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# Effect of soil properties on the toxicity of Pb: Assessment of the appropriateness of guideline values

A. Romero-Freire[a,b,*], F.J. Martin Peinado[a], C.A.M. van Gestel[b]

[a] Department of Soil Science, University of Granada, Avd. Fuente Nueva, 18002 Granada, Spain
[b] Department of Ecological Science, Faculty of Earth and Life Sciences, VU University, De Boelelaan 1085, 1081HV Amsterdam, The Netherlands

## HIGHLIGHTS

- Pb bioavailability was assessed in 7 soils with different properties.
- Pb toxicity measured in aqueous extracts (plants, bacteria) and with soil respiration.
- Pb toxicity was affected by pH, carbonate content and organic carbon content.
- *L. sativa* root elongation was more sensitive to Pb than microbial parameters.
- Current regulatory guideline values can underestimate or overestimate Pb toxicity.

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## ABSTRACT

Soil contamination with lead is a worldwide problem. Pb can cause adverse effects, but its mobility and availability in the terrestrial environment are strongly controlled by soil properties. The present study investigated the influence of different soil properties on the solubility of lead in laboratory spiked soils, and its toxicity in three bioassays, including *Lactuca sativa* root elongation and *Vibrio fischeri* illumination tests applied to aqueous extracts and basal soil respiration assays. Final aim was to compare soil-dependent toxicity with guideline values. The *L. sativa* bioassay proved to be more sensitive to Pb toxicity than the *V. fischeri* and soil respiration tests. Toxicity was significantly correlated with soil properties, with soil pH, carbonate and organic carbon content being the most important factors. Therefore, these variables should be considered when defining guideline values.



## 1. Introduction

Lead is a non-essential metal, without metabolic function, that is ubiquitous in the soil. Although it is one of the less mobile heavy metals, lead is easily accumulated in plants when bioavailable [1]. Its average concentration in the Earth's crust is 16 mg $kg^{-1}$ [2], whereas natural uncontaminated soils have a mean value of 40 mg $kg^{-1}$ [3]. Due to its low mobility, Pb tends to accumulate mainly in the top soil layers [4]. Finding the range of lead concentrations in agricultural, urban and industrial soils well defined to different countries [5]. Around the world, different authors identified a range of Pb concentrations from 0.5 mg $kg^{-1}$ [6] to 16,338 mg $kg^{-1}$ [7]. Lead has become widely distributed across the globe as a result of man's actions [8], which can cause adverse effects on human health and the environment [9]. The majority of soil Pb comes from anthropogenic sources [10], including historic ones (e.g., road gasoline emissions). Nowadays, Pb is used widely in buildings, lead-acid batteries, bullets and shot, weights, solder, pewter, and fusible alloys [8]. These different uses resulted in a registered accumulation of Pb in surface soils exposed to various pollution sources at some sites to levels as high as 135,000 mg $kg^{-1}$ [11].

Due to its low mobility, properties and constituents of soils are the main factors that explain the different levels of lead toxicity to soil organisms [12]. Nevertheless the different risk levels proposed by different countries largely do not take into account soil properties and also cover a wide range of soil concentrations. Moreover these values are based on different national strategies in environmental policies which is reflected in a large variation among soil guideline values [13]. Guideline concentrations for soil Pb vary enormously, with reference values in relation to land use, e.g., residential areas, ranging from 100 mg $kg^{-1}$ in Norway [14] to 300 mg $kg^{-1}$ in Australia [15] and 350 mg $kg^{-1}$ in China [16]. Other authors propose risk levels that do consider soil

* Corresponding author at: Department of Soil Science, University of Granada, Avd. Fuente Nueva, Granada, 18002, Spain. Tel.: +34 958 241276; fax: +34 958 244160.
*E-mail address:* anaromerof@ugr.es (A. Romero-Freire).

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properties. Rodrigues et al. [17], for example, mention soil threshold total concentrations for Pb depending on $Al_{ox}$ concentrations from 51–411 mg $kg^{-1}$ at $Al_{ox}$ = 50 mmol $kg^{-1}$ to 82–789 mg $kg^{-1}$ at $Al_{ox}$ = 150 mmol $kg^{-1}$. The Netherlands propose 85 mg $kg^{-1}$ as a target value corresponding with a negligible risk and 530 mg $kg^{-1}$ as the intervention level, independent of soil use but taking into account soil properties (standard soil: 25% clay, 10% organic matter) [18]. In Andalusia, values proposed for agricultural soils are 350 mg $kg^{-1}$ at $pH_{H2O} < 7$ or 500 mg $kg^{-1}$ at pH > 7 [19].

The chemistry of Pb in soils is affected by three main factors: specific adsorption to different solid phases, precipitation of sparingly soluble compounds, and the formation of relatively stable complexes or chelates with the soil organic matter [20]. Pb accumulation and distribution in soils is dependent on mineral grain sizes [21]. Soil pH is another important factor contributing to the potential retention of Pb by the formation of Pb precipitates at high pH [22,23]. Soil pH is also controlling the solubility of metal hydroxides, metal carbonates and phosphates, hydrolysis, ion-pair formation, organic matter solubility, as well as the surface charge of iron and aluminum oxides and clay edges [24–26]. Moreover, some authors have reported Pb fixation by soil organic matter as an important factor [27,28], explaining the accumulation of lead in the upper horizon of soils where organic matter contents is highest [29,30].

Since total metal concentration bears little information on the potential risk [31], the use of biological tests is a key tool for determining the bioavailability and toxicity of metal(oid)s. Bioassays and toxicity tests therefore are crucial for assessing the actual ecological risk and to support legislation regarding contaminated soils [32]. Furthermore, a battery of toxicity tests using a number of representative species of different taxonomic groups is needed to obtain proper insight into the potential hazard of a chemical for the ecosystem [33]. In addition, it is important to assess how soil properties may affect the toxicity of metals and whether the influence of soil properties is consistent across different species of test organisms. Bioassays may provide direct insight into the exposure of organisms to potentially toxic elements [34]. But it is good to mention that, although they are quick and reproducible, standard laboratory bioassays usually overestimate metal bioavailability because of the lack of chemical equilibration [35,36]. Moreover toxicity of salt spiked soils may be aggravated compared to that of field-contaminated soils because of the associated pH decrease and increased salinity [64]. Therefore, the toxicity response in laboratory spiked soils is usually higher than in situ contaminated soils. Since laboratory toxicity data generally are used, this may lead to more strict safety levels for environmental risk assessment. However, the application of the safety levels gathered for legislation could be unsuitable from a diagnostic point of view, because toxicity tests usually measure the toxic effects of individual metals on a limited number of soils and organisms. Moreover, the complexity increases in the field because pollution often is caused by a mixture of elements which can mask the effects attributed to the studied metal [37,60]. The aim of this study is to determine the influence of different soil properties on the availability of lead in laboratory-spiked soils and to assess its toxicity in three different toxicity tests, using different organisms and processes. By choosing test concentrations corresponding with threshold values for Pb defined in different Spanish regulations, results from this study could be used to validate risk limits for Pb in soils.

## 2. Materials and methods

### 2.1. Test soils

Seven soils with different properties, representing most of the main soil groups in Spain were selected (Table 1). The main parameters analyzed were: soil pH (pHS, measured using a soil:water ratio of 1:2.5), electrical conductivity (EC), texture, organic carbon (OC) content, water holding capacity (WHC), cation exchange capacity (CEC), specific area (SA) and calcium carbonate content ($CaCO_3$). These properties were determined according to official methods of analysis [38]. Moreover free and amorphous iron, aluminum and manganese oxides were analyzed according to Holmgren [39] and Schwertmann and Taylor [40], respectively.

Total soil lead concentration (PbT) was determined after acid digestion of the soils in strong acids ($HNO_3$ + HF). Water extractable Pb concentration (PbW) was determined in soil:water extracts (1:1 ratio) obtained by shaking for 24 h, followed by centrifuging for 20 min at 3500 rpm and extraction with 10 cm Rhizon MOM (Metal and Organic Matter) soil moisture samplers. In all cases, Pb concentration was measured by Inductively Coupled Plasma-Mass Spectrometry using an ICP-MS NEXION 300D spectrometer. The accuracy of the method was corroborated by analyses of standard reference material SRM 2711; the mean (±SD) experimental value for Pb was 1138 ± 11.0 mg $kg^{-1}$ ($n$ = 6) and the certified value was 1162 ± 31.0 mg $kg^{-1}$.

### 2.2. Soil spiking

Soils were contaminated in the laboratory with lead (II) nitrate ($Pb(NO_3)_2$: PanReac-ApliChem, 98% purity) in increasing concentrations according to the reference values proposed by the Junta de Andalusia [19] (500–1000–2000 mg $kg^{-1}$) and adding two more levels of extreme pollution (4000–8000 mg $kg^{-1}$). Furthermore an uncontaminated sample (control) was included. The contamination was performed by spiking individual samples of 50 g of soil with aqueous Pb solutions, in triplicate. After spiking, the soils were moistened to 60% of their water holding capacity (WHC). The soils were incubated for 4 weeks at 25 ± 1 °C and 60% air humidity, with a light/dark cycle of 10/14 h. Soil moisture content was checked and if needed readjusted weekly. The incubation period chosen allows stabilization of the Pb added, and duration was based on similar studies by other authors [41–43].

Total Pb concentrations in the soils after incubation were measured by XRF with a NITON XLt 792 instrument. In all cases, the measured concentrations differed less than 10% from the total estimated concentrations (background value + added Pb). Furthermore a saturation extraction with soil:water ratio 1:1 was performed. Soil solutions were obtained after 24 h of shaking followed by extraction with a 10 cm Rhizon MOM. In the obtained water extracts, pH (pHW), electrical conductivity, and water-extractable lead concentrations (PbW) were measured. All measurements were performed within 48 h of obtaining the extracts.

### 2.3. Toxicity tests

Three toxicity tests were selected for this study:

1. Seed germination/Root elongation Toxicity test, according to US EPA [44] recommendations. This test assesses the phytotoxic effects on seed germination and initial seedling growth [45]. In Petri dishes 15 seeds of *Lactuca sativa* were incubated in 5 ml of aqueous extract from the Pb-spiked soils. The dishes were placed in an incubator at 25 ± 1 °C and the number of seeds germinated and the length of the developing roots were measured after 120 h. The endpoint calculated was root elongation reduction (LsR) compared to the control.
2. Microtox® test [46] based on the reduction of the amount of light emitted by a non- pathogenic strain of the luminescent marine bacterium *Vibrio fischeri* upon exposure to a toxic sample [47]. The test was performed in a Microtox 500 analyser from Microbics Corporation, according to a modification of Microtox Basic