Lead transfer into the vegetation layer growing naturally in a Pb-contaminated site

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Abstract The lead was one of the main elements in the glazes used to colour ceramic tiles. Due to its presence, ceramic sludge has been a source of environmental pollution since this dangerous waste has been often spread into the soil without any measures of pollution control. These contaminated sites are often located close to industrial sites in the peri-urban areas, thus representing a considerable hazard to the human and ecosystem health. In this study, we investigated the lead transfer into the vegetation layer (Phragmites australis, Salix alba and Sambucus nigra) growing naturally along a Pb-contaminated ditch bank. The analysis showed a different lead accumulation among the species and their plant tissues. Salix trees were not affected by the Pb contamination, possibly because their roots mainly develop below the contaminated deposit. Differently, Sambucus accumulated high concentrations of lead in all plant tissues and fruits, representing a potential source of biomagnification. Phragmites accumulated large amounts of lead in the rhizomes and, considering its homogeneous distribution on the site, was used to map the contamination. Analysing the Pb concentration within plant tissues, we got at the same time information about the spread, the history of the contamination and the relative risks. Finally, we discussed the role of natural recolonizing plants for the soil pollution mitigation and their capacity on decreasing soil erosion and water run-off.

Keywords Pb · Soil contamination · Phytoscreening · Plant uptake · Pollution spread · Environmental risk

Introduction

The industrial development radically changed the landscape, modifying ecological equilibria and introducing new potential impacts for human health and environment. Among the different impacts produced, soil pollution is a persistent effect of past industrial activities, inadequate waste disposal, mining, military tests or accidents (FAO 2015).
Heavy metals are significant environmental pollutants, and their toxicity represents a problem of increasing significance. Arsenic, cadmium, chromium, copper, lead, nickel and zinc can cause risks for human health and the environment (Jaishankar et al. 2014).

According to the World Health Organization, 0.2% of deaths and 0.6% of DALYs (disability-adjusted life years) can be attributable to the lead exposure (WHO 2009). A considerable health risk may be urban soils in areas with an old habitation history which can be polluted by anthropogenic Pb sources, in particular building materials as tiles or Pb-based paints (Warraven et al. 2016).

Although the lead was banned in several manufacturing processes (European Commission 2007), the presence of this metal in tile glazes still represents today a risk for human health, in particular children’s intellectual function (Jacobs et al. 2002; Lanphear et al. 2005).

An additional exposure hazard was the use of sludge from the ceramic industry with a high Pb concentration as filling material for civil engineering, which is therefore in close contact with natural/agricultural soils (European Commission 2013). The consequence was the soil contamination in the vicinity of these industries (European Commission 2016), which are frequently located in peri-urban areas, thus representing a considerable hazard for the human and ecosystems health.

The spatial survey of heavy metal distribution in contaminated soil represents the first step of the risk assessment (Stewart and Hursthouse 2018), and it is essential to identify the sources of pollution and define appropriate protection and remediation strategies. The vegetation which naturally recolonizes these soils can be used to assess the spatial diffusion of the contamination (Algreen et al. 2014; Yan et al. 2015) thanks to its capacity to uptake heavy metals and translocate them into their tissues (Tangahu et al. 2011; Viehweger 2014).

An additional end up of the vegetation analysis is the possibility, in some cases, to reconstruct the history of contamination and the associated exposure risks. Different functional plant traits (Wullschleger et al. 2014) may be used to characterize polluted sites. A multispecific sampling of plant species with a different growth form allows to get additional information arising from the different species autecology (Brunetti et al. 2009). For example, the root spread/depth combined with the plant age could support the dating of a contamination event. Considering the variability of the uptake and transfer to plant tissues (Thakur et al. 2016; Viehweger 2014), the sampling strategy must be carefully evaluated and properly applied to different plant species according to their ecological characteristic.

Furthermore, it is important to study the vegetation growing in polluted sites to further reduce the risks related to the spread of contamination because plants are the primary producers in the ecosystem food chain and are eaten directly by animals or humans, which may transfer contaminants far from the original sources.

The main objective of this study was to investigate the diffusion of Pb contamination along a ditch bank in a peri-urban area, using the natural vegetation as a proxy of the spatial distribution of the source. In fact, a previous survey carried out by the Regional Agency for Environmental Protection showed lead contamination in the ditch collector downstream a ceramic industry plant.

We analysed three plant species growing over the ditch bank with the aim to (1) assess the Pb transferred into the different plant tissues, (2) investigate the spatial distribution of contamination and (3) evaluate the pros and cons of the vegetation presence in the ditch bank, with regard to both the risk of metal transfer to the food chain and the protection against the substrate erosion responsible for the sediment and water contamination.

Materials and methods

Site description

The study area is located in Gualdo Tadino (43°16’2.09”N, 12°45’20.59”E—Italy) along the creek “Categge”, a natural drainage ditch, for a length of 180 m (Fig. 1). In this area, the Regional Agency for Environmental Protection found a soil sample characterized by Pb concentration 30 times higher than the threshold fixed by the law for industrial areas (Decree Law 152/2006). Considering the site morphology, the accredited hypothesis was that the ceramic sludge was used to consolidate the banks of the local ditch. Among the species that colonized the banks, the three
selected for this study were the common reed (*Phragmites australis*), the willow (*Salix alba*) and the elderberry (*Sambucus nigra*), respectively, an herbaceous perennial species typical of wetlands, a woody tree and a shrub.

We used an aerial orthophotograph time series, provided online by the Umbria Region Geoportal (http://www.umbriageo.regione.umbria.it/), to analyse the land cover evolution of the study area (Fig. 2).

**Soil and plant sampling**

To characterize the Pb contamination in the two main ditch banks present in the site (W–E and N–S directions), five soil cores of 30 cm depth were collected along the two axes (plot 5–6 and 1c–3c) close to the intersection points (Fig. 1). To avoid the effect of possible cross-contamination due to the surface transport of soil particles and due to the plant’s litter, the first 10 cm has been removed.

To investigate the lead transfer into the vegetation layer, we analysed the Pb concentration of different organs sampled from the plants growing inside six 16 m² plots distributed along the “Categge” ditch banks (West–East axis) at a distance of 30 m apart (Fig. 1). We sampled, at the end of spring 2014, leaves and current-year branches of *S. alba* (4 plants, 4 plots), leaves, current-year branches and fruits of *S. nigra* (3 plants, 2 plots) and leaves, culms and rhizomes of *P. australis* (11 samples, all plots). Additional *P. australis* rhizomes were also sampled in the 3 plots (1c–3c) realized along the secondary ditch (North–South axis), resulted from the preliminary characterization as not contaminated (control plots). After collection, rhizomes were washed to remove soil particles: preliminarily under running tap water and then into 500-ml jars filled with 250 ml of deionized water placed in oscillator (3 cycles of 15 min at 400 oscillations per minute). All plant samples were dried at a temperature of 70 °C and then chopped with a blade mill.

The Pb concentrations of samples were determined by ICP-AES (Inductively Coupled Plasma–Atomic emission spectrometry) after a microwave-assisted acid digestion, according to the standard references UNI EN 13805:2002 and UNI EN 14083:2003. The Pb concentrations were then reported on a dry matter basis.

Nonparametric Kruskal–Wallis method was used to compare the differences in Pb concentration among the species and tissues (*p* < 0.05).
Spatial analysis of the Pb contamination

*Phragmites australis*, present in all plots, was selected to analyse the spatial distribution of the contamination. To this purpose, only rhizomes were considered.

The spatial analysis of the Pb concentration in *P. australis* rhizomes was performed by an ordinary kriging interpolation of the spatial positions of sampling points by a GIS software (ArcGIS v.10.5) using a spherical semivariogram model.

Results

Landscape evolution

In the last 50 years, the landscape of the study area changed radically, passing from strictly agricultural land use to the industrial one. The aerial photograph of 1954–1955, available online at the Umbria Region Geoportal, shows that the area was devoid of spontaneous vegetation because of the intense agricultural activity.

In the aerial image of the year 1997 (Fig. 2), it is possible to identify the nucleus of *P. australis* stand colonizing the ditch bank. The growth of the spontaneous vegetation could be related to the land use change of the area, from the agricultural to the industrial one, as attested by the presence of an industrial settlement in the lower right corner of the image. In the year 2000 (Fig. 2), a new industrial settlement appears in the lower central part of the image.

In the image of 2005 (Fig. 2), the herbaceous vegetation and shrubs were removed, while trees were left on the site. We suppose that the removal of the vegetation was associated with the deposit of the ceramic sludge, enriched in Pb, possibly with the objective to increase the height of the bank in order to protect the agricultural field from the seasonal flooding events. After this phase, as shown in the image of 2008 (Fig. 2), herbaceous and shrub vegetation colonized again the ditch bank, covering the contaminated sludge deposit.

Pb concentration in the soil

The results of the soil analyses (Table 1) showed that the main ditch (plots 5–6 W–E direction) was highly
contaminated, while the secondary one not (plots 1c–3c). The values in non-contaminated plots (control) were close to the limit fixed for public use of green areas, while the values of the contaminated plots resulted in the highest range of Pb contamination reported in the literature (Gupta et al. 2013; Rotkit-tikhun et al. 2006).

Pb distribution within the plant tissues

The results of the chemical analysis of the different plant organs showed a wide range of Pb concentrations (Fig. 3).

In *P. australis*, the highest Pb concentration was found in rhizomes, with an average concentration of 415 ± 206 mg kg$^{-1}$, while culms and leaves presented a similar lower Pb content (14.6 ± 17.3 and 7.2 ± 4.3 mg kg$^{-1}$, respectively) (Fig. 3).

*S. nigra* leaves and the branches showed a similar Pb content (8.7 ± 0.9 and 13.7 ± 12.7 mg kg$^{-1}$, respectively), while fruits were characterized by a lower Pb concentration (0.9 ± 0.4 mg kg$^{-1}$) (Fig. 3). *S. alba*, instead, branches showed a higher Pb concentration than leaves, 3.6 ± 0.7 and 2.1 ± 0.7, respectively.

The among-species comparison (within the same tissues) showed a higher Pb content in *S. nigra* than in *S. alba*, for both leaves and branches. *Salix alba* presents, in fact, a lower Pb content than the common Pb concentration (7 mg kg$^{-1}$, red line in Fig. 3) reported for plants (Lambers et al. 2008) (Fig. 3). *Phragmites australis* showed higher values than *Salix alba* in leaves; instead, the differences among the culms/branches were not significant because of the high variability. The rhizome of *P. australis* showed the highest values compared to all the other species/tissues.

Pb distribution in the horizontal layer

The Pb concentration in rhizomes was used to define a map of the contamination degree along the two ditch banks of the study area. Pb concentration of rhizomes presented a high spatial variability (Fig. 4), and the spatial pattern clearly separates the contaminated plots (along the W–E axis) from the non-contaminated plots (along the S–N axis) plots.

Among the contaminated plots, plot 4 shows the highest Pb values moving towards the extremes of the axis; the lead concentration tends to decrease (Fig. 4).

### Table 1: Lead concentration of the soil collected in one point along the banks deposit

| Plot                          | Sampling depth (cm) | Pb (mg kg$^{-1}$) |
|-------------------------------|---------------------|-------------------|
| Control (1c–3c)               | 10–30               | 102 ± 24          |
| Contaminated (5–6)            | 10–30               | 31,809 ± 5743     |

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![Fig. 3](https://example.com/f3.png)
Discussion

The species analysed in this study belong to three plant’s functional types: trees (S. alba), shrubs (S. nigra) and perennial herbaceous species (P. australis). These species show therefore a different size, permanence of structure and architecture that affect their interactions with the contaminants present in the soil (Wullschleger et al. 2014).

These different characteristics may explain the variability observed in the Pb uptake.

Among the species included in this work, the willow has the largest size and, since the lead transfer occurs by the apoplastic pathway, the greater biomass acts as a buffer which decreases the Pb concentration within plant tissues (Pourrut et al. 2013).

Vysloužilová et al. 2003 found similar Pb concentrations in Salix spp. growing on lead-contaminated soil (2029 mg kg\(^{-1}\)): 10–20 mg kg\(^{-1}\) in twigs and 7–27 mg kg\(^{-1}\) in leaves.

Even Tlustoš et al. 2007 reported a similar low Pb content: 1–16 mg kg\(^{-1}\) in twigs and 3–99 mg kg\(^{-1}\) in leaves, with a soil Pb content of 2297 mg kg\(^{-1}\).
The lower Pb level in *S. alba* tissues sampled in this study can be explained from the addition of the sludge deposit after the plant and roots development. In fact, the tree vegetation (including *Salix alba* trees) was growing along the ditch bank before the accumulation of the sludge, and we suppose that their roots do not extensively explore the contaminated deposit, mainly developing below it.

*Salix alba* roots can, in addition, reach greater depths than *S. nigra* and *P. australis*, easily exploring the soil layers below the contaminated sludge (Canadell et al. 1996).

Furthermore, differently from *S. alba*, *P. australis* and *S. nigra* were removed before the sludge disposal on the ditch bank, and they colonized again the area after this disturbance, growing directly on the contaminated deposit.

According to this different growth form and site history, *S. nigra* resulted in higher Pb levels than *S. alba* in both leaves and branches. A small amount of Pb was also transferred to the fruits of *Sambucus* (0.9 ± 0.4 mg kg\(^{-1}\)) resulting in higher value than that reported in the literature (0.2 mg kg\(^{-1}\)) for the plant growing on Pb-contaminated soil (26–42 mg kg\(^{-1}\)) (Al Sayegh Petkovsek et al. 2015). Because the fruits of *S. nigra* are eaten by birds, the presence of this species in contaminated sites can involve a risk of biomagnification (Thakur et al. 2016).

The higher Pb concentration observed in rhizomes (415 ± 206 mg kg\(^{-1}\)), compared to similar lower values found in leaves and culms of *P. australis*, shows how plant species accumulate Pb in the roots and only a small fraction is translocated to aerial parts (Pourrut et al. 2011). In fact, plants make at roots level a physical barrier constituted by the endodermis that plays an important function for the plant tolerance to Pb, accumulating large amounts of lead in the underground organs (Pourrut et al. 2013). The lead translocation to shoots is limited and it is accumulated in the belowground biomass, in particular rhizomes (Vymazal and Brˇezinová 2016).

These results are in accordance with Marchiol et al. (2013) which found a Pb concentration of 150–200 mg kg\(^{-1}\) in rhizomes of *P. australis* growing on a soil with a Pb concentration between 2624 and 7371 mg kg\(^{-1}\).

The ability to store large amounts of Pb in rhizomes, in combination with the low Pb translocation from belowground to aboveground tissues, was also observed in *P. australis* from Windham et al. (2001) and Bernardini et al. (2015). This characteristic represents a suitable trait of the species to be used in the phytoremediation for decreasing the risk of the contamination diffusion into the environment and the metal transfer into the food chain.

*Phragmites australis* is an herbaceous plant able to make very dense stands with a high above- and belowground biomass, respectively, 1.7 kg m\(^{-2}\) and 8 kg m\(^{-2}\) (Tripathee and Schäfer 2014), which is comparable to the yearly biomass production of a *Salix alba* shrub (6.8 kg) (Mleczek et al. 2010). In addition, thanks to its capacity to quickly colonize bare substrates, it can be useful to reduce the soil erosion and water run-off in contaminated sites (Ahmad et al. 2016).

**Conclusion**

This study showed that, among the different species present at the site, the shrub species (*S. nigra*) transferred more Pb to the aboveground tissues with respect to the tree species (*S. alba*), in relation to their morphology and site history. The herbaceous perennial species (*P. australis*) shows similar Pb concentration in the aboveground biomass, but a much higher value in rhizomes.

The phytoscreening analysis based on these root tissues confirmed the soil contamination and allowed to define the spread of soil contamination in the area.

According to the results obtained in this study, the ability of *P. australis* to store a significant amount of Pb in an extensive root system (rhizomes), in combination with the capacity to quickly form extensive stands, suggests that this species can be employed for the in situ stabilization of contaminated soils, in particular for the securing of contaminated materials before their removal. In fact, it can reduce the substrate erosion and the water run-off and the consequent contamination of the surrounding environment.

The analysis of the vegetation naturally growing in the contaminated site allowed to investigate the spread and history of the soil contamination in the nearby area and the health risk associated with Pb transfer to plant tissues.

Furthermore, this information allows selecting the suitable species for phytoremediation applications.
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