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1 Floristic survey, trace element transfers between soil and vegetation and human health risk at an urban  
2 industrial wasteland.

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10

## 11 Highlights

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

19

20

21

22 Abstract:

23

24

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

37 Key words:

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

40

41

## 42 **1 Introduction**

43 Due to human activities, large quantities of potentially toxic elements have been released into the  
44 environment since the end of the 19th century (Zhang & Wang, 2020). These activities have led to the  
45 formation of many marginal sites containing various organic and inorganic compounds in soil (Panagos  
46 et al., 2013). The presence of these compounds may impair the ecosystem services provided by the soil  
47 of these sites (Morel et al., 2015), such as provisioning services (raw materials, food) or regulating  
48 services (water quality, carbon storage, maintaining biodiversity, Jónsson & Davíðsdóttir, 2016). The  
49 cost of contamination management leads to a lack of interest in these sites and urban management that  
50 favours the artificialization of agricultural land rather than the rehabilitation of these soils (Vidal-Baudet  
51 & Rossignol, 2018). Approximately 690 000 industrial sites with past or current polluting activities were  
52 identified in the European Union (EU) in 2018 (Paya Perez & Rodriguez Eugenio, 2018), while the  
53 Géorisques database (<https://www.georisques.gouv.fr/>) listed 16 000 sites in France in 2022.  
54 Contamination is due to various sources, such as industrial production and commercial services (36%),  
55 the oil industry (17%), municipal waste (15%) and industrial waste (9%) (Paya Perez & Rodriguez  
56 Eugenio, 2018). The most common contaminants affecting soil are trace elements (TEs) (Panagos et al.,  
57 2013). Being nonbiodegradable, TEs tend to accumulate in the environment and could impact the  
58 environment and human health (Ali et al., 2013), even at very low concentrations (Arora et al., 2008).

59 Most TEs are strongly bound to the soil; however, there is always a spreading risk due to the mobile  
60 fraction that can be transferred into the ecosystem (Qasim & Motelica-Heino, 2014). Moreover, if some  
61 TEs, such as Zn, Cu or Mn, are essential for the health and development of plants and animals (Alloway,  
62 2013), excessive contents may have adverse impacts. Some other TEs without known biological  
63 function (e.g., Cd, Pb) present high toxicity for most organisms. The effect of TEs depends on their  
64 contents and physico-chemical properties, Zn, Pb, Cd, Cu and Ni being frequently studied elements, as  
65 they are considered to have the greatest impact on organisms (Diatta et al., 2003). They can provoke  
66 damage to cells (Kabata-Pendias, 2011) and disrupt cellular processes such as photosynthesis among  
67 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

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

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

105

106 **2 Materials and methods**

107 **2.1 Study site**

108 The study site is a 2 ha industrial wasteland located in the city of Vieux-Charmont (47°31'15.8"N,  
109 6°50'23.8"E) in eastern France. This wasteland is surrounded by dwellings, a pond created in 1957, the  
110 river “La savoureuse” and 2 operational factories (Fig. 1). Several activities have taken place in the  
111 factories since 1871, such as watchmaking, heat treatment and manufacture of piston pins, cars and  
112 precision parts. The site was used as an uncontrolled landfill for industrial and household wastes, found  
113 buried and on the surface. The wasteland was closed to the public in 2007 after soil analysis revealed  
114 contamination by organic (PAH, PCB) and inorganic (Zn, Pb, Cd, As, Cu, Ni, Cr, Hg) compounds.



115

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

119 **2.2 Floristic survey**

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

122 considered open zones (OZ, zones 5 to 8) with low woody cover, as representative as possible of the  
123 diversity of the accessible parts of the site (Fig. S1). Each zone was homogenous in terms of vegetation,  
124 cover, stratification and human intervention. Between 10 and 20 quadrats of 0.2 m<sup>2</sup> were randomly  
125 delimited on each zone, and the species present on these surfaces were determined as well as their cover  
126 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,  
127 87.5%, respectively) (Wikum & Shanholtzer, 1978; Anne et al., 2021). Based on these data, four  
128 diversity indices were calculated: species richness, Shannon index (eq. 1), Simpson index (eq. 2) and  
129 Hill index (eq. 3).

$$130 \quad H' = - \sum (p_i \cdot \log(p_i)) \quad (\text{eq. 1})$$

$$131 \quad \lambda = 1 - (\sum(p_i^2)) \quad (\text{eq. 2})$$

$$132 \quad \text{Hill} = 1 - \left( \frac{\lambda - 1}{\exp(H')} \right) \quad (\text{eq. 3})$$

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

### 135 2.3 Soil sampling and analyses

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

145 Soil samples collected for total element fraction measurements were also used to ensure at least 3  
146 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<sub>2</sub> 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*.

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

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

173 the exposure duration (years); BW is the body weight (kg); AT is the average time (years), equivalent  
174 to ED for noncarcinogenic contaminants or a lifetime (70 years) for carcinogenic contaminants (MEEM,  
175 2017).

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

$$181 \quad HQ = \frac{DI}{TRV} \quad (eq. 5)$$

$$182 \quad CR = DI \times TRV \quad (eq. 6)$$

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

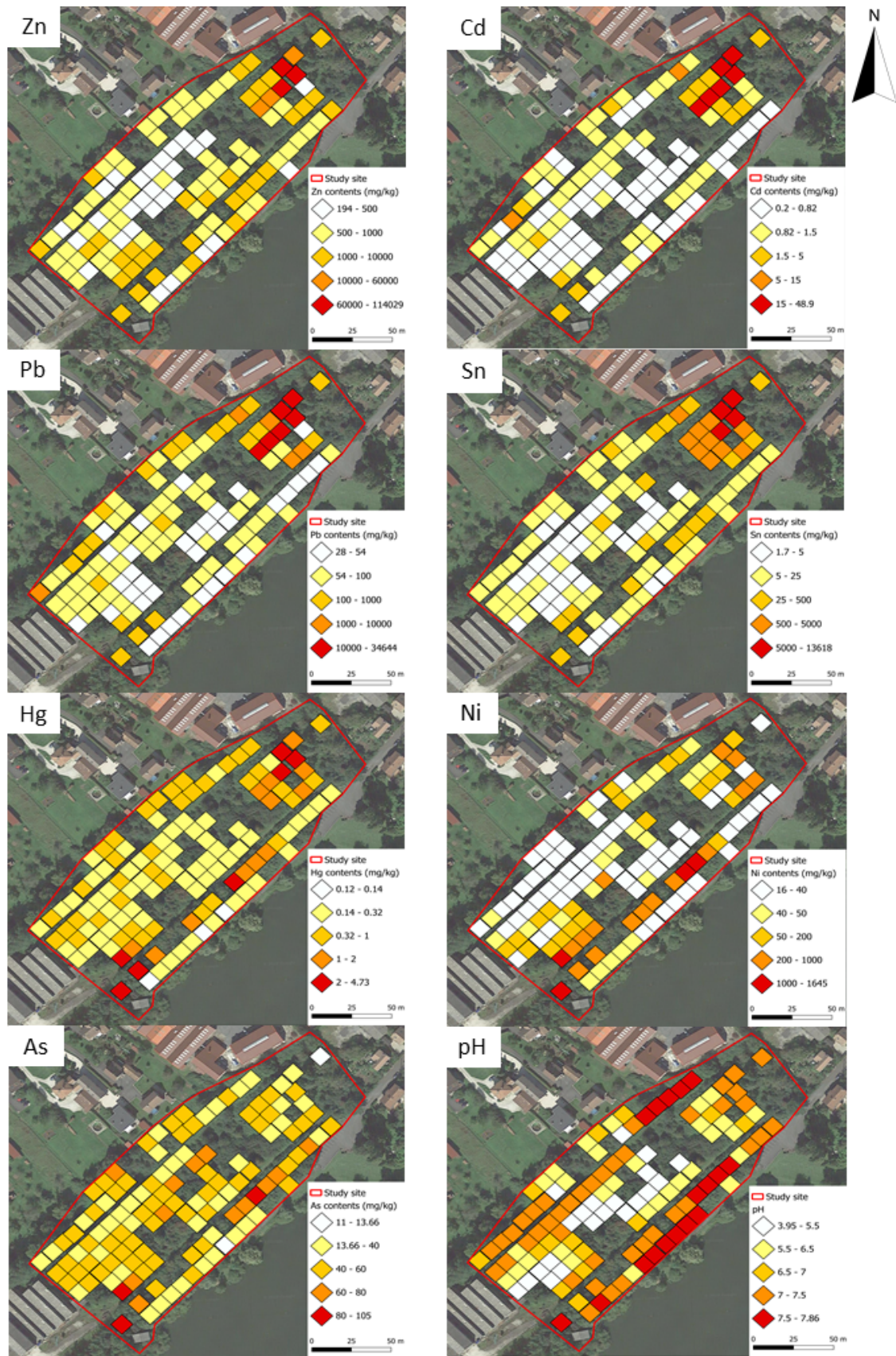
## 187 2.6 Mapping and statistical analysis

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

### 199 3 Results

#### 200 3.1 Soil TE contents

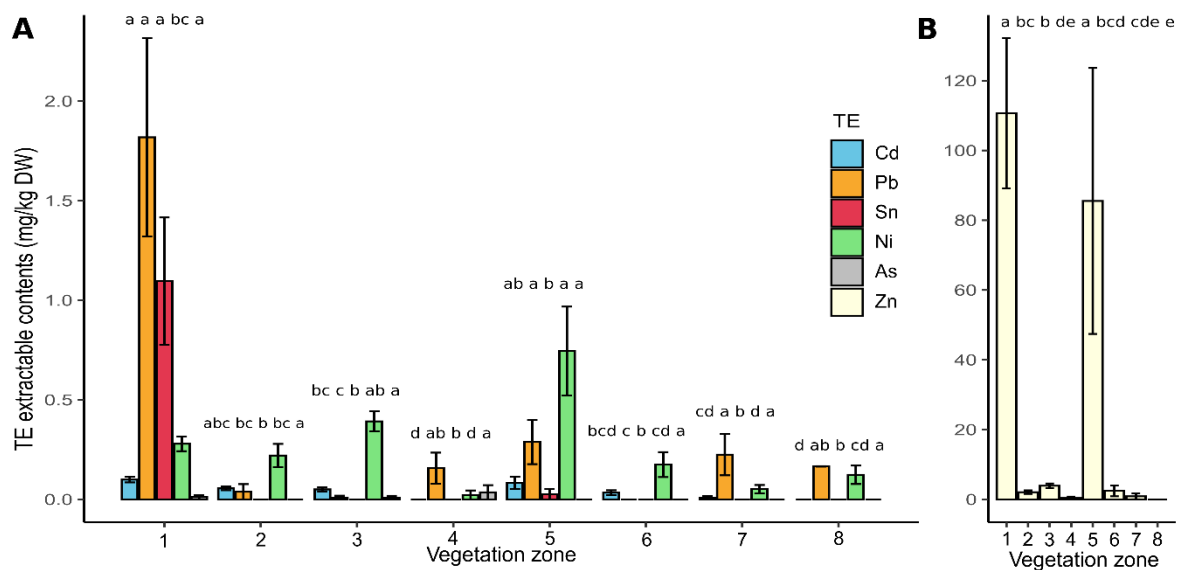
201 The main contaminants found at the site were Zn, Pb, Cd, Sn, Hg, Ni and As (Fig. 2), which were  
202 heterogeneously distributed throughout the investigated area. Indeed, in most of the wasteland, the  
203 measured contents varied from 200 to 10 000 mg.kg<sup>-1</sup> dry weight (DW) for Zn, 0.2 to 10 mg.kg<sup>-1</sup> DW  
204 for Cd, 30 to 2 500 mg.kg<sup>-1</sup> DW for Pb, 2 to 1 830 mg.kg<sup>-1</sup> DW for Sn and 0.12 to 2 mg.kg<sup>-1</sup> DW for  
205 Hg. However, the contaminant map highlighted a heavily contaminated zone of almost 500 m<sup>2</sup> in the  
206 northeast of the site that contained the highest amounts of Zn (36 000–115 000 mg.kg<sup>-1</sup> DW), Pb  
207 (13 500–34 600 mg.kg<sup>-1</sup> DW), Cd (17–50 mg.kg<sup>-1</sup> DW), Sn (3 200–13 600 mg.kg<sup>-1</sup> DW), and Hg (1.4–  
208 4.5 mg.kg<sup>-1</sup> DW). Concerning Ni and As, the contents varied from 16 to 1650 mg.kg<sup>-1</sup> DW and 11 to  
209 105 mg.kg<sup>-1</sup> DW, respectively. The highest contents for these two elements were localized in the south  
210 of the site, close to the factory and the pond. In addition, the site was also contaminated with Cr (26-  
211 1475 mg.kg<sup>-1</sup> DW), Cu (24–1800 mg.kg<sup>-1</sup> DW), Fe (13 000–224 000 mg.kg<sup>-1</sup> DW) and Mn (250–2300  
212 mg.kg<sup>-1</sup> DW). Cu was localized in the most contaminated area of the site (northeast), and Cr, Fe and Mn  
213 were localized in the southern part of the site, similar to Ni and As. The pH values were heterogeneous  
214 throughout the site, although they remained close to neutral, with values that varied from 6.4 to 7.3 in  
215 the most contaminated zone (Fig. 2). The other part of the site had pH values that varied from 4 to 7.9,  
216 and the lowest pH value (4–5.5) was localized along a line from the southwest of the site to the centre.



217

218 *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*  
 219 *plots described in Fig. S1 in the industrial wasteland. For TE contents, the first levels of the scale (white square) range from*  
 220 *the minimum values found on the site to twice the world soil average values defined by Kabata-Pendias (2011), except for Zn,*  
 221 *where the minimum value was too high.*

222 The analysis of the bioavailable fractions further revealed this high heterogeneity for both the UZ (zones  
 223 1 to 4) and OZ (zones 5 to 8) (Fig. S1 & 3). Zones 1 and 5 were statistically the most contaminated  
 224 zones, with Zn extractable contents of 110 and 85 mg.kg<sup>-1</sup> DW, respectively, while the other zones had  
 225 Zn contents of < 4 mg.kg<sup>-1</sup> DW (Fig. 3B). Furthermore, the same distribution was observed in zone 1  
 226 for Sn, with a mean value of 1.1 mg.kg<sup>-1</sup> DW; this value was higher than that in the other zones, with a  
 227 content of 0.026 in zone 5 and contents below the limits of quantification (< LQ) in the other zones.  
 228 Zone 5 had the highest contents of Ni, with a value of 0.74 mg.kg<sup>-1</sup> DW, which was statistically higher  
 229 than that in the other zones (except zone 3), where the contents were in the range of 0.02-0.39 mg.kg<sup>-1</sup>  
 230 DW. For other TEs, although zones 1 and 5 had the highest Pb and Cd contents, the differences in  
 231 content were lower. Indeed, the contents varied from 0.01 to 0.1 mg.kg<sup>-1</sup> DW for Cd (< LQ for zones 4  
 232 and 8), from 0.01 to 1.8 mg.kg<sup>-1</sup> DW for Pb (< LQ for zone 6) and from 0.01 to 0.036 mg.kg<sup>-1</sup> DW for  
 233 As (< LQ for zones 2, 5, 6, 7 and 8) (Fig. 3A).



234

235 *Fig. 3. Mean CaCl<sub>2</sub> extractable contents (mg.kg<sup>-1</sup> DW) of Cd, Pb, Sn, Ni, As (A) and Zn (B) in the 8 floristic survey zones and*  
 236 *standard errors. Letters in common indicate statistically similar groups at p < 0.05 according to Kruskal-Wallis and post hoc*  
 237 *pairwise comparison tests.*

### 238 3.2 Plant diversity

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

241

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

244

245

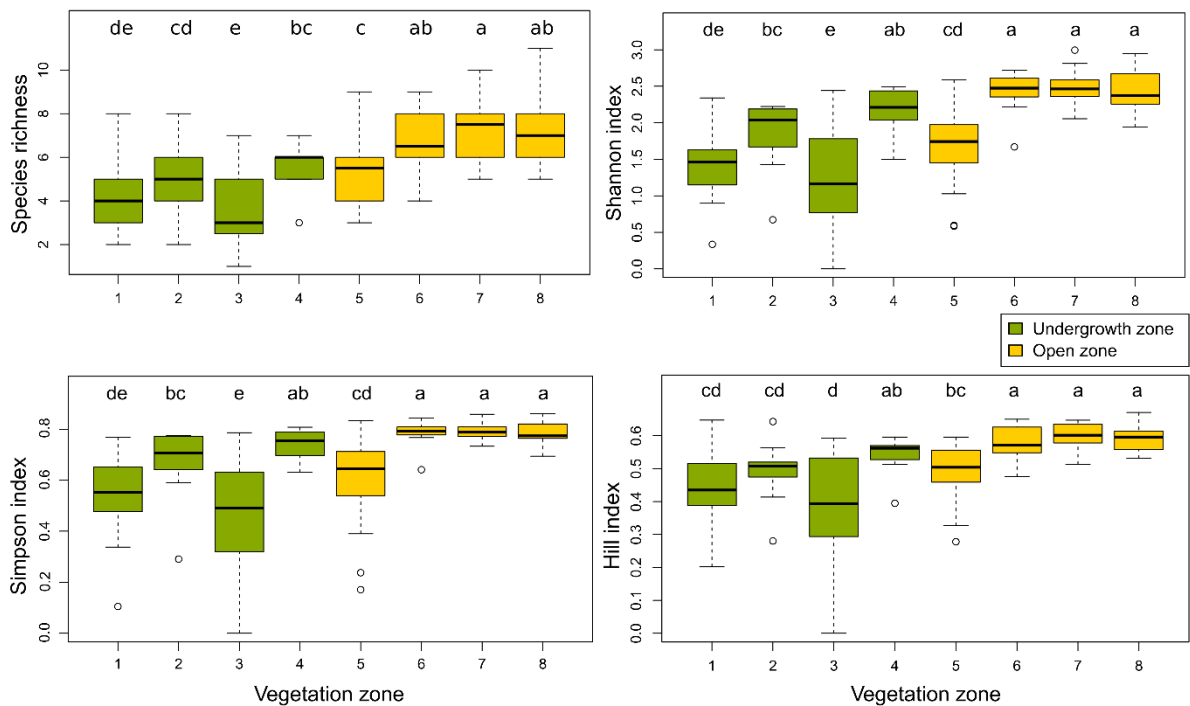
246

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

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

249  
 250 The main families of surveyed species were Asteraceae, Poaceae and Fabaceae (13, 10 and 5 species,  
 251 respectively), which represented almost 40% of the herbaceous species, as well as Salicaceae and  
 252 Rosaceae (6 species each), which represented 50% of shrubs and tree species. However, some families  
 253 were also well represented due to the frequency of certain member species. Indeed, *Hedera helix* was  
 254 the only species observed from the Araliaceae family, but its frequency was 42.7%. Similarly, the  
 255 Brassicaceae family was exclusively represented by *A. petiolata* with a frequency of 40.9%. These two  
 256 plants, along with the Rosaceae species *Rubus fruticosus* (frequency of 54.5%), were the most frequent  
 257 herbaceous species on the site. Conversely, 25 species were observed only once and had a frequency of  
 258 less than 1%, such as the herbaceous species *Dipsacus fullonum* (Caprifoliaceae), *Hypericum humifusum*  
 259 (Hypericaceae) and *Verbascum blattaria* (Scrophulariaceae) and the tree species *Tilia platyphyllos*  
 260 (Malvaceae), *Prunus serotina* (Rosaceae) and *Salix alba* (Salicaceae).

261 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  
 262 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  
 263 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  
 264 in the UZ and from 0.48 to 0.59 in the OZ (Fig. 4).



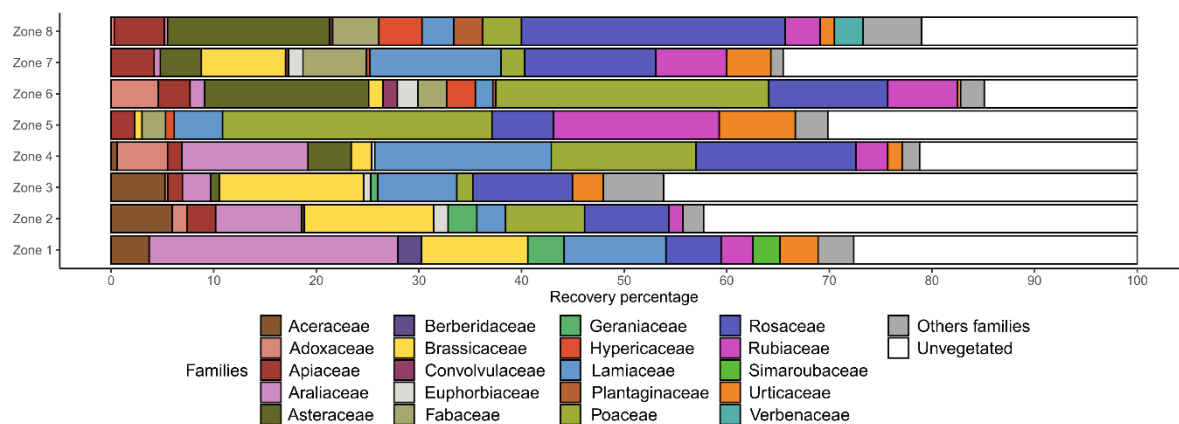
265

266 *Fig. 4. Boxplots of Shannon, Simpson and Hill indices and species richness in the 0.2 m<sup>2</sup> quadrats of the 8 survey zones. Letters*  
 267 *in common indicate statistically similar groups at p < 0.05 according to Kruskal–Wallis and post hoc pairwise comparison*  
 268 *tests.*

269 Overall, the OZ had statistically higher biodiversity indices than the UZ (p values < 0.01), except for  
 270 zone 4, which was the least contaminated part of the UZ, and zone 5, which was the most contaminated  
 271 part of the OZ. We also observed that the biodiversity indices of zone 4 were all statistically higher than  
 272 those of zones 1 and 3. This was also the case for zone 2 but only for the Shannon and Simpson indices.  
 273 Concerning the OZ, the most contaminated area (zone 5) had a systematically lower biodiversity for  
 274 each index than the three other zones. In addition, the TE extractable contents and the biodiversity  
 275 indices for the UZ had significant anticorrelations with Zn (from -0.28 to -0.45) and Ni (from -0.39 to -  
 276 0.57). For the OZ, there were not only anticorrelations with Zn (from -0.44 to -0.6) and Ni (from -0.48  
 277 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).

278 In addition to these relationships between biodiversity and TE extractable contents, the plant  
 279 communities also differed from one zone to another (Fig. 5). Indeed, in the UZ, the Adoxaceae,  
 280 Apiaceae, Asteraceae, Euphorbiaceae and Poaceae families appeared in zones 2, 3 and 4 but not in zone  
 281 1, which was the most contaminated. Moreover, the Rosaceae family represented almost 5.4% of the  
 282 plants in zone 1, unlike the other zones, where it represented between 8.2 and 15.6%.





283

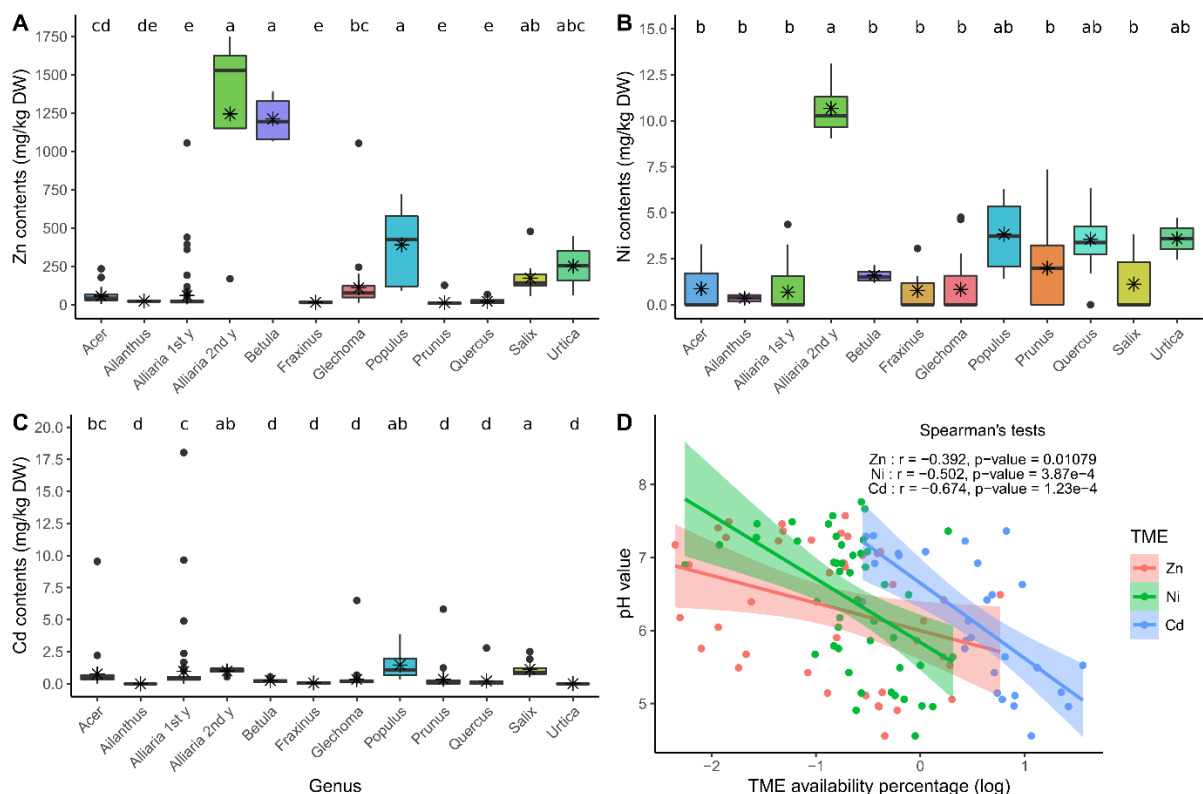
284 Fig. 5. Plant communities present in the floristic survey zones expressed as a recovery percentage of the 37 families identified  
 285 and unvegetated parts.

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<sup>-1</sup> 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

304 plant genus considered. Indeed, *Alliaria* (second-year plants), *Betula*, *Populus*, *Salix*, *Glechoma* and  
 305 *Urtica* contained an average of 1250, 1210, 390, 174, 111 and 253 mg.kg<sup>-1</sup> DW of Zn, respectively.  
 306 Those values were significantly higher than those for the *Ailanthus*, *Alliaria* (first-year plants), *Fraxinus*,  
 307 *Prunus* and *Quercus* genera, where the contents were between 13 and 60 mg.kg<sup>-1</sup> DW. For Ni, second-  
 308 year *Alliaria* had a mean content of 10.7 mg.kg<sup>-1</sup> DW, which was statistically higher than that of the  
 309 other plant genera (except *Populus*, *Quercus* and *Urtica*), where the values varied from 0.36 to 2 mg.kg<sup>-1</sup>  
 310 <sup>1</sup> DW. Concerning Cd, *Alliaria* (second-year plants), *Populus* and *Salix* had mean contents of 1.00, 1.46  
 311 and 1.13 mg.kg<sup>-1</sup> DW, respectively, which were statistically higher than those of other genera (except  
 312 *Acer*), where the values were between 0.09 and 0.96 mg.kg<sup>-1</sup> (< LQ for *Ailanthus* and *Urtica*). The data  
 313 also showed high Mn contents in the leaves of *Quercus* and *Acer* species, with an average of 400 (max  
 314 = 1 350) and 280 mg.kg<sup>-1</sup> (max = 2 400), respectively. In contrast, other genera showed mean Mn  
 315 contents that varied from 15 to 150 mg.kg<sup>-1</sup> with a maximal value of 550 mg.kg<sup>-1</sup>.

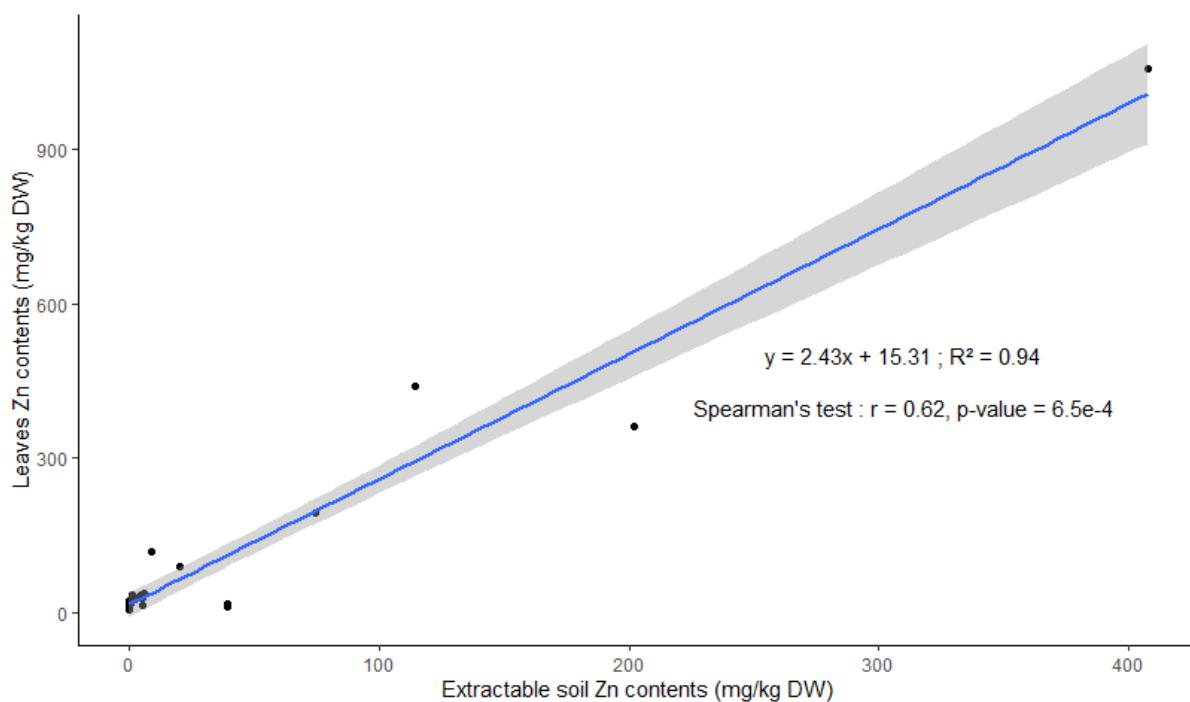


316

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

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

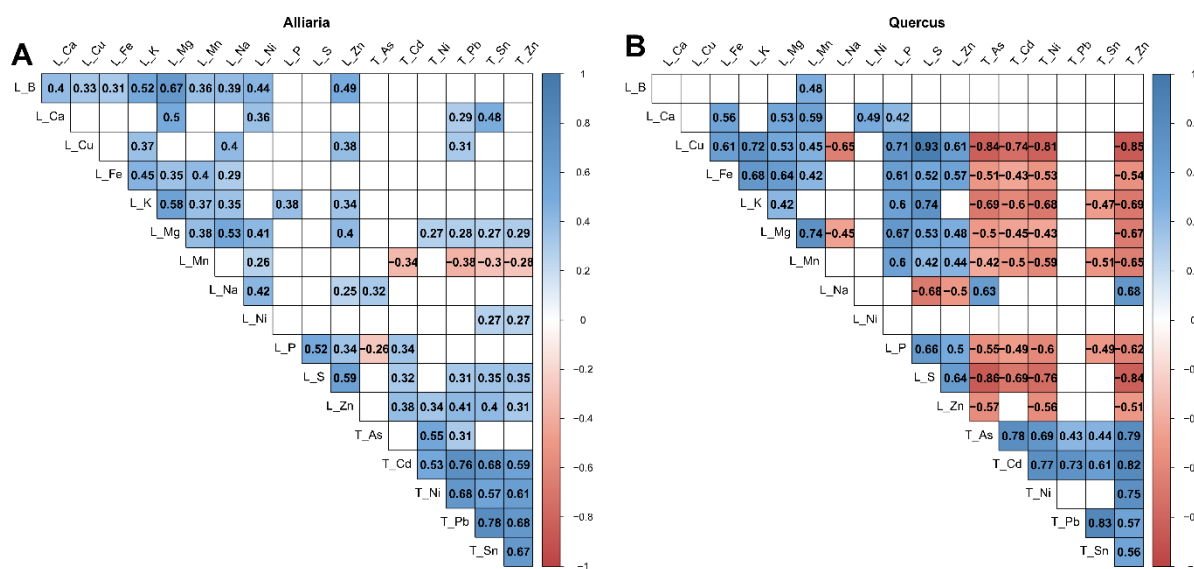
330 In addition to the high contaminant contents found in *A. petiolata*, the data showed that Zn leaf contents  
 331 in first-year plants were representative of soil extractable Zn contents. Indeed, as highlighted in Fig. 7,  
 332 the scatter plot of these two parameters showed that the leaf contents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



336  
 337 Fig. 7. Scatter plot of the first-year *Alliaria petiolata* Zn leaf contents depending on Zn CaCl<sub>2</sub> extractable contents of soil and  
 338 Spearman correlation test between these two parameters. The 95% confidence interval is represented around the relationship.

339 3.4 TE toxicity and risk assessment for plants and humans

340 The correlations between the nutrient contents in leaves and the TE total contents in soil for the *Alliaria*  
 341 and *Quercus* genera are compared in Fig. 8. These 2 genera were compared because they had the lowest  
 342 (*Alliaria*) and the highest (*Quercus*) correlation values of all the genera studied (presented in Fig. S2).  
 343 Concerning *Alliaria*, few anticorrelations were found between Cd, Pb, Sn and Zn soil contents and Mn  
 344 leaf contents and between As soil contents and P leaf contents. Correlation coefficients were low, with  
 345 r values of -0.28 to -0.38 for Mn and -0.26 for P. For other nutrients, there were no highlighted links,  
 346 but there were positive correlations with Mg (r = 0.27 to 0.29 for Ni, Pb, Sn and Zn soil contents), S (r  
 347 = 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  
 348 soil contents). Soil TE contents were more strongly related to nutrient contents for the *Quercus* genus  
 349 than for *Alliaria*. Indeed, Cu, Fe, K, Mg, Mn, P, S and Zn plant contents were negatively correlated with  
 350 As, Cd, Ni and Zn soil contents (and with Sn for K, Mn and P). The correlation coefficients were higher  
 351 for *Quercus* than *Alliaria*, with r values that varied from -0.43 to -0.69 for Fe, K, Mg, P and Zn and from  
 352 -0.69 to -0.86 for Cu and S.



353  
 354 Fig. 8. Correlation matrix of nutrient leaf contents and total TE contents for the *Alliaria* (A) and *Quercus* (B) genera. The  
 355 Spearman correlation coefficient is shown only for tests with a p value < 0.05. L: Leaf contents; T: Total contents in soil.

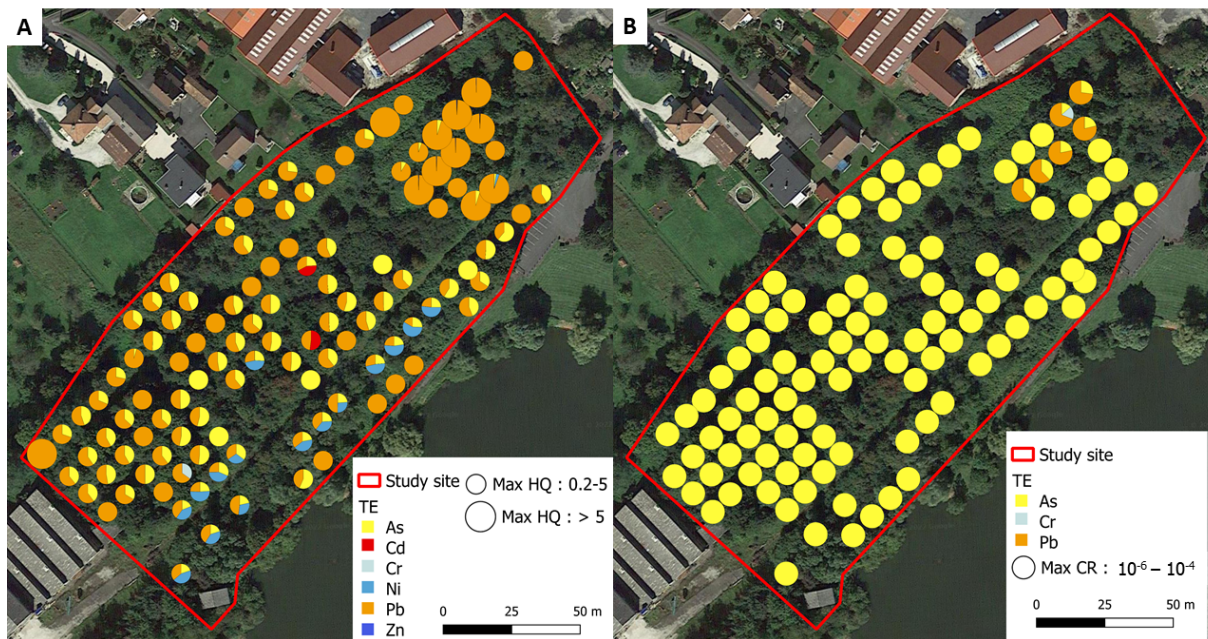
356 For other nutrients, the data did not show noteworthy links with soil contents, with only 2 positive  
 357 correlations between Na leaf contents and As and Zn soil contents (r = 0.63 and 0.68, respectively). In

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  $3.91 \times 10^{-5}$ , except in the most  
377 contaminated area, where Pb (6 areas) and Cr (1 area) also had high CR values that reached  $6.21 \times 10^{-5}$   
378 and  $1.51 \times 10^{-5}$ , 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  $1.32 \times 10^{-5}$ ). 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

## 390 4 Discussion

### 391 4.1 TE contamination and its impact on floristic diversity and plants

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

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

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

420 As a consequence, the TE contents in soils explained most of the variation observed for the four  
421 biodiversity indices. Indeed, while the lower biodiversity in the UZ than in the OZ was related to the  
422 area type, the environmental impacts of contaminants were highlighted by the lower biodiversity indices  
423 found in the most contaminated UZ (except zone 3) and OZ areas. These decreases were related to the  
424 modifications of the plant communities resulting in the presence of fewer but more abundant species.  
425 Indeed, TEs are well known to inhibit plant growth and alter plant communities by selecting tolerant  
426 species (Muszynska & Labudda, 2019), thus inducing a reduction in plant biodiversity (Alsherif et al.,  
427 2022). More specifically, our data showed that Zn and Ni had the greatest impact on biodiversity for  
428 both the UZ and OZ based on the anticorrelations with extractable contents. Cd, Sn and Pb also showed  
429 anticorrelation but only for OZ. Zone 3 showed a high percentage of bare soil and therefore low  
430 biodiversity indices (with high variability), while contaminant levels were comparable with that in zones  
431 2 and 4. This could be explained by the heterogeneous pH values in this part of the site, varying from  
432 3.95 (minimal pH value on site) to 7, with pH being one of the major soil parameters influencing metal-  
433 tolerant plant communities, as shown by Conesa et al. (2007).

434 In addition to influencing biodiversity, TE contents also modified nutrient uptake, as shown by  
435 anticorrelations between TE contents in soil and Cu, Fe, K, Mg, Mn, P, S, Zn contents in the leaves of  
436 *Quercus* species (Fig. 8). These differences can be explained by the mechanisms involved in the uptake  
437 of nonessential TEs by plants. Indeed, these elements can enter the plant through nonspecific pathways  
438 by substituting other divalent cations, such as  $\text{Ca}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Fe}^{2+}$  and  $\text{Zn}^{2+}$  (Rascio & Navari-Izzo,  
439 2011; Tibbett et al., 2021), and the effect depends on the element and the plant considered (Boechat et  
440 al., 2016). Indeed, for the most accumulative species found at the site, *A. petiolata*, few effects were  
441 shown and only for Mn uptake. The tolerance and accumulation capacities are usually explained by the  
442 overexpression of genes encoding transmembrane transporters, proteins involved in the responses to  
443 oxidative stress and synthesis of metal chelators in accumulator species (Rascio & Navari-Izzo, 2011;



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

#### 447 4.2 TE accumulation in plants with a special emphasis on *Alliaria petiolata*

448 Overall, Zn, Cd and Ni were the most accumulated elements in plant leaves at the site because the CaCl<sub>2</sub>  
449 extractable fraction of these elements was higher than that for the other elements, e.g., Pb and Sn. The  
450 anticorrelations between the availability percentage and the pH value in soil for Zn, Cd and Ni supported  
451 this assumption. Indeed, the soil pH value has a strong influence on the availability of these  
452 contaminants, and a decrease in the pH value induces an increase in the extractable CaCl<sub>2</sub> content, as  
453 previously described by other authors ([Kashem & Singh, 2001](#); [Spurgeon et al., 2006](#); [Gandois et al.,](#)  
454 [2010](#)). Therefore, the pH indirectly affected Zn, Cd and Ni plant contents by solubilizing them in soil.

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

470 Fe, Cu and Mn were also found to be transferred to the leaves, which seems rational since these are  
471 essential elements for plants (Fernando et al., 2016; Lange et al., 2017; Naranjo-Arcos et al., 2017).  
472 However, Mn values exceeded the normal leaf content range of 30-300 mg.kg<sup>-1</sup> DW described by  
473 Kabata-Pendias (2011), especially in samples from the genera *Acer* and *Quercus*. Comparable Mn  
474 concentrations have been reported by Stanković et al. (2011), with mean values that ranged between 370  
475 and 550 and 67 and 285 mg.kg<sup>-1</sup> DW for *Quercus petraea* and *Acer campestre* species, respectively,  
476 species that are also present at our study site. Fernando et al. (2016) found Mn contents that reached  
477 2600 mg.kg<sup>-1</sup> DW for *Acer rubrum* (similar to our data) and even higher maximal contents in *Acer*  
478 *saccharum*, with a value of 4300 mg.kg<sup>-1</sup> DW. Concerning the *Quercus* genus, Huang et al. (2014) found  
479 contents of 800 mg.kg<sup>-1</sup> DW, with a maximal value of 2700 mg.kg<sup>-1</sup> DW in *Quercus alba* on an Mn  
480 mining site, which was almost twice as high as the values found for *Quercus* species at our study site.  
481 All of these data highlight that accumulation capacities of Mn, Zn and Cd can vary greatly from one  
482 species to another, even within the same genus.

483 Hg, Sn and Cr were not found in the leaves of any of the tree, shrub or herbaceous species studied. These  
484 results are consistent with the contents of these elements found in the soil CaCl<sub>2</sub> extractable fraction,  
485 which were less than the LQ for most of the samples. Several studies on the chemical distributions of  
486 Pb and As have highlighted that the exchangeable fraction of these elements was lower than that of other  
487 TEs, such as Cd or Zn (Pichtel et al., 2000; Qasim et al., 2014; Gankhurel et al., 2020).  
488 Indeed, depending on the soil, Pb and As could be bound to carbonate, Fe-Mn oxides, organic matter  
489 and/or in residual fractions that are harder to extract and therefore less mobile (Angelova et al., 2004;  
490 Qasim & Motelica-Heino, 2014) and hence poorly transferred to plant tissues.

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

497 2.1 mg.kg<sup>-1</sup> for Cd at contaminated sites. Furthermore, the translocation ratio (ratio between shoot and  
498 root contents) was higher than 1 for Zn, Cd and Ni (Grabner et al., 2011; Drozdova et al., 2019),  
499 indicating that this species has the capacity to concentrate these three contaminants in its aerial parts.  
500 Moreover, the use of *A. petiolata* in phytoextraction strategies has been suggested by Drozdova et al.  
501 (2019), and the maximal Zn and Ni values found in the present study were much higher than the  
502 previously published data, supporting this potential. In addition, this plant is fast growing and produces  
503 a high biomass, which has interesting implications for phytoextraction (Grabner et al., 2011).

504 *A. petiolata* was also demonstrated to be a relevant Zn bioindicator, being distributed throughout the  
505 site. Indeed, Zn accumulation in the leaves of this species was correlated with the CaCl<sub>2</sub> extractable  
506 fractions. These features are usually described to consider a plant as a metal bioindicator (Kabata-  
507 Pendias, 2011).

#### 508 4.3 Impact of TEs on human health

509 The HQ and CR calculated for several parts of the site showed that the TE contents could have an impact  
510 on human health depending on the soil and plant ingestion scenarios considered. For most of the areas,  
511 a possible risk was identified due to Pb and As based on the HQ and CR values. These two elements are  
512 known to be particularly toxic to humans either by disturbing metabolism or being carcinogenic (Turdi  
513 & Yang, 2016). The element that presented the highest risk at the site for both HQ and CR is Pb,  
514 especially in the soil ingestion scenario, due to its high total contents in soil relative to its toxicity.  
515 Indeed, even if this element was the second most abundant after Zn, it would remain the greatest threat  
516 since its toxicity is a thousand times higher than that of Zn. However, in our study site, the low  
517 bioavailability of Pb prevented it from accumulating in plants to levels that are dangerous for humans  
518 in the scenarios considered; nevertheless, the maximal contents found reached the tolerable limit for the  
519 crops described by Kabata-Pendias (2011), which varies from 0.5 to 10. The low contents available for  
520 plants have often been reported in the literature for this element, as previously explained in section 4.2.  
521 Although Pb is the element with the highest risk values at the site, As is the element that most frequently  
522 exceeded the boundary between no risk and possible risk. Although this element had no HQ or CR

523 values high enough to identify it as a risk, there is a possible risk in almost all the areas studied. This is  
524 explained by the high levels of As throughout the site, with more than 60% of the areas studied having  
525 total As contents in soil that exceed 40 mg.kg<sup>-1</sup> DW. Indeed, these values are almost 6 times higher than  
526 the world soil average of 6.8 mg.kg<sup>-1</sup> identified by [Kabata-Pendias \(2011\)](#).

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

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

547

## 548 **5 Conclusions**

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

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