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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.

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Keywords (separated by '-') Pb - Soil contamination - Phytoscreening - Plant uptake - Pollution spread - Environmental risk

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Footnote Information

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

4 **Rocco Pace** · **Dario Liberati** · **Paolo Sconocchia** · **Paolo De Angelis**

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the soil pollution mitigation and their capacity on 34  
decreasing soil erosion and water run-off. 35

**Keywords** Pb · Soil contamination · 36  
Phytoscreening · Plant uptake · Pollution spread · 37  
Environmental risk 38

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**Introduction** 39

The industrial development radically changed the **AQI-10** 41  
landscape, modifying ecological equilibria and intro- 42  
ducing new potential impacts for human health and 43  
environment. Among the different impacts produced, 44  
soil pollution is a persistent effect of past industrial 45  
activities, inadequate waste disposal, mining, military 46  
tests or accidents (FAO 2015).

47 Heavy metals are significant environmental pollu- 96  
48 tants, and their toxicity represents a problem of 97  
49 increasing significance. Arsenic, cadmium, chro- 98  
50 mium, copper, lead, nickel and zinc can cause risks 99  
51 for human health and the environment (Jaishankar 100  
52 et al. 2014).

53 According to the World Health Organization, 0.2% 101  
54 of deaths and 0.6% of DALYs (disability-adjusted life 102  
55 years) can be attributable to the lead exposure (WHO 103  
56 2009). A considerable health risk may be urban soils in 104  
57 areas with an old habitation history which can be 105  
58 polluted by anthropogenic Pb sources, in particular 106  
59 building materials as tiles or Pb-based paints (Wal- 107  
60 raven et al. 2016).

61 Although the lead was banned in several manufac- 108  
62 turing processes (European Commission 2007), the 109  
63 presence of this metal in tile glazes still represents 110  
64 today a risk for human health, in particular children's 111  
65 intellectual function (Jacobs et al. 2002; Lanphear 112  
66 et al. 2005).

67 An additional exposure hazard was the use of 113  
68 sludge from the ceramic industry with a high Pb 114  
69 concentration as filling material for civil engineering, 115  
70 which is therefore in close contact with natural/ 116  
71 agricultural soils (European Commission 2013). The 117  
72 consequence was the soil contamination in the vicinity 118  
73 of these industries (European Commission 2016), 119  
74 which are frequently located in peri-urban areas, thus 120  
75 representing a considerable hazard for the human and 121  
76 ecosystems health.

77 The spatial survey of heavy metal distribution in 122  
78 contaminated soil represents the first step of the risk 123  
79 assessment (Stewart and Hursthouse 2018), and it is 124  
80 essential to identify the sources of pollution and define 125  
81 appropriate protection and remediation strategies. The 126  
82 vegetation which naturally recolonizes these soils can 127  
83 be used to assess the spatial diffusion of the contam- 128  
84 ination (Algreen et al. 2014; Yan et al. 2015) thanks to 129  
85 its capacity to uptake heavy metals and translocate 130  
86 them into their tissues (Tangahu et al. 2011; 131  
87 Viehweger 2014).

88 An additional end up of the vegetation analysis is 132  
89 the possibility, in some cases, to reconstruct the 133  
90 history of contamination and the associated exposure 134  
91 risks. Different functional plant traits (Wullschleger 135  
92 et al. 2014) may be used to characterize polluted sites. 136  
93 A multispecific sampling of plant species with a 137  
94 different growth form allows to get additional infor- 138  
95 mation arising from the different species autecology 139  
140

(Brunetti et al. 2009). For example, the root spread/ 96  
depth combined with the plant age could support the 97  
dating of a contamination event. Considering the 98  
variability of the uptake and transfer to plant tissues 99  
(Thakur et al. 2016; Viehweger 2014), the sampling 100  
strategy must be carefully evaluated and properly 101  
applied to different plant species according to their 102  
ecological characteristic. 103

104 Furthermore, it is important to study the vegetation 105  
growing in polluted sites to further reduce the risks 106  
related to the spread of contamination because plants 107  
are the primary producers in the ecosystem food chain 108  
and are eaten directly by animals or humans, which 109  
may transfer contaminants far from the original 110  
sources.

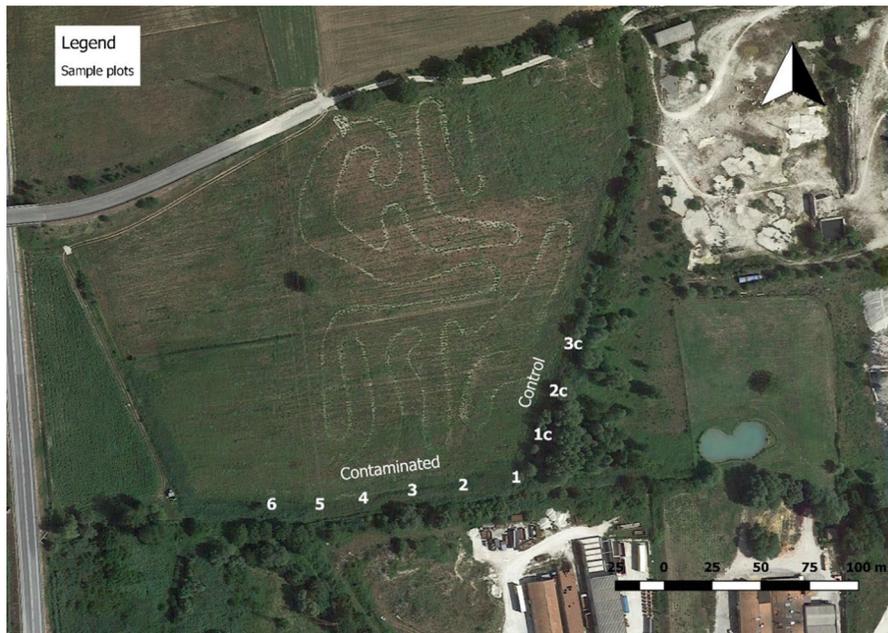
111 The main objective of this study was to investigate 112  
the diffusion of Pb contamination along a ditch bank in 113  
a peri-urban area, using the natural vegetation as a 114  
proxy of the spatial distribution of the source. In fact, a 115  
previous survey carried out by the Regional Agency 116  
for Environmental Protection (ARPA Umbria, unpub- 117  
lished results) showed lead contamination in the ditch 118  
collector downstream a ceramic industry plant.

119 We analysed three plant species growing over the 120  
ditch bank with the aim to (1) assess the Pb transferred 121  
into the different plant tissues, (2) investigate the 122  
spatial distribution of contamination and (3) evaluate 123  
the pros and cons of the vegetation presence in the 124  
ditch bank, with regard to both the risk of metal 125  
transfer to the food chain and the protection against the 126  
substrate erosion responsible for the sediment and 127  
water contamination. 128

## 129 Materials and methods

### 130 Site description

131 The study area is located in Gualdo Tadino 132  
133 (43°16'2.09"N, 12°45'20.59"E—Italy) along the creek 134  
135 "Categge", a natural drainage ditch, for a length of 136  
137 180 m (Fig. 1). In this area, the Regional Agency for 138  
139 Environmental Protection found a soil sample char- 140  
acterized by Pb concentration 30 times higher than the 131  
threshold fixed by the law for industrial areas (Decree 132  
Law 152/2006). Considering the site morphology, the 133  
accredited hypothesis was that the ceramic sludge was 134  
used to consolidate the banks of the local ditch. 135  
Among the species that colonized the banks, the three 136  
137 138 139 140



**Fig. 1** Sampling plots in the experimental site

141 selected for this study were the common reed  
 142 (*Phragmites australis*), the willow (*Salix alba*) and  
 143 the elderberry (*Sambucus nigra*), respectively, an  
 144 herbaceous perennial species typical of wetlands, a  
 145 woody tree and a shrub.

146 We used an aerial orthophotograph time series,  
 147 provided online by the Umbria Region Geoportal  
 148 (<http://www.umbriageo.regione.umbria.it>), to anal-  
 149 yse the land cover evolution of the study area (Fig. 2).

150 Soil and plant sampling

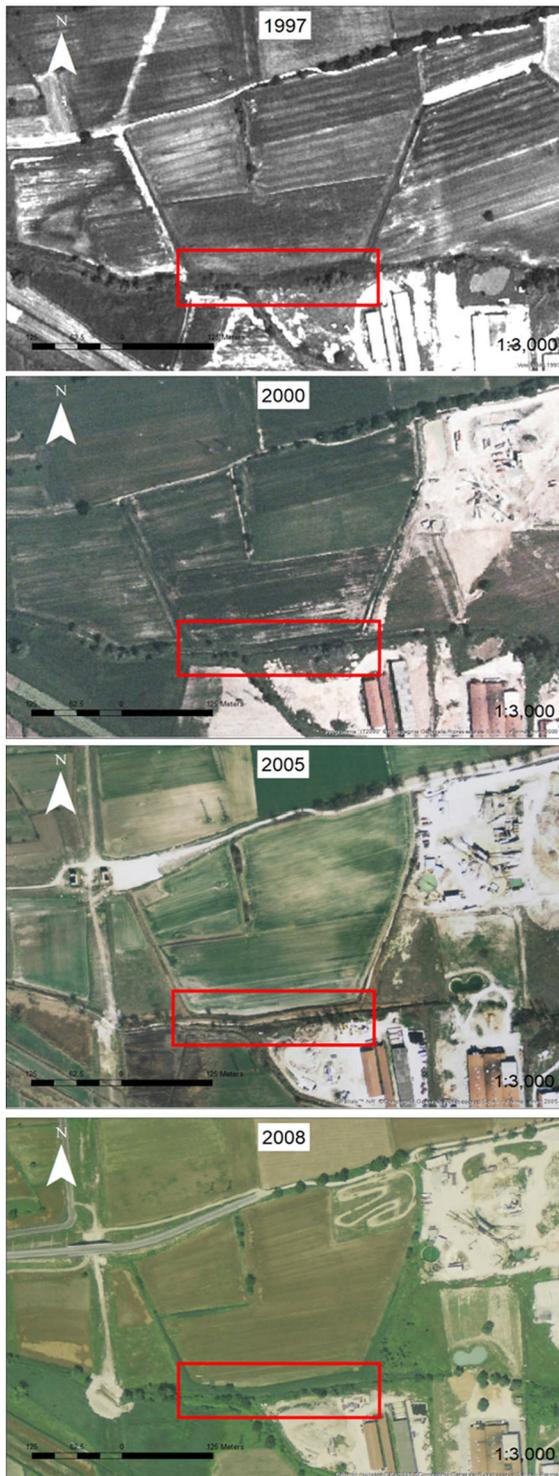
151 To characterize the Pb contamination in the two main  
 152 ditch banks present in the site (W–E and N–S  
 153 directions), five soil cores of 30 cm depth were  
 154 collected along the two axes (plot 5–6 and 1c–3c)  
 155 close to the intersection points (Fig. 1). To avoid the  
 156 effect of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

159 To investigate the lead transfer into the vegetation  
 160 layer, we analysed the Pb concentration of different  
 161 organs sampled from the plants growing inside six  
 162 16 m<sup>2</sup> plots distributed along the “Categge” ditch  
 163 banks (West–East axis) at a distance of 30 m apart  
 164 (Fig. 1). We sampled, at the end of spring 2014, leaves  
 165 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. aus-*  
*tralis* 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 ( $p < 0.05$ ).



**Fig. 2** Orthophotograph time series. In order: 1997, 2000, 2005 and 2008. The red rectangle points out the contaminated axis

Spatial analysis of the Pb contamination 190

*Phragmites australis*, present in all plots, was selected to analyse the spatial distribution of the contamination. To this purpose, only rhizomes were considered. 191  
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193

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. 194  
195  
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**Results** 199

Landscape evolution 200

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 Geoportale, shows that the area was devoid of spontaneous vegetation because of the intense agricultural activity. 201  
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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. 208  
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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. 218  
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Pb concentration in the soil 229

The results of the soil analyses (Table 1) showed that the main ditch (plots 5–6 W–E direction) was highly 230  
231

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

Plot	Sampling depth (cm)	Pb (mg kg <sup>-1</sup> )
Control (1c–3c)	10–30	102 ± 24
Contaminated (5–6)	10–30	31,809 ± 5743

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; Rotkitikhun 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<sup>-1</sup>, while culms and leaves presented a similar lower Pb content (14.6 ± 17.3 and 7.2 ± 4.3 mg kg<sup>-1</sup>, 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<sup>-1</sup>, respectively), while fruits were characterized by a

lower Pb concentration (0.9 ± 0.4 mg kg<sup>-1</sup>) (Fig. 3). *S. alba*, instead, branches showed a higher Pb concentration than leaves, 3.6 ± 0.7 and 2.1 ± 0.7, respectively.

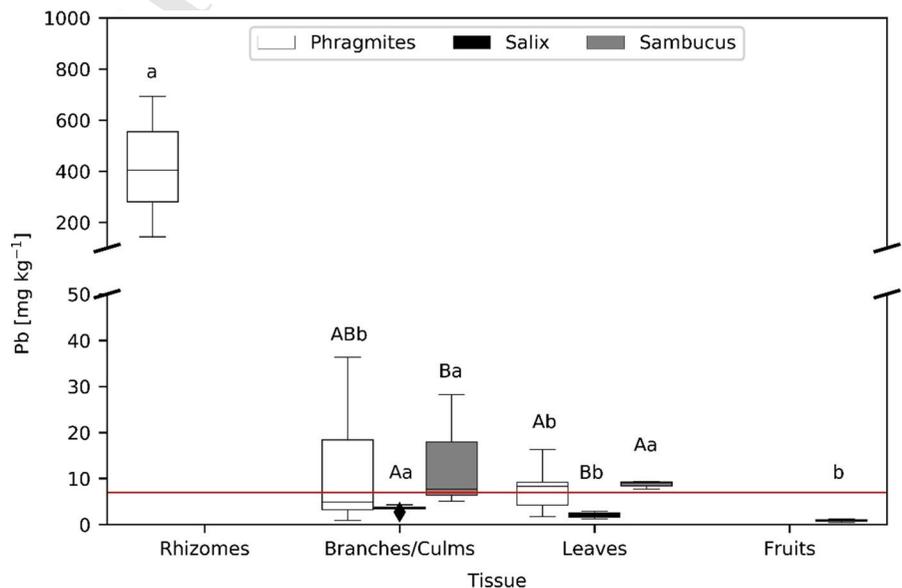
The among-species comparison (within the same tissues) showed a higher Pb content in *S. nigra* than in *S. alba*, for both leaves and branches. *Salix alba* presents, in fact, a lower Pb content than the common Pb concentration (7 mg kg<sup>-1</sup>, red line in Fig. 3) reported for plants (Lambers 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).

**Fig. 3** Lead content in plant tissues. The red line indicates the common Pb concentration in plant tissue, according to Lambers et al. (2008). Within each species, significant differences among the Pb concentration of different tissues are indicated by different lower-case letters; for each tissue (leaves and non-photosynthetic aboveground tissues), differences among-species are indicated by different capital letter

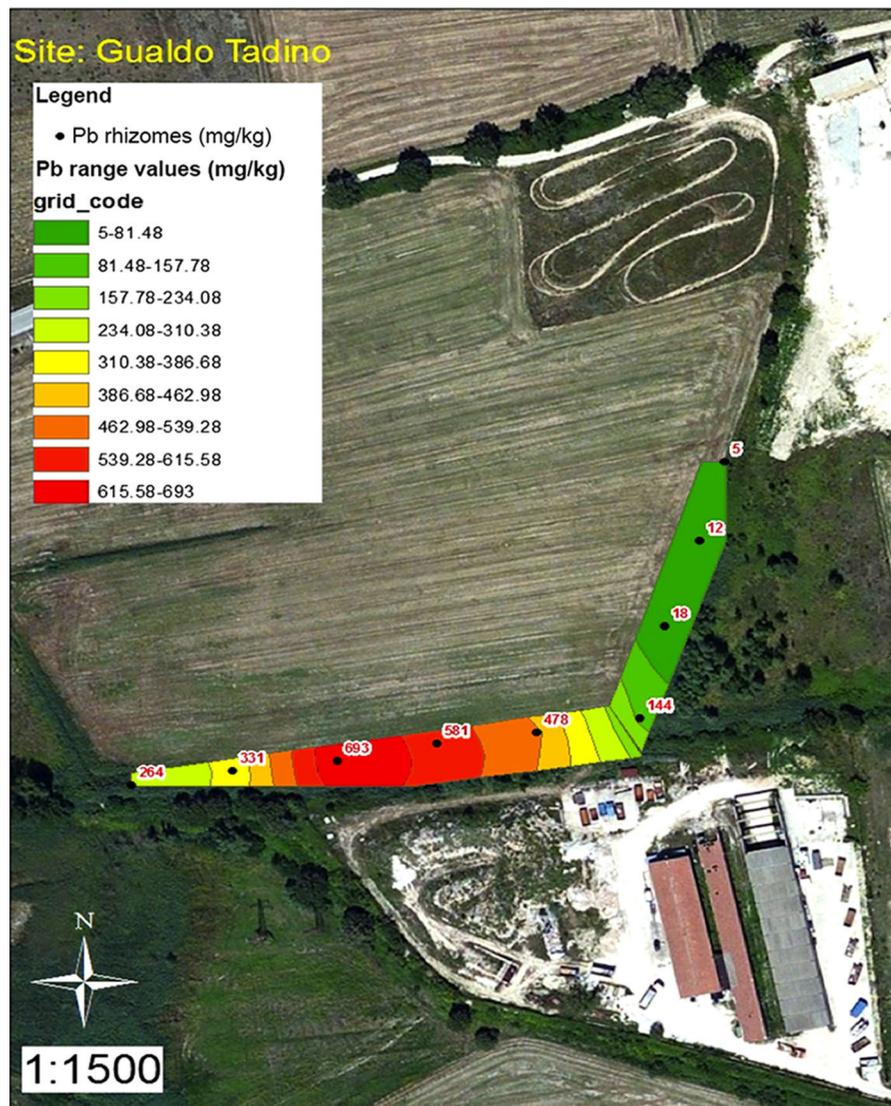


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**Fig. 4** Map shows the average Pb content per plot of *Phragmites australis* rhizomes along the axis East–West and North–South (black dots). The highest Pb values are measured in plot 4

278 **Discussion**

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

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

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

293 Vysloužilová et al. 2003 found similar Pb concentrations  
 294 in *Salix* spp. growing on lead-contaminated soil (2029  
 295 mg kg<sup>-1</sup>): 10–20 mg kg<sup>-1</sup> in twigs and  
 296 7–27 mg kg<sup>-1</sup> in leaves.

297 Even Tlustoš et al. 2007 reported a similar low Pb  
 298 content: 1–16 mg kg<sup>-1</sup> in twigs and 3–99 mg kg<sup>-1</sup> in  
 299 leaves, with a soil Pb content of 2297 mg kg<sup>-1</sup>.

300 The lower Pb level in *S. alba* tissues sampled in this  
 301 study can be explained from the mechanical of the  
 302 sludge deposit and roots architecture. In fact, the tree  
 303 vegetation (including *Salix alba* trees) was growing  
 304 along the ditch bank before the accumulation of the  
 305 sludge, and we suppose that their roots do not  
 306 extensively explore the contaminated deposit, mainly  
 307 **AQ3** developing below it.

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

312 Furthermore, differently from *S. alba*, *P. australis*  
 313 and *S. nigra* were removed before the sludge disposal  
 314 on the ditch bank, and they colonized again the area  
 315 after this disturbance, growing directly on the con-  
 316 taminated deposit.

317 **AQ4** According to this different growth form and site  
 318 history, *S. nigra* resulted in higher Pb levels than *S.*  
 319 *alba* in both leaves and branches. A small amount of  
 320 Pb was also transferred to the fruits of *Sambucus*  
 321 ( $0.9 \pm 0.4 \text{ mg kg}^{-1}$ ), resulting in higher value than  
 322 that reported in the literature ( $0.2 \text{ mg kg}^{-1}$ ) for the  
 323 plant growing on Pb-contaminated soil  
 324 ( $26\text{--}42 \text{ mg kg}^{-1}$ ) (Al Sayegh Petkovsek et al. 2015).  
 325 Because the fruits of *S. nigra* are eaten by birds, the  
 326 presence of this species in contaminated sites can  
 327 involve a risk of biomagnification (Thakur et al. 2016).

328 The higher Pb concentration observed in rhizomes  
 329 ( $415 \pm 206 \text{ mg kg}^{-1}$ ), compared to similar lower  
 330 values found in leaves and culms of *P. australis*,  
 331 shows how plant species accumulate Pb in the roots  
 332 and only a small fraction is translocated to aerial parts  
 333 (Pourrut et al. 2011). In fact, plants make at roots level  
 334 a physical barrier constituted by the endodermis that  
 335 plays an important function for the plant tolerance to  
 336 Pb, accumulating large amounts of lead in the  
 337 underground organs (Pourrut et al. 2013). The lead  
 338 translocation to shoots is limited and it is accumulated  
 339 in the belowground biomass, in particular rhizomes  
 340 (Vymazal and Březinová 2016).

341 These results are in accordance with Marchiol et al.  
 342 (2013) which found a Pb concentration of  
 343  $150\text{--}200 \text{ mg kg}^{-1}$  in rhizomes of *P. australis* growing  
 344 on a soil with a Pb concentration between 2624 and  
 345  $7371 \text{ mg kg}^{-1}$ .

346 The ability to store large amounts of Pb in  
 347 rhizomes, in combination with the low Pb transloca-  
 348 tion from belowground to aboveground tissues, was

also observed in *P. australis* from Windham et al. 349  
 (2001) and Bernardini et al. (2015). This characteristic 350  
 represents a suitable trait of the species to be used in 351  
 the phytoremediation for decreasing the risk of the 352  
 contamination diffusion into the environment and the 353  
 metal transfer into the food chain. 354

*Phragmites australis* is an herbaceous plant able to 355  
 make very dense stands with a high above- and 356  
 belowground biomass, respectively,  $1.7 \text{ kg m}^{-2}$  and 357  
 $8 \text{ kg m}^{-2}$  (Tripathee and Schäfer 2014), which is 358  
 comparable to the yearly biomass production of a *Salix* 359  
*alba* shrub ( $6.8 \text{ kg}$ ) (Mleczek et al. 2010). In addition, 360  
 thanks to its capacity to quickly colonize bare 361  
 substrates, it can be useful to reduce the soil erosion 362  
 and water run-off in contaminated sites (Ahmad et al. 363  
 2016). 364

**Conclusion** 365

This study showed that, among the different species 366  
 present at the site, the shrub species (*S. nigra*) 367  
 transferred more Pb to the aboveground tissues with 368  
 respect to the tree species (*S. alba*), in relation to their 369  
 morphology and site history. The herbaceous perenni- 370  
 al species (*P. australis*) shows similar Pb concen- 371  
 tration in the aboveground biomass, but a much higher 372  
 value in rhizomes. 373

The phytoscreening analysis based on these root 374  
 tissues confirmed the soil contamination and allowed 375  
 to define the spread of soil contamination in the area. 376

According to the results obtained in this study, the 377  
 ability of *P. australis* to store a significant amount of 378  
 Pb in an extensive root system (rhizomes), in combi- 379  
 nation with the capacity to quickly form extensive 380  
 stands, suggests that this species can be employed for 381  
 the in situ stabilization of contaminated soils, in 382  
 particular for the securing of contaminated materials 383  
 before their removal. In fact, it can reduce the 384  
 substrate erosion and the water run-off and the 385  
 consequent contamination of the surrounding 386  
 environment. 387

The analysis of the vegetation naturally growing in 388  
 the contaminated site allowed to investigate the spread 389  
 and history of the soil contamination in the nearby area 390  
 and the health risk associated with Pb transfer to plant 391  
 tissues. 392

Furthermore, this information allows selecting the 393  
 suitable species for phytoremediation applications. 394

Author Proof

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