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Are plants useful as accumulation indicators of metal bioavailability?

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Abstract

The use of accumulation bioindicator to assess metal bioavailability has mainly concerned individual species. This work addresses this issue at the plant community level.

30 Metal content within different species from plant communities found at three contaminated and one uncontaminated site were compared. Results showed that for two contaminated sites, leaf metals concentrations were comparable to those in plants from control site, i.e. approx (mg/kg) 0.1 Cd, 0.2 Cr, 9.2 Cu, 1.8 Ni, 0.5 Pb and 42 Zn. Only plants from the third site showed higher metal contents, ranging from 1.5- to 8-fold those of the control community.

35 This contrasted with ammonium acetate-EDTA extractions, which indicated a very high “availability” of metals at the three sites, as compared to the control site. Thus, metal content in plant communities provided accurate information on actual transfer toward the ensemble of vegetation, which could be used to establish site-specific “fingerprints” of metal bioavailability.

40

Capsule: Plant communities as accumulation bioindicators of trace metal bioavailability in contaminated soils.

Keywords: Soil contamination; Phytoavailability; Bioindication; Metallurgical landfill

45 **1. Introduction**

During the last two decades there has been an increasing awareness of the potential adverse effects of soil pollution by trace metals (Adriano, 2001). As a consequence, assessing soil metal contamination is today of major interest for governments and regulators who are concerned with public health and sustainable development policies (Fairbrother et al., 2007).

50 A number of standardized procedures have been developed for rapid, precise and reliable measurement of total metal content in soils (AFNOR, 1994, 2001, 2002). Nevertheless, it is obvious that metal toxicity in soils depends on complex geochemical and biological interactions (van Gestel, 2008) that cannot be anticipated by the sole knowledge of total content. Thus, it is widely admitted that simply measuring total metal concentrations does not
55 provide enough information for an accurate risk assessment (McLaughlin et al., 2000).

In fact, when studying metal-enriched soils, one of the main questions is whether contaminants can be transferred to living organisms, i.e. whether these metals are bioavailable (Schekel et al., 2009). Indeed, a number of chemical extraction methods have been developed for assessing the likelihood of a metal's bioavailability. Basically, these methods use more or
60 less strong extractants, either in single step protocols (Beckett, 1989, Houba et al., 1990, 1996) or in sequential extraction procedures (Tessier et al., 1989, Quevauvillier et al., 1997). However, although these approaches have brought significant knowledge about interactions between metals and soil components, they seldom can predict the fraction of metal actually interacting with living organisms (McLaughlin et al., 2000, van Gestel, 2008, Wilson et al.,
65 2009, Mourier et al., 2011). Consequently, development of supplemental methods to evaluate the bioavailability of metals remains necessary (Moreno-Jimenez et al., 2011). From this standpoint, the use of bioindicators (Pérès et al., 2011, Le Guédard et al., 2012) is a simple and efficient complementary approach to chemical extraction procedures.

Bioindicators are organisms that help study the level (“accumulation bioindicators”)

70 and/or impact (“effect bioindicators”) of pollutants in the environment. Measuring the content
of metals or other toxic compounds in these living organisms is of great interest to assess the
actual bioavailability of contaminants. During the last decade, a few animal species have been
shown to be efficient accumulation indicators of soil contamination by trace metals, e.g. the
snail *Helix aspersa* (Gomot de Valfleury and Pihan, 2000), earthworm *Eisenia foetida*
75 (*Nahmani et al., 2007*) and collembolan *Proistoma minuta* (*Nursita et al., 2009*). Likewise, the
use of plants as accumulation indicators is a rather old concept, and several species are now
considered as good bioindicators of soil contamination, e.g. *Taraxacum officinale* (*Kuleff and*
Djingova, 1984, Simon et al., 1996), *Capsella bursa-pastoris* (*Aksoy et al., 1999*) or *Populus*
alba (*Madejon et al., 2004*). In fact, when compared to animal bioindicators, plants have a
80 number of advantages. They are sessile organisms and their mineral nutrition is closely
dependant on the soil characteristics. They are ubiquitous organisms colonizing virtually all
terrestrial environments, even those that are highly contaminated. Their classification is well
established and their taxonomic recognition is often unproblematic. Lastly, plants are easy to
collect and generally produce enough biomass to allow chemical analyses. However, using a
85 single plant species to assess metal bioavailability is open to criticism (*Mertens et al., 2005*).
This is mainly because metal content in plants can be both element- and species-dependent
(*Baker, 1981, Harada and Hatanaka, 2000, Broadley et al., 2001*). Thus the indication of
metal bioavailability by plants needs further investigation before being adopted in routine
assessments of soil quality.

90 In this work, we postulated that performing metal analyses for communities of plants,
rather than a single species evaluation, could be a more comprehensive and effective method
to judge contaminated soils with respect to metal bioavailability. To assess this approach,
metal concentrations in a set of different plant species representative of local plant
communities from three contaminated sites, and an uncontaminated control site, were

95 measured. Metal bioavailability, as estimated by phytoaccumulation data, was compared with
“metal potential mobility” determined by a chemical extraction procedure. Results are
discussed in relation to the concept of bioavailability in the area of risk assessment for
contaminated soils.

100 **2. Materials and method**

2.1. Site descriptions

Three former metallurgical landfills of varying ages were studied. The first one (“Dor”
site) was situated near Saint-Etienne (Loire, France). This foundry waste site extended over
approximately 2 ha with a dense plant cover with many trees and bushes. Although there was
105 not much historical data on this site, according to the tree sizes and the vegetation cover we
assumed it had been abandoned for at least 60 years. The second landfill (“Lay” site) was
located in the same commune, but about 5 km away. It had been used from about 1916 to the
beginning of the sixties and was subsequently abandoned. The Lay’s entire area extended
over 4.5 ha with a relatively even vegetation cover with grasses, shrubs and trees (Remon et
110 al., 2005). The third landfill (“Usi” site) was located near Lyon (Rhône, France), about 30 km
away from the two other sites. The landfill was just behind a steel and iron factory which was
still active. The Usi landfill had been used from about 1850 to 2001, and extended over nearly
15 ha. The vegetation cover on this landfill was not homogeneous: some areas were still
periodically disturbed by heavy vehicle traffic and were scarcely colonized by plants or not at
115 all. However, there were other undisturbed areas (perhaps for several years) that showed
greater plant diversity with relatively homogeneous vegetation cover, mainly constituted of
ruderal and pioneer species.

In addition to the three metallurgical landfills, an uncontaminated natural site (Nat site)
was included in the study, as a soil control.

120

2.2. Soil analysis

For each site, 9 to 19 random soil samples were collected at a depth of 0 to 20 cm, in vegetated places. Samples were dried at 60°C for 48 h and sieved to 2 mm before analysis.

125 Measurements of pH values were carried out in deionised water with a soil/water ratio of 1:2 following the NF ISO 10390 procedure (AFNOR, 2005). Metal extraction was conducted with aqua regia according to the NF X 31-151 procedure (AFNOR, 1994) for “pseudo-total fraction” (i.e. considering metals in silicates are not leachable), and with ammonium acetate–EDTA according to the NF X 31-120 procedure (AFNOR, 2003) for “potentially available fraction”.

130 Concentrations of metals in various extracts were measured by inductively coupled plasma optical emission spectrometry (ICP-OES) using a Horiba Jobin-Yvon apparatus.

2.3. Plant analysis

135 For each site studied, a botanical survey of the plant community was carried out and the 8 to 10 most abundant plant species were identified (Tab. I). For the Usi site, only undisturbed areas, with an homogeneous plant cover, were studied. Leaf samples of each species were collected from 6 to 10 randomly chosen locations over the study areas, resulting in a total of 48 to 90 leaf samples from each site. Plant sampling was performed in the beginning of June.

140 Leaf samples were thoroughly washed in tap water and rinsed with distilled water. They were then dried at 40°C to constant weight and ground up to pass through a 2 mm sieve. Metal extraction was effectuated by digestion using hot HNO₃, according to Zarcinas et al. (1987). Metal concentrations in the extracts were measured by ICP-OES.

2.4. Accuracy and quality control of metal analysis

145 The detection limits for metal analysis by ICP-OES were 0.06, 0.14, 1.17, 2.05, 2.38 and
15.51 $\mu\text{g l}^{-1}$ for Zn, Cd, Cr, Cu, Pb and Ni, respectively. The analytical precision was checked
by measuring in triplicate about 20% of the samples. The relative standard deviation routinely
was between 1 and 8%, and never higher than 10%.

For the quality assurance of soil analysis for total metals, certified reference soils NCS
150 DC 73323 and 73006 from the China National Analysis Center for Iron and Steel were used.
They were also included in the batch of samples submitted to aquae regia extraction. Average
recoveries (n=6) were 83, 73, 76, 85, 104 and 99% for Zn, Cd, Cr, Cu, Pb and Ni,
respectively.

For the quality assurance of plant analysis, the certified reference CTA-OTL-1 (Oriental
155 Tobacco Leaves from the Bulgarian Institute for Plant Protection) was employed. Average
recoveries (n=6) were 98, 87, 110, 97, 106 and 103% for Zn, Cd, Cr, Cu, Pb and Ni,
respectively.

2.5. Statistical analysis

160 Because of the non-normal distributions of both metal contents in soils and in plants, we
used the median and the median absolute deviation (MAD) as descriptors of the central
tendency and dispersion of data distributions. Inter-group comparisons were performed using
the distribution-free Kruskal-Wallis test. Post-hoc comparisons were done, when necessary,
by pair wise Wilcoxon rank sum tests using the Holm's *p*-value-adjustment method. All
165 calculations were performed using the R 2.13.0 program (R Development Core Team, 2010).
Differences were considered significant if $p < 0.05$.

3. Results

3.1. "Pseudo-total" and "potentially available" metal concentrations in metallurgical soils

170 Table II presents pH values and “pseudo-total” metal contents of the soils studied. An
overview of these results show that a first clear-cut difference between metallurgical and
natural soils resided in soil pH. Median values ranged between 7.9 and 8.3 for the
metallurgical landfills, in other words “moderately alkaline”. It was 3.9 for the natural control
soil, or “extremely acidic”. Evidently, as expected, soils from metallurgical landfills had
175 much higher metal contents than the natural soil. This was particularly noticeable for Ni, Cr
and Cu whose median concentrations were respectively 14- to 71-fold, 10- to 54-fold and 8-
to 26-fold higher in metallurgical soils than in the control. Although to a lesser extent, soils
from metallurgical landfills were also clearly contaminated with Pb and Zn (median levels
respectively 4.5- to 15-fold and 2- to 10-fold higher than in the natural soil). For Cd, only the
180 Usi site showed a very high contamination, while the Dor and Lay sites were not significantly
different from the control, despite a few extreme values in some instances.

Results presented in Table II also show that “pseudo-total” metal contents in
metallurgical soils were highly heterogeneous, as indicated by the MAD values which were
always very close or even higher than the medians. This high variability, combined with the
185 relatively low number of samples collected on each site (n=12 to 19) and the use of
distribution-free statistics, makes it difficult to determine if significant differences exist
between the three metallurgical sites. Nevertheless the Lay site had overall lower metal
content than the Dor and Usi sites. Furthermore, at least for Cr and Ni these differences were
statistically significant. Likewise, the Usi site had significantly higher Cd and Cu levels than
190 the Dor and Lay sites. It had also a higher Zn content, but this divergence was only significant
in comparison with the Lay site.

When “potentially available” metal contents (ammonium acetate-EDTA extractable
fractions) were considered, the patterns of contamination were slightly altered (Tab. III).
Despite the very high “pseudo-total” metal concentrations in the metallurgical soils, their

195 ammonium acetate-EDTA extractable metal fractions were relatively low and, by comparison with the Nat site no significant difference was evidenced for Cd, Cr and Ni. In fact, only Cu and Zn were significantly higher in the three metallurgical soils, with levels respectively 7- to 10-fold and 3- to 26-fold those of the natural site. For Pb, only the Lay site showed significantly higher extractable levels than the control site.

200 Comparisons between the three metallurgical soils (Tab. III) confirmed the trend previously observed for pseudo-total contents, indicating that the Usi site had overall higher amounts of available Cd, Cu and Zn. However, this difference was not statistically significant for Cu. They also confirmed the great variability in extractable metal content as shown by the high values for MAD.

205

3.2. Metal accumulation by plants

Metal build up in plants was measured in leaf samples taken from the eight to ten most abundant species representative of the plant community observed on each site. For every selected species six to ten separate samples were collected at various locations over the sites and analyzed for their metal content. Results are shown in figure 1.

210 As a general rule, leaf metal concentrations were relatively variable from one species to the next on a given site. This was particularly marked for the metallurgical landfills (Fig. 1, Dor, lay, Usi) where several species or groups of species were systematically separated from the others because of their different level in one or several metals. On the control site, differences in metal content between species were less obvious (Fig. 1, Nat) and, except for Cd, they were not statistically significant. Anyway, because of high inter-specific variability on contaminated sites, it was obvious that metal contents in an individual species couldn't be considered as representative of metal transfer toward the ensemble of vegetation as a whole.

To overcome this problem, the overall data of leaf metal contents in each species were

220 pooled, to get an overview of metal transfer toward the plant community that was
representative for each site. Results (Tab. IV) demonstrated that Cr content in the plant
communities from de Dor, Lay and Usi sites were respectively 3.5-fold, 4.9-fold and 8.3-fold
higher than that measured in the Nat site. Concerning the other metals, the plant communities
from the “Dor” and “Lay” sites had not significantly higher contents than that of the “Nat”
225 site. Surprisingly, plant communities from the “Dor” and “Lay” sites had even significantly
lower contents in Cd, Cu, Pb and Zn than the community from the natural uncontaminated
site. Contrarily, plant community originated from the “Usi” site showed higher leaf
concentrations in all metals but Cd (no significant difference with the Nat site) by comparison
with the other studied sites. Aforementioned, this was particularly marked for Cr, but also for
230 Ni whose foliar concentration was about 6-fold higher in the plant community from the “Usi”
site than in the community from the “Nat” site.

4. Discussion

The concept of metal bioavailability is rather complicated (Semple et al., 2004), and it is
235 virtually impossible to give it a clear and precise definition (Harmsen, 2007). Nonetheless, it
is recognized today that bioavailability of metals in soil is a dynamic process (Peijnenburg et
al., 1997, Lanno et al., 2004, Hodson et al., 2011) involving at least two distinct notions: the
stability of thermodynamic equilibriums of metals at the water/solid phases interface, also
called “bioaccessibility” or “environmental availability”, and the physiological uptake
240 processes by target organisms, also called “bioavailability” or “environmental bioavailability”
(Semple et al., 2004, Harmsen, 2007). Consequently, by integrating these two aspects,
bioavailability can be operationally defined in terms of internal concentrations in living
organisms, which is indeed an important issue in the area of risk assessment.

From this point of view, the aim of this work was to implement a methodology based on

245 vegetation analysis, to assess metal bioavailability in contaminated soils. We assumed that
analysing a set of differing plant species that were representative of the plant community at
the study sites was an efficient strategy to obtain a general insight into metal bioavailability.
To test this assumption we studied metal contents in soils (both in their “pseudo-total” and
“extractable” forms) and in native vegetation from three metallurgical soils of various ages
250 and one natural uncontaminated soil.

4.1. Metal concentrations in plant communities from foundry waste sites are not related
to “pseudo-total” or “extractable” metals in soil

Determination of pseudo-total metals concentrations in soils from foundry waste dumps
255 showed they were highly contaminated, with metal levels ranging from 2- to 70-fold those
measured in the control natural soil. Moreover, measurements of metal concentrations in the
ammonium acetate-EDTA extractable fractions suggested that significant amounts of Cu, Zn
and, in a lesser extent Pb, were highly “available”. This was particularly noticeable for Cu,
whose extracted levels from the three metallurgical soils were even higher than total contents
260 normally found in unpolluted soils (median value in French soil approx. 13 mg/kg, Baize,
2000). At the opposite end, despite very high pseudo-total concentrations, Cd, Cr and Ni
appeared to be no more “available” in the contaminated soils than in the natural soil. Thus,
while results of aquae regia extractions indicated that the metal contaminated soils were
clearly distinct from the natural soil because of their very high total metal contents, results of
265 ammonium acetate – EDTA extractions pointed out that only Cu, Zn and Pb should pose a
risk because of their high availability. Conversely, the very low content of Cr, Cd and Ni in
the extractable fractions suggested a very low availability of these metals and consequently, a
low risk of transfer.

In view of these results we expected to find higher contents in Cu, Pb and Zn, and lower

270 contents in Cd, Cr and Ni in native plants colonizing metallurgical sites when compared to
plants from natural site. However we did not find any clear relationship between leaf metal
concentrations and soil's levels. For instance, plants from the Lay and Dor sites had
significantly lower concentrations in Cd, Cu, Pb and Zn than those from the Nat site, albeit
275 soils from metal waste dumps had significantly higher contents in these metals, both in total
and extractable fractions. Conversely, plants from metallurgical sites had significantly higher
Cr contents than those from the natural site although Cr "availability", as estimated by
ammonium acetate-EDTA extraction, was about the same in metallurgical and natural sites.
Calculations of Kendall's τ coefficients correlation and associated p-values confirmed that,
whatever the metal considered, there was no correlation neither between pseudo total metal
280 contents in soils and metal contents in plants ($\tau < 0.6$; $0.17 < p < 0.62$), nor between extractable
metals in soils and metal in plants ($\tau < 0.3$; $0.37 < p < 0.83$). Thus, as already emphasized by a
number of authors for different types of soil and contamination levels (McLaughlin et al.,
2000, Murphy et al., 2000, Remon et al., 2005, van Gestel, 2008, Mourier et al., 2011, Lopes
et al., 2012), these results confirm that neither "pseudo-total" soil concentrations nor
285 "extractable" concentrations give enough information to foresee actual metal bioavailability
on contaminated soils.

It must be noticed that extraction methods using chelating agents, such as EDTA or
DTPA, were first developed (Viro, 1955, Lindsay and Norvell, 1978) to assess trace metal
phytoavailability in agricultural unpolluted soils, with the aim of detecting potential
290 micronutrient deficiencies. In others words, although these methods are routinely used for
extracting metals from contaminated soils (Beckett, 1989, Ure, 1996), they have inherently
not been designed to assess metals bioavailability in highly polluted matrices.

4.2 Bioavailability of metals is lower in metallurgical industrial sites abandoned earlier

295 In this work we demonstrated that plant communities from the different study sites had differing metal content, suggesting differences in metal bioavailability. Comparison with the control Nat site lead to the conclusion that for the Dor and Lay sites the bioavailability of all metal but Cr, is equal or lower than that observed on a natural uncontaminated soil. At the opposite end of the spectrum, for the Usi site the bioavailability of all metals, except for Cd, is

300 significantly higher. It is interesting to note that two out the three metal contaminated soils exhibit a low metal bioavailability while the third one, albeit of very similar origin, displays a high bioavailability. The goal of this work was not to study the underlying physico-chemical or biological differences between the sites considered, but simply to compare metal bioavailability. However, because the main differences between the three metallurgical

305 landfills reside in the age of the deposits and in the degree of development of the plant cover, we can suppose that both these factors may be related to the decrease in metal bioavailability. Actually, with time, beside the progressive leaching of the most labile metals, both weathering and plant development lead to an increase in clay and organic matter content, which are essential components controlling metal speciation. Thus, slow and progressive

310 pedogenic processes occurring on metallurgical waste deposits could play a key role in the decrease of “environmental availability” of metals. Also, it is known for a long time (Antonovics et al., 1971, Baker, 1987) that the selection pressure exerted by metals on contaminated sites may promote the emergence of tolerant races or ecotypes from normal populations (McNair, 1993, Remon et al., 2007). Although there are numerous strategies to

315 cope with excess metals (Baker, 1981), it is possible that the plant communities colonizing older metallurgical dumps have evolved towards a capacity to exclude metals, due to a natural increase in the number of excluder species (i.e species which maintain low leaf metal content under a wide range of soil concentrations, by preventing either metals uptake or their

translocation from root to shoot). Thus, such a selection of excluder species would lead to a
320 decrease in the “environmental bioavailability” of metals. Consequently, ageing and
successional changes in plant communities (Wang et al., 2011) could promote a decrease in
both environmental “availability” and “bioavailability”, hence leading to a decrease in metal
bioavailability as a whole.

325 4.3 Metals content in plant communities is a “chemical fingerprint” of metal
bioavailability

For an evaluation of soil quality, it is important to have reference values (i.e. values for
the metal content from plants growing in uncontaminated soil) to interpret field data. The
concept of reference values for plant mineral content was first introduced by Höhne (1962)
330 and Duvigneaud and Denaeys-de Smet (1970) with respect to macronutrients contents in
plants (Ca, Mg, N, P, K). This concept was brought a step further by Markert (1992) who
proposed, by analogy to the “Reference Man” established by the International Commission on
Radiological Protection, to define the “Reference Plant”, i.e. average content of all the
inorganic elements found in plants. Thus according to Markert (1992), the following values
335 could be considered for leaf metal concentrations in plants from uncontaminated
environment: 0.08 mg kg⁻¹ Cd, 1.50 mg kg⁻¹ Cr, 8.90 mg kg⁻¹ Cu, 1.50 mg kg⁻¹ Ni, 0.84 mg
kg⁻¹ Pb and 40.60 mg kg⁻¹ Zn. It is noteworthy that these values are in close agreement with
those of Harada and Hatanaka (2000) suggested for Japanese plants. These reference values
can thus be used to establish the baseline of the “chemical fingerprint” (Markert, 1992) for the
340 native vegetation for any kind of soil.

To illustrate this approach, figure 2 shows the relative deviations (RD) in metals content
of plants from the sites studied in this paper, in comparison with the Markert’s “Reference
Plant”. Plants taken from the “Nat” site are very similar to the “Reference Plant”, with relative
deviations in terms of metal concentrations varying between -85 and +50%. Likewise, plants

345 originating from the “Lay” and “Dor” sites are closely related to the “Reference Plant”, with
slightly lower or higher leaf metal levels ($-92\% < RD < +27\%$). This confirms that their metal
bioavailability is low, and anyway not significantly higher than in a normal situation, despite
very high total and extractable levels in soils. Conversely, plants from the “Usi” site show
350 systematically higher metal contents than the “Reference Plant”, resulting in positive values
of the RD ($+22\% < RD < 550\%$) for all the analyzed metals. This is particularly noticeable for
Ni, whose relative leaf concentration is 550% with respect to “Reference Plant”. This clearly
confirms that metal bioavailability is dramatically higher in the “Usi” soil than in normal
uncontaminated soil.

355 **5. Conclusion**

There is no doubt today (AFNOR, 2008) that there is a need for developing
complementary methods to chemical extraction procedures, which could be used to monitor
soil’s quality with respect to metal bioavailability. In this context we hypothesized that plant
communities living on contaminated sites might be suitable accumulation bioindicators.

360 Results presented in this study illustrate that analysis of the ensemble of vegetation as a whole
indeed clearly differentiated the studied sites and provided a characteristic fingerprint of metal
bioavailability.

It must be kept in mind that such an evaluation of bioavailability gives information
about real field situations, i.e the equilibrium state between the abiotic and biotic
365 compartments at a given time. Consequently any modification of one or the other of these
compartments, either due to natural changes or for site redevelopment purposes, can alter the
bioavailability of metals and thus, their fate in the environment, in such a way that cannot be
anticipated.

Also, it is obvious that the accuracy of the results is closely dependent on the choice of

370 the “Reference Plant”. In this work we used the data of Markert (1992) but in a risk
assessment procedure these values could be revisited at a country or even regional scale.
Whatever the reference value is, our results show that the analyses of plant communities, in
terms of metal contents, could be a useful additional tool to complete soil chemical analyses,
to more accurately evaluate metal bioavailability.

375 Evidently, the small number and types of sites studied, means that an expansion of this
approach to other areas and differing polluted sites is an excellent path for further research.
Another question that merits examining, concerns the respective effects of soil ageing and
successional changes in plant communities, in the decrease (or increase) in the bioavailability
of metals on vegetated contaminated sites.

380

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Table I: selected species representative of the plant communities collected on the three metallurgical landfills (Dor, Lay and Usi) and on the control uncontaminated site (Nat). 0 or 1 indicates presence or absence.

Species	Family	Code name*	Site			
			Dor	Lay	Usi	Nat
<i>Acer platanoides</i>	Aceraceae	ACPL	1	1	0	0
<i>Acer pseudoplatanus</i>	Aceraceae	ACPS	0	0	0	1
<i>Ailanthus altissima</i>	Simaroubaceae	AIAL	0	0	1	0
<i>Artemisia campestris</i>	Asteraceae	ARCA	0	1	0	0
<i>Chelidonium majus</i>	Papaveraceae	CHMA	1	0	0	0
<i>Clematis vitalba</i>	Ranunculaceae	CLVI	1	0	0	0
<i>Cornus sanguinea</i>	Cornaceae	COSA	0	1	0	0
<i>Crataegus monogyna</i>	Rosaceae	CRMO	0	1	0	1
<i>Elytrigia campestris</i>	Poaceae	ELCA	0	1	0	0
<i>Galium aparine</i>	Rubiaceae	GAAP	0	1	0	0
<i>Geranium pyrenaicum</i>	Geraniaceae	GEPY	1	0	0	0
<i>Hedera helix</i>	Araliaceae	HEHE	1	0	0	1
<i>Lactuca serriola</i>	Asteraceae	LASE	0	0	0	1
<i>Lactuca virosa</i>	Asteraceae	LAVI	0	0	1	0
<i>Melilotus albus</i>	Fabaceae	MEAL	0	1	1	0
<i>Oenothera biennis</i>	Onagraceae	OEBI	0	0	1	0
<i>Plantago arenaria</i>	Plantaginaceae	PLAR	0	0	1	0
<i>Populus nigra</i>	Salicaceae	PONI	0	0	1	0
<i>Reseda lutea</i>	Resedaceae	RELU	0	0	1	0
<i>Robinia pseudoacacia</i>	Fabaceae	ROPS	1	1	0	1
<i>Rubus sp.</i>	Rosaceae	RUSP	1	1	0	1
<i>Scrophularia canina</i>	Scrophulariaceae	SCCA	0	0	1	0
<i>Taxus baccata</i>	Taxaceae	TABA	1	0	0	0
<i>Teucrium scorodania</i>	Lamiaceae	TESC	0	0	0	1
<i>Urtica dioica</i>	Urticaceae	URDI	0	0	0	1
<i>Verbascum densiflorum</i>	Scrophulariaceae	VEDE	0	0	1	0
<i>Vicia sativa</i>	Fabaceae	VISA	0	1	0	0

*Code names refer to Figure 1.

Table II: pH and “pseudo-total” metal contents (extracted with aqua regia) in soils from three metallurgical landfills (Dor, Lay and Usi) and one control uncontaminated site (Nat). n=12, n=15, n=19 and n=3 for Dor, Lay, Usi and Nat, respectively. Metal contents are in mg.kg⁻¹

540 DW.

Site	Distribution parameter	pH	Cd	Cr	Cu	Ni	Pb	Zn
Dor	10 th centile	7.1	bdl	292	52	144	23	46
	Median	8.3^a	0.3^a	1623^a	208^a	1357^a	178^{a,b}	298^{a,b}
	90 th centile	8.5	71.2	7221	1120	5146	3159	9222
	MAD	0.4	0.5	1937	230	1704	224	315
Lay	10 th centile	7.7	bdl	106	59	68	171	78
	Median	7.9^a	bdl	305^b	158^a	260^b	581^a	206^a
	90 th centile	8.1	2.2	662	203	503	1513	394
	MAD	0.2	0.0	225	61	219	606	128
Usi	10 th centile	7.7	6.7	530	149	327	71	139
	Median	8.1^a	14.0^b	1392^a	502^b	755^a	601^a	1152^b
	90 th centile	8.8	83.7	3900	1602	1705	12018	7740
	MAD	0.4	10.3	1171	321	460	657	1440
Nat	10 th centile	3.9	0.2	31	19	17	38	103
	Median	3.9^b	0.2^a	33^c	19^c	19^c	39^b	108^a
	90 th centile	4.1	0.4	34	19	19	39	110
	MAD	0.0	0.0	2	0	0	1	3

*In a given column, values followed by the same letters are not significantly different at p<0.05

545 **Table III:** “potentially available” metal contents (extracted with ammonium acetate-EDTA) in soils from three metallurgical landfills (Lay, Dor and Usi) and one control uncontaminated site (Nat). n=12, n=15, n=19 and n=3 for Dor, Lay, Usi and Nat, respectively. Metal contents are in mg.kg⁻¹ DW.

Site	Distribution parameter	Cd	Cr	Cu	Ni	Pb	Zn
Dor	10 th centile	0.07	0.08	13.6	4.1	0	16
	Median	0.20^{a,b}	0.97^a	25.4^a	8.7^a	9^a	30^{a,b}
	90 th centile	29.67	3.95	101.8	48.9	75	706
	MAD	0.16	0.64	16.7	9.2	14	29
Lay	10 th centile	0.01	0.02	11.7	1.5	46	10
	Median	0.22^a	0.02^b	29.5^a	3.6^b	298^b	20^a
	90 th centile	0.33	0.51	37.3	6.0	1248	53
	MAD	0.07	0.00	7.3	2.3	328	11
Usi	10 th centile	0.10	0.19	10.6	1.6	11	6
	Median	0.58^b	0.26^a	39.2^a	4.3^{a,b}	109^{b,c}	156^b
	90 th centile	11.30	1.13	86.0	12.7	2684	544
	MAD	0.64	0.09	35.3	4.4	80	166
Nat	10 th centile	0.11	0.40	3.7	1.2	14	5
	Median	0.12^{a,b}	0.46^{a,b}	3.8^b	1.4^{a,b}	16^{a,c}	6^c
	90 th centile	0.15	0.52	4.1	1.9	22	6
	MAD	0.01	0.10	0.1	0.3	3	1

550 *In a given column, values followed by the same letters are not significantly different at p<0.05

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Table IV: Metal contents in leaves of plant species representative of communities grown on three metallurgical landfills (Lay, Dor and Usi) and one control uncontaminated site (Nat). Number of representative species were 8 (Dor), 10 (Lay), 9 (Usi) and 8 (Nat). Number of leaf samples were n=80, n=100, n=88 and n=48 for Dor, Lay, Usi and Nat, respectively. Metal contents are in mg.kg⁻¹ DW.

Site	Distribution parameter	Cd	Cr	Cu	Ni	Pb	Zn
Dor	10 th centile	0.04	0.22	1.72	0.63	bdl	11.76
	Median	0.07^a	0.70^a	3.31^a	1.91^a	0.07^a	23.59^a
	90 th centile	0.33	2.29	6.19	10.99	0.26	59.40
	MAD	0.03	0.47	1.54	1.73	0.11	11.43
Lay	10 th centile	0.04	0.30	1.70	0.23	bdl	11.64
	Median	0.06^b	1.07^b	3.64^a	0.72^b	0.12^b	20.22^b
	90 th centile	0.16	4.03	6.83	3.30	0.87	31.64
	MAD	0.02	0.98	1.54	0.68	0.15	9.93
Usi	10 th centile	0.00	0.52	7.44	1.49	bdl	32.38
	Median	0.15^c	1.83^c	11.84^b	9.75^c	1.27^c	62.40^c
	90 th centile	0.62	8.00	20.15	60.84	3.81	427.12
	MAD	0.14	1.62	4.17	12.36	1.16	44.63
Nat	10 th centile	0.06	0.12	6.13	0.88	0.31	19.82
	Median	0.12^c	0.22^d	9.20^c	1.80^a	0.53^d	41.70^d
	90 th centile	1.76	0.37	13.42	4.03	0.66	116.60
	MAD	0.09	0.10	2.34	1.23	0.13	30.79

*In a given column, values followed by the same letters are not significantly different at p<0.05

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Figure caption

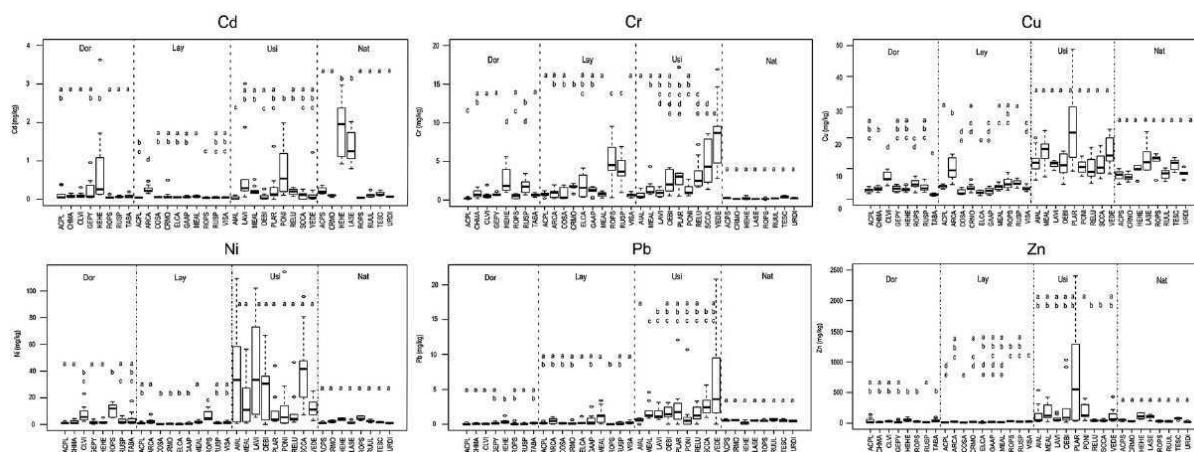
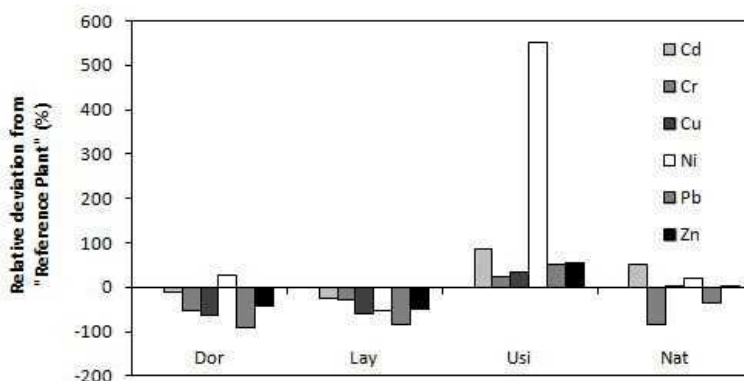


Figure 1: Distribution of metal content in the vegetation of three metallurgical sites (Lay, Dor, Usi) and one control uncontaminated site (Nat). Measured were performed on leaves taken from the 8 to 10 most abundant species of each site, 10 individuals were sampled for each species. For each site, values with the same letter are not significantly different at $p=0.05$. For code names, see Table I.



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Figure 2: Fingerprints of metals contents of plant communities from three metallurgical landfills (Lay, Dor and Usi) and one control natural site (Nat). Results expressed as relative deviation from the “Reference Plant” of Markert (1992).