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Highlights

- The floristic composition of a wasteland was determined under heterogeneous levels of trace element contamination.
- Trace element contamination reduced biodiversity by selecting tolerant species.
- The dominant species, *Alliaria petiolata*, is a potential Zn bioindicator with the highest leaf Zn, Ni and Cd uptake.
- Pb and As are the elements that presented the greatest risk to human health in the case of accidental soil ingestion.

Abstract:

This study aimed to determine the trace element accumulation in the soil and plants in an industrial wasteland and to estimate the extent of transfer to humans to measure the effects on and risks to vegetation and human health and find bioindicator plants representative of the levels of the main contaminants. In areas with the highest extractable trace element levels, we observed decreases in plant biodiversity explained by the disappearance of several families, favouring the coverage of tolerant species, such as *Urtica dioica* and *Hedera helix*. Trace elements were also found in the leaves of several plants, especially in a dominant species that is poorly studied, *Alliaria petiolata*. Indeed, this species had the highest contents of Zn (1750 $mg.kg^{-1}$ DW), Ni (13.1 $mg.kg^{-1}$ DW), and Cd (18 $mg.kg^{-1}$ DW) found at the site and is a potential Zn bioindicator since its leaf contents were also representative of the Zn extractable contents in soil ($R^2 = 0.94$). The hazard quotient and carcinogen risk revealed that most of the site had an identified or possible risk, mainly due to Pb and As. Native species, especially *A. petiolata*, could be used in phytoextraction to manage and limit these human and environmental risks.

Key words:

Industrial wasteland; *Alliaria petiolata*; Trace elements; Contaminated soils; Bioindicator; Human health; Hazard quotient; Carcinogen risk; Phytomanagement.

1 Introduction

Due to human activities, large quantities of potentially toxic elements have been released into the environment since the end of the 19th century (Zhang & Wang, 2020). These activities have led to the formation of many marginal sites containing various organic and inorganic compounds in soil (Panagos et al., 2013). The presence of these compounds may impair the ecosystem services provided by the soil of these sites (Morel et al., 2015), such as provisioning services (raw materials, food) or regulating services (water quality, carbon storage, maintaining biodiversity, Jónsson & Davíðsdóttir, 2016). The cost of contamination management leads to a lack of interest in these sites and urban management that favours the artificialization of agricultural land rather than the rehabilitation of these soils (Vidal-Baudet & Rossignol, 2018). Approximately 690 000 industrial sites with past or current polluting activities were identified in the European Union (EU) in 2018 (Paya Perez & Rodriguez Eugenio, 2018), while the Géorisques database (https://www.georisques.gouv.fr/) listed 16 000 sites in France in 2022. Contamination is due to various sources, such as industrial production and commercial services (36%), the oil industry (17%), municipal waste (15%) and industrial waste (9%) (Paya Perez & Rodriguez Eugenio, 2018). The most common contaminants affecting soil are trace elements (TEs) (Panagos et al., 2013). Being nonbiodegradable, TEs tend to accumulate in the environment and could impact the environment and human health (Ali et al., 2013), even at very low concentrations (Arora et al., 2008).

Most TEs are strongly bound to the soil; however, there is always a spreading risk due to the mobile fraction that can be transferred into the ecosystem (Qasim & Motelica-Heino, 2014). Moreover, if some TEs, such as Zn, Cu or Mn, are essential for the health and development of plants and animals (Alloway, 2013), excessive contents may have adverse impacts. Some other TEs without known biological function (e.g., Cd, Pb) present high toxicity for most organisms. The effect of TEs depends on their contents and physico-chemical properties, Zn, Pb, Cd, Cu and Ni being frequently studied elements, as they are considered to have the greatest impact on organisms (Diatta et al., 2003). They can provoke damage to cells (Kabata-Pendias, 2011) and disrupt cellular processes such as photosynthesis among primary producers (Prasad & Strzałka, 1999; Warsi et al., 2023), cause oxidative stress, generate

68 neurotoxic effects, disrupt the function of numerous enzymes, and increase cancer risk (Ali et al., 2013;
69 Turdi & Yang, 2016).

70 As primary producers, plants are some of the first organisms to suffer from high TE contents in soils.
71 TE transfer from soil to plants constitutes crucial information for understanding the environmental
72 impacts of these pollutants. Ions enter plants through their roots and are then either directly stored or
73 translocated to the shoots through xylem vessels and more specifically deposited in vacuoles
74 (Verbruggen et al., 2009; Rascio & Navari-Izzo, 2011). Sequestration in vacuoles is a way to cope with
75 high metal concentrations and limit their impact on metabolic processes, increasing plant tolerance to
76 TE-contaminated soils (Jabeen et al., 2009). In this way, TEs could be transferred into food chains and
77 will accumulate through networks to become potentially harmful for plant and animal health, including
78 that of humans (Aycicek et al., 2008; Rajaganapathy et al., 2011).

79 In the context of the rehabilitation of industrial territories, it is necessary to manage marginal sites, often
80 localized around urban areas, to restore their value, in particular through ecosystem services and hence
81 the development of new uses. Proper management of soil pollution requires knowledge of the risks and
82 impacts of contaminants on human health and the environment. For this purpose, plants can be vectors
83 of information about the contamination degree and toxic effects (Diatta et al., 2003). The capacity of a
84 plant to be used as a bioindicator mostly depends on its capacity to take up and accumulate contaminants
85 to a level representative of soil contents (Kabata-Pendias, 2011; Favas et al., 2012). The risk for human
86 health associated with exposure to contaminants can be assessed by measuring the hazard quotient (HQ)
87 and carcinogenic risks (CR) according to scenarios of human ingestion of soil and/or plants from a
88 contaminated site (Antoine et al., 2017; Dehghani et al., 2018). These indexes provide information about
89 the human risk associated with each contaminant present at a site and therefore about the management
90 priorities for the elements present.

91 In this context, the aims of the present study are as follows: i) Locate and quantify TEs in soil and
92 determine their effect on the floristic diversity of an industrial wasteland. This diversity will be assessed
93 by a floristic survey as well as the measurement of the vegetation cover of species. ii) Determine the
94 accumulation of TEs in plants to estimate the potential of using indigenous plants as bioindicators of

contamination as well as measuring the TE effect on nutrient accumulation. For this purpose, the leaf contents of TEs and their relationship with the soil contents will be measured. iii) Calculate the human HQ and CR for individuals exposed to contaminants by soil ingestion and consumption of edible plants. Contaminant levels in soil and plant leaves will be used for the calculation based on the elemental toxicity and exposure scenarios.

We hypothesized that the variable level of TEs on the site induced the presence of different plant communities by reducing biodiversity and selecting tolerant species in heavily contaminated areas. TEs can also have toxic effects on plants and pose a risk for human health in the event of exposure. On the other hand, among the tolerant species at the site, some could be bioindicators with potential to be used in management decisions.

2 Materials and methods

2.1 Study site

The study site is a 2 ha industrial wasteland located in the city of Vieux-Charmont (47°31'15.8"N, 6°50'23.8"E) in eastern France. This wasteland is surrounded by dwellings, a pond created in 1957, the river “La savoureuse” and 2 operational factories (Fig. 1). Several activities have taken place in the factories since 1871, such as watchmaking, heat treatment and manufacture of piston pins, cars and precision parts. The site was used as an uncontrolled landfill for industrial and household wastes, found buried and on the surface. The wasteland was closed to the public in 2007 after soil analysis revealed contamination by organic (PAH, PCB) and inorganic (Zn, Pb, Cd, As, Cu, Ni, Cr, Hg) compounds.

Fig. 1. The industrial wasteland of Vieux-Charmont (Doubs, France). (A) Location of the Vieux-Charmont wasteland (in red) and its environment. (B) Aerial picture of the Vieux-Charmont wasteland entrance. (C) Aerial picture of the Vieux-Charmont wasteland and its environment.

2.2 Floristic survey

From June to July 2020, a floristic survey of the herbaceous layer was carried out in 8 zones; 4 zones had intense woody cover and were named undergrowth zones (UZ, zones 1 to 4), and 4 zones were

considered open zones (OZ, zones 5 to 8) with low woody cover, as representative as possible of the diversity of the accessible parts of the site (Fig. S1). Each zone was homogenous in terms of vegetation, cover, stratification and human intervention. Between 10 and 20 quadrats of 0.2 m² were randomly delimited on each zone, and the species present on these surfaces were determined as well as their cover value based on the Braun-Blanquet scale and cover estimation (+, 1, 2, 3, 4, 5 for 0.1, 2.5, 15, 37.5, 62.5, 87.5%, respectively) (Wikum & Shanholtzer, 1978; Anne et al., 2021). Based on these data, four diversity indices were calculated: species richness, Shannon index (eq. 1), Simpson index (eq. 2) and Hill index (eq. 3).

$$H' = -\sum(pi \cdot \log(pi)) \quad \text{(eq. 1)}$$

$$\lambda = 1 - \left(\sum(pi^2)\right) \quad \text{(eq. 2)}$$

$$\text{Hill} = 1 - \left(\frac{\lambda - 1}{\exp(H')}\right) \quad \text{(eq. 3)}$$

pi being the cover value of species i divided by the total plant cover value. The Simpson and Hill indices are subtracted from 1; thus, for each of the four indices, an increase in value means higher diversity.

2.3 Soil sampling and analyses

In May 2021, soil was sampled in 125 zones (10 m x 10 m) that cover all accessible parts of the site (Fig. S1). A composite sample was collected in each zone by mixing 4 samples taken from the corners and 1 sample taken in the middle of the zone at a depth of 0-30 cm. The samples were then stored in plastic bags and air dried before analysis. The pH value was determined directly on the soil samples using a field Soil Stick pH meter (Spectrum Technologies, Inc., Aurora, USA) (Tano et al., 2020). The total element content was measured on a 500 mg dry soil sample using inductively coupled plasma atomic emission spectrometry (ICP–AES, Thermo Fischer Scientific, Inc., Waltham, USA) after acid digestion (DigiPREP system, SCP Sciences, Courtaboeuf, France) using a mixture of 2 ml of 67% nitric acid, 6 ml of 34% hydrochloric acid and 2 ml of 48% hydrofluoric acid (Ciadamidaro et al., 2019).

Soil samples collected for total element fraction measurements were also used to ensure at least 3 measurements of the extractable fraction in each floristic survey zone (the number of samples depended

147 on the surface). These samples are related to one of the 125 zones described in Fig. S1 and are considered
148 representative of the zone. 2.5 g of dried soil was extracted with 25 ml of 0.01 M $CaCl_2$ solution under
149 constant agitation at 100 rpm for 3 h (Houba et al., 1996). Then, the samples were filtered, acidified at
150 1% (v/v) and quantified by ICP–AES.

151 ## 2.4 Plant sampling

152 Leaf samples were collected from the genera *Acer*, *Ailanthus*, *Alliaria* (on first- or second-year plants),
153 *Betula*, *Fraxinus*, *Glechoma*, *Populus*, *Prunus*, *Quercus*, *Salix* and *Urtica*, which are well represented
154 herbaceous plants, shrubs and trees found throughout the site during two campaigns in summer 2018
155 and autumn 2021. A maximum of one sample per species was sampled in the 125 zones previously
156 described (Fig. S1). The samples were stored in plastic bags at 4°C until washing with distilled water to
157 remove soil particles. Samples were then dried in an oven at 70°C and crushed for 3 min in a mixer mill
158 (MM 200, Retsch, Éragny, France) at 30 rps before analysis. The plant element concentration was
159 measured in 100 mg samples using ICP–AES after acid digestion using a mixture of 2 ml of 67% nitric
160 acid and 0.5 ml of 35% oxygen peroxide (Assad et al., 2017).

161 ## 2.5 Measurement of the human health risk

162 Based on the contaminant contents in the total soil fraction and in edible plant leaves, the daily intake
163 (DI; eq. 4) of contaminants was calculated according to the French national methodology of
164 management of polluted sites and soils (MEEM, 2017). Two exposure scenarios were considered in the
165 present study: (1) ingestion of 91 mg of soil per day (Stanek et al., 2001) while frequenting the site 148
166 days a year for 6 years by a 15 kg child and (2) ingestion of 37.5 g of edible plant leaves per day
167 (Barbillon et al., 2019), 26 days a year, corresponding to 7% of the total leaf consumption from the site,
168 for 6 years by a 15 kg child. Two plants with edible leaves are considered, *Alliaria petiolata* and *Urtica*
169 *dioica*.

170 $$DI\ (mg.kg^{-1}.day^{-1}) = \frac{C\ x\ IR\ x\ EF\ x\ ED}{BW\ x\ (AT\ x\ 365)} \qquad (eq.\ 4)$$

171 C is the concentration of the material ingested (mg.kg^{-1} of dry weight for soil and mg.kg^{-1} of fresh weight
172 for leaves); IR is the intake rate of material (kg.day^{-1}); EF is the exposure frequency (day.year^{-1}); ED is

the exposure duration (years); BW is the body weight (kg); AT is the average time (years), equivalent to ED for noncarcinogenic contaminants or a lifetime (70 years) for carcinogenic contaminants (MEEM, 2017).

The hazard quotient (HQ; eq. 5) for threshold effects and the carcinogenic risk (CR; eq. 6) for non-threshold effects were calculated for each contaminant studied when the toxicological reference value (TRV) was available (As, Cd, Cr, Cu, Hg, Ni, Pb and Zn for threshold effects and As, Cr and Pb for non-threshold effects). In accordance with the national methodology (MEEM, 2017), the DI values were compared to TRV, as described in Table S1.

$$HQ = \frac{DI}{TRV} \qquad (eq.\ 5)$$

$$CR = \text{DI x TRV} \qquad (\text{eq. } 6)$$

For HQ and CR, the sum of the values for the soil and plant scenarios in each soil sampling area are considered for interpretation. According to the methodology, there is no risk for HQ values < 0.2 and CR values < 10^{-6}, a possible risk for HQ values between 0.2 and 5 and CR values between 10^{-6} and 10^{-4} and an identified risk for HQ values ≥ 5 and CR values ≥ 10^{-4} (MEEM, 2017).

2.6 Mapping and statistical analysis

Mapping was performed with QGIS (ver. 3.16) using Google background maps. The analyses were performed using R (ver. 4.1.1) with the packages *agricolae* (De Mendiburu, 2021), *corrplot* (Wei & Simko, 2021), *cowplot* (Wilke, 2020), *ggplot2* (Wickam, 2016), *Hmisc* (Harrell, 2022), and *vegan* (Oksanen et al., 2022). For all the tests performed, results were considered statistically significant at a p value < 0.05. The Shannon and Simpson diversity indices were calculated using *vegan*. The normality of the data was first tested using Shapiro–Wilk and Levene tests. Comparisons of means were made using Kruskal–Wallis tests for biodiversity indices and extractable TE contents by vegetation zone as well as for foliar TE contents between genera. Spearman's test was used to assess the correlation between biodiversity indices and TE extractable contents, between pH values and TE extractable percentage and between soil total contents of TEs and nutrient contents in plant leaves. The graphs were made using the *ggplot2* package, and the correlation matrix was made using the *corrplot* package.

3 Results

3.1 Soil TE contents

The main contaminants found at the site were Zn, Pb, Cd, Sn, Hg, Ni and As (Fig. 2), which were heterogeneously distributed throughout the investigated area. Indeed, in most of the wasteland, the measured contents varied from 200 to 10 000 $mg.kg^{-1}$ dry weight (DW) for Zn, 0.2 to 10 $mg.kg^{-1}$ DW for Cd, 30 to 2 500 $mg.kg^{-1}$ DW for Pb, 2 to 1 830 $mg.kg^{-1}$ DW for Sn and 0.12 to 2 $mg.kg^{-1}$ DW for Hg. However, the contaminant map highlighted a heavily contaminated zone of almost 500 m^2 in the northeast of the site that contained the highest amounts of Zn (36 000–115 000 $mg.kg^{-1}$ DW), Pb (13 500–34 600 $mg.kg^{-1}$ DW), Cd (17–50 $mg.kg^{-1}$ DW), Sn (3 200–13 600 $mg.kg^{-1}$ DW), and Hg (1.4–4.5 $mg.kg^{-1}$ DW). Concerning Ni and As, the contents varied from 16 to 1650 $mg.kg^{-1}$ DW and 11 to 105 $mg.kg^{-1}$ DW, respectively. The highest contents for these two elements were localized in the south of the site, close to the factory and the pond. In addition, the site was also contaminated with Cr (26-1475 $mg.kg^{-1}$ DW), Cu (24–1800 $mg.kg^{-1}$ DW), Fe (13 000–224 000 $mg.kg^{-1}$ DW) and Mn (250–2300 $mg.kg^{-1}$ DW). Cu was localized in the most contaminated area of the site (northeast), and Cr, Fe and Mn were localized in the southern part of the site, similar to Ni and As. The pH values were heterogeneous throughout the site, although they remained close to neutral, with values that varied from 6.4 to 7.3 in the most contaminated zone (Fig. 2). The other part of the site had pH values that varied from 4 to 7.9, and the lowest pH value (4–5.5) was localized along a line from the southwest of the site to the centre.

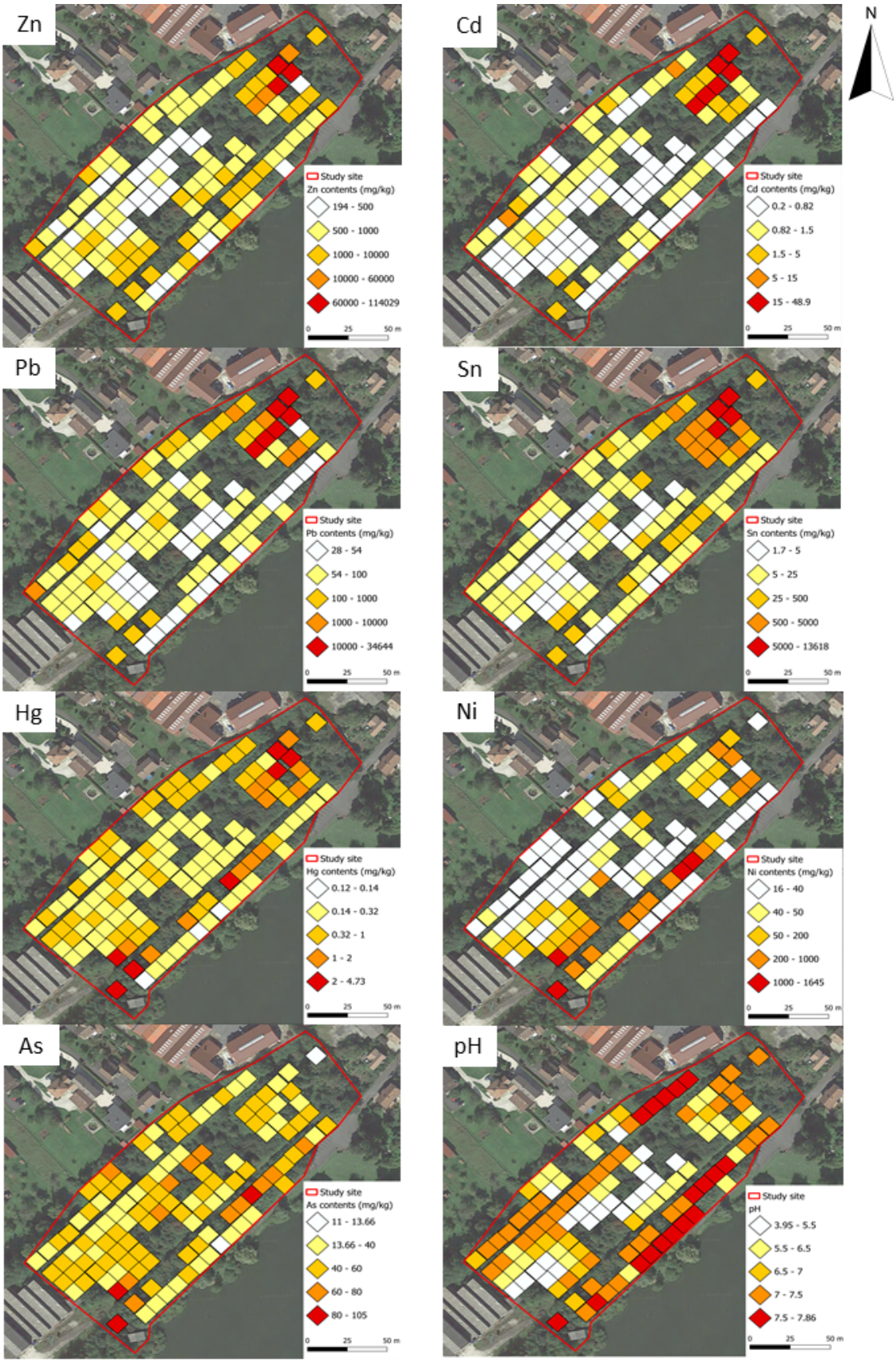

Fig. 2. Maps of total contents of the main contaminants (Zn, Cd, Pb, Sn, Hg, Ni, As) and pH value localized at the soil sampling plots described in Fig. S1 in the industrial wasteland. For TE contents, the first levels of the scale (white square) range from the minimum values found on the site to twice the world soil average values defined by Kabata-Pendias (2011)*, except for Zn, where the minimum value was too high.*

The analysis of the bioavailable fractions further revealed this high heterogeneity for both the UZ (zones 1 to 4) and OZ (zones 5 to 8) (Fig. S1 & 3). Zones 1 and 5 were statistically the most contaminated zones, with Zn extractable contents of 110 and 85 $mg.kg^{-1}$ DW, respectively, while the other zones had Zn contents of < 4 $mg.kg^{-1}$ DW (Fig. 3B). Furthermore, the same distribution was observed in zone 1 for Sn, with a mean value of 1.1 $mg.kg^{-1}$ DW; this value was higher than that in the other zones, with a content of 0.026 in zone 5 and contents below the limits of quantification (< LQ) in the other zones. Zone 5 had the highest contents of Ni, with a value of 0.74 $mg.kg^{-1}$ DW, which was statistically higher than that in the other zones (except zone 3), where the contents were in the range of 0.02-0.39 $mg.kg^{-1}$ DW. For other TEs, although zones 1 and 5 had the highest Pb and Cd contents, the differences in content were lower. Indeed, the contents varied from 0.01 to 0.1 $mg.kg^{-1}$ DW for Cd (< LQ for zones 4 and 8), from 0.01 to 1.8 $mg.kg^{-1}$ DW for Pb (< LQ for zone 6) and from 0.01 to 0.036 $mg.kg^{-1}$ DW for As (< LQ for zones 2, 5, 6, 7 and 8) (Fig. 3A).

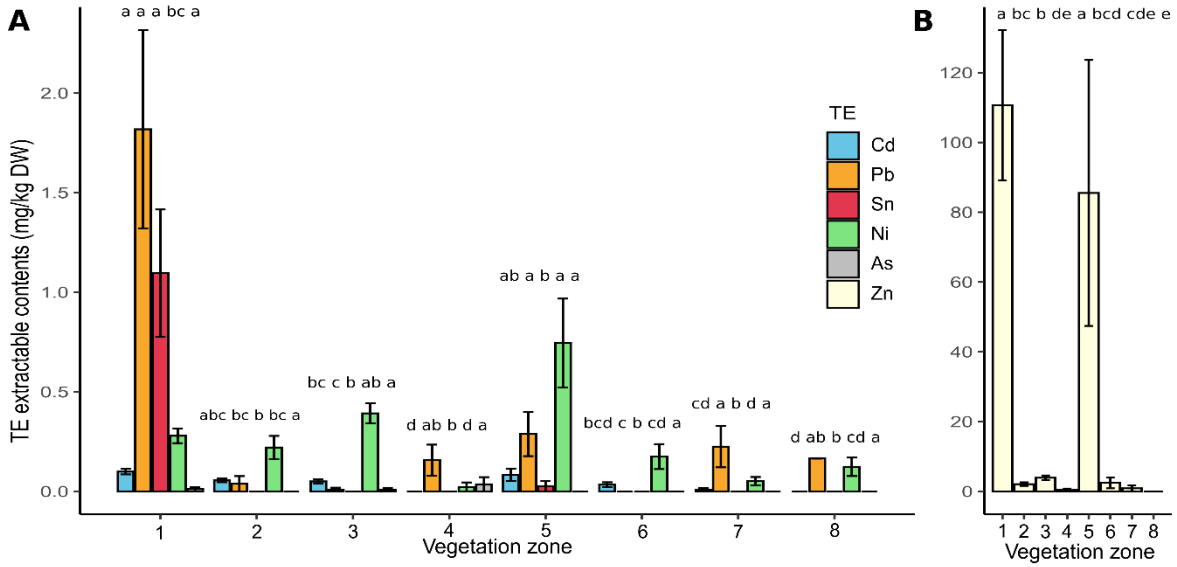

Fig. 3. Mean $CaCl_2$ extractable contents ($mg.kg^{-1}$ DW) of Cd, Pb, Sn, Ni, As (A) and Zn (B) in the 8 floristic survey zones and standard errors. Letters in common indicate statistically similar groups at $p < 0.05$ according to Kruskal–Wallis and post hoc pairwise comparison tests.

3.2 Plant diversity

The floristic survey, conducted over a total area of 4860 m², and the vegetation samples led to the identification of 96 species (including 72 herbaceous species) belonging to 40 families (Tables 1 & 2).

242 *Table 1. Identification and frequency of species from the herbaceous layer on the Vieux-Charmont wasteland. Frequencies*
243 *were measured based on the number of quadrats in the floristic survey.*

Family	Species	Frequency	Family	Species	Frequency
Aceraceae	*Acer campestre*	23.6	Juglandaceae	*Juglans regia*	0.9
Aceraceae	*Acer pseudoplatanus*	4.5	Lamiaceae	*Galeopsis tetrahit*	21.8
Adoxaceae	*Sambucus nigra*	15.5	Lamiaceae	*Glechoma hederacea*	29.1
Apiaceae	*Aethusa cynapium*	1.8	Lamiaceae	*Lamium maculatum*	10.0
Apiaceae	*Daucus carota*	8.2	Lamiaceae	*Stachys sylvatica*	0.9
Apiaceae	*Heracleum sphondylium*	1.8	Malvaceae	*Malva moschata*	1.8
Apiaceae	*Torilis japonica*	8.2	Malvaceae	*Malva alcea*	0.9
Aquifoliaceae	*Ilex aquifolium*	1.8	Oxalidaceae	*Oxalis stricta*	2.7
Araliaceae	*Hedera helix*	42.7	Papaveraceae	*Chelidonium majus*	0.9
Asteraceae	*Achillea millefolium*	4.5	Plantaginaceae	*Linaria vulgaris*	8.2
Asteraceae	*Artemisia vulgaris*	3.6	Plantaginaceae	*Plantago lanceolata*	0.9
Asteraceae	*Cirsium arvense*	1.8	Poaceae	*Agrostis capillaris*	14.5
Asteraceae	*Cirsium vulgare*	3.6	Poaceae	*Arrhenaterum elatius*	12.7
Asteraceae	*Crepis capillaris*	6.4	Poaceae	*Avena fatua*	2.7
Asteraceae	*Erigeron annuus*	26.4	Poaceae	*Brachypodium sylvaticum*	0.9
Asteraceae	*Erigeron canadensis*	6.4	Poaceae	*Dactylis glomerata*	19.1
Asteraceae	*Jacobea vulgaris*	2.7	Poaceae	*Elymus caninus*	4.5
Asteraceae	*Lactuca serriola*	3.6	Poaceae	*Elystrigia repens*	1.8
Asteraceae	*Lapsana communis*	5.5	Poaceae	*Holcus lanatus*	6.4
Asteraceae	*Picris hieracioides*	0.9	Poaceae	*Poa trivialis*	9.1
Asteraceae	*Tanacetum vulgare*	8.2	Poaceae	*Triticum aestivum*	3.6
Asteraceae	*Tripleurospernum inodorum*	1.8	Polygonaceae	*Reynoutria japonica*	0.9
Berberidaceae	*Berberis aquifolium*	1.8	Rosaceae	*Crataegus monogyna*	20.9
Brassicaceae	*Alliaria petiolata*	40.9	Rosaceae	*Crataegus laevigata*	8.2
Campanulaceae	*Campanula patula*	6.4	Rosaceae	*Geum urbanum*	8.2
Cannabaceae	*Humulus lupulus*	0.9	Rosaceae	*Potentilla reptans*	2.7
Caprifoliaceae	*Dipsacus fullonum*	0.9	Rosaceae	*Prunus avium*	1.8
Caryophyllaceae	*Silene vulgaris*	0.9	Rosaceae	*Prunus spinosa*	4.5
Convolvulaceae	*Convolvulus sepium*	5.5	Rosaceae	*Prunus domestica*	2.7
Cornaceae	*Cornus sanguinea*	7.3	Rosaceae	*Rubus fruticosus*	54.5
Dryopteridaceae	*Dryopteris filix-mas*	1.8	Rosaceae	*Rubus caesius*	5.5
Euphorbiaceae	*Euphorbia dulcis*	8.2	Rubiaceae	*Galium aparine*	23.6
Fabaceae	*Lathyrus pratensis*	0.9	Rubiaceae	*Galium mollugo*	18.2
Fabaceae	*Melilotus albus*	6.4	Rubiaceae	*Galium rotundifolium*	0.9
Fabaceae	*Robinia pseudoacacia*	2.7	Rubiaceae	*Galium palustre*	11.8
Fabaceae	*Trifolium pratense*	4.5	Scrophulariaceae	*Verbascum blattaria*	0.9
Fabaceae	*Vicia cracca*	2.7	Simaroubaceae	*Ailanthus altissima*	6.4
Fabaceae	*Vicia sepium*	0.9	Solanaceae	*Solanum nigrum*	0.9
Fagaceae	*Quercus robur*	0.9	Taxaceae	*Taxus baccata*	1.8
Geraniaceae	*Geranium robertianum*	4.5	Urticaceae	*Urtica dioica*	24.5
Geraniaceae	*Geranium pusillum*	0.9	Verbenaceae	*Verbena officinalis*	2.7
Hypericaceae	*Hypericum humifusum*	0.9	Vitaceae	*Parthenocissus quinquefolia*	3.6
Hypericaceae	*Hypericum perforatum*	14.5			

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Table 2. Shrubs and tree species identified by leaf sampling on the Vieux-Charmont wasteland. Frequencies were measured based on the 125 zones used for soil and plant samplings.

Family	Species	Frequency (%)
Betulaceae	*Betula verrucosa*	0.8
Fagaceae	*Quercus petraea*	2.4
Malvaceae	*Tilia platyphyllos*	0.8
Oleaceae	*Fraxinus excelsior*	4
Rosaceae	*Prunus serotina*	0.8
Salicaceae	*Populus nigra*	0.8
Salicaceae	*Populus tremula*	3.2
Salicaceae	*Salix alba*	0.8
Salicaceae	*Salix fragilis*	1.6
Salicaceae	*Salix purpurea*	4
Salicaceae	*Salix viminalis*	0.8

The main families of surveyed species were Asteraceae, Poaceae and Fabaceae (13, 10 and 5 species, respectively), which represented almost 40% of the herbaceous species, as well as Salicaceae and Rosaceae (6 species each), which represented 50% of shrubs and tree species. However, some families were also well represented due to the frequency of certain member species. Indeed, *Hedera helix* was the only species observed from the Araliaceae family, but its frequency was 42.7%. Similarly, the Brassicaceae family was exclusively represented by *A. petiolata* with a frequency of 40.9%. These two plants, along with the Rosaceae species *Rubus fruticosus* (frequency of 54.5%), were the most frequent herbaceous species on the site. Conversely, 25 species were observed only once and had a frequency of less than 1%, such as the herbaceous species *Dipsacus fullonum* (Caprifoliaceae), *Hypericum humifusum* (Hypericaceae) and *Verbascum blattaria* (Scrophulariaceae) and the tree species *Tilia platyphyllos* (Malvaceae), *Prunus serotina* (Rosaceae) and *Salix alba* (Salicaceae).

The species richness ranged from 3.7 to 5.6 in the UZ (zones 1 to 4) and from 5.4 to 7.4 in the OZ (zones 5 to 8); the Shannon index, from 1.17 to 2.09 in the UZ and from 1.61 to 2.39 in the OZ; the Simpson index, from 0.44 to 0.72 in the UZ and from 0.58 to 0.77 in the OZ; and the hill index, from 0.38 to 0.54 in the UZ and from 0.48 to 0.59 in the OZ (Fig. 4).

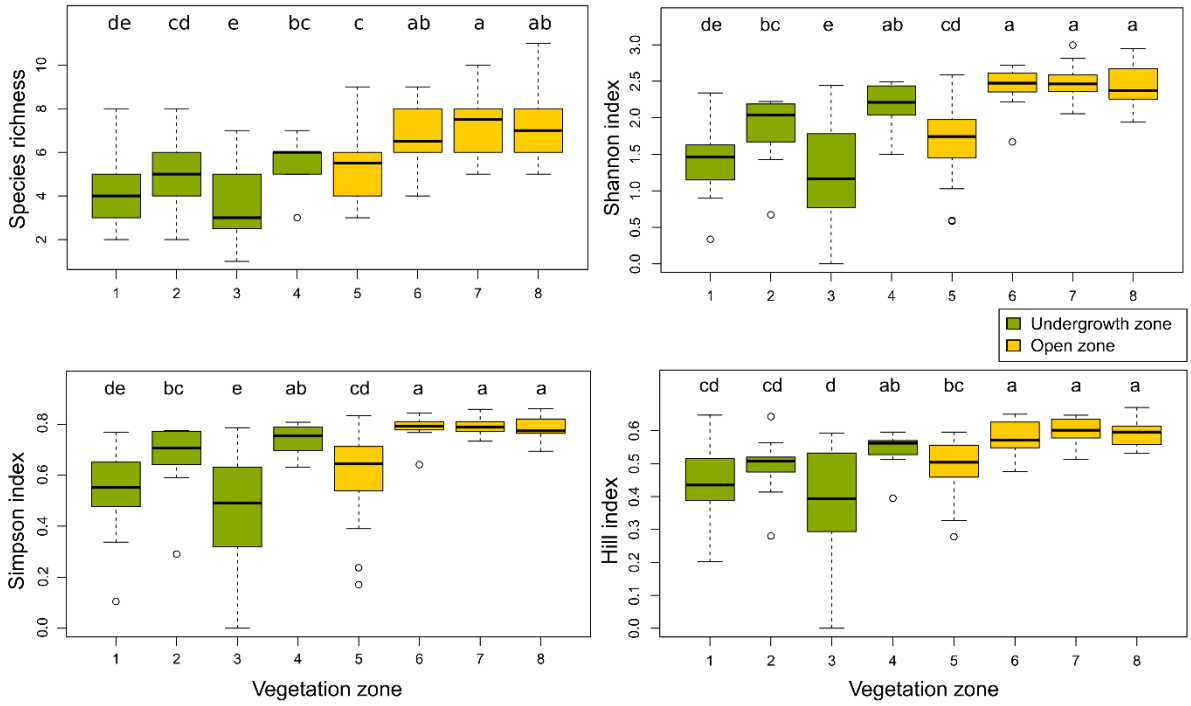
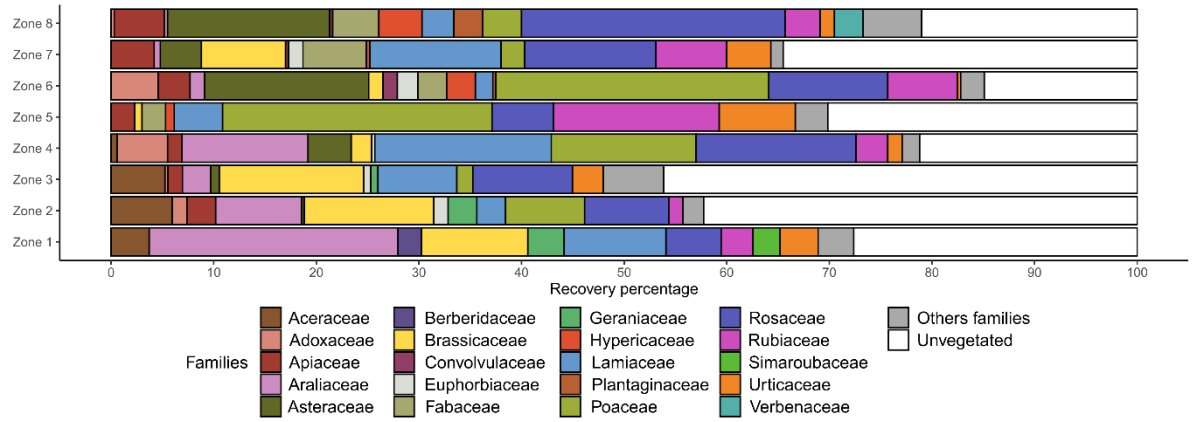


Fig. 4. Boxplots of Shannon, Simpson and Hill indices and species richness in the 0.2 m² quadrats of the 8 survey zones. Letters in common indicate statistically similar groups at $p < 0.05$ according to Kruskal–Wallis and post hoc pairwise comparison tests.

Overall, the OZ had statistically higher biodiversity indices than the UZ (p values < 0.01), except for zone 4, which was the least contaminated part of the UZ, and zone 5, which was the most contaminated part of the OZ. We also observed that the biodiversity indices of zone 4 were all statistically higher than those of zones 1 and 3. This was also the case for zone 2 but only for the Shannon and Simpson indices. Concerning the OZ, the most contaminated area (zone 5) had a systematically lower biodiversity for each index than the three other zones. In addition, the TE extractable contents and the biodiversity indices for the UZ had significant anticorrelations with Zn (from -0.28 to -0.45) and Ni (from -0.39 to -0.57). For the OZ, there were not only anticorrelations with Zn (from -0.44 to -0.6) and Ni (from -0.48 to -0.62) but also with Cd (from -0.44 to -0.6), Pb (from -0.36 to -0.61) and Sn (from -0.47 to -0.68).

In addition to these relationships between biodiversity and TE extractable contents, the plant communities also differed from one zone to another (Fig. 5). Indeed, in the UZ, the Adoxaceae, Apiaceae, Asteraceae, Euphorbiaceae and Poaceae families appeared in zones 2, 3 and 4 but not in zone 1, which was the most contaminated. Moreover, the Rosaceae family represented almost 5.4% of the plants in zone 1, unlike the other zones, where it represented between 8.2 and 15.6%.



283

284 *Fig. 5. Plant communities present in the floristic survey zones expressed as a recovery percentage of the 37 families identified*
285 *and unvegetated parts.*

286 In contrast, Berberidaceae and Simaroubaceae, each represented by only one species at the site, *Ilex*
287 *aquifolium* and *Ailanthus altissima,* respectively, were found only in zone 1. The Araliaceae family, also
288 represented by only one species, *H. helix*, represented a quarter of the vegetation (24.25%) in zone 1,
289 while it represented 2.75 to 12.3% of the vegetation in other areas. Concerning the OZ, Asteraceae and
290 Convolvulaceae were not found in the most contaminated zone (zone 5) but were present in the other
291 zones. The Rosaceae family was also less represented in the most contaminated area, with a cover
292 percentage of 6%, compared with 11.6 to 25.7% for the other zones. Conversely, Rubiaceae and
293 Urticaceae were more represented in zone 5, accounting for 16.15 and 7.4% of the vegetation,
294 respectively, in comparison to other zones, where the values varied from 3.4 and 6.9 for Rubiaceae and
295 from 0.3 to 4.3 for Urticaceae. Globally, except for the most contaminated zones (zones 1 and 5), the
296 families identified were similar between the UZ (except Geraniaceae in zone 4 and Urticaceae in zone
297 2) and the OZ (except Adoxaceae and Plantaginaceae in zone 7 and Euphorbiaceae and Verbenaceae in
298 zone 8).

299 ## 3.3 TE uptake by the vegetation

300 *A. petiolata* was the plant species with the highest maximal Zn, Ni and Cd contents, with 1750, 13.1 and
301 18 $mg.kg^{-1}$ DW, respectively (Fig. 6). The data also highlighted that the second-year *A. petiolata*
302 accumulated significantly more of these TEs than the first-year plants. For Cd, the maximal content
303 value was found in first-year plants. The Zn, Ni and Cd contents in leaves were highly dependent on the

plant genus considered. Indeed, *Alliaria* (second-year plants), *Betula*, *Populus*, *Salix*, *Glechoma* and *Urtica* contained an average of 1250, 1210, 390, 174, 111 and 253 mg.kg^{-1} DW of Zn, respectively. Those values were significantly higher than those for the *Ailanthus*, *Alliaria* (first-year plants), *Fraxinus*, *Prunus* and *Quercus* genera, where the contents were between 13 and 60 mg.kg^{-1} DW. For Ni, second-year *Alliaria* had a mean content of 10.7 mg.kg^{-1} DW, which was statistically higher than that of the other plant genera (except *Populus*, *Quercus* and *Urtica*), where the values varied from 0.36 to 2 mg.kg^{-1} DW. Concerning Cd, *Alliaria* (second-year plants), *Populus* and *Salix* had mean contents of 1.00, 1.46 and 1.13 mg.kg^{-1} DW, respectively, which were statistically higher than those of other genera (except *Acer*), where the values were between 0.09 and 0.96 mg.kg^{-1} (< LQ for *Ailanthus* and *Urtica*). The data also showed high Mn contents in the leaves of Quercus and Acer species, with an average of 400 (max = 1 350) and 280 mg.kg^{-1} (max = 2 400), respectively. In contrast, other genera showed mean Mn contents that varied from 15 to 150 mg.kg^{-1} with a maximal value of 550 mg.kg^{-1}.

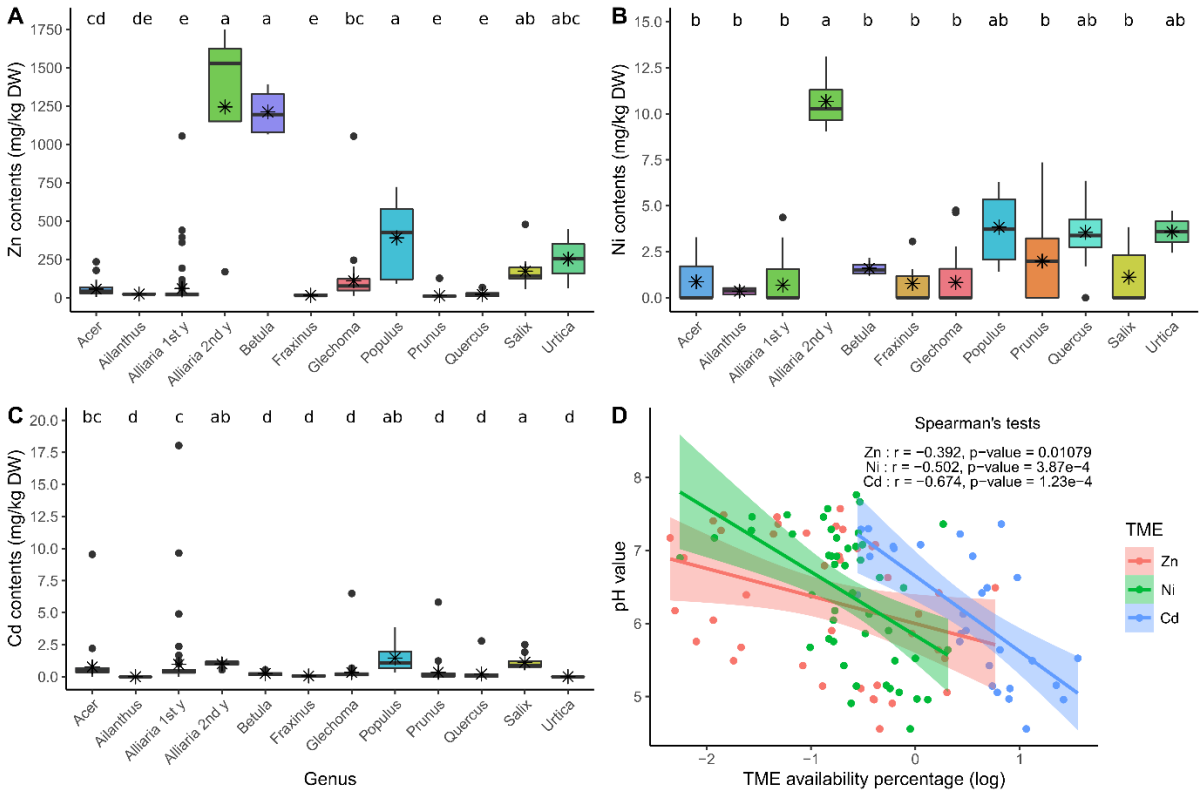


Fig. 6. Leaf contents of Zn (A), Ni (B) and Cd (C) in the main plant genera and TE $CaCl_2$ extractable percentage as a function of pH value (D). The mean value of TE contents in each plant genera is represented by stars in the boxplots. Samples from first-year plants (1st y) and second-year plants (2nd y) are separated for the Alliaria genus. The TE availability is expressed as a logarithm of the percentage of TE availability (extractable contents of TE divided by total contents of TE). The 95% confidence interval is represented in a scatter plot around the equation lines.

The mean Fe and Cu contents in leaves were 82 $mg.kg^{-1}$ DW (max = 370) and 7 $mg.kg^{-1}$ DW (max = 42), respectively, for all the genera. Finally, for As, Pb, Cr, Hg and Sn contents in plant leaves, most of the samples had values < LQ. However, the data still showed that Pb reached 10.7 $mg.kg^{-1}$ DW and As reached 4.2 $mg.kg^{-1}$ DW for *Alliaria*. We also showed that pH values were anticorrelated with Zn, Ni and Cd availability percentages (Fig. 6), indicating that the extractable contents increased with decreasing pH values. We also highlighted that the anticorrelation was highest for Cd (r = -0.67), followed by Ni (r = -0.5) and finally Zn (r = -0.39). No correlations were found between the pH and the available percentages of As, Pb and Sn.

In addition to the high contaminant contents found in *A. petiolata*, the data showed that Zn leaf contents in first-year plants were representative of soil extractable Zn contents. Indeed, as highlighted in Fig. 7, the scatter plot of these two parameters showed that the leaf contents were strongly related to the extractable contents since the R^2 value of the relationship was 0.94. Furthermore, there was a positive correlation between these two parameters with an r value of 0.62. This link was observed only for first-year plants.

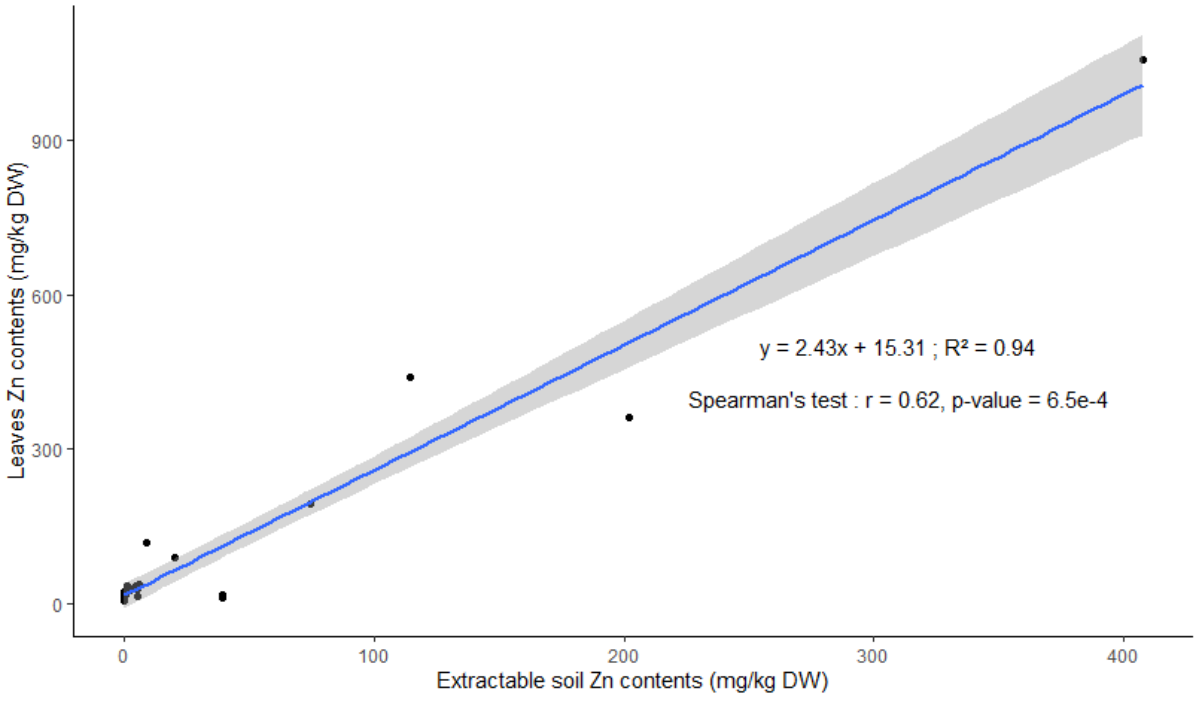


Fig. 7. Scatter plot of the first-year Alliaria petiolata Zn leaf contents depending on Zn $CaCl_2$ extractable contents of soil and Spearman correlation test between these two parameters. The 95% confidence interval is represented around the relationship.

3.4 TE toxicity and risk assessment for plants and humans

The correlations between the nutrient contents in leaves and the TE total contents in soil for the *Alliaria* and *Quercus* genera are compared in Fig. 8. These 2 genera were compared because they had the lowest (*Alliaria*) and the highest (*Quercus*) correlation values of all the genera studied (presented in Fig. S2). Concerning *Alliaria*, few anticorrelations were found between Cd, Pb, Sn and Zn soil contents and Mn leaf contents and between As soil contents and P leaf contents. Correlation coefficients were low, with r values of -0.28 to -0.38 for Mn and -0.26 for P. For other nutrients, there were no highlighted links, but there were positive correlations with Mg (r = 0.27 to 0. 29 for Ni, Pb, Sn and Zn soil contents), S (r = 0.31 to 0.35 for Cd, Pb, Sn and Zn soil contents) and Zn (r = 0.31 to 0.41 for Cd, Ni, Pb, Sn and Zn soil contents). Soil TE contents were more strongly related to nutrient contents for the *Quercus* genus than for *Alliaria*. Indeed, Cu, Fe, K, Mg, Mn, P, S and Zn plant contents were negatively correlated with As, Cd, Ni and Zn soil contents (and with Sn for K, Mn and P). The correlation coefficients were higher for *Quercus* than *Alliaria,* with r values that varied from -0.43 to -0.69 for Fe, K, Mg, P and Zn and from -0.69 to -0.86 for Cu and S.

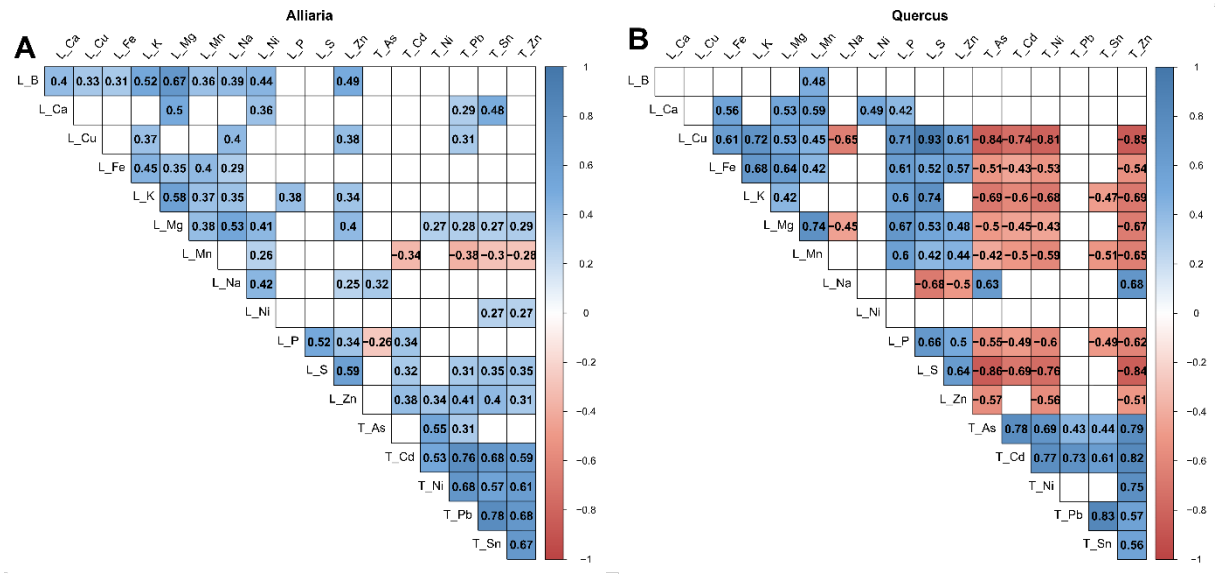


Fig. 8. Correlation matrix of nutrient leaf contents and total TE contents for the Alliaria (A) and Quercus (B) genera. The Spearman correlation coefficient is shown only for tests with a p value < 0.05. L: Leaf contents; T: Total contents in soil.

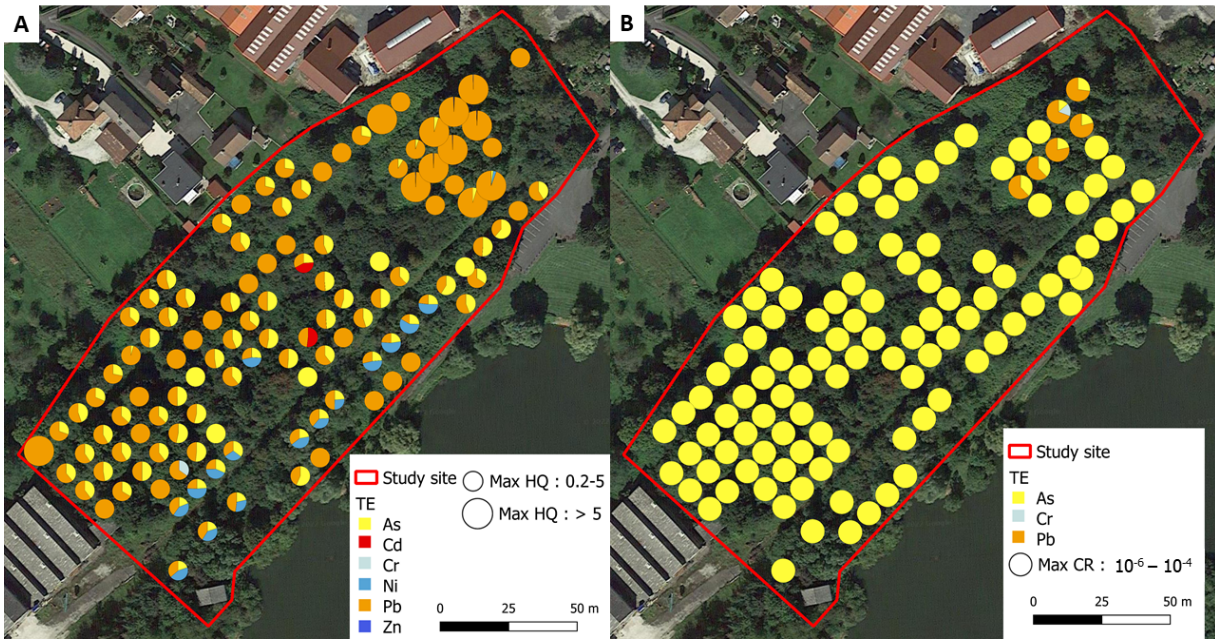
For other nutrients, the data did not show noteworthy links with soil contents, with only 2 positive correlations between Na leaf contents and As and Zn soil contents (r = 0.63 and 0.68, respectively). In

358 general, the anticorrelations with nutrients were higher for As, Ni and Zn than for Cd and higher for Cd
359 than for Sn.

360 Based on the TE contents in the plants and soil TE contents, the HQ and CR were calculated, and their
361 values were mapped (Fig. 9). For the soil and leaf ingestion scenarios, most of the sampling areas had a
362 maximal HQ between 0.2 and 5 across the site (117/125 areas) and were considered to have a possible
363 risk for human health. Pb and As were the elements with the highest number of areas with possible risk
364 (101 and 87 areas, respectively), followed by Ni (19 areas), with the areas of risk mainly distributed
365 close to the pond; other elements had lower numbers of areas with possible risk with 6 areas for Zn, 5
366 areas for Cd and 2 areas for Cr (Fig. 9A). In comparison, only 8 areas had values < 0.2 for all elements
367 and were therefore considered to have no risk (data not shown). In addition, 11 sampling areas had
368 maximal HQ values between 5 and 170 and were therefore considered to have an identified risk (Table
369 S3). One of these areas with high HQ values was localized close to the factory (west), while the others
370 were all in the most contaminated part of the site (north-east). Pb was the only element with HQ > 5 and
371 therefore justifies the risk in these areas. However, in the most contaminated area, a possible risk was
372 also detected for As in 7 areas with HQ values between 0.2 and 5, as well as for Zn (6 areas), Cd (3
373 areas), Ni (3 areas) and Cr (1 area).

374 Concerning CR, there was no risk identified in 14 areas (values < 10^{-6}). All the other areas (111 areas)
375 had a possible risk due to values ranging from 10^{-6} to 10^{-4} (Fig. 9B). The elevated CR values were often
376 due to As throughout the site, with a maximal value that reached $3.91x10^{-5}$, except in the most
377 contaminated area, where Pb (6 areas) and Cr (1 area) also had high CR values that reached $6.21x10^{-5}$
378 and $1.51x10^{-5}$, respectively.

379 A comparison of the two scenarios showed that soil ingestion contributed more than plant consumption
380 to the HQ and CR values (Table S2). Indeed, for the plant consumption scenario, only 4 HQ values
381 exceeded 0.2 for Cd and Cr (max = 0,61 for Cd), and only 1 CR value exceeded 10^{-6} for Cr (CR =
382 $1.32x10^{-5}$). Concerning the soil ingestion scenario, HQ values exceeded 0.2 in 109 areas for Pb (11 areas
383 with HQ > 5), followed by As (86 areas), Ni (18 areas), Zn (6 areas) and Cd (3 areas), and Cr values
384 exceeded 10^{-6} in 110 areas for As and 6 areas for Pb.



385

386 *Fig. 9. Location and ranges of HQ (A) and CR (B) values calculated at the study site. For each sampling area, a pie chart*
387 *compared the HQ or CR values associated with each element. The size of the pie chart represents the HQ or CR value of the*
388 *element with the highest risk. The values below 0.2 for the HQ and 10^{-6} for the CR are not shown on the map.*

389

4 Discussion

4.1 TE contamination and its impact on floristic diversity and plants

Among the main contaminants described in Fig. 2, all had maximal content values at the site that far exceeded the average TE values in surface soil described by Kabata-Pendias (2011). Indeed, the average values commonly reported were 70, 0.41, 27, 2.5, 0.07, 29 and 6.83 $mg.kg^{-1}$ for Zn, Cd, Pb, Sn, Hg, Ni and As, respectively. Therefore, this contamination had an effect on the floristic composition of the study site. Indeed, the herbaceous families Asteraceae, Poaceae and Fabaceae and tree family Salicaceae, which were the dominant plant families at the study site, have also been reported as the main families at a naturally recolonized industrial site contaminated with Mn, Fe, Cu and Zn in France (Zapata-Carbonell et al., 2019). More specifically, 23 species were shared by the two industrial wastelands. These families have also been described as dominant in several other sites contaminated by various TEs, such as As, Pb and Cd in Mexico (Salas-Luévano et al., 2017) and Zn, Cu, Ni and Cr in Cameroon (Anne et al., 2021), although with fewer shared species (2 and 1, respectively). Our results confirm that TE tolerance, regardless of the element considered, is common in species belonging to these families.

However, the composition of the plant communities found on the wasteland differed between the different areas studied. Indeed, two factors structured these communities. The first factor is the type of area, the plant communities are not the same between open and undergrowth areas. This is a logical conclusion since the plants composing the ecosystems have ecological requirements for development, such as light intensity, temperature or humidity (Syed et al., 2018). The second factor is the level of contamination, highlighted in zones 1 and 5, which were the most contaminated UZ and OZ, respectively. The disappearance of species sensitive to TEs along a contamination gradient on a wasteland has already been reported by Dazy et al. (2009). However, our data also showed a decrease in the vegetation cover of some families, even the most represented ones such as Asteraceae, Fabaceae and Rosaceae. In contrast, coverage for some families, especially Araliaceae, Rubiaceae and Urticaceae, increased with increasing levels of contamination. This is explained by the replacement of species that do not tolerate high levels of TEs by new species and by an increase in the abundance of other species,

such as *U. dioica* and *H. helix*, that are more tolerant. Therefore, the contamination level led to a selection of communities based on tolerance to contaminants. As hypothesized, the level of contaminants at the site was a factor that modified the composition of plant communities.

As a consequence, the TE contents in soils explained most of the variation observed for the four biodiversity indices. Indeed, while the lower biodiversity in the UZ than in the OZ was related to the area type, the environmental impacts of contaminants were highlighted by the lower biodiversity indices found in the most contaminated UZ (except zone 3) and OZ areas. These decreases were related to the modifications of the plant communities resulting in the presence of fewer but more abundant species. Indeed, TEs are well known to inhibit plant growth and alter plant communities by selecting tolerant species (Muszynska & Labudda, 2019), thus inducing a reduction in plant biodiversity (Alsherif et al., 2022). More specifically, our data showed that Zn and Ni had the greatest impact on biodiversity for both the UZ and OZ based on the anticorrelations with extractable contents. Cd, Sn and Pb also showed anticorrelation but only for OZ. Zone 3 showed a high percentage of bare soil and therefore low biodiversity indices (with high variability), while contaminant levels were comparable with that in zones 2 and 4. This could be explained by the heterogeneous pH values in this part of the site, varying from 3.95 (minimal pH value on site) to 7, with pH being one of the major soil parameters influencing metal-tolerant plant communities, as shown by Conesa et al. (2007).

In addition to influencing biodiversity, TE contents also modified nutrient uptake, as shown by anticorrelations between TE contents in soil and Cu, Fe, K, Mg, Mn, P, S, Zn contents in the leaves of *Quercus* species (Fig. 8). These differences can be explained by the mechanisms involved in the uptake of nonessential TEs by plants. Indeed, these elements can enter the plant through nonspecific pathways by substituting other divalent cations, such as Ca^{2+}, Cu^{2+}, Mg^{2+}, Fe^{2+} and Zn^{2+} (Rascio & Navari-Izzo, 2011; Tibbett et al., 2021), and the effect depends on the element and the plant considered (Boechat et al., 2016). Indeed, for the most accumulative species found at the site, *A. petiolata*, few effects were shown and only for Mn uptake. The tolerance and accumulation capacities are usually explained by the overexpression of genes encoding transmembrane transporters, proteins involved in the responses to oxidative stress and synthesis of metal chelators in accumulator species (Rascio & Navari-Izzo, 2011;

Corso & Garcia de la Torre, 2020). The genomic study of *A. petiolata* would allow us to better understand the mechanisms involved in these metal-accumulating species, which should receive attention.

4.2 TE accumulation in plants with a special emphasis on *Alliaria petiolata*

Overall, Zn, Cd and Ni were the most accumulated elements in plant leaves at the site because the $CaCl_2$ extractable fraction of these elements was higher than that for the other elements, e.g., Pb and Sn. The anticorrelations between the availability percentage and the pH value in soil for Zn, Cd and Ni supported this assumption. Indeed, the soil pH value has a strong influence on the availability of these contaminants, and a decrease in the pH value induces an increase in the extractable $CaCl_2$ content, as previously described by other authors (Kashem & Singh, 2001; Spurgeon et al., 2006; Gandois et al., 2010). Therefore, the pH indirectly affected Zn, Cd and Ni plant contents by solubilizing them in soil.

Therefore, of the major contaminants studied at the site, Zn, Cd and Ni were the most heavily accumulated in plants. In particular, the following genera tended to accumulate one or more of these contaminants: *Alliaria* (Zn, Cd, Ni), *Betula* (Zn), *Populus* (Zn, Cd), *Salix* (Zn, Cd) and *Urtica* (Zn). The *Betula*, *Populus* and *Salix* genera are known to contain species with accumulation capacities for Zn and/or Cd (Margui et al., 2007; Migeon et al., 2009; Bissonnette et al., 2010; Mrnka et al., 2012; Dmuchowski et al. 2014), including several species found at the study site (*P. nigra, P. tremula, S. alba, S. fragilis, S. purpurea, S. viminalis*) (Marmiroli et al., 2011). Migeon et al. (2009) found Zn contents varying from 200 to 950 $mg.kg^{-1}$ DW in the genera *Betula*, *Salix* and *Populus* at a Zn-, Pb- and Cd-contaminated site, which is similar to our data. However, much higher contents can be found in the literature, as for the species *Salix aquatica grandis*, which can reach 2800 $mg.kg^{-1}$ DW for Zn and 128 $mg.kg^{-1}$ DW for Cd (Ciadamidaro et al. 2019). These genera are therefore frequently studied in phytoremediation because of their interesting capacities (Mleczek et al., 2010; Lewis et al., 2015; Dadea et al., 2017; Kubatova et al., 2022; Yi et al., 2022; Yin et al., 2022). In contrast, the other genera contained low leaf contents, especially *Ailanthus*, *Fraxinus* and *Prunus*, as found previously (Migeon et al., 2009).

Fe, Cu and Mn were also found to be transferred to the leaves, which seems rational since these are essential elements for plants (Fernando et al., 2016; Lange et al., 2017; Naranjo-Arcos et al., 2017). However, Mn values exceeded the normal leaf content range of 30-300 $mg.kg^{-1}$ DW described by Kabata-Pendias (2011), especially in samples from the genera *Acer* and *Quercus*. Comparable Mn concentrations have been reported by Stanković et al. (2011), with mean values that ranged between 370 and 550 and 67 and 285 $mg.kg^{-1}$ DW for *Quercus petraea* and *Acer campestre* species, respectively, species that are also present at our study site. Fernando et al. (2016) found Mn contents that reached 2600 $mg.kg^{-1}$ DW for *Acer rubrum* (similar to our data) and even higher maximal contents in *Acer saccharum*, with a value of 4300 $mg.kg^{-1}$ DW. Concerning the *Quercus* genus, Huang et al. (2014) found contents of 800 $mg.kg^{-1}$ DW, with a maximal value of 2700 $mg.kg^{-1}$ DW in *Quercus alba* on an Mn mining site, which was almost twice as high as the values found for *Quercus* species at our study site. All of these data highlight that accumulation capacities of Mn, Zn and Cd can vary greatly from one species to another, even within the same genus.

Hg, Sn and Cr were not found in the leaves of any of the tree, shrub or herbaceous species studied. These results are consistent with the contents of these elements found in the soil $CaCl_2$ extractable fraction, which were less than the LQ for most of the samples. Several studies on the chemical distributions of Pb and As have highlighted that the exchangeable fraction of these elements was lower than that of other TEs, such as Cd or Zn (Pichtel et al., 2000; Qasim et motelica-Heino, 2014; Gankhurel et al., 2020). Indeed, depending on the soil, Pb and As could be bound to carbonate, Fe-Mn oxides, organic matter and/or in residual fractions that are harder to extract and therefore less mobile (Angelova et al., 2004; Qasim & Motelica-Heino, 2014) and hence poorly transferred to plant tissues.

The dominant species of the site, *A. petiolata,* which belongs to the Brassicaceae family, showed the highest levels of TEs in its leaves. The Brassicaceae family is known to contain 25% of the metal-hyperaccumulating species described (Gall & Rajakaruna, 2013; Suman et al., 2018), which are more tolerant to metal contamination. *A. petiolata* does not seem to differ from this general feature. To our knowledge, few studies have been conducted on TE contents in this species, with lower values reported than is our study for Zn. Grabner et al. (2011) found maximal contents of 393 $mg.kg^{-1}$ DW for Zn and

2.1 $mg.kg^{-1}$ for Cd at contaminated sites. Furthermore, the translocation ratio (ratio between shoot and root contents) was higher than 1 for Zn, Cd and Ni (Grabner et al., 2011; Drozdova et al., 2019), indicating that this species has the capacity to concentrate these three contaminants in its aerial parts. Moreover, the use of *A. petiolata* in phytoextraction strategies has been suggested by Drozdova et al. (2019), and the maximal Zn and Ni values found in the present study were much higher than the previously published data, supporting this potential. In addition, this plant is fast growing and produces a high biomass, which has interesting implications for phytoextraction (Grabner et al., 2011).

A. petiolata was also demonstrated to be a relevant Zn bioindicator, being distributed throughout the site. Indeed, Zn accumulation in the leaves of this species was correlated with the $CaCl_2$ extractable fractions. These features are usually described to consider a plant as a metal bioindicator (Kabata-Pendias, 2011).

4.3 Impact of TEs on human health

The HQ and CR calculated for several parts of the site showed that the TE contents could have an impact on human health depending on the soil and plant ingestion scenarios considered. For most of the areas, a possible risk was identified due to Pb and As based on the HQ and CR values. These two elements are known to be particularly toxic to humans either by disturbing metabolism or being carcinogenic (Turdi & Yang, 2016). The element that presented the highest risk at the site for both HQ and CR is Pb, especially in the soil ingestion scenario, due to its high total contents in soil relative to its toxicity. Indeed, even if this element was the second most abundant after Zn, it would remain the greatest threat since its toxicity is a thousand times higher than that of Zn. However, in our study site, the low bioavailability of Pb prevented it from accumulating in plants to levels that are dangerous for humans in the scenarios considered; nevertheless, the maximal contents found reached the tolerable limit for the crops described by Kabata-Pendias (2011), which varies from 0.5 to 10. The low contents available for plants have often been reported in the literature for this element, as previously explained in section 4.2.

Although Pb is the element with the highest risk values at the site, As is the element that most frequently exceeded the boundary between no risk and possible risk. Although this element had no HQ or CR

values high enough to identify it as a risk, there is a possible risk in almost all the areas studied. This is explained by the high levels of As throughout the site, with more than 60% of the areas studied having total As contents in soil that exceed 40 $mg.kg^{-1}$ DW. Indeed, these values are almost 6 times higher than the world soil average of 6.8 $mg.kg^{-1}$ identified by Kabata-Pendias (2011).

On the other hand, for Cd and Cr, although most of the areas had HQ and CR values < 0.2, the contents in leaves of *A. petiolata* presented a potential risk for humans in the case of consumption for 4 areas at the site. Human exposure to these elements via consumption of metal-accumulating plants in contaminated sites has already been described as potentially toxic (Naz et al., 2020). Concerning Cr, the risk values exceeded 0.2 for only a few parts of the site because most plant sample contents were below the LQ. Nevertheless, even low contents (18 to 25 $mg.kg^{-1}$ DW) were sufficient to consider a possible risk, indicating that this TE should be taken into consideration even at low levels in plants as should its bioavailability. Cd is considered one of the elements with the most toxic effects on human, animal and plant biological processes (Diatta et al., 2003; Kabata-Pendias, 2011). Antoine et al. (2017) reported that Cd was responsible for higher HQ than As, Al, and Pb in several food crops tested. Our data also showed that this element had the highest risk in the plant exposure scenario. This can be explained by the efficient mechanisms for the uptake and translocation of Cd in plants, although it is not essential, which leads to high concentrations in aerial plant parts (Naz et al., 2020). Consequently, although few areas of the site showed a possible risk for Cd, its bioavailability needs to be monitored, especially under high pH variation, as in our study site. Indeed, pH can greatly increase the Cd contents accumulated in aerial plant parts and thus the risk from consumption.

Overall, the modification of nutrient contents in plants and the measurement of HQ and CR showed that TE contamination had an effect on the environment at the study site. These results highlight the human and environmental risks and the necessity to manage the study site and similar marginal lands to restore interest and uses to these sites.

5 Conclusions

The soils of the industrial wasteland located in Vieux-Charmont presented contamination with several TEs, with heterogeneous TE contents and pH values. This has led to the presence of environments with similar abiotic conditions but with modified floristic compositions. Moreover, this study highlighted differences in the accumulation of TEs with the vegetation of the site, depending on the genus considered. One of these species, *A. petiolata*, is also a potential bioindicator of Zn that could provide information about the mobility and spreading risk of this element in ecosystems, especially since the species grows easily in contaminated environments. The accumulation capacities of this species, as well as others on the site, notably those belonging to *Salix*, *Populus* and *Betula*, allow us to consider the possibility of using these indigenous species for managing the site by phytoremediation techniques such as phytoextraction to reduce the quantity of contaminants in the soil. These techniques are low-cost and non-invasive ways to use plants to manage site contamination (Peer et al., 2005). Moreover, it may be possible to use marginal lands for economic purposes and to restore some ecosystem services. The level of contamination in the soil and vegetation as well as the measurement of the associated HQ and CR also make it possible to decide and locate the necessary actions for the future of the site. Indeed, the measurement of human health risks is fundamental because the long-term objective is to open a part of the site as an educational park with communication about the research conducted for the public.

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