Lead contamination from shooting activities: bioavailability, bioaccessibility and hydrological controls on Pb geochemical partitioning in soil

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Abstract

Lead (Pb) contamination in soils is a persistent environmental and human health concern in areas affected by shooting activities. This study provides an integrated assessment of Pb geochemical behaviour, plant interactions, and human bioaccessibility in waterlogged alluvial wetland soils from Soglitelle (southern Italy), a natural reserve historically impacted by hunting activities. The results show that, despite elevated anthropogenic inputs, Pb is largely retained in stable soil fractions associated with Fe/Mn oxides and residual phases, limiting its mobility and immediate bioaccessibility. However, environmental drivers such as flooding and plant growth can modify Pb partitioning, increasing the proportion of more labile forms. Pb translocation to plant aerial tissues was not observed, with leaf contamination mainly attributable to surface deposition, while in vitro bioaccessibility tests indicated moderate potential human exposure. Overall, this study demonstrates that Pb contamination in shooting-impacted soils is not static, underscoring the importance of integrating geochemical fractionation, environmental processes, and bioaccessibility for accurate and reliable risk assessment.

Keywords: soil Pb contamination, shooting activities, metal speciation, lead bioavailability and bioaccessibility, flooding effects.

1. Introduction

Lead (Pb) remains a pervasive soil contaminant of global concern due to its persistence, lack of biological function, and well-established toxicity to humans and ecosystems (Albores-Garcia et al., 2021, Li et al., 2019). Despite reductions in major emissions such as leaded gasoline, industrial emissions, paints, pipes, battery recycling, and, notably, hunting and shooting activities legacy and diffuse sources continue to sustain Pb contamination in soils and environment, where it can persist for decades and act as a long-term exposure reservoir (Kabata-Pendias, 2010, Flora et al., 2012, Wani et al., 2015).

Among anthropogenic sources, shooting and hunting activities represent a significant but often underestimated contributor to localised Pb contamination. Although freshly deposited metallic Pb is relatively immobile, it undergoes progressive weathering through oxidation, carbonation, and dissolution processes, leading to the formation of secondary Pb phases that are more mobile and potentially bioavailable (Ahmad et al., 2012, Sanderson et al., 2018, Ma et al., 2008, Scheetz and Rimstidt, 2009, Chrastný et al., 2010). Reported weathering rates of Pb shot pellets range from 5–17% over periods of 6–25 years (approximately 0.2–2.8% yr^{-1}), although significantly faster transformations may occur depending on climatic conditions and pellet size (Hardison et al., 2004).

The environmental risk associated with Pb in soils cannot be adequately assessed based on total concentrations alone, but is instead governed by its speciation, geochemical partitioning, mobility, and bioavailability. Assessing the bioavailable fraction is thus essential for a realistic evaluation of contamination risk (Rocco et al., 2018b). These properties are strongly controlled by soil physicochemical parameters, including pH, redox potential, organic matter content, cation exchange capacity, and mineral composition, which regulate Pb interactions with carbonates, clay minerals, Fe–Mn oxides, and organic phases (Rieuwerts et al., 1998, Kabata-Pendias, 2010). In addition, environmental drivers such as soil moisture, temperature, and CO_2 partial pressure can significantly modulate Pb solubility and transformation pathways by affecting soil pH, redox conditions, and dissolved organic matter, thereby controlling Pb speciation and mobility (McLaren et al., 2009, Sauvé et al., 1998). In general, metallic Pb exhibits low solubility in soils due to its strong affinity for solid phases; however, under specific environmental conditions, its mobilization can be substantially enhanced.

In shooting-range environments, soil properties and environmental conditions jointly control Pb leaching and release. Acidic conditions, elevated organic matter content, and increased soil moisture have been shown to promote Pb dissolution and mobility (Hardison et al., 2004, Ahmad et al., 2012, Sanderson et al., 2018). These processes are particularly relevant in systems subjected to dynamic hydrological conditions, where alternating wetting–drying cycles and periodic flooding can further enhance mineral dissolution, modify redox conditions, and redistribute Pb among geochemical fractions.

This is especially critical in natural and protected areas impacted by illegal shooting activities, where Pb inputs are diffuse, unmanaged, and continuously accumulated over time. In such environments, environmental variability associated with hydrological fluctuations can strongly influence Pb transformation processes and ultimately its bioavailability and ecological risk.

The present study focuses on a Pb-contaminated soil located in the Soglitelle–Villa Literno natural reserve (Campania region, southern Italy) (Figure 1), an area characterised by agricultural use, ecological value, and for long time exposed to illegal shooting activity, as well as periodic hydrological fluctuations associated with flooding events. The objectives are to: (i) assess the degree of soil contamination; (ii) evaluate Pb mobility, bioavailability, and bioaccessibility through chemical extractions and plant uptake measurements; and (iii) investigate the influence of dynamic hydrological conditions on Pb distribution among soil geochemical fractions.

Figure 1. Geographic and environmental setting of the Soglitelle wetland (Campania region, southern Italy), located along the Tyrrhenian coastal plain near the mouth of the Volturno River, between Caserta and Naples. The inset map shows the regional context within Italy. The study area is part of a low-lying coastal wetland–lagoon system characterized by saline/brackish conditions, shallow water bodies, and evaporitic features influenced by marine and fluvial inputs. Representative field images (right) illustrate salt flats, seasonal ponds, and a hunting hide used for waterfowl hunting.

2. Materials and methods

2.1 Study area

The study area, known as Soglitelle, covers approximately 1600 m^2 and is located in the Volturno Plain, near the left bank of the Volturno River and about 2 km from the coast, between the municipalities of Castel Volturno and Villa Literno in Campania Region (southern Italy). The plain is an extensive lowland formed by Holocene alluvial sediments partially overlie pyroclastic deposits and Plio-Pleistocene lacustrine, palustrine, and/or marine sediments (Ermice, 2002). The plain is bounded by the Roccamonfina volcano to the north, the carbonate mountains of the Campanian Apennines to the east, the Phlegrean Fields volcanic district to the south, and Tyrrhenian Sea to the west (Ermice, 2002). The area has a Mediterranean climate, with an average annual temperature of 16.8 °C, and an average annual rainfall of approximately 991 mm, mainly concentrated between October and April. Land use is predominantly agricultural, with irrigated fields devoted to forage crops production (Ermice, 2002).

The Soglitelle area is located within the Litorale Domitio Flegreo designated in 2014 by Italian Parliament as a Regional Interest Priority Site (RIPS) due to the presence of 2551 "potentially polluted" sites (Capra et al., 2014). Soglitelle is characterised by a wetland system consisting of shallow artificial ponds constructed in the 1970s for hunting purposes and used until 2005, when they were seized to combat poaching and subsequently, declared a protected area (Usai, 2023). The site is of high naturalistic value, serving as a wintering area for migratory bird species (Usai, 2023). The artificial ponds are supplied by artesian wells and are interconnected by canals with a freshwater pond and a nearby saline lagoon (Usai, 2023). During the summer months, the ponds tend to dry out (Usai, 2023), resulting in pronounced dry-wet cycles across the study area. According to the USDA soil classification, soils at the study site are Terric Hoplohemists. Preliminary investigations also identified a discrete accumulation of metallic lead, attributed to the historical use of lead pellets (bullets and shot) as hunting ammunition.

2.2 Field experiment and sampling design

In 2014, the site was selected as pilot area within the EU-LIFE Ecoremed project (LIFE11/ENV/IT/275) and was subjected to an environmental restoration programme based on phytoremediation. *Eucalyptus camaldulensis* (Dehnh.) was planted at spacing of 2 x 1 m. This species was chosen as a test plant due to its high tolerance to elevated concentrations of potentially toxic elements (PTEs) in soil, its ability to accumulate PTEs in easily harvestable organs (leaves), its rapid biomass production, and its low palatability to grazing animals (Fiorentino et al., 2018, Abhilash et al., 2012). Before planting *E. camaldulensis*, soil samples were collected at two depths, 0-30 cm (A) and 30-60 cm (B), from twenty-one georeferenced sampling points arranged in a 15 x 5 m grid across the Soglitelle field, yielding a total of 42 samples. After three years of plants growth, surface soil (0-30 cm) and *Eucalyptus* leaves were collected from seven plots (VLL9, VLL12, VLL13, VLL14, VLL15, VLL16 and VLL17) to assess Pb bioavailability and plant uptake. These plots were selected based on their total soil lead concentrations.

Both initial and final soil samplings were conducted under dry soil conditions (spring/summer period).

2.3 Soil characterization

Soil samples were air-dried to constant weight and sieved to < 2 mm in accordance with the official procedures published by Ministero delle Politiche Agricole e Forestali (MiPAAF, 2000, Violante, 2000), prior to the determination of moisture content at 105 °C. No intact shot was visible in the 2 mm soil fraction, but the presence of small fragments cannot be excluded.

Soil pH was measured in water using 1:2.5 soil:water ratio, and electrical conductivity was determined at 1:5 soil:water ratio. Total organic carbon (TOC) was analysed using the Walkley and Black method. Cation exchange capacity (CEC) was determined using the $BaCl_2$ - triethanolamine (TEA) at pH 8.1 and carbonate content was measured using a Dietrich–Fruehling pressure calcimeter method. Particle size distribution was determined by the Andreasen pipette method, following wet sieving and sedimentation in aqueous media after oxidation of organic matter with H_2O_2. The different forms of Fe, Al and Si in the < 2 mm soil fraction were determined using acid ammonium oxalate extraction (Schwertmann, 1964) and dithionite–citrate–bicarbonate (Holmgren, 1967) dissolution techniques.

The pseudototal concentration of 14 PTEs (As, Be, Cd, Co, Cr, Cu, Hg, Ni, Pb, Se, Sb, Tl, V and Zn), identified as priority pollutants under Italian legislation (Legislative Decrees 152/2006 and DL 46/2019), were determined using microwave assisted aqua regia digestion (EPA 3051A/2007) and analysed by inductively coupled plasma-atomic emission spectrometry (Perkin Elmer ICP-AES Optima 7300DV; EPA6020A/2007). Pseudototal concentration refers to the fraction extracted by aqua regia, excluding element associated with the silicate minerals (Petrović et al., 2022, Adamo et al., 2024).

2.4 Assessment of PTEs mobility and bioavailability

The bioavailable metal pools in soil, before and after plants growth, were determined using two different extraction methods (Rocco et al., 2018b). Readily bioavailable PTEs were extracted using

1 M NH_4NO_3 (soil:solution ratio 1:2.5 w/v, extraction time 2 h) (DIN19730, 1997); and the potentially bioavailable fraction was extracted using 0.05 M EDTA (pH 7) at a 1:10 w/v soil:solution ratio for 1 h (EUR 19774 EN/2001). Metal concentrations in the NH_4NO_3 and EDTA extracts were determined by Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES Optima 7300DV, Perkin Elmer).

To assess Pb uptake and translocation in plants, *Eucalyptus* leaves were collected 3 years after the start of the experiment. Leaves were washed with distilled water, oven-dried at 65 °C to constant weight, and ground for chemical analysis. To distinguish Pb translocated to aerial tissues from Pb deposited on leaves surface, half of the collected leaves from each plant were analysed without washing. The total metal content in leaf tissues was determined following microwave assisted acid digestion in HNO_3, and metal concentrations in the digestion solutions were measured by Inductively Coupled Plasma-Mass Emission Spectroscopy (ICP-MS Elan 9000, Perkin Elmer).

2.5 Determination of Pb geochemical distribution under dry and flooding conditions

As the study site is periodically subjected to flooding, the impact of changing hydrological conditions on Pb geochemical distribution was assessed through a laboratory experiment. Two soil samples (VLL9 and VLL14) were selected based on their initial total Pb concentrations (412 and 252 mg kg^{-1}, respectively).

To simulate the flooding conditions, ultrapure deionised water was added to the soils at a 1:10 soil:water (w/v) ratio until full saturation. Soil redox potential (E_h) was monitored throughout the experiment using a platinum E_h electrode (HI 3111, HANNA instrument) inserted into the soil. Measured E_h values were corrected against a ZoBell solution reference. The initial redox potential was approximately +300 mV, and after four weeks of submergence, reducing condition were stabilized at E_h approximately 0 mV. At this stage, both flooded (wet) and dry soil samples were analysed for BCR four-step sequential extraction procedure, developed by the Measurement and Testing Programme of the European Commission, to fractionate Pb into operationally defined geochemical forms (Rocco et al., 2018a, Rocco et al., 2018b, Ure et al., 1993). The procedure included 0.11 M HOAc – Step 1 (soluble, exchangeable and associated to carbonates fractions); 0.1 M NH_2OH HCl – Step 2 (reducible fraction); H_2O_2/1 M NH_4OAc – Step 3 (oxidable fractions); aqua regia – Step 4 (Residual fraction).

Lead concentrations in the extracts were determined by atomic absorption spectroscopy (AAS - Analyst 700, Perkin Elmer). Iron and manganese were analysed in the extracts from steps 1, 2 and 3. Soil pH was measured at the beginning and at the end of the flooding simulation experiment.

Wet soil samples were not dried prior to sequential extraction to avoid re-oxidation processes. Soil moisture content was determined in parallel using separate aliquots of the wet samples.

2.6 Bioaccessibility analysis

Three soil samples were selected based on their initial total Pb content (VLL5: 106; VLL9: 412; VLL14: 252 mg kg^{-1}) for an *in vitro* bioaccessibility test, aimed at estimating the potential human bioavailability of Pb. This method measures the fraction of a pollutant solubilised from soil under simulated gastrointestinal conditions (Kim et al., 2002).

In this study, the simple bioavailability extraction test (SBET), a simplified *in vitro* method, was used to determine Pb bioaccessibility (Kim et al., 2002, USEPA, 2007). Synthetic gastric fluid was prepared by dissolving 60.06 g glycine in 2 L of deionised water and adjusting to pH 1.5 with 12.1 M HCl. One hundred millilitres of the artificial gastric fluid were added to centrifuge tubes containing 1.00 g of soil sample. The tubes were fully immersed and placed in a water bath at 37 °C, under rotation at 30 rpm for 1 h. After extraction, the suspensions were filtered through 0.45 µm membrane filters, and the final pH was checked to ensure it not deviate by more than ±0.5 pH units from the initial pH value. Lead concentrations in the extracts were determined by flame atomic adsorption spectroscopy (FAAS) using an AAnalyst 700 (Perkin Elmer) instrument.

The bioaccessible fraction (BAF, %) of Pb was calculated as the percentage of Pb solubilised in the simulated gastric fluid ($Pb_{bioaccessible}$, mg kg^{-1}) relative to the pseudototal Pb concentration (Pb_{total}, mg kg^{-1}) in the soil sample, according to the following equation:

$$BAF\ (\%) = \frac{Pb_{bioaccessible}}{Pb_{total}} x100$$

2.7 Data analysis

All data are reported as mean values ± relative standard deviation based on three replicates. Statistically differences were evaluated using a two-tailed t-test, with significance at $P \leq 0.05$.

Correlation analyses were performed between the results of the single extraction procedures, the SBET bioaccessibility data and the corresponding pseudototal metal concentrations.

The coefficient of variation (CV) was used to compare the variability of Pb and V pseudototal concentrations. CV values were calculated only when mean concentrations were positive and above zero. CVs were not calculated when mean values were below the detection limits.

The degree of soil contamination was assessed using the geoaccumulation index (I_{geo}) (Loska et al., 2003, Müller, 1981). The I_{geo} is a single element index that evaluates environmental contamination by comparing current metal concentrations with preindustrial background levels, and is calculated using the following equation:

$$I_{geo} = \log_2 (C_n/1.5\ B_n)$$

Where C_n is the measured concentration of the metal in soil, B_n is the corresponding geochemical background value, and the factor 1.5 accounts for natural fluctuations in metal concentration in soil environment, including minor anthropogenic influences (Loska et al., 2003). For the study area, geochemical background values were set at 20 mg kg^{-1} for Pb and 150 mg kg^{-1} for V (ISPRA, 2010, Lima, 2014). According to Müller (Müller, 1981), the I_{geo} interpretation is: class 0, practically uncontaminated ($I_{geo} \leq 0$); class 1, uncontaminated to moderately contaminated ($0 < I_{geo} < 1$); class 2, moderately contaminated ($1 < I_{geo} < 2$); class 3, moderately to heavily contaminated ($2 < I_{geo} < 3$); class 4, heavily contaminated ($3 < I_{geo} < 4$); class 5, heavily to very heavily contaminated ($4 < I_{geo} < 5$); class 6, very heavily contaminated ($I_{geo} \geq 5$).

3. Results and discussion

3.1 Soil physico-chemical properties

Soil physico-chemical properties were analysed at depths of 0-30 cm and 30-60 cm. As no significant differences were observed between depths, only data for the 0-30 cm layer are reported in Table 1. Soglitelle site is characterized by clay loam soil with 41 % of the soil in the clay fraction. The soil pH-H_2O is sub-alkaline (pH 8.00 ± 0.05) consistent with the relatively high carbonate content (101 ± 0.7 g kg^{-1}). Electrical conductivity is 1.4 ± 0.7dS m^{-1} (equivalent to 12.2 dS m^{-1} at soil saturation), indicating a moderate to high salinity stress for plant growth and agricultural production (Hardie and Doyle, 2012, Kamran et al., 2020, Mori and Di Mola, 2012) . These results are consistent with the coastal location of the site and the presence of saline groundwater outcrop (Corniello and Ducci, 2014).

The soil is also characterised by high organic carbon content (OC) and cation exchange capacity (CEC), with values of 81.2±21.3 g kg^{-1} and 71.9±0.2 cmol(+) kg^{-1}, respectively (Mori and Di Mola, 2012). These properties reflect the strong capacity of the soil to retain nutrients and metals.

The results from the dissolution experiments reported in Table 2 can be used as indicators of allophanic materials and iron-rich phases (Dahlgren and Ugolini, 1991), which often constitute a significant fraction of the soil colloids in volcanic systems (Arnalds et al., 2007). The very high Al/Si molar ratio indicates that the concentration of allophane is negligible in the studied soils.

The Fe$_o$/Fe$_d$ ratios suggest a relatively stable contribution of amorphous and poorly crystalline iron oxides, alongside crystalline iron oxide phases, across the studied soils. According to the relationship proposed by Childs (Childs, 1985) (Fe$_{est}$ = 1.7 * Fe$_{ox}$ %), the ferrihydrite content is also relatively consistent, averaging approximately 3% .

Table 1. Physico-chemical properties of surface soils samples (0-30 cm) collected at the Soglitelle site (mean ± SD, n= 21).

Soil properties	**mean ± SD**
Sandy (%)	25
Silt (%)	34
Clay (%)	41
pH (1:2.5 H_2O)	8.00±0.05
EC (dS m^{-1})	1.4±0.7
Total carbonates (g kg^{-1})	101.3±0.7
OC (g kg^{-1})	81.2±21.3
CEC (cmol(+) kg^{-1})	71.9±0.2

EC= Electrical conductibility; OC = Organic carbon; CEC = cation exchange capacity

Table 2. Ammonium-oxalate- and dithionite–citrate–bicarbonate-extractable Si, Al and Fe in soils from Soglitelle site (mean, SD, min and max values, n=5). X$_o$ and X$_d$ denoted the Si, Al and Fe fractions extractable by ammonium-oxalate and dithionite–citrate–bicarbonate, respectively; Fe$_{est}$ is the estimated ferrihydrite content; Al$_o$-Al$_d$/Si$_o$ is the Al/Si molar ratio used as indicator of allophanic materials).

Variable	**Mean**	**Standard deviation**	**Minimum**	**Maximum**
Si$_o$ (%)	0.01	0.00	0.01	0.01
Al$_o$ (%)	0.18	0.04	0.13	0.21
Fe$_o$ (%)	0.04	0.01	0.04	0.05
Al$_d$ (%)	0.04	0.03	0.00	0.05
Fe$_d$ (%)	0.24	0.16	0.00	0.33
Al$_o$-Al$_d$/Si$_o$	20.3	1.20	18.8	21.8
Al$_o$+1/2 Fe$_o$	0.18	0.10	0.10	0.28
Fe$_o$/Fe$_d$	0.61	0.05	0.55	0.65
Fe$_{est}^b$ (%)	3.42	0.69	2.82	4.29

^{b}Fe$_{est}$= 1.7 Fe$_o$

3.2 PTEs content in soil and I_{geo} of Pb and V

In all soil samples, the pseudototal concentrations of As, Be, Cd, Co, Cr, Cu, Hg, Ni, Se, Sb, Tl and Zn never exceeded the Italian Action Levels for agricultural land use recently established under

Italian environmental law (D. Lgs 46/2019). Since only lead and vanadium exceeded these limits, only data for these two elements are reported.

Descriptive statistics of pseudototal Pb and V concentrations in soil samples collected at the investigated pilot sites are reported in Table 3. For each element, the table reports, the Italian Action Levels for agricultural land use (D. Lgs46/2019) and the local Soil Background Reference values for V and Pb (ISPRA, 2010, Lima, 2014). Both surface (A) and sub-surface (B) soils showed a potential contamination by Pb (A: mean 174±76 mg kg^{-1}; B: mean 138±86 mg kg^{-1}) and V (A: mean 147±10 mg kg^{-1}; B: mean 148±15 mg kg^{-1}). Maximum concentrations found were 412 mg kg^{-1} in A and 435 mg kg^{-1} in B for Pb, and 173 mg kg^{-1} in A and 172 mg kg^{-1} in B for V.

Concentration of both Pb and V were generally above the Italian legal limits for agricultural soils (100 mg kg^{-1} for Pb and 90 mg kg^{-1} for V; D. Lgs 46/2019). The only exception was Pb in sub-surface soils, where the 67% of the samples exceeded the legal threshold. A predominantly superficial accumulation of Pb is commonly observed when contamination originates from shooting activities (Chrastný et al., 2010), whereas subsurface contamination may be related to the formation of secondary Pb minerals that are susceptible to leaching processes (Hardison et al., 2004).

The large differences between the maximum and minimum Pb concentrations (A: max 412 mg kg^{-1} and min 106 mg kg^{-1}; B: max 435 mg kg^{-1} and min 55 mg kg^{-1}), together with the high coefficient of variation (CV: 44% for A and 62% for B), indicate marked heterogeneity and strong spatial variability, typical of non-natural contamination sources. Similar results of high spatial variability in soil pollutants have been reported by Rocco et al. (Rocco et al., 2016) and Agrelli et al. (Agrelli et al., 2020) for other sites in the Campania Region polluted by anthropogenic activities.

In contrast, V concentrations varied within a much narrower range (132 - 173 mg kg^{-1} in A and 111 - 172 mg kg^{-1} in B) and exhibited low CV values (7% in A and 10% in B), suggesting a predominantly geogenic origin. This interpretation is supported by comparison with the Soil Background Reference values of 150 mg kg^{-1} defined by ISPRA (ISPRA, 2010) for the study area, according to which none of the samples can be classified as polluted by V.

Based on geoaccumulation index (I_{geo}) calculations, the study area resulted from moderately to heavily contaminated by Pb (A: $1.81 < I_{geo} < 3.78$; B: $0.87 < I_{geo} < 3.86$) and uncontaminated by V ($I_{geo} < 0$) in both surface and subsurface soils (Figure 2). These results confirm the geogenic origin of V and the anthropogenic origin of Pb contamination at the study site.

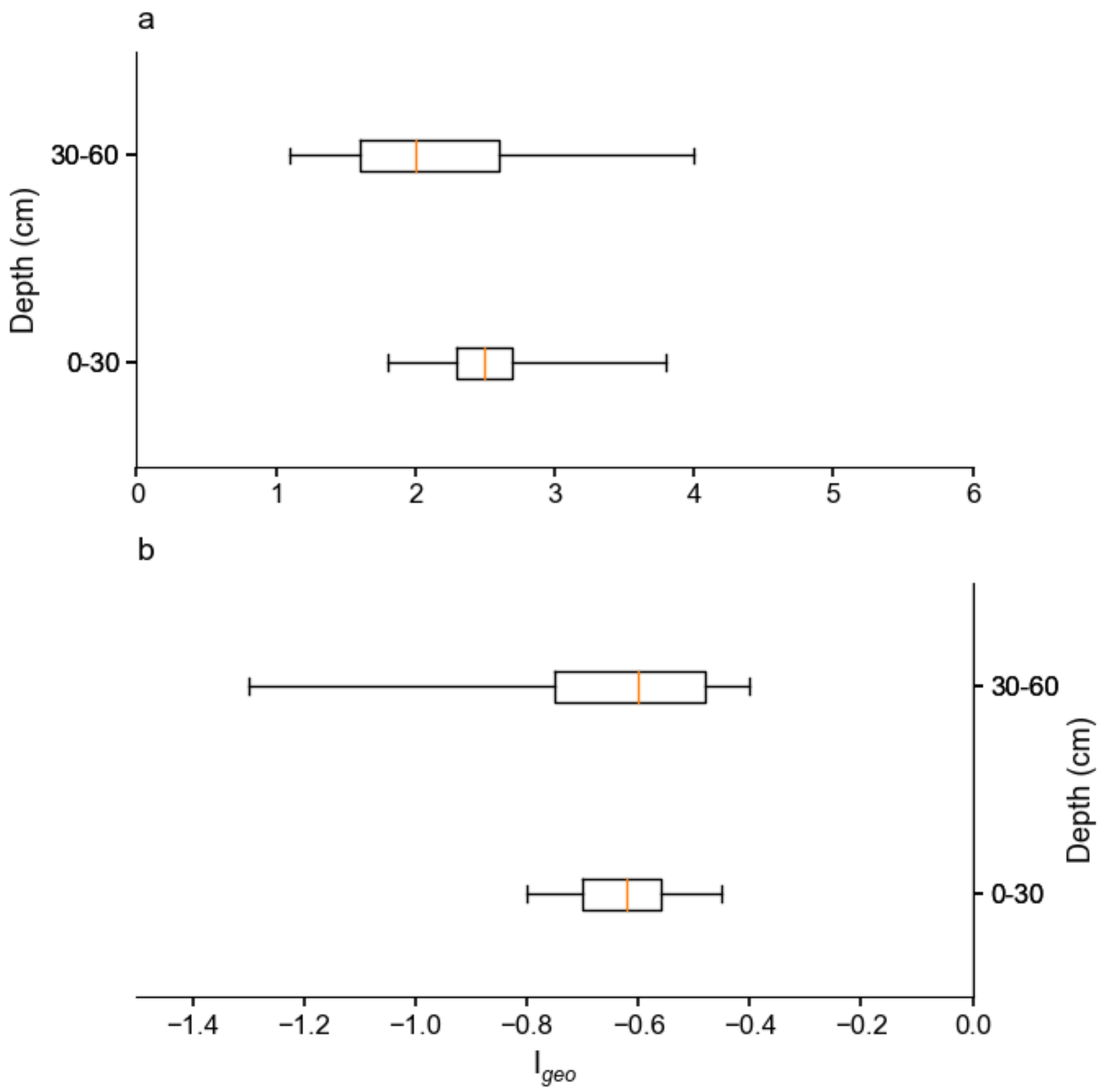

Figure 2. Geoaccumulation index (I_{geo}) values of Pb and V in soils collected at 0-30 and 30-60 cm depth at the study site. Boxes represent the interquartile range (25th – 75th percentiles), the horizontal line indicate the median, and whiskers show the minimum and maximum values.

Table 3. Descriptive statistics of pseudototal Pb and V concentrations (mg kg^{-1}) in soil from the Soglitelle site, based on 21 samples per depth interval.

	Pb	**V**
	0-30 cm	
Mean	174	147
Median	153	146
Min	106	132
Max	412	173

CV%	44	7
	30-60 cm	
Mean	138	148
Median	109	147
Min	55.0	111
Max	435	172
CV%	62	10
Italian limit*	**100(20)**	**90(150)**

*D.Lgs 46/2019; values in brackets indicate local Soil Background Reference values according to ISPRA, 2010 and Lima et al., 2014

3.3 Metals mobility, bioavailability and uptake

Lead and vanadium content in surface (A) and subsurface (B) soil samples collected at the Soglitelle site before plant growth were analysed in 1 mol L^{-1} NH_4NO_3 and 0.05 mol L^{-1} EDTA extractions (Table 4). As expected, the amounts of Pb and V extracted in EDTA were consistently much higher than those extracted in NH_4NO_3. Specifically, the fraction extracted by 1 mol L^{-1} NH_4NO_3 ranged from 0 to 0.22 % of the pseudototal Pb content and from 1 to 2 % of V pseudototal content, whereas EDTA extracted between 11 and 70 % of pseudototal Pb, and between 6 and 11 % of V. The different extraction efficiencies of NH_4NO_3 and EDTA are related to the distinct chemical nature and mechanisms of action of the two extractants. EDTA is a non-specific chelating agent capable of mobilising both labile and non-labile metal fractions. In particular, it can solubilise metals specifically adsorbed onto mineral surfaces and those associated with organic and organometallic complexes through the formation of stable chelates. In addition, EDTA can partially mobilise metals occluded within oxides and secondary clay minerals. In contrast, metal extraction by NH_4NO_3 mainly occurs through cation exchange and desorption processes, which are promoted by the high ionic strength of the NH_4NO_3 solution (Adamo et al., 2024, Rocco et al., 2018b).

The amounts of Pb extracted by EDTA in this study are consistent with literature data from soil contaminated by shooting activities (Chrastný et al., 2010), indicating that a substantial fraction of soil Pb is potentially mobile and bioavailable. Moreover, in many Soglitelle soil samples, the Pb concentration extracted by 1 mol L^{-1} NH_4NO_3 exceeded the Pb trigger value of 0.1 mg kg^{-1} adopted by several European countries for agricultural soils (BBodSchV, 1999, ASP, 2004, Carlon, 2007) highlighting a potential risk of Pb transfer to plants.

No significant linear correlations were observed between Pb pseudototal concentration and EDTA or NH_4NO_3 extractable contents (data not shown). This lack of correlation may indicate the presence of metallic Pb shot fragments in the sieved soil samples. Such fragments can substantially influence pseudototal Pb concentrations while contributing little to the extractable Pb pool, as metallic Pb is poorly mobilised by both extraction procedures.

The EU-MAT (formerly BCR) sequential extraction procedure fractionates the total metal content in soils into four operationally defined pools: step 1: the CH_3COOH-extractable or easily extractable fraction (presumably soluble, exchangeable, and carbonate-bound metals); step 2: the reducible fraction comprising metals associated with easily reducible Mn and Fe oxides; step 3: the oxidizable fraction, which includes metals bound to organic matter and sulphides; and step 4: the residual fraction, consisting mainly of metals incorporated within the silicate mineral lattice. Metals mobility and bioavailability decrease progressively in the order: step 1 > step 2 > step 3 > step 4 (Rocco et al., 2018b, Adamo et al., 2024, Ure and Davidson, 2002).

As shown in Figure 3, EU-BCR sequential extractions performed on selected soil samples under dry (oxic) conditions (VLL9: Pb 412 mg kg^{-1}; VLL14: Pb 252 mg kg^{-1}) indicated that Pb was mainly associated with easily reducible mineral phases (Step 2: 28 and 49%) and residual (step 4: 65 and 38 %). A small portion of Pb was associated with the oxidisable fraction (Step 3: 7 and 13 %). The residual fraction may include Pb species strongly bound to organic matter and Fe-oxides (Tai et al., 2013, Okkenhaug et al., 2016), as well as mineral forms such as lead phosphate, lead sulphate, lead sulphite, lead chloride phosphate (Kelebemang et al., 2017), in addition to metallic Pb(0) derived from shot fragments (Vantelon et al., 2005). Only a very small fraction of Pb was extracted in Step 1 (acetic acid: 0.4 for VLL9 and 0.2 % for VLL14), indicating very low Pb mobility under current soil conditions. These results are consistent with the high content of non-crystalline Fe oxides measured in the field (Table 2). Previous studies have reported a strong affinity of Pb for Fe and Mn oxides (Sanderson et al., 2018), as well as the formation of oxidized Pb species from metallic Pb, which subsequently dissolved during the reducible step of sequential extraction (Chrastný et al., 2010). This behaviour is due to the reducing conditions and low pH of the extractant used in Step 2. Although carbonate-bound Pb is often reported as the dominant fraction in shooting-range soils, due to the formation of cerussite and hydrocerussite, which are extracted in Step 1 of the EU-BCR procedure (Fayiga and Saha, 2016, Kelebemang et al., 2017), this was not observed in our study. The alkaline soil pH may have limited the formation of Pb carbonates, favouring instead the persistence of Pb oxides as the predominant mineral form of Pb in the investigated soils (Ma et al., 2007).

After plant growth, V was not analysed due to its geogenic origin and low bioavailable fraction. Lead concentrations extracted with NH_4NO_3 from the seven selected plots (Table 5) were comparable before (pre) and after (post) plant growth, with mean values of 0.14±0.08 mg kg^{-1} (pre) and 0.15±0.16 mg kg^{-1} (post). In contrast, the potentially available Pb fraction extracted with EDTA showed a significantly increase after plant growth, with mean values rising from 63.9±12.2 mg kg^{-1} (Pre) to

91.8±17.4 mg kg^{-1} (Post). These results indicate that the development of the vegetation over the time enhanced Pb mobility and availability in the soil, increasing the potential risks to the surrounding environment, including Pb leaching into groundwater and uptake by plants and, consequently, posing a greater risk to human health. The observed increase in Pb bioavailability is likely related to plant-induced changes in soil properties, such as pH and organic carbon content, and to the re-equilibration processes of Pb in soil among different polls (Agrelli et al., 2020, Rocco et al., 2018a, Rocco et al., 2018b, Huynh et al., 2010, Hammer and Keller, 2002). Moreover, the plausible soil acidification occurring after plant growth may have accelerated Pb weathering processes, promoting the transformation of Pb oxides into EDTA-extractable species such as cerussite and hydrocerussite. These reactions require the presence of hydrogen ions (Ma et al., 2007, Chrastný et al., 2010) , and therefore are expected to proceed more slowly under alkaline conditions typical of the Soglitelle soil (Ma et al., 2007).

No significant changes were observed in the Pb fraction extracted with ammonium nitrate before and after plant growth. This suggests that any plant-induced soil acidification was not sufficient to enhance the solubilization, despite potentially favouring the formation or transformation of Pb into less soluble carbonate-associated forms. Although no increase was detected in the readily bioavailable Pb fraction (NH_4NO_3-extractable), the observed increase in the potentially bioavailable pool (EDTA-extractable) remains of concern. This larger potentially mobilizable Pb reservoir could be released in the presence of organic ligands or under further soil acidification, thereby increasing its availability for plant uptake. In addition, this pool may act as a source that replenishes the readily bioavailable Pb fraction depleted by plant uptake, through mobilisation driven by organic ligands or additional acidification (Agrelli et al., 2020).

The uptake and translocation of Pb into the *Eucalyptus* leaves are reported in Table 6. When leaves were analysed without washing, the mean Pb concentration was 0.44±0.36 mg kg^{-1} (with a maximum value of 0.67 mg kg^{-1}). In contrast, after washing, Pb concentrations in the leaves were almost always below detection limit (BDL, 0.05 µg kg^{-1}). Only sample VLL16 showed a detectable Pb concentration after washing (0.35 mg kg^{-1}). These results indicate that *Eucalyptus* did not significantly translocate Pb to the aerial part of the plants. Instead, it was mainly present as surfaces deposition on the leaves, confirming the high selectivity of *Eucalyptus* against the uptake of the non-essential elements and highlighting the potential for plant contamination via aerial deposition at the study site. Although Pb naturally occurs in all plants, it has no known essential role in plant metabolism and is typically present at very low concentrations in plant tissues (Kabata-Pendias, 2010). In the present study, although Pb is mainly present on the surface of leaves, it may still represent a potential risk for living organisms. Airborne (or deposited) Pb can be taken up by food crops through foliar absorption and subsequently internalised by plant cells. It has been estimated that up to 95 % of the total Pb content in plants may derive from aerial/atmospheric deposition onto leafy surfaces rather than from root uptake (Isermann, 1977, Dalenberg and Van Driel, 1990, Bi et al., 2009, Kabata-Pendias, 2010, Srivastava et al., 2022). This aspect should be carefully considered in the restoration and management of contaminated sites located in agricultural areas, such as the one investigated here, in addition to risks related to PTEs bioavailability in soil for food and forage crops.

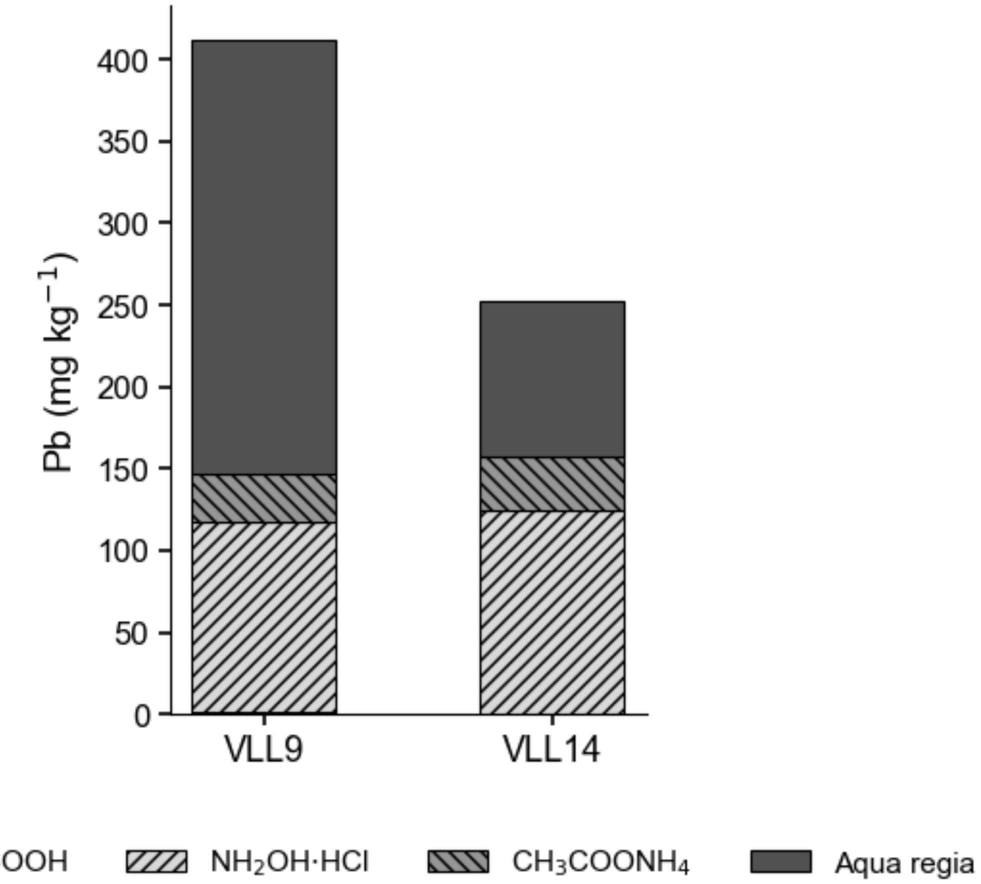

Figure 3. Amounts of Pb extracted by BCR sequential extraction procedure in VLL9 and VLL14 soil samples, expressed as mg kg^{-1}.

Table 4. Pb and V concentrations extracted by NH_4NO_3 and EDTA, and aqua regia (mg kg^{-1}) in soils from Soglitelle site.

	NH_4NO_3		**EDTA**		**Pseudototal**	
	Pb	**V**	**Pb**	**V**	**Pb**	**V**
			0-30 cm			
VLL1	BDL	3.18	33.2	15.2	111	152
VLL2	0.17	3.21	28.2	12.7	125	146
VLL3	0.17	2.80	31.6	11.1	130	146
VLL4	0.05	2.80	35.8	13.1	143	156
VLL5	0.06	2.29	30.6	11.2	106	140
VLL6	0.08	2.16	55.6	11.5	138	150

VLL7	0.05	1.98	67.5	9.07	169	134
VLL8	0.11	2.05	73.5	9.59	170	163
VLL9	0.26	2.08	60.8	10.2	412	149
VLL10	0.04	2.32	42.7	10.2	151	154
VLL11	BDL	2.63	64.2	11.8	181	157
VLL12	0.13	2.27	59.4	10.1	153	139
VLL13	0.01	2.33	73.6	10.9	188	146
VLL14	0.10	2.20	85.5	9.75	252	138
VLL15	0.20	2.79	47.2	11.5	156	173
VLL16	0.20	3.54	60.0	12.7	345	151
VLL17	0.10	2.55	61.0	10.8	199	146
VLL18	0.17	2.01	43.3	8.57	137	147
VLL19	0.02	2.27	30.5	8.81	160	132
VLL20	0.01	1.66	29.9	8.05	124	138
VLL21	0.14	1.41	39.0	7.46	112	135
			30-60 cm			
VLL22	0.12	2.79	47.2	16.2	99	172
VLL23	0.09	3.01	36.0	13.5	147	129
VLL24	0.02	2.26	16.0	11.6	55	149
VLL25	0.06	2.81	41.1	18.7	106	171
VLL26	0.01	1.57	24.5	10.8	65	162
VLL27	0.01	1.51	23.2	10.1	75	147
VLL28	0.24	1.55	22.9	7.96	109	111
VLL29	BDL	1.15	42.2	10.9	125	144
VLL30	0.25	2.42	39.1	18.9	117	167
VLL31	0.04	2.52	27.5	13.6	95	162
VLL32	0.04	2.15	41.0	18.2	71	159
VLL33	0.11	2.37	78.4	11.7	435	134
VLL34	0.21	2.47	29.2	12.2	264	135
VLL35	0.08	2.26	46.1	10.4	190	135
VLL36	0.05	2.12	65.5	8.44	223	144
VLL37	BDL	3.12	52.6	13.5	115	149
VLL38	0.03	1.39	73.3	10.8	105	146
VLL39	BDL	2.04	43.6	8.71	107	140
VLL40	BDL	2.24	59.9	9.15	117	133
VLL41	0.02	1.32	46.8	10.2	190	151
VLL42	0.05	1.84	38.8	9.10	94	164

BDL = below detection limit (0.01 mg kg^{-1})

Table 5. NH_4NO_3 and EDTA extractable Pb concentrations (mg kg^{-1}) measured before (Pre) and after (Post) phytoremediation with *Eucaliptus camedulendis* (n = 7, mean ± SD).

	NH_4NO_3	**EDTA**
	Pre	
Mean±SD	0.14±0.08	63.9±12.2
Min	BDL	47.2
Max	0.26	85.5
	Post	

Mean±SD	0.15±0.16	91.8±19.6*
Min	BDL	72.1
Max	0.39	126

*Significant at 0.05 probability level
BDL = below detection limit (0.01 mg kg^{-1})

Table 6. Pb concentration (mg kg^{-1}) in washed and unwashed *E. camedulendis* leaves

	Washed	**Unwashed**
VLL9	BDL	BDL
VLL12	BDL	BDL
VLL13	BDL	BDL
VLL14	BDL	BDL
VLL15	BDL	0.02
VLL16	0.35	0.67
VLL17	BDL	0.62

BDL = below detection limit (0.05μg kg^{-1})

3.4 Comparison of Pb distribution between the two watering regimes

A considerable and statistically significant change ($P < 0.05$) in Pb distribution was observed when comparing EU-BCR sequential extractions performed on VLL9 and VLL14 soil samples under oxic (dry soil) and hypoxic (submerged soil) conditions. During the flooding simulation, soil pH increased from 8.2 to 8.5, indicating a slight alkalinisation under hypoxic conditions. This pH shift, together with redox changes induced by submersion, was accompanied by a redistribution of Pb among geochemical fractions.

As shown in Figure 4 a and b, Pb concentrations in step 1 (the soluble and easily exchangeable fraction) increased under hypoxic conditions, rising from 0.4 to 1.2 % in VLL9 and from 0.2 to 1 % in VLL14. This shift indicates a higher Pb solubility and mobility during periodic flooding, thereby potentially increasing the risk of both plant uptake and leaching into seawater.

Pb extractability also increased under hypoxic conditions in the steps 2 (reducible) and 3 (oxidizable). Specifically, Step 2 increased from 28 to 38 % in VLL9; while Step 3 increased from 7 to 14 % in VLL9 and from 13 to 17 % in VLL14. The only exception was Step 2 in VLL14 where Pb concentrations remained nearly unchanged between oxic and hypoxic conditions (49% and 48%, respectively).

The overall increase in Pb extracted in the Step 1 and 2 under submerged conditions is consistent with findings of Ma et al. (Ma et al., 2007), who reported an increased transformation of metallic Pb into Pb oxides and carbonates under weathering processes where a water layer surrounds metallic Pb. Such conditions promote gases diffusion around Pb particles, including CO_2 produced during organic matter degradation, promoting secondary Pb mineral formation (Ponnamperuma, 1972, Kelebemang et al., 2017). In this context, the observed slight increase in soil pH under hypoxic conditions is also consistent with alkalinisation associated with the formation of these secondary Pb minerals (Ma et al., 2007), rather than the acidification typically expected in submerged soils, where pH often tends toward neutrality (pH 7) (Ponnamperuma, 1972).

Under experimental conditions, reducing process did not lead to significant dissolution of Fe oxides (Figure 4 c and d). In contrast, manganese showed a decreased in the reducible fraction after submersion (Step 2) (Figure 4 e and f), while Fe content remained unchanged. Although Pb concentrations in Step 2 increased (Figure 4 a and b), no evidence of Pb release from Fe oxide reducible fractions was observed during submersion. Instead, Pb redistribution likely involved a shift from the residual fraction (which was the only fraction that decrease after submersion) towards the first and third steps fractions.

At alkaline pH, soil surfaces are typically negatively charged, which can favour re-adsorption of released cations (Ajmone-Marsan et al., 2019), potentially contributing to Pb redistribution among the phases. These findings agree with previous study reporting limited Fe-oxides dissolution under similar pH and redox conditions (Gotoh and Patrick, 1974). Even if the minimum redox potential was not fully reached, Fe-oxides dissolution in alkaline soils generally occurs only under much more reducing conditions (low redox potentials). Therefore, Pb release from Fe oxide–associated fractions during submersion at the study site appears unlikely.

It should be noted that, under natural conditions, floodwaters at the study site are brackish, whereas the experimental submersion was conducted using deionised water (von Fraunhofer, 1969, Tylecote, 1983). This aspect should be further investigated through field-based sampling under natural submerged conditions, since reproducing all variables controlling Pb corrosion (e.g. water chemistry, dissolved oxygen, hydrodynamics) in laboratory experiments remains challenging (von Fraunhofer, 1969).

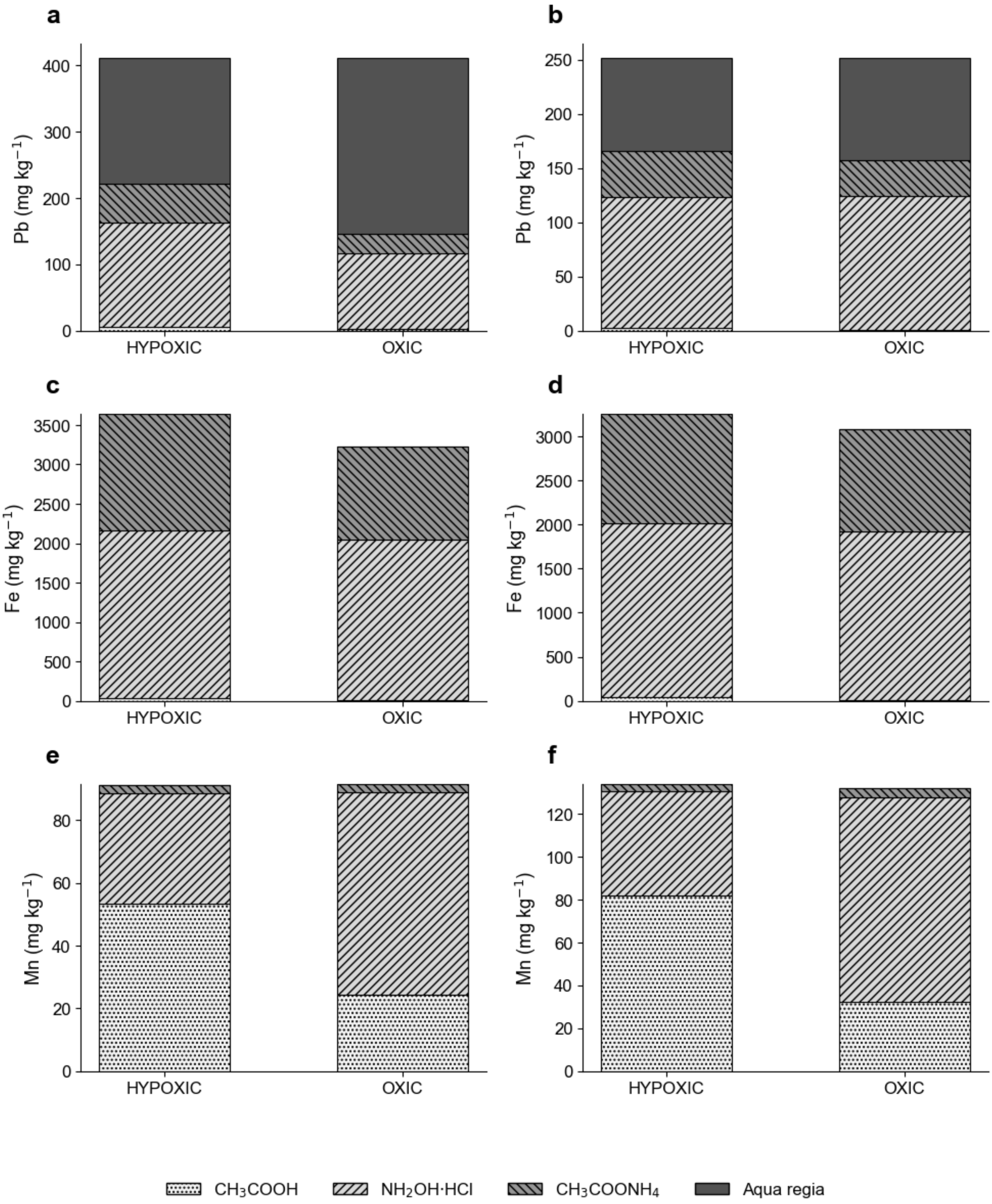

Figure 4. Sequentially extracted Pb (a-b), Fe (c-d) and Mn (e-f) fractions (mg kg^{-1}) determined by the BCR extraction procedure in VLL9 (a, c, e) and VLL14 (b, d, f) soil samples under dry (oxic) and flooding (hypoxic) conditions.

3.5 Bioaccessibility in vitro

The post-ingestion bioavailability of Pb was investigated using the *in vitro* simple bioaccessibility extraction test (SBET). SBET is a static gastric model we used to estimate the potential human bioaccessibility of metals from contaminated soils (Hu et al., 2011, USEPA, 2008). In this study, SBET was used to estimate the oral bioaccessibility of Pb in soils from the study site. The method involves leaching soil samples in a simulated human gastric fluid to mimic conditions in the stomach following accidental soil ingestion (Luo et al., 2012a, Luo et al., 2012b). Pseudototal Pb concentrations and SBET-extractable Pb for three selected soil samples (VLL5, VLL9 and VLL14) are reported in Table 7. Pseudototal Pb concentrations were 106±3.7, 412±4.2 and 252±2.5mg kg^{-1}, respectively. The amounts of Pb released under simulated gastric conditions were 34.1±2.7 mg kg^{-1} for VLL5, 60.2±6.8 mg kg^{-1} for VLL9 and 60.4±9.6 mg kg^{-1} for VLL14. These values correspond to bioaccessible fractions (BAF) of 32, 15 and 24 %, respectively.

The bioaccessible Pb concentrations were comparable to the EDTA-extractable Pb contents measured for the same soil samples, suggesting that the EDTA-extractable fraction reflects the Pb fraction mobilizable under gastric conditions. Overall, the Pb bioaccessibility observed in this study was lower than that reported for other shooting range soils in the literature, where BAF bioaccessibility fractions (BAF) often exceed 75 % (Sanderson et al., 2018). This difference is likely related to the high presence of strongly immobilized Pb in the Soglitelle soils, as indicated by the large residual fraction identified through sequential extractions (paragraph 3.4). Consequently, a substantial portion of Pb is present in forms that are poorly bioaccessible, in contrast to soils where Pb is predominantly associated with more labile phases such as oxides and carbonates (Sanderson et al., 2018). Consistent with this interpretation, Cao and co-authors (Cao et al., 2009) reported a marked decrease in Pb bioaccessibility in shooting-range soils amended with phosphate, attributed to the possible formation of pyromorphite, a highly stable Pb phosphate mineral that would fall within the residual fraction of a sequential extraction. No significant linear correlation was found between pseudototal Pb concentrations and SBET-extractable Pb. In contrast, a highly significant negative linear correlation was observed between pseudototal Pb concentration and BAF (R^2 = 0,9999), indicating that increasing total Pb concentrations do not correspond to increased bioaccessibility. This suggests that higher pseudototal Pb levels at the study site do not necessarily imply an increased risk to human health via soil ingestion. Considering the highest bioaccesible Pb concentration measured in the Soglitelle soil samples(approximately 60 mg kg^{-1}), and the provisional tolerable weekly intake for Pb established by JECFA (0.025 mg kg^{-1} body weight) (JECFA, 2011) , assuming accidental soil ingestion as the sole source of Pb exposure, an intake of approximately 0.42 g of soil per kg of body weight per week would be required to exceed the risk threshold. This value is substantially higher than the upper percentile rate for combined soil and dust ingestion in adults (200 mg day^{-1}) (USEPA, 2017). Therefore, under current conditions, the soils at the study site do not appear to pose a significant risk to human health.

Table 7. Pb bioaccessibility determined by simple bioaccessibility extraction test (SBET) using synthetic gastric fluid expressed as bioaccessible Pb concentration (mg kg^{-1}) and bioaccessible fraction (BAF, %) for soils from the Soglitelle site (mean ± SD).

	Pb bioaccessibility		
	SBET (mg kg^{-1})	BAF (%)	Total content
VLL5	34.1±2.7	32	106±3.7
VLL9	60.2±6.8	15	412±4.2
VLL14	60.4±9.6	24	252±2.5

4. Conclusions

Site characteristics, including soil properties, pedoclimatic conditions and vegetation, strongly influence the weathering, mobility, bioavailability and oral bioaccessibility of Pb in soils affected by hunting activities. In particular, alkaline pH appears to slow weathering processes responsible for the formation of more mobile and bioavailable Pb species and reduces the likelihood of Fe oxides reduction under low redox conditions induced by periodic flooding, limiting the release of Pb associated with this soil fraction. However, flooding events promote the redistribution of Pb among different geochemical phases within the soil.

In this context, *Eucalyptus* does not appear to be a suitable option for phytoremediation or phytostabilisation. Although, *Eucalyptus* plants absorb only small amounts of Pb, they may enhance its mobility. The resuspension of contaminated soil particles could pose a risk of surface contamination for nearby food and forage crops. However, given the low bioaccessibility of Pb, this does not appear to represent a direct risk to human health. Nevertheless, plant activity and wet-dry cycles may increase Pb bioaccessibility over time. The application of soil amendments aimed at strongly immobilizing Pb could be an effective strategy to reduce its mobility and bioaccessibility at the studied site, although their long-term effectiveness may be diminished by repeated wet–dry cycles.

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Competing interests

The authors declare no competing interests.

Author contributions

C.R., P.A., N.F., and M.F. conceived the study. C.R., D.A., and P.A. developed the methodology. C.R. collected the experimental data, with assistance from D.A. Data analysis was performed by C.R., D.A., and D.J.W. The manuscript was written by all authors.

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