Agriculture Organization of The United Nations - FAO (2018) *Soil Pollution: a hidden reality*. FAO: Rome. <http://www.fao.org/3/I9183EN/i9183en.pdf>. Accessed 20 December 2018
- Ferreyroa GV, Lagorio MG, Trinelli MA, Lavado RS, Molina FV (2017) Lead effects on *Brassica napus* photosynthetic organs. *Ecotoxicol Environ Saf* 140:123-130. <https://doi.org/10.1016/j.ecoenv.2017.02.031>
- Fountain MT, Hopkin SP (2005) *Folsomia candida* (collembola): A "Standard" Soil Arthropod\*. *Annu. Rev. Entomol* 50:201-222. <https://doi.org/10.1146/annurev.ento.50.071803.130331>
- Frank JJ, Poulakos AG, Tornero-Velez R, Xue J (2019) Systematic review and meta-analyses of lead (Pb) concentrations in environmental media (soil, dust, water, food, and air) reported in the United States from 1996 to 2016. *Sci Total Environ* 694:133489. <https://doi.org/10.1016/j.scitotenv.2019.07.295>

- Garcia MVB, Roembke J, Martius C (2004) Proposal for an artificial soil substrate for toxicity tests in tropical regions. In: 25<sup>th</sup> Annual Meeting of Society of Environmental Toxicology and Chemistry - SETAC, Portland
- Gardi C, Angelini M, Barceló S, Comerma J, Gaistardo CC, Rojas AE, Jones A, Krasilnikov P, Mendonça-Santos ML, Montanarella L, Muñiz Ugarte O, Schad P, Rodríguez MIV, Vargas R, da Silva MR (2015) Atlas de Solos de América Latina e do Caribe, Comissão Europeia – Serviço de Publicações da União Europeia, L-2995 Luxembourg, p 176. (in Portuguese)
- Guevara YZC, Souza JJLL, Veloso GV, Veloso RW, Rocha PA, Abrahão WAP, Fernandes Filho EI (2018) Reference Values of Soil Quality for the Rio Doce Basin. Rev. Bras. Ciência do Solo 42:1-16. <https://doi.org/10.1590/18069657rbc20170231>
- Gupta DK, Corpas FJ, Palma JM (2013) Heavy Metal Stress in Plants. Springer, Berlin. <https://doi.org/10.1007/978-3-642-38469-1>
- Haouas Z, Sallem A, Zidi I, Hichri H, Mzali I, Mehdi M (2014) Hepatotoxic effects of lead acetate in rats: histopathological and cytotoxic studies. J Cytol Histol 5:256. <https://doi.org/10.4172/2157-7099.1000256>
- Harter RD (1983) Effect of soil pH on adsorption of lead, copper, zinc, and nickel, Soil Sci. Soc. Am. J. 7:47-51. <https://doi.org/10.2136/sssaj1983.03615995004700010009x>
- Hossain MS, Abdelrahman M, Tran CD, Nguyen KH, Chu HD, Watanabe Y, Hasanuzzaman M, Mohsin SM, Fujita M, Tran LSP (2020) Insights into acetate-mediated copper homeostasis and antioxidant defense in lentil under excessive copper stress. Environ Pollut 258:113544. <https://doi.org/10.1016/j.envpol.2019.113544>
- Ibrahim NM, Eweis EA, El-Beltagi HS, Abdel-Mobdy YE (2012) Effect of lead acetate toxicity on experimental male albino rat. Asian Pac J Trop Biomed 2:41-46. [https://doi.org/10.1016/s2221-1691\(11\)60187-1](https://doi.org/10.1016/s2221-1691(11)60187-1)
- International Organizations for Standardization – ISO (1999) Soil quality: inhibition of reproduction of *Collembola* (*Folsomia candida*) by soil pollutants. ISO 11267, ISO, Geneva
- International Organizations for Standardization – ISO (1998) Soil quality-effects of pollutants on earthworms (*Eisenia fetida*): part 2, determination of effects on reproduction. ISO 11268-2, ISO, Geneva
- International Organizations for Standardization – ISO (2005) Soil quality: determination of the effects of pollutants on soil flora: part 2, effects of contaminated soil on the emergence and early growth of higher plants. ISO 11269-2, ISO, Geneva
- International Organizations for Standardization – ISO (2003) Soil quality - effects of pollutants on Enchytraeidae (*enchytraeus* Sp.) - determination of effects on reproduction and survival. ISO 16387, ISO, Geneva
- Jie X, Yin W, Yong-Ming L, Jing S, Xin K (2009) Effects of cooper, lead and zinc in soil on egg development and hatching of *Folsomia candida*. J Insect Sci 16:51-55. <https://doi.org/10.1111/j.1744-7917.2009.00253.x>
- Kabata-Pendias A (2004) Soil-plant transfer of trace elements: an environmental issue. Geoderma 122:143-149. <https://doi.org/10.1016/j.geoderma.2004.01.004>
- Kabata-Pendias A, Szteke B (2015) Trace elements in abiotic and biotic environments. CRC Press, New York

- Kader M, Lamb DT, Mahbub KR, Megharaj M, Naidu R (2016) Predicting plant uptake and toxicity of lead (Pb) in long-term contaminated soils from derived transfer functions. *Environ Sci Pollut Res* 23:15460-15470. <https://doi.org/10.1007/s11356-016-6696-z>
- Kim JM, To TK, Matsui A et al (2017) Acetate-mediated novel survival strategy against drought in plants. *Nat Plants* 3:17097. <https://doi.org/10.1038/nplants.2017.97>
- Li C, Zhu Z, Wang Y, Guo Q, Wang C, Zhong P, Tan Z, Yang R (2020) Lead acetate produced from lead-acid battery for efficient perovskite solar cells. *Nano Energy* 69:104380. <https://doi.org/10.1016/j.nanoen.2019.104380>
- Li M, Zhang R, Guo Y, Huan Y, Xi J, Bai Z, Ya X (2018) Introducing lead acetate into stoichiometric perovskite lewis acid-based precursor for improved solar cell photovoltaic performance. *J Alloys Compd* 767:829-837. <https://doi.org/10.1016/j.jallcom.2018.07.190>
- Liao M, Chen CL, Zeng LS, Huang CY (2007) Influence of lead acetate on soil microbial biomass and community structure in two different soils with the growth of Chinese cabbage (*Brassica chinensis*). *Chemosphere* 66:1197-1205. <https://doi.org/10.1016/j.chemosphere.2006.07.046>
- Lock K, Janssen CR (2003) Multi-generation toxicity of zinc, cadmium, copper and lead to the potworm *Enchytraeus albidus*. *Environ Pollut* 117:89-92. [https://doi.org/10.1016/S0269-7491\(01\)00156-7](https://doi.org/10.1016/S0269-7491(01)00156-7)
- Malavolta E (1980) Elementos de nutrição mineral de plantas. Agronômica Ceres, São Paulo
- Menta C, Maggiani A, Vattuone Z (2006) Effects of Cd and Pb on the survival and juvenile production of *Sinella coeca* and *Folsomia candida*. *Eur J Soil Biol* 42:181-189. <https://doi.org/10.1016/j.ejsobi.2006.01.001>
- Neuhauser EF, Loehr RC, Milligan DL, Malecki MR (1985) Toxicity of metals to the earthworm *Eisenia fetida*. *Biol Fertil Soils* 1:149-152. <https://doi.org/10.1007/BF00301782>
- National Toxicology Program - NTP (2016) Report on Carcinogens, Fourteenth Edition.; Research Triangle Park, NC: U.S. Department of Health and Human Services, Public Health Service. <https://ntp.niehs.nih.gov/go/roc14>. Accessed 10 December 2020
- Päivöke AE (2002) Soil lead alters phytase activity and mineral nutrient balance of *Pisum sativum*. *Environ Exp Bot* 48:61-73. [https://doi.org/10.1016/S0098-8472\(02\)00011-4](https://doi.org/10.1016/S0098-8472(02)00011-4)
- Penha JG, Carvalho GS, de Abreu LB, Ribeiro BT, de Souza CET, Marques JJ (2017) Procedimentos para quantificação de elementos-traço por espectrofotometria de absorção atômica em matrizes de interesse ambiental. Editora UFLA, Lavras. <https://doi.org/10.13140/RG.2.2.24948.55685>
- Peijnenburg W, Capri E, Kula C, Liess M, Luttk R, Montforts M, Nienstedt K, Römbke J, Sousa JP, Jensen J (2012) Evaluation of Exposure Metrics for Effect Assessment of Soil Invertebrates. *Crit Rev Environ Sci Technol* 42:1862-1893. <https://doi.org/10.1080/10643389.2011.574100>
- Pierangeli MAP, Guilherme LRG, Curi N, Silva MLN, Oliveira LR, Lima JD (2001a) Efeito do pH na adsorção-dessorção de chumbo em Latossolos brasileiros. *Rev Bras Cienc Solo* 25:269-277. <http://dx.doi.org/10.1590/s0100-06832001000200003>

- Pierangeli MAP, Guilherme LRG, Oliveira LR, CURI N, SILVA MLN (2001b) Efeito da força iônica da solução de equilíbrio sobre a adsorção/dessorção de chumbo em Latossolos brasileiros. *Pesq Agropec Bras* 36:1077-1084. <https://doi.org/10.1590/S0100-204X2003000600010>
- Pohanish RP (2017) *Sittig's handbook of toxic and hazardous chemicals and carcinogens*. Elsevier, Amsterdam
- Pourrut B, Shahid M, Dumat C, Winterton P, Pinelli E (2011) Lead uptake, toxicity, and detoxification in plants. *Rev Environ Contam Toxicol* 213:113-136. [https://doi.org/10.1007/978-1-4419-9860-6\\_4](https://doi.org/10.1007/978-1-4419-9860-6_4)
- Rees N, Fuller R (2020) *The Toxic Truth: Children's Exposure to Lead Pollution Undermines a Generation of Future Potential*. 2<sup>nd</sup> ed. UNICEF and Pure Earth. <https://www.unicef.org/media/73246/file/The-toxic-truth-children%E2%80%99s-exposure-to-lead-pollution-2020.pdf>. Accessed 20 December 2020
- Rehman ZU, Khan S, Brusseau ML, Shah MT (2017) Lead and cadmium contamination and exposure risk assessment via consumption of vegetables grown in agricultural soils of five-selected regions of Pakistan. *Chemosphere* 168:1589-1596. <https://doi.org/10.1016/j.chemosphere.2016.11.152>
- Romero-Freire A, Martin Peinado FJ, van Gestel CAM (2015) Effect of soil properties on the toxicity of Pb: Assessment of the appropriateness of guideline values. *J Hazard Mater* 289:46–53. <https://doi.org/10.1016/j.jhazmat.2015.02.034>
- Sandifier RD, Hopkin SP (1997) Effects of temperature on the relative toxicities of Cd, Cu, Pb, and Zn to *Folsomia candida* (Collembola). *Ecotoxicol Environ Saf* 37:125-130. <https://doi.org/10.1006/eesa.1997.1536>
- Sharma P, Dubey RS (2005) Lead toxicity in plants. *Braz J Plant Physiol* 17:35–52. <http://dx.doi.org/10.1590/S1677-04202005000100004>
- Selwyn L (2017) *How to Test For Sulfur in Materials Using Lead Acetate Test Paper – Canadian Conservation Institute (CCI) Notes 17/5 (ISSN 1928-1455)*. Government of Canada, Canadian Conservation Institute. <https://www.canada.ca/en/conservation-institute/services/conservation-preservation-publications/canadian-conservation-institute-notes/test-sulfur-acetate-paper.html>. Accessed 10 December 2020
- Smolders E, Oorts K, Peeters S, Lanno R, Cheyns K (2015) Toxicity in lead salt spiked soils to plants, invertebrates and microbial processes: Unraveling effects of acidification, salt stress and ageing reactions. *Sci Total Environ* 536:223-231. <https://doi.org/10.1016/j.scitotenv.2015.07.067>
- Statsoft INC (2004) *Statistica: data analysis software system, version 7*. Tulsa. <http://www.statsoft.com/>. Accessed 20 December 2018
- Sobolev D, Begonia M (2008) Effects of heavy metal contamination upon soil microbes: lead-induced changes in general and denitrifying microbial communities as evidenced by molecular markers. *Int J Environ Res Public Health* 5:450-456. <https://doi.org/10.3390/ijerph5050450>
- Soil Survey Staff (2014) *Keys to soil taxonomy, 12th edn*. U.S. Department of Agriculture, Natural Resources Conservation Service, Washington
- Teixeira WG, Viana JHM, Donagemma GK (2017) *Manual de Métodos de Análise de Solo, 3 ed*. Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA), Brasília. <https://www.infoteca.cnptia.embrapa.br/handle/doc/1085209>. Accessed 20 December 2020

U.S. Environmental Protection Agency - USEPA (2007) Method 3051A (SW-846): Microwave Assisted Acid Digestion of Sediments, Sludges, and Oils. USEPA, Washington

Verma J, Kumari S, Dhasmana A (2020) Toxic effect of Lead on earth's life. IJIRT. 7:673-676. <https://doi.org/10.6084/m9.figshare.12472106>

Wani AL, Ara A, Usmani JA (2015) Lead toxicity: a review. Interdiscip Toxicol 8:55–64. <https://doi.org/10.1515/intox-2015-0009>

Walkley A, Black IA (1934) An examination of the Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. Soil Sci 37:29-38.

Wierzbicka M, Antosiewicz D (1993) How lead can easily enter the food chain - a study of plant roots. Sci Total Environ. 134, 423-429. [https://doi.org/10.1016/S0048-9697\(05\)80043-9](https://doi.org/10.1016/S0048-9697(05)80043-9)

World Health Organization - WHO (2019) Exposure to Lead: A Major Public Health Concern, WHO/CED/PHE/EPE/19.4.7. <https://apps.who.int/iris/bitstream/handle/10665/329953/WHO-CED-PHE-EPE-19.4.7-eng.pdf?ua=1>. Accessed 20 December 2020

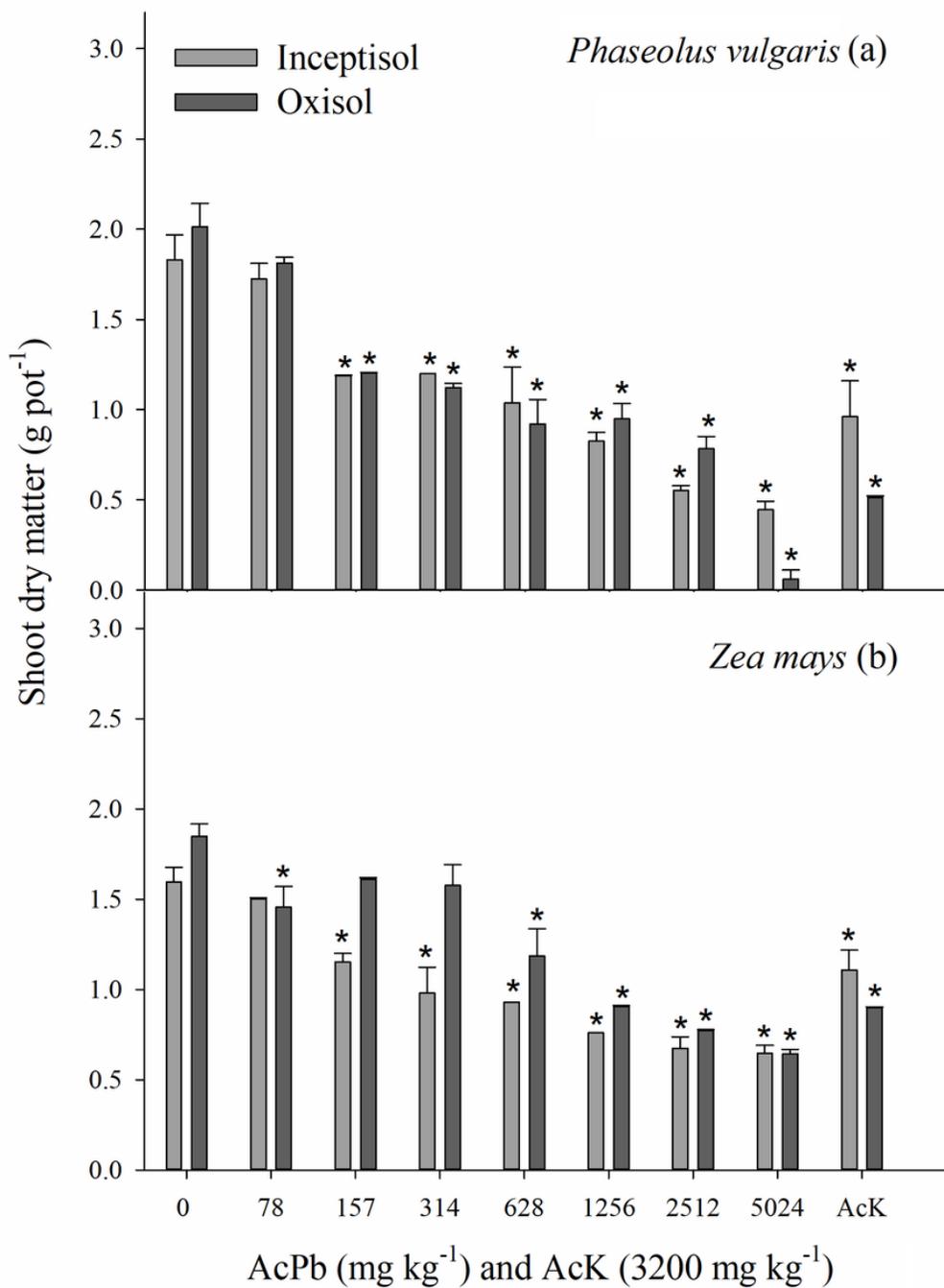
Xu J, Wang Y, Luo Y M, Song J, Ke X (2009) Effects of copper, lead and zinc in soil on egg development and hatching of *Folsomia candida*. Insect Science 16:51-55. <https://doi.org/10.1111/j.1744-7917.2009.00253.x>

Zeng LS, Liao M, Chen CL, Huang CY (2007) Effects of lead contamination on soil enzymatic activities, microbial biomass, and rice physiological indices in soil-lead-rice (*Oryza sativa* L.) system. Ecotoxicol Environ Saf 67:67-74. <https://doi.org/10.1016/j.ecoenv.2006.05.001>

Zhang L, Muccillo VBS, Van Gestel CA (2019a) A combined toxicokinetics and toxicodynamics approach to investigate delayed lead toxicity in the soil invertebrate *Enchytraeus crypticus*. Ecotoxicol Environ Saf 169:33-39. <https://doi.org/10.1016/j.ecoenv.2018.10.084>

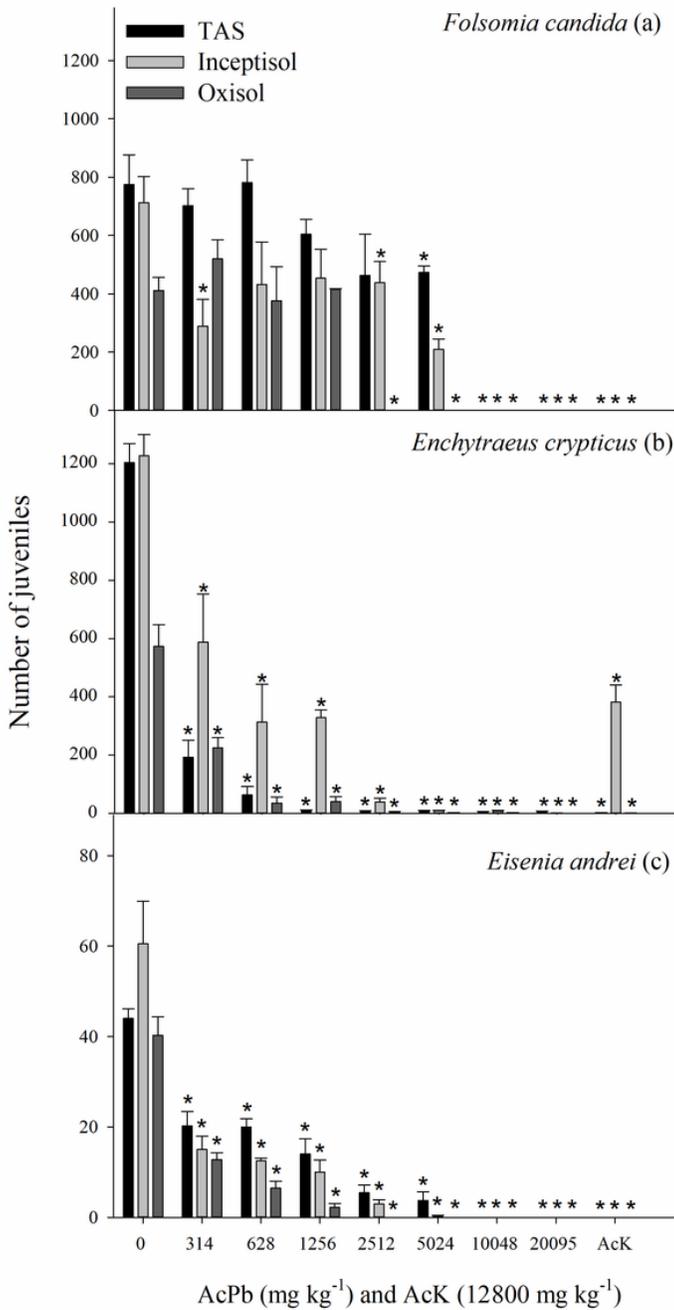
Zhang L, Verweij RA, Van Gestel CAM (2019b) Effect of soil properties on Pb bioavailability and toxicity to the soil invertebrate *Enchytraeus crypticus*. Chemosphere 217:9-17. <https://doi.org/10.1016/j.chemosphere.2018.10.146>

## Figures



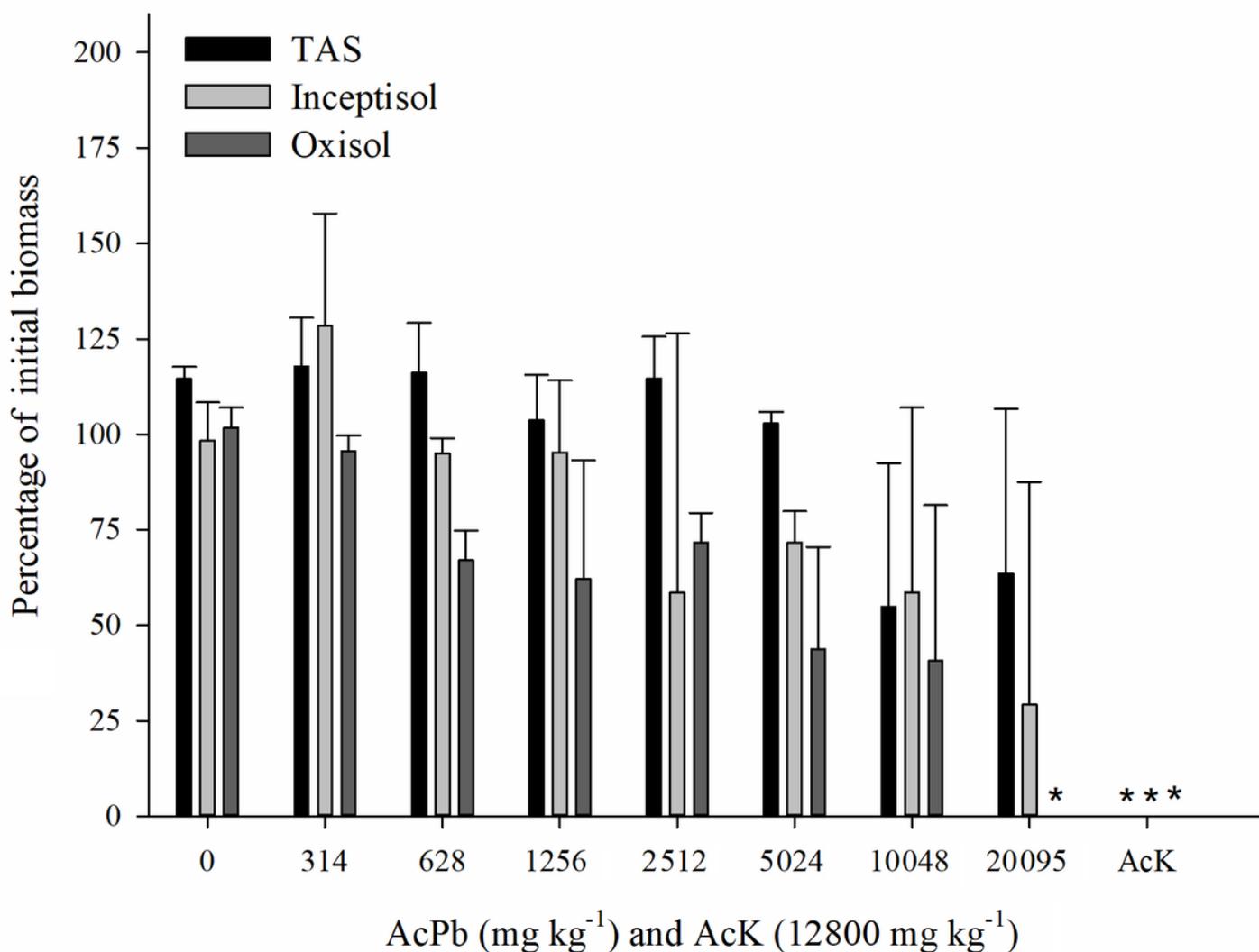
**Figure 1**

Shoot dry matter (SDM) production (average  $\pm$  standard deviation,  $n=4$ ) of *Phaseolus vulgaris* (a) and *Zea mays* (b) when exposed to an Oxisol and an Inceptisol spiked with increasing concentrations of lead acetate (AcPb) or potassium acetate (in a concentration of 5320 mg kg<sup>-1</sup>; AcK) in a higher plant growth test. \* - Shoot dry matter significantly different from respective control (0 mg kg<sup>-1</sup>; Dunnett's test,  $p \leq 0.05$ )



**Figure 2**

Number of juveniles (average  $\pm$  standard deviation,  $n = 4$  to  $5$ ) of *Folsomia candida* (a), *Enchytraeus crypticus* (b), and *Eisenia andrei* (c) when exposed to an Oxisol, an Inceptisol, and a Tropical Artificial Soil (TAS) spiked with increasing concentrations of lead acetate (AcPb) or potassium acetate (in a concentration of  $12121 \text{ mg kg}^{-1}$ ; AcK) in laboratory reproduction tests. \* - Number of juveniles significantly different compared from respective control ( $0 \text{ mg kg}^{-1}$ ; Dunnett's test,  $p \leq 0.05$ )



**Figure 3**

Percentage of the initial biomass (average  $\pm$  standard deviation, n=4) of surviving adults of *E. andrei* after being exposed to an Oxisol, an Inceptisol and a Tropical Artificial Soil (TAS) spiked with increasing concentrations of Pb acetate (AcPb) or potassium acetate (AcK) in laboratory reproduction tests. \* - Percentage significantly different from the respective control (Dunnett's test,  $p \leq 0.05$ )

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Supplementarymaterial.docx](#)