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Wijayawardena, M. A. Ayanka; Megharaj, Mallavarapu; Naidu, Ravi; "Bioaccumulation and toxicity of lead, influenced by edaphic factors: using earthworms to study the effect of Pb on ecological health". Published in Journal of Soils and Sediments Vol. 17, Issue 4, p. 1064-1072 (2017)

**Available from**: http://dx.doi.org/10.1007/s11368-016-1605-0

This is a post-peer-review, pre-copyedit version of an article published in Journal of Soils and Sediments. The final authenticated version is available online at:
http://dx.doi.org/10.1007/s11368-016-1605-0

**Accessed from:** http://hdl.handle.net/1959.13/1347359

Journal of Soils and Sediments

# Bioaccumulation and toxicity of lead, influenced by edaphic factors: using earthworms to study the effect of Pb on ecological health

--Manuscript Draft--

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| **Manuscript Number:** | JSSS-D-16-00493 |
| **Full Title:** | Bioaccumulation and toxicity of lead, influenced by edaphic factors: using earthworms to study the effect of Pb on ecological health |
| **Article Type:** | Research Article |
| **Section/Category:** | Soils |
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| **Order of Authors Secondary Information:** | |
| **Funding Information:** | CRCCARE — Dr M. A. Ayanka Wijayawardena |
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# Bioaccumulation and toxicity of lead, influenced by edaphic factors: using earthworms to study the effect of Pb on ecological health

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**Abstract**

**Purpose:** Lead (Pb) is an emerging contaminants with no known biological function that cause harmful adverse effects on ecological and human health. We tried to evaluate how protective the current soil regulatory levels are for Pb towards safeguarding the ecological health. In order to achieve this, our study evaluated the effect of soil texture and pH on the toxicity and availability of lead to earthworms in soils varying in soil properties.

**Materials and methods:** The earthworm *Eisenia fetida* was exposed to Pb in three soils with different physico-chemical characteristics. Pb solutions were homogenously mixed with soil to obtain concentrations ranging from 0 to 10000 mg/kg Pb dry soil. Avoidance behaviour, weight loss, and mortality, were measured in this study to calculate the $EC_{50}$ and $LC_{50}$ values.

**Results and discussion:** Weight loss and mortality in earthworms due to Pb toxicity were in the order: acidic > neutral > alkaline soil. The $EC_{50}$ values resulting in 50% decrease in worm weight over control for Pb in acidic, neutral and alkaline soils were 460, 3606 and 5753 mg/kg soil, respectively. Thus, the acidic soil recorded an $EC_{50}$ well below the soil guideline value for Pb. Whereas, the $LC_{50}$ values resulting in 50% mortality in worms over control were 1161, 4648, 7851 mg/kg respectively, for acidic, neutral and alkaline soils. The Pb concentrations in earthworms ranged from 0.2 to 740 mg/kg wet weight. Soils with low clay content and acidic to neutral pH values demonstrated an increased Pb toxicity in earthworms compared to the soils with alkaline pH.

**Conclusions:** The worm weight loss is more sensitive parameter than the mortality. This study emphasise that the soil regulatory levels for Pb are not protective of worms in acidic soils. Therefore care should be taken when using the current regulatory limits to assess and predict the safety of a contaminated site with acidic soils towards the ecological health.

**Keywords** Bioaccumulation • Earthworm • Lead • Soil guideline value • Toxicity

## 1 Introduction

Lead contamination and the resulting decrease of usable pristine land has gained both social, political as well as economical attention (Cao et al. 2009, Cartwright et al. 1977, Jiang et al. 2010). Lead has no known metabolic function and is toxic to human as well as ecological health. Lead distribution and toxicity has gained attention worldwide as this heavy metal can be ingested by children in particular via hand-to-mouth behaviour, and in the long term leads to serious nutritional, neurological and physiological damage and also lowers intellectual ability(Khan & Khan 1999, Saper et al. 2008, Smith et al. 2011). Soil parent materials mainly depict the background concentrations of heavy metals in soils. However, anthropogenic activities including mining, smelting and industrial activities as well as some agricultural practices (Kidd et al. 2007) give rise to, and mostly exceeds the naturally occurring concentrations(Akmal & Jianming 2009, Woolf et al. 2007). It is very important to be aware of and monitor the concentration and the bioavailable fraction of Pb in any soil subjected to direct or indirect human contact. Assuming the total metal content is 100% bioavailable in any soil is a widely misused concept often used to the date. This overly conservative approach leads to over predictions of the actual estimates of exposure and leads to unnecessary financial costs to remediate sites that do not require immediate remediation. If a proper understanding of edaphic factors such as pH, CEC and distribution of a metal between soil phases and how these soil properties determine metal bioavailability and toxicity can be acquired, this knowledge can be utilized towards proper risk assessment and remediation processes. This can save time, money as well as man power. An exception for the general regulatory dictate that all metal content is 100% bioavailable is for the US EPA soil guideline values for Pb which are based on a relative bioavailability adjustment factor(US EPA 2000).

It is not prudent to predict metal bioavailability and toxicity using any single soil property because many soil physico-chemical factors are inter-related. Dissolved organic and inorganic ligand- exchange have been demonstrated to impact the reactions between soil and heavy metals (Harter & Naidu 2001). Pb bioavailability to soil invertebrates are reported to be mainly driven by soil physico-chemical properties such as pH, CEC iron and aluminium oxides contents, and dissolved organic carbon (Bradham et al. 2006, Yang et al. 2003).

Earthworms are soil dwelling invertebrates which help maintain ecological health via their major role in maintaining soil fertility. Earthworms accumulate toxic metals and other contaminants and acts as possible bio-indicators of heavy metal contaminations (Bradham et

al. 2006, Fujii & Kaneko 2009, Ming et al. 2012, Morgan & Morgan 1988, Park et al. 2011). They accumulate contaminants present in the soil and dissolved in pore water through ingestion and direct dermal contact. Many organismal parameters such as accumulation of chemical substances, avoidance responses, reproductive capacity and mortality are indicators used in earthworm assays.

Lead has shown to be bioavailable and toxic to earthworms (Conder & Lanno 2000, Davies et al. 2003b). However there are many uncertainties pertaining with regards to what extent this toxicity differs when the soil physical/chemical characteristics differ (Lanno et al. 2004, Wijayawardena et al. 2012). Therefore the current research focuses on the use of the batch sorption kinetics and correlation techniques as well as mortality data and bioaccumulation of Pb in earthworms towards gaining a better understanding on the soil edaphic factors influencing the Pb toxicity and bioaccumulation in earthworms.

The current study focuses on: (1) studying the bioavailability and toxicity of Pb to earthworm *E. fetida* in three South Australian soils with different $k_d$ values, and (2) assessing the ability of this earthworm to bioaccumulate Pb.

## 2 Materials and methods

### 2.1 Soil invertebrates

*E. Fetida* used in the experiment were obtained from a local vermiculturist at the Free Range Worm Farm, Davoren Park, South Australia. The worms were incubated in in the uncontaminated test soils for 2 weeks before starting the assay in a controlled temperature room maintained at 20°C, with a regulated light (500 lux)-dark cycle 8:16 h (Cáceres et al. 2010) .

### 2.2 Test soils and their characterization

Composite soil sampling was carried out in South Australia from three different sites. The soil sampling sites were selected based on the criteria; (1) The soil Pb levels has to be below NEPM EIL and HIL values (2) The physical/chemical properties has to differ between the sites. The soil samples were collected from the top 0-20 cm layer. Soil samples were air dired

at temperatures <37 °C. They were then stored at ambient temperature for handling and analysis. The soils were analyzed for soil physico-chemical properties such as electrical conductivity (EC), organic matter content, soil texture, pH, cation exchange capacity (CEC) and total heavy metals (Wijayawardena et al. 2014). Soil was equilibrated with water at 1:5 ratio for 1 hour and the suspension was measured with a pH/conductivity meter (smartCHEM-LAB, TPS, Australia) to determine soil pH and EC. The dichromate oxidation method was used to measure the soil organic matter content (Walkley & Black 1934) and the hydrometer method was used to determine soil texture (Gee et al. 1986). CEC and exchangeable cations were estimated by percolation of 1 mol/L ammonium acetate solution, pH = 7 (Chapman 1965). The inorganic elemental concentrations were measured by ICP-MS (Inductively-coupled Plasma Mass Spectrometry) (model 7500c, Agilent Technologies, Tokyo, Japan). Citrate/dithionite extraction method was used to determine oxides of Fe, Al and Mn in the soils (1987). Pb concentration data were fitted to adsorption isotherms to calculate Freundlich adsorption coefficients ($K_d$) (Naidu et al. 1994).

### 2.3 Chemical treatment of soils

Pb concentrations in soil was achieved between 0-10000 mg Pb/kg dry soil by spiking the uncontaminated soils with Pb nitrate solutions and mixing thoroughly to achieve homogeneity. 750 g of these spiked soils were placed in plastic containers (1 L). Appropriate amounts of deionized water was added to the soils to maintain at 70% of water-holding capacity. Triplicates per Pb exposure concentration was used in all experiments. Unspiked soils were used as controls.

### 2.4 Earthworm toxicity test

The toxicity experiment was carried out according to the method described by Lokke and Van Gestel (1998). These tests were conducted using adult worms with prominent clitellum. The worms were rinsed thoroughly with deionized water to remove any soil or debris adhering to them, softly blotted dried using absorbent paper and depurated for 24 h. The filter papers were removed twice daily and earthworms were cleaned and placed carefully back on the filter papers to prevent coprophagy. Earthworms were placed in groups of ten in each of

the triplicate jars with lead treated and untreated soils. The jar lids were pierced with enough number of holes to allow ample aeration for the worms. All the jars were maintained in a constant temperature at 20°C, with a controlled light (500 lux)-dark cycle 8:16 h (Cáceres et al. 2010). Number of live worms were counted after 14 days of exposure. The $LC_{50}$ values were determined after 14 days of exposure by plotting log concentration of Pb against the probit-transformed percentage mortality values.

### 2.5 Determination of total Pb

After 24 hour depuration the worms were rinsed thoroughly with milliQ water and blotted with absorbent paper. Then they were weighed and humanely dispatched by rapid temperature reduction. Next they were digested using concentrated nitric acid and the resulting solution was diluted using milliQ water followed by filteration through 0.45 µm cellulose acetate syringe filters (Wijayawardena et al. 2012). Lead content in earthworms was determined using ICP-MS.

### 2.6 Chemical reagents

Lead nitrate [$Pb(NO_3)_2$], was purchased from Sigma-Aldrich (Sydney). Concentrated nitric acid (Mallinckrodt Chemicals, USA) was used for the dissolution of earthworms. The chemical analysis was validated using NIST SRM 2711 (Montana soil).

### 2.7 Statistical analysis

$EC_{50}$ (concentration that causes 50% reduction in worm biomass) and $LC_{50}$ (concentration causing 50% mortality in worms) values were estimated using Minitab Version 16. Analysis of variance (ANOVA) and SPSS 19 software were used to compare the weight loss and mortality of worms in lead treated and control soils. Treatment means were compared using Tukey's HSD (Honestly Significant Difference). Variability in data was expressed as the standard deviation and a $p<0.05$ level of probability considered to be statistically significant. One-tailed Pearson correlation was tested using SPSS 19 software to study any correlations between soil properties and biological endpoints.

## 3 Results

### 3.1 Soil analysis

Physical/chemical properties of the three soils are summarized in the Table 1 and 2. The CEC of Pb treated soils ranged from 1.63 to 8.56 cmol/kg and the pH of the soils ranged from 4.96 to 8.45. Organic carbon content ranged from 2.4 to 3.1 %.

### 3.2 Toxicity test

Initially, the worms in soil with Pb up to 1730 mg/kg (acidic soil), 5622 mg/kg (neutral soil) and 6651 mg/kg (in alkaline soil) burrowed completely into the soil within an hour. However, by the second day all the worms in 8766 – 9344 mg/kg Pb spiked alkaline soil containers had moved on to the soil surface showing avoidance behavior, and their bodies were coiled and extended. Worms exposed to Pb concentrations >2344 mg/kg Pb in neutral soil showed anterior bulging whilst the posterior segments remained very thin.

### 3.3 Earthworm weight loss and mortality

Earthworm weight loss and mortality (Fig. 1) at 14 days were significantly impacted by Pb exposure (ANOVA, $p<0.05$). Weight loss was significantly affected by Pb concentrations of 1470 mg/kg in acidic soil, 5622 mg/kg in neutral soil and 9344 mg/kg in alkaline soil (Tukey, $p< 0.05$). The calculated $EC_{50}$ values were 460, 3606 and 5753 mg/kg for acidic, neutral and alkaline soils, respectively. The $LC_{50}$ values for Pb are 1161, 4648, 7851 mg/kg for acidic, neutral and alkaline soils, respectively.

The relationships between the *E. fetida* assay endpoints and soil physico-chemical properties were recorded (Table 3). The fit of Pb sorption data to the Freundlich model to obtain adsorption coefficients is shown in Fig. 2.

### 3.4 Lead analysis of worm samples

Lead concentrations in tissues of worms exposed to Pb treated soils are presented in Fig. 3. The Pb body burden of the earthworms varied greatly between the three soils. Increase in Pb concentrations in the worm tissues were observed with an increase in soil Pb concentration. Thus, when soils spiked with 0 – 1900 mg/kg Pb are compared, body burden of worms exposed to acidic soil is greater (2.2 – 740 mg/kg worm tissue) than neutral (0.4 – 106 mg/kg worm tissue) and alkaline (0.2 – 85 mg/kg worm tissue) soils.

The earthworm bioaccumulation factors (BAF, the ratio of total Pb in earthworm, mg/kg to total lead in soil, mg/kg) were greater at lower total soil Pb concentrations whereas these decreased at higher soil Pb concentrations. The acidic soil exhibited higher bioaccumulation factors (BAF) for Pb than the neutral and alkaline soils. Thus, the BAF in acidic soils ranged between 0.1 - 0.7 whereas for the neutral soil the BAF range was 0.02-0.09 and for the alkaline soil 0.03-0.08.

## 4 Discussion

All the soils used in this study were sandy (84 - 94% sand). The organic matter and clay contents were low in the test soils. We investigated the bioaccumulation, bioavailability and the toxicity of Pb to earthworms in these three soils with different soil properties. The toxicity of Pb to *E. fetida* in soils followed the order: acidic soil > neutral soil > alkaline soil. No mortality was observed in the unspiked (i.e. control) soils. Mortality ranged from 0-100% in Pb spiked soils indicating the substantial lethality of Pb to earthworms. The earthworms grown in similar concentrations of Pb spiked soils exhibited different levels of mortality. This implies the different physico-chemical properties in the soils have accounted for varying levels of Pb bioavailability and thereby resulted in different degrees of toxicity to earthworms. The toxicity of Pb to *E. fetida* in terms of $LC_{50}$ values reported in the available literature are 5259 mg/kg dry soil with neutral pH and total organic carbon amount of 2.8% (Davies et al. 2003a), and $LC_{50}$ of 5941 mg/kg dry soil for Pb was found by Neuhauser et al.(1985) for an artificial soil consisting of 10% finely ground sphagnum peat, 20% kaolinite clay, 69% fine sand and 1% pulverized calcium carbonate (all measured on a dry weight basis) with a pH of 6.0. In the current study (Fig. 1) neutral soil which had total carbon of 2.0% displayed a $LC_{50}$ value of 4648 mg/kg which is a relatively closer value to the previous

studies. The minor discrepancy in toxicity in our study soils compared to $LC_{50}$ values in neutral pH soils in the above sited literature might have been be due to the differences in composition of the soils which resulted in differences in the soil properties.

A significant biomass loss was observed in earthworms exposed to increasing levels of Pb (Fig.1). Worm $EC_{50}$ values were observed at lower soil Pb levels to that of $LC_{50}$ values indicating weight loss to be a more sensitive parameter of Pb toxicity. In the Pb treatments, even at 306 mg/kg concentration in acidic soil, 53% weight loss was observed. The differences in weight loss of the worms may be most probably due to their avoidance behaviour (non-feeding) exhibited during exposure to Pb in soils. Earthworms have sensory receptors which can detect chemicals in soils and their locomotor ability enables them to avoid any unfavourable environmental conditions which threatens their existence (Edwards & Bohlen 1996). As a result they avoided these Pb contaminated soils even at lower metal concentrations.

Earthworms in test jars where 100% mortality occurred were excluded from the tissue Pb concentration testing. This was done to avoid comparing the changes in the Pb concentration values arising from contaminated soil contained in earthworm guts to changes in tissue Pb concentrations through oral and dermal uptake. The concentration of Pb in surviving worms after 14 days for the acidic soil ($R^2$=0.97), neutral soil ($R^2$=0.94) and alkaline soil ($R^2$=0.86) significantly increased (Tukey $p<0.05$) with soil Pb concentrations (Fig. 3). There are two hypotheses to explain this scenario. One is that the uptake of Pb into earthworms occur passively where Pb diffuses across epidermis and gastric epithelium along a concentration gradient of high to low in to the worm cells. The second hypothesis is the critical Pb concentration which triggers the activation of intracellular and intercellular signaling cascades which in turn triggers protective mechanisms to limit the Pb intake has not yet reached in the worms (Morgan & Morgan 1988). However, the mortality observed in these three soils suggests that the detoxification mechanisms have become ineffective. Davis N.A (Davies et al. 2003a) postulated that organisms that are not affected by increased levels of metals are either (1) does not bio accumulate these metals or (2) effectively convert it to a less harmful form or (3) are able to eliminate the metals from their bodies effectively. However Fig. 3 shows that Pb bio accumulate in earthworm tissues. In neutral soil and alkaline soils there were no mortality even up to 3000 mg Pb/kg soil which suggest that they might be bioaccumulating Pb up to these concentrations in a relatively nontoxic form.

However in contrast to this worm mortality was observed even around 300 mg Pb/kg dry acidic soil. This suggests the changes in mortality may be more Pb bioavailability dependent rather than related to the soil total metal concentrations. This phenomenon is can be better explained with the results of this study which showed >200 mg Pb/kg worm fresh weight was bio accumulated in earthworms (Fig. 3) grown in acidic soil with 306 mg Pb/kg soil whereas in neutral and alkaline soils the bioaccumulation was <200 mg/kg Pb worm fresh weight at Pb levels of 2164 mg /kg and 3569 mg /kg in soil respectively. The high bioavailability of Pb to earthworms in acidic soils must have caused disruptions in the regulatory mechanisms functioning inside earthworms to detoxify the Pb. This must have caused earthworm mortality at relatively low Pb concentrations compared to that of neutral and alkaline soils. Morgan and Morgan(1989) and Ireland(1979) have suggested that Pb can be excreted following the excretion pathways operating for calcium (Ca) by substituting Ca in the normal excretion from the calciferous glands. This is mainly due to the similarity of these two cations which often compete for same binding sites in organisms (Barton et al. 1978). Toxic and lethal effects become evident when the internal Pb concentrations suddenly surpasses the critical Pb concentrations that can be safely detoxified under normal body conditions crippling the biological defence mechanisms. The internal concentrations (IC) of *E. fetida* exposed to the control (i.e. un-spiked) soils were 2.2, 0.4 and 0.2 mg/kg Pb in the acidic, neutral and alkaline soils, respectively. Earthworm IC after exposure to Pb-spiked soils ranged widely, from 18 to 740 mg/kg. The seven-fold increase of IC in acidic soils spiked with ~1750 mg/kg over that of neutral soil spiked with Pb ~1750 mg/kg and other similar increases in IC in acidic soil indicate that soil edaphic factors are responsible for modifying Pb bioavailability. This finding is consistent with those of Bradham et al.(2006) which shows Pb uptake by earthworms is heavily dependent on soil properties. The Pb-spiked acidic soil showed the highest BAF values ranging between 0.1-0.7. This is in agreement with earlier reports that pH is one of the important factors influencing metal bioavailability to earthworms. Suave et al. have shown once the pH decreases below 6.5 there is elevation in Pb solubility (Sauve et al. 1998). Soil pH plays a role in ionization of pH- dependent ion exchange sites on metal oxide clay minerals and organic matter causing changes to cationic metal bioavailability (Bradham et al. 2006). Therefore, surfaces of sand and clay are likely to become negatively charged with an increase in pH, which can attract the positively charged $Pb^{2+}$ cation thereby increasing Pb sorption. This is shown by the soil pH showing a linear

relationship to the Pb adsorbed to the soil. This was proven further by the linear relationship seen between soil pH and the Freundlich adsorption coefficient in the current study (r=0.83).

It is crucial to examine to what extent the soil edaphic factors modify the Pb toxicity and bioaccessibility to *E. fetida*. In order to achieve this relationship between worm mortality/weight loss and soil properties were investigated using simple linear correlation coefficients (r) (Table 3).

The current study show the very strong linear relationship between soil pH and $EC_{50}$ and soil pH and $LC_{50}$ (r=0.999, $p<0.05$ for both). Acidic pH values have been proven to increase the solubility of cationic metals such as Pb resulting a high bioavailability of these metals to organisms (Harter 1983, Harter & Naidu 2001). Other studies have also demonstrated the bioaccumulation of metals by earthworms increasing with the decrease in the soil pH (Peijnenburg & Jager 2003). The pH critically affects both CEC and metal-complexing capacity of organic constituents (McBride et al. 1997). Naidu and Harter (1998) observed that organics play a vital role in metal sorption. This can be seen in the correlations observed between total carbon content with $EC_{50}$ and $LC_{50}$ (r=0.747 and r=0.800) in the current study.

The mobility and bioavailability of Pb is affected by it binding to soil constituents. Harter postulated that a portion of sorbed metals strongly bound to soil probably depicts its specific sorption sites (1983). The binding of Pb is low in sandy and low clay soils than soils with high clay, silt or organic matter(Appel et al. 2008). In one review Harter and Naidu (2001) argued that the low molecular weight organics might be playing a substantial role in metal solubility given that these organics can be decomposed quite rapidly by soil microorganisms increasing the metal bioavailability. Hydrous oxides of Al, Fe and Mn rich in pH-dependent cation exchange sites binds effectively with Pb (Manceau et al. 1992) and lead to lower Pb bioavailability. Pb adsorption to the soil can be correctly presented by the Freundlich adsorption coefficient ($K_d$) and is used in the current study (Fig. 2 and Table 3).

No significant relationships were found between pH, total carbon, citrate dithionite-extractable Al or Mn. The % clay content was significantly related to CDE-Mn (Citrate dithionite- extractable Mn) (r=0.99, $p<0.05$) and a significant negative relationship was seen between % clay and CDE-Al (-0.99, $p<0.05$). Naidu et al. (1994) demonstrated increase of soil clay content resulted in increased $K_d$ values and hence elevated metal adsorption resulting in decrease of metal content in the solution.

The way the Pb was behaving in different soils resulting in varying solubility and bioavailability values in organisms is similar to the way active ingredients which are the compounds responsible for the therapeutic effect inside a veterinary or medicinal formulations behave giving rise to different bioavailabilities in humans or animal biological systems. Depending on the particle sizes, binding characteristics and affinity of excipients (supporting material lacking in therapeutic value) in a formulation the release of active ingredient inside the animal or human changes. Similarly depending on soil physico-chemical characteristics the release of Pb differs. Although soil is a more complex heterogeneous mixture which is highly unpredictable as opposed to the heavily controlled composition of a drug this similarity in the behavior of Pb to the active ingredient in the therapeutic drug with regards to their interaction with surrounding physico-chemical matrices and the biological systems is fascinating.

The Ecological Investigation Levels (EILs) or soil guideline values for lead set by the USA, Australia, The Netherlands and Canada range from 11-1700 mg Pb $kg^{-1}$ soil (Table 4). There is therefore a wide variation in these soil guideline values for Pb between different nations which may well be due to differences in their methods/models used to derive EIL values. The $EC_{50}$ values for Pb for earthworms in soils in the current study range from 460 – 5753 mg/kg soil. The acidic soil showed a significant toxicity with 53% weight loss in worms even at the 306 mg/kg Pb level. Considering the fact that Pb exhibited significant toxicity and bioaccumulation in earthworms of this study even at concentrations lower than the guideline values employed by some of the above nations, the usefulness of these values for risk assessment of soils similar to the ones used in this study is questionable.

## 5 Conclusions

Different responses were seen in the bioavailability, toxicity and lethality of earthworms in this experiment to similar concentrations of Pb in soils with different edaphic factors. These differences were due to Pb complexing, undergoing sorption and dissolution processes to differing degrees due to the differences in the physico-chemical matrices in soil. This study demonstrates that the toxicity of Pb to *E. fetida* in soil varies considerably depending on soil properties. Lead bioavailability and toxicity to earthworms in sandy soils with a low clay was

severe in the acidic soil, followed by the neutral and more alkaline soils. Earthworms exposed to Pb bioaccumulated Pb in their tissues.

Soil properties are very important factors which modify metal bioavailability and should be considered in the ecological risk assessment process so as not to over-estimate contaminant risk. For example in this study, the acidic soil recorded an $EC_{50}$ well below the soil guideline value for Pb. Therefore study emphasise that the soil regulatory levels for Pb are not protective of ecological health if we consider organisms such as earthworms living in acidic soils. Therefore there is an increasing need for studies conducted on soils differing in their edaphic factors using many ecological receptors to test whether the soil regulatory levels are able to reliably protect the ecosystems dwellers which they are claiming to protect.

**Acknowledgements** We would like to sincerely thank the Centre for risk assessment and remediation, University of South Australia for the laboratory facilities and Cooperative Research Centre for Contamination Assessment and Remediation of the Environment (CRC CARE) postgraduate scholarship for funding the research. The authors would like to acknowledge C. Danidu Kudagamage for help with the *E. fetida* experiments and Professor Alan Baker for proof reading the manuscript.

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**Table 1:** Selected physical/chemical properties of soils

| Location (South Australia) | Soil type | pH | Electrical conductivity (µS/m) | Organic carbon (%) | Sand (%) | Silt (%) | Clay (%) | CEC (cmol/kg) | Total N % | $K_d$[a] | Background Pb levels (mg/kg) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Mount Lofty | Kurosol | 4.96 | 20.7 | 2.8 | 89.1 | 5.4 | 5.4 | 1.63 | 0.08 | 50 | 34.2 |
| Adelaide hills | Chromosol | 6.94 | 167.1 | 2.4 | 84.1 | 7.9 | 7.9 | 8.56 | 0.11 | 112 | 29.3 |
| Warooka | Calcarosol | 8.45 | 120.8 | 3.1 | 94.2 | 0 | 5.8 | 4.82 | 0.22 | 439845 | 3.61 |

[a] Freundlich adsorption coefficient

**Table 2:** Citrate dithionite-extractable cations and their ranges (%)

| Location (South Australia) | Fe | Al | Mn |
|---|---|---|---|
| Mount Lofty | 0.036-0.038 | 0.043-0.048 | 0.001 |
| Adelaide hills | 0.022 | 0.017-0.022 | 0.010-0.012 |
| Warooka | 0.021-0.022 | 0.044-0.046 | 0.004 |

**Table 3:** Relationship between experimental end points and soil properties for earthworms in Pb spiked soils[a]

| Soil property[b] | Linear correlation coefficients (r) | |
|---|---|---|
| | $EC_{50}$ | $LC_{50}$ |
| pH | .999* | .999* |
| Total carbon (%) | .747 | .800 |
| Kd | .807 | .854 |

[a] $*p<0.05$

[b] $K_d$ = Adsorption coefficient

n = 3

**Table 4**: Ecological (EIL) and human (HIL) health investigational levels of lead in soil (mg/kg dry soil)

| Ecological investigational levels | | | | | | | Health investigational levels in standard residential soil | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| USEPA[a] (mg/kg dry weight in soil) | | | | NEPM[b] (mg/kg) | Netherlands[c] (mg/kg) | Canada[d] (mg/kg) | USEPA[a] (mg/kg) | NEPM[b] (mg/kg) | Netherlands[c] (mg/kg) | Canada[d] (mg/kg) |
| Plants | Soil | invertebrates | Avian | Mammalian | Interim urban | | | | | |
| 120 | 1700 | | 11 | 56 | CA[e] | 530 | 300 | 400 | 300 | 530 | 140 |

Notes: NC- Not calculated

[a]U.S. Environmental Protection Agency 2010, Regional Screening Levels (RSL), USA.

[b]National Environment Protection Council 1999, Schedule B1: Investigation levels for soil and groundwater.

[c]Soil Remediation Circular 2009, Netherlands.

[d]Canadian Council of the Ministers of the Environment 1999, Soil quality guidelines, Canada.

[e]CA- Calculated according to the site-specific assessment characteristics

Figure 1

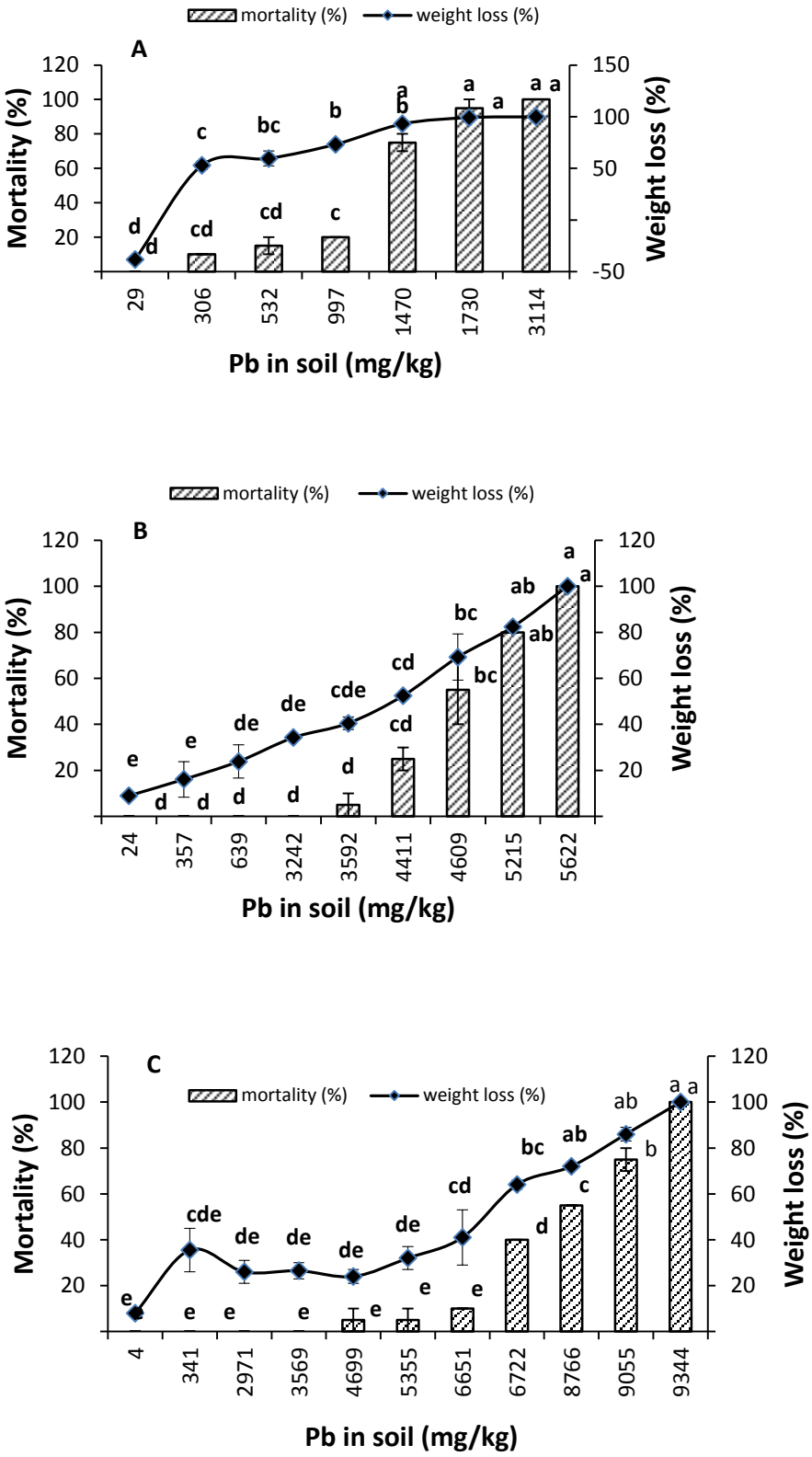


**Fig. 1**: Mean weight loss (%) and mortality (%) measured for *E. fetida* exposed to a range of increasing concentrations of Pb A, acidic soil; B, neutral soil; C, alkaline soil. Identical letters indicate statistically identical values.

Figure 2

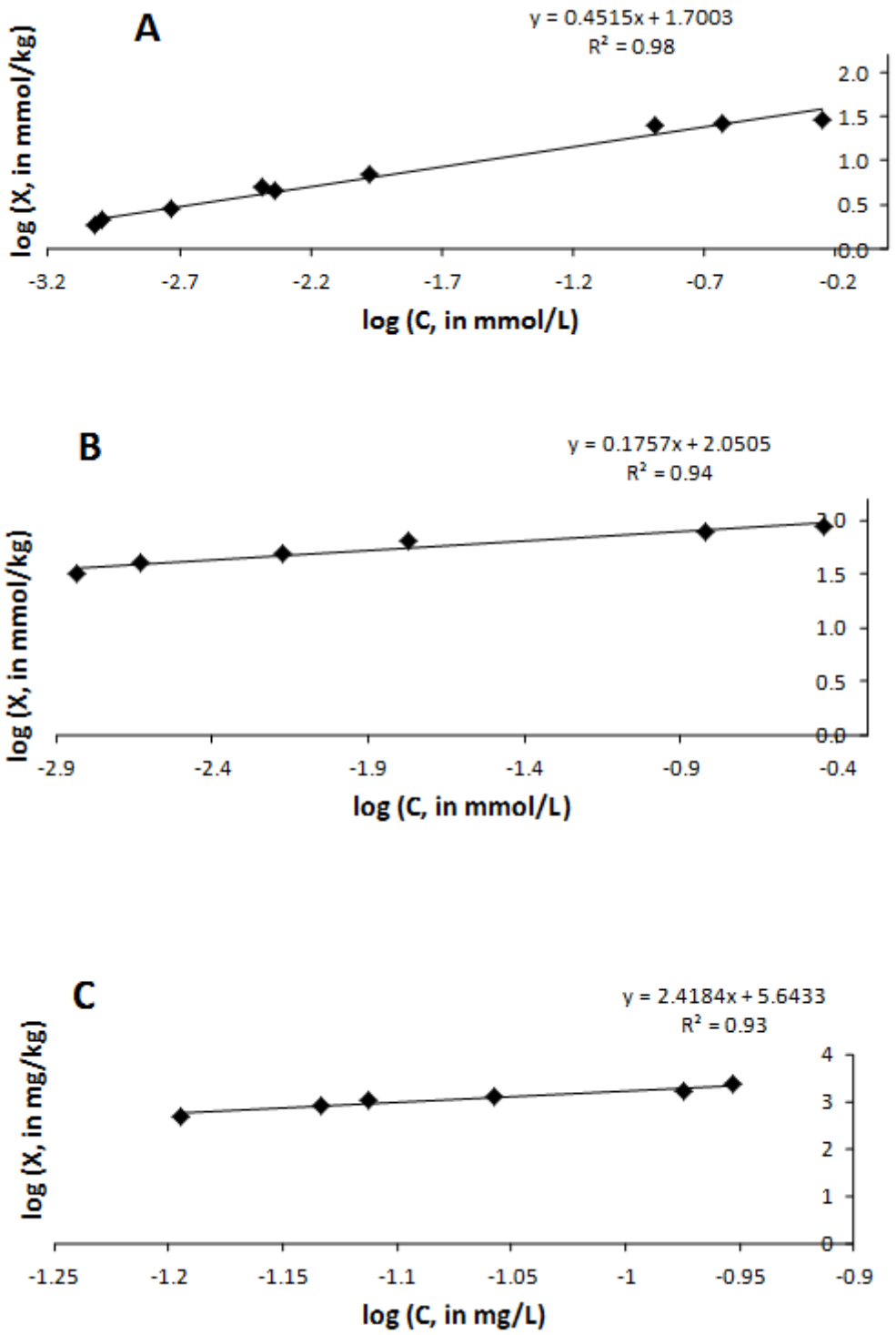


**Fig. 2**: The fit of Pb sorption data to Freundlich model A, acidic soil; B, neutral soil; C, alkaline soil. X is the amount of Pb adsorbed on soil and C is the equilibrium concentration of Pb.

Figure 3

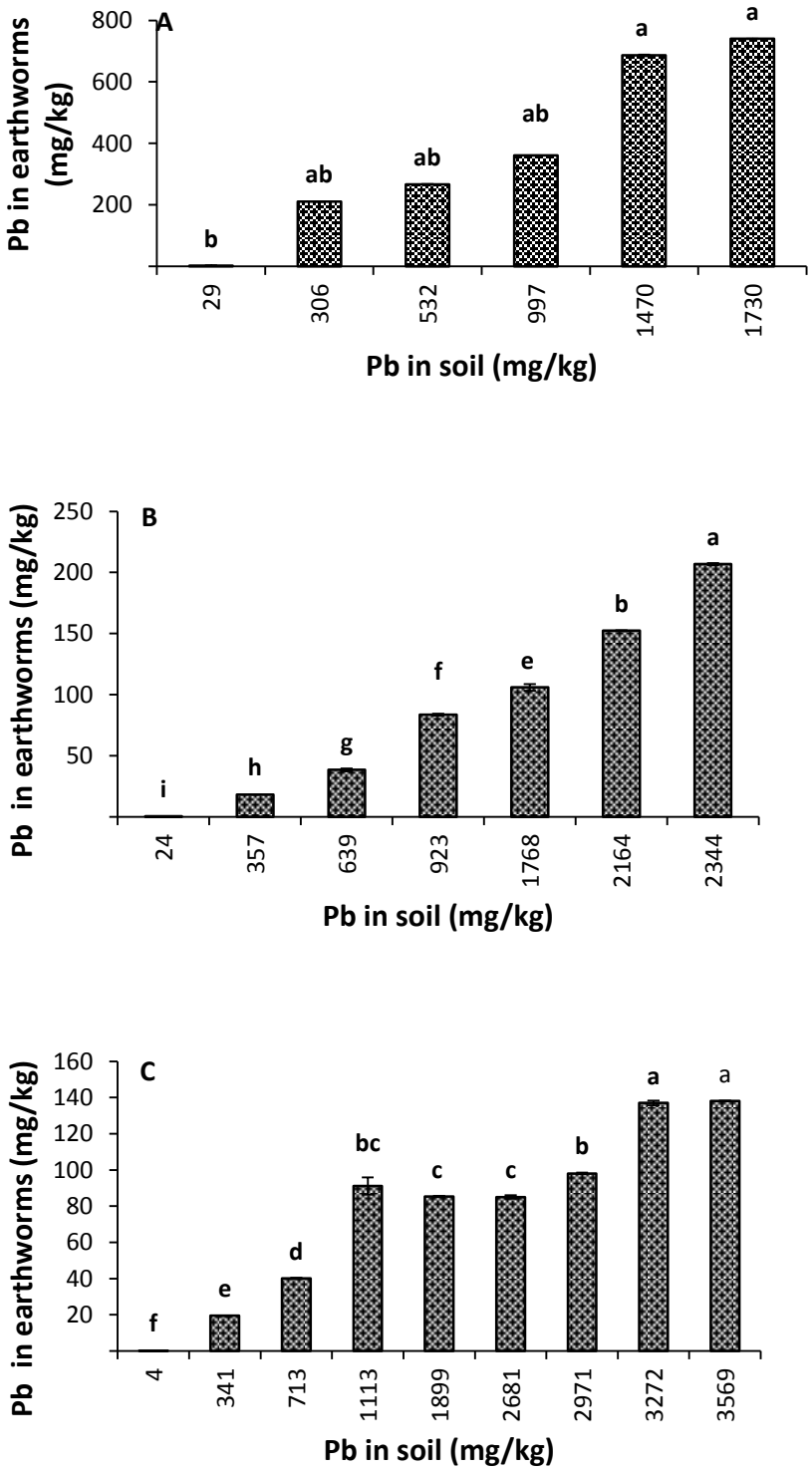


**Fig. 3:** Mean body concentrations of Pb (mg/kg worm fresh weight) in *E. fetida* exposed to Pb spiked soils; A, acidic soil; B, neutral soil; C, alkaline soil. Identical letters indicate statistically identical values