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Title: The phytoextraction power of *Cichorium intybus* L. on metal-contaminated soil: focus on time- and cultivar-depending accumulation and distribution of cadmium, lead and zinc.

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1 **Abstract**

2 This study is focused on the evaluation of the accumulation of Cd, Pb and Zn in five cultivars of
3 *Cichorium intybus* L. (chicory) which were produced on contaminated agricultural soil. Over a growth
4 period of 211 days, the roots and leaves were collected in four stages and then analyzed by flame
5 atomic absorption spectrometry to measure the concentration and distribution of the target metals in
6 these two chicory organs considering the weather and the nature of the cultivar. For all cultivars, sharp
7 decreases of Pb (from 165 mg kg⁻¹ to 3 mg kg⁻¹), Cd (from 11 mg kg⁻¹ to 5 mg kg⁻¹) and Zn
8 concentrations (from 157 mg kg⁻¹ to 40 mg kg⁻¹) in the roots were highlighted over time. The data
9 collected enabled the calculation of the variation of the bioconcentration factor, the biological
10 absorption coefficient and the translocation factor for Cd, Pb and Zn. These parameters were then
11 correlated with the distribution of the fresh biomass of leaves and roots and several indicators such as
12 chlorophyll content, flavonols, anthocyanin and nitrogen balance index were measured. The study
13 concludes with the discussion on the ability of chicory to clean up contaminated agricultural soil. The
14 current investigation has shown: i) a translocation of Cd (and Zn to a lesser extent) from the roots to
15 the leaves; ii) an increase in the level of anthocyanins with the increase of the metal trace elements
16 concentration in the leaf, while the content of chlorophyll and the nitrogen balance index decrease,
17 which could be linked to the phenomenon of senescence; iii) an ability of the chicory to reduce the
18 bioavailable pool of the three metal trace elements studied, in particular for Cd.

19

20 **Key words:** Metal; Chicory; Bioavailability; Plant pigments; Remediation factor

21

22 1. Introduction

23 Soil pollution by metal(loid)s remains a major environmental concern in the whole world. These
24 contaminants accumulate quickly in living organisms (fauna and flora) and often reach toxic
25 concentrations that affect the soil functioning, the ecosystem, and the human health due to their long-
26 term persistence in the environment. Human activities (e.g. mining and smelting of metal ores) are the
27 main sources that contributed to increase the environmental anthropogenic pollution by metal(loid)s
28 (Alloway, 1995, 2013). The most famous contaminated area in the Hauts-de-France region (France) is
29 located in the former coal mining and ore processing sites (Metaleurop Nord and Nyrstar). For more
30 than a century, the main pollutants released by these two metallurgical plants were lead (Pb), cadmium
31 (Cd), and zinc (Zn), defined as metal trace elements (MTE) in the current study. The total
32 concentration of these MTE and their bioavailability in the surrounding agricultural soils is sometimes
33 problematic since a high proportion of the agricultural crops for foodstuffs are not conform with the
34 European legislation (Douay et al., 2013; Pruvot et al., 2006).

35 Many *ex-situ* and *in-situ* methods have been developed to manage polluted soils (Gong et al.,
36 2018) (e.g. encapsulation, soil washing, vitrification...). While these methods allow effective soil
37 treatment, these technologies are often costly, time consuming and unsuitable for managing the soil of
38 large polluted sites (Liu et al., 2018). Soil phytoremediation is a much more viable alternative because
39 of its ease of implementation and the low costs it implies. This technology refers to the use of plants
40 and amendments and/or microorganisms that can be added to improve the soil quality through several
41 processes such as the degradation of organic matter, the degradation of pollutants as well as their
42 extraction or stabilization (Ali et al., 2013). Among these processes, phytoaccumulation consists in
43 metal(loid)s accumulation in the plant organs after their extraction from the polluted soils. Some of
44 them namely nickel (Ni), copper (Cu), manganese (Mn) and Zn are considered as essential for the
45 growth of plants and so, they can accumulate them (Bhargava et al., 2012). Some others like Cd, Pb,
46 silver (Ag), arsenic (As), antimony (Sb) or mercury (Hg) are not essential and can cause phytotoxic
47 effects (Finnegan and Weihua, 2012; Sheoran et al., 2016; Shtangeeva et al., 2011). However,
48 metallophytes are able to concentrate some of these metal(loid)s from contaminated soils within their
49 organs without necessarily significantly alter their growth and development (Rossini-Oliva et al.,

50 2018). Therefore, organ of these plants can be used through many applications to develop the bio
51 economy and so ingrate the circular economy concept (Deyris et al., 2019; Hazotte et al., 2017;
52 Gomes, 2012; Guérin et al., 2021; Hechelski et al., 2018; Janus et al., 2015; Oustrière et al., 2019;
53 Waterlot et al., 2019).

54 *Cichorium intybus* L. (chicory) adapts to wide temperature ranges, different types of soils and is able
55 to grow along the roadside, in industrial sites, urban parks and wasteland, even under high level of
56 contamination (Askoy, 2008). Several studies have shown that chicory is capable, to some extent, of
57 accumulating MTE (Cd, Pb and Zn) when produced on moderately contaminated soil (Mandzhieva et
58 al., 2016; Sekara et al., 2005; Zeng et al., 2019). Moreover, Zeng et al. (2019) showed that
59 translocation of metals to shoots depended on the nature of metals and on seedling and mature periods.

60 The purpose of this work is (i) to assess the dynamic of the MTE accumulation (Pb, Cd and Zn)
61 by the roots and shoots of various cultivars of chicory produced on severely contaminated agricultural
62 soils sampled near the former Metaleurop Nord plant, (ii) to examine the variation of several plant
63 pigments (chlorophyll, anthocyanins, flavonols and nitrogen balance index) and (iii) to evaluate the
64 ability of chicory in the remediation of contaminated soils by Cd, Pb and Zn.

65

66 2. Materials and Methods

67 2.1. Soil sampling, experimental set-up, and plants sampling

68 Contaminated soil (300 kg) was sampled from the ploughed layer (0-25 cm) of an agricultural plot
69 (latitude: 50° 26' 15.9" N; longitude: 3° 01' 04.9" E) located not far away from the former Metaleurop
70 Nord plant (France) in November 2018. This soil is a clay sandy loam dominated by silt and it is
71 characterized by the following parameters: pH=7.7, Total N: 1200 mg kg⁻¹; Total C: 18.2 g kg⁻¹; C/N:
72 15.2; P₂O₅: 160 mg kg⁻¹; Organic matter: 45 g kg⁻¹; CEC: 15 cmol⁺ kg⁻¹ (Hechelski et al., 2020;
73 Nsanganwimana et al., 2015). After homogenization, five containers (outside length: 40 cm; outside
74 width: 30 cm; outside height: 25 cm) were filled with 20 kg of air-dried contaminated soil. The soil
75 moisture experimental device was maintained at 40% field capacity with tap water in greenhouse at
76 20-25 °C. In each of containers, 7 seeds of chicory were sown in 2 rows. Five cultivars, providing by
77 Florimond Desprez company ([3](https://www.florimond-desprez.com/rep/en/nl/groupe-florimond-</p></div><div data-bbox=)

78 desprez-2.html), were considered (C1, C2, C3, C4 and C5) in the current study. Chicories were
79 samples after 74, 105, 156 and 211 days after sowing, taking one plant out of every two times. Shoots
80 and roots of chicory were then separated, crushed, and dried at 105 °C, then stored in acid-washed
81 plastic bottles until digestion.

82

83 **2.2. Digestion of plant and soil**

84 **2.2.1. Soil digestion and extractable-MTE in soils**

85 300 mg of soil were digested by a mixture of 37% HCl (4.5 mL) and 70% HNO₃ (1.5 mL). The
86 suspension was heated at 120 °C for 90 min in a hot-block digester (**HotBlock™ Environmental**
87 **Express®SC100, Charleston, USA**). Ultra-pure water was then added until reaching 25 mL and the
88 mixture was filtered over an acetate Millipore membrane (0.45 µm porosity). The resulting solutions
89 were stored in acid-washed plastic bottles at 4 °C prior to analysis. Calcium chloride (0.01 M) and a
90 mixture of Low Molecular Weight Organic Acids (LMWOAs) were used to evaluate the labile pool
91 MTE in soils, the bioavailable MTE in short and long run ([Waterlot et al., 2017](#); [Waterlot and](#)
92 [Hechelski, 2019](#)).

93 **2.2.2. Plant digestion**

94 300 mg of dry plant sample (roots or leaves) were added to 5 mL of 70% HNO₃. The resulting
95 suspension was heated at 95 °C for 90 min using the hot-block digester. After heating, 5 mL of H₂O₂
96 (30%) were slowly added in the cooled solution and the mixture was heated at 90±5 °C for 180 min.
97 Ultra-pure water was then added until reaching a total volume of 25 mL, the mixture was filtered over
98 an acetate Millipore membrane (0.45 µm porosity) and stored at 4 °C prior to analysis.

99 **2.2.3. Chemical and statistical analyses**

100 The concentration of MTE (Pb, Cd and Zn) in soil and plant digests were determined by flame or
101 electrothermal atomic absorption spectrometry (**AA-6800, Shimadzu, Tokyo, Japan**) following the
102 recommendations described in the literature ([Waterlot et Douay, 2009](#); [Savio et al., 2010](#)). **The limit of**
103 **detection was 2.1 µg Cd L⁻¹, 41 µg Pb L⁻¹, 1.5 µg Zn L⁻¹ using flame as atomizer and 0.06 µg Pb L⁻¹**
104 **using graphite furnace as atomizer** ([Waterlot et al., 2013](#); [Waterlot and Hechelski, 2019](#)). **INCTL-**
105 **OBTL-5 (Virginia tobacco leaves n=3) was used as reference material for the quality control. The**

106 digestion and analysis procedures yielded good average recovery values of 90-108 %, depending on
107 MTE (Obtained concentrations: [Cd] = 2.36±0.12 mg kg⁻¹; [Pb] = 2.11±0.21 mg kg⁻¹; [Zn] = 56.2±2.2
108 mg kg⁻¹; Certified concentrations: [Cd] = 2.64±0.14 mg kg⁻¹; [Pb] = 2.01±0.31 mg kg⁻¹; [Zn] =
109 52.4±1.8 mg kg⁻¹).

110 Chlorophyll content (µg cm⁻²), Flavonols Index (relative units) and Anthocyanins Index (relative
111 units) and were measured using a plant pigment content meter (Dualex® Scientific+, Force-A, Orsay,
112 France) on green leaves at the base of the rosette. Moreover, the Dualex device provided a Nitrogen
113 Balance Index (NBI) as reported in Rusinowski et al. (2019). Data collected were compared with those
114 obtained from chicories grown in the same conditions on a control unpolluted soil considered as a
115 reference soil (R). From the data set (n₁ = 120; n₁ = 6n with n=20), analyses were performed using the
116 software program XLSTAT 2021 (Addinsoft, Paris, France). Significant differences between reference
117 and/or cultivars were determined using a one-way ANOVA. Normality and homoscedasticity of
118 residuals were met for all tests. When significant differences occurred between reference and/or
119 cultivars, multiple comparisons of mean values were made using post-hoc Tukey HSD tests.
120 Differences were considered statistically significant at p<0.05.

121

122 **2.3. Indices calculation**

123 **2.3.1. Bioconcentration factor (BCF)**

124 The BCF is the ability of plants to accumulate MTE with respect to their concentration in soils
125 (Wang et al., 2019). In the current study, the BCF is the ratio of MTE concentration in the roots to
126 those in the soil substrate (Eq. 1; Mirzaei et al., 2021):

$$127 \quad BCF = \frac{C_{MTERP}}{C_{MTE S}} \quad (1)$$

128 where C_{MTERP} is the MTE concentration in the root of plants and C_{MTE S} is the MTE concentration of
129 in soils.

130 **2.3.2. Biological absorption coefficient (BAC)**

131 The BAC is related to the bioaccumulation of MTE in the aerial part of plants. This index is
132 defined as the ratio between the MTE concentrations in the shoots and the soil substrate (Eq. 2, [Zehra
133 et al., 2020](#)).

$$134 \quad BAC = \frac{C_{MTESP}}{C_{MTE S}} \quad (2)$$

135 where C_{MTESP} is the MTE concentration in the shoot of plants and $C_{MTE S}$ is the MTE concentration in
136 soils.

137 2.3.3. Translocation factor (TF)

138 The TF indicate the ability of plants to translocate MTE from roots to leaves ([Zehra et al., 2020](#))
139 and is defined as follows (eq 3):

$$140 \quad TF = \frac{C_{MTE L}}{C_{MTE R}} \quad (\text{eq 3})$$

141 where $C_{MTE L}$ is the concentration of MTE in leaves and $C_{MTE R}$ is the concentration of MTE in roots.

142

143 3. Results and discussion

144 3.1. Metal concentration in soil-chicory system

145 The concentrations of Pb, Cd and Zn in the studied agricultural soil were $757.1 \pm 35.4 \text{ mg kg}^{-1}$,
146 $10.7 \pm 0.5 \text{ mg kg}^{-1}$, $857.4 \pm 74.0 \text{ mg kg}^{-1}$ ($n = 9$), respectively. On average, these concentrations are
147 20-fold, 25-fold and 12-fold higher than those in the loessic reference soils ([Sterckeman et al., 2002](#)).
148 Compared to the ploughed horizons of cultivated plots around Noyelles-Godault plants, Pb and Zn
149 concentrations in the studied agricultural soil were the highest (45% and 33%, respectively) whereas
150 Cd was quite similar (10.7 vs 9.8 ; [Sterckeman et al., 2002](#)). The 0.01M CaCl_2 -extractable
151 concentrations were $0.04 \pm 0.01 \text{ mg Pb kg}^{-1}$, $0.28 \pm 0.07 \text{ mg Cd kg}^{-1}$ and $0.56 \pm 0.11 \text{ mg Zn kg}^{-1}$ ($n =$
152 9). The extractable-MTE concentrations measured in the mixture of LMWOAs were $5.34 \pm 1.07 \text{ mg}$
153 Pb kg^{-1} , $1.83 \pm 0.12 \text{ mg Cd kg}^{-1}$ and $172 \pm 13 \text{ mg Zn kg}^{-1}$ ($n = 9$). These results highlight the poor
154 labile pool of these MTE. The bioavailable fraction of MTE in short run was below 0.1%. In long run,
155 it was 0.7% for Pb, 17% for Cd and 20% for Zn. This means that a potential decrease of the total Cd
156 and Zn will be done, but obviously it will take time.

157 [Figure 1](#) shows the mean of MTE concentrations in the roots and shoot of chicory over time. Pb-
158 concentrations in the roots of chicory at the first sampling (74 days) ranged from 101 to 165 mg.kg⁻¹,
159 depending on cultivars. These concentrations were 10-fold higher than those measured in the shoots of
160 cultivars C1, C2 and C5 and 6-fold higher for C4. From the second sampling up to the sampling at the
161 end of the experiment, the concentration measured in the roots of chicory drastically decreased (from
162 60% to 85%) to reach a plateau, ranging from 3.21 to 9.07 mg Pb kg⁻¹ for the two last samplings,
163 which depends on cultivars. In the same time, slight modifications in the Pb concentrations measured
164 in the shoots of chicory may be noted but without any relations with the decrease of Pb concentrations
165 in the roots. These results are not surprising knowing that Pb is a non-phytoavailable MTE and
166 suggests a dilution of Pb in the roots over the growing period. **Indeed, the ratio of roots biomass**
167 **between the second and the first samplings (105 and 74 days, respectively) is approximately 14.**
168 However, these results are somewhat at odds with those described in Zeng et al. (2019). The mean Pb
169 concentrations measured by the authors in the roots and shoots of chicory were around 50 and 25 mg
170 kg⁻¹, although the Pb concentration in their soil were 10-fold lower at least than in the current study.
171 This result means that the labile pool of Pb is a crucial parameter to consider. The extractable-MTE
172 concentration using the mixture of LMWOAs and 0.01 M CaCl₂ represented 1% and 0.01% of the
173 total Pb concentration, respectively, as reported in previous studies (Hechelski et al., 2020;
174 Nsanganwimana et al., 2015). These data correlated well with the fact that the Pb concentrations in
175 both studied chicory' organs are low despite the high total Pb concentrations in the soil substrate.

176 Variations in Cd and Zn concentrations in roots and shoots of chicory over time are presented in
177 [Figures 1B and 1C](#). The concentrations of Cd and Zn in the roots of chicory decreased over time (from
178 50 to 60% and 64 to 78%, respectively) and slightly increased at the fourth sampling (211 days). In
179 contrast, concentration of the two metals increased over the growth period, from 25 to 147% for Cd
180 and from 5.5 to 43% for Zn. As shown on [Figures 1B and 1C](#), the Cd and Zn accumulations in plant
181 organs at the end of the experiments were higher in shoots than in roots whatever the cultivars are. 211
182 days after sowing, the Cd and Zn concentrations in the shoots of chicory were up to 50 mg kg⁻¹ DW
183 and 172 mg kg⁻¹ DW, respectively. These concentrations were, respectively, 7-fold and 3.4-fold higher
184 than those in chicory' roots. These results correlated well with those obtained for Zn by Zeng et al.

185 (2019) but are quite different for Cd. No significant difference was highlighted by the authors between
186 the Cd concentrations measured in shoots and in roots in seedling and mature periods. It has been
187 reported in previous studies that the MTE accumulation capacities are affected by interspecific
188 differences, environment conditions (Grant et al. 2008; Shi and Cai 2009) and the available Cd and the
189 total Cd concentration, which was 8-fold higher in the current study than in Zeng et al. (2019).

190

191 **3.2. Bioaccumulation factor, biological absorption coefficient and translocation factor**

192 MTE-accumulation, MTE-translocation, and MTE-distribution in the upper and underground
193 parts of plants were studied by means of three indices named bioconcentration factor (BCF: root-to-
194 soil metal concentration ratio), biological adsorption coefficient (BAC: plant leaves-to-soil metal
195 concentration ratio) and translocation factor (TF: leaves-to-root metal concentration ratio). The
196 utilization of these indexes was recommended to define the ability of plant models in the
197 phytoextraction and phytostabilization of MTE (Zhou et al., 2020). According to Alagić et al. (2015),
198 a value of BCF greater than 1 ($BCF > 1$) indicates a good bioaccumulation of MTE in the roots of
199 plants and if these plants have BAC values greater than 1 ($BAC > 1$), they are a suitable option for
200 bioaccumulation of MTE in their shoots. Moreover, if $TF > 1$, the good ability of plants to translocate
201 MTE from their roots to their aboveground parts make them as suitable for phytoextraction purpose
202 (Vamelari et al., 2010). In contrast, for $BCF > 1$ and $TF < 1$, the potential of plants to be MTE-tolerant
203 is great and so, they have potential for introducing as phytostabilization purpose (Vamelari et al.,
204 2010). The three parameters were reported in Table 1 for each sample. The Pb-BCF values were low,
205 ranging from 0.13 to 0.22 at the first sampling, and then, decreased sharply to reach 0.01 from the
206 third sampling up to the last one. This trend was not cultivar-dependent. As shown in Table 1, the Pb-
207 BAC values are very low over time (ranging from 0.01 to 0.03) whatever the cultivar meaning that
208 chicories should not be suitable for Pb-phytoaccumulation. However, depending on time, the Pb-TF
209 values were higher than 1 and were cultivar-dependent (from 1.44 to 3.53 at 156 days and from 0.86 to
210 1.34 at 211 days). These trends correlated well with the fact that the Pb-concentration remained quite
211 constant from the second harvest to the end of the experiment. Caution should be taken when
212 interpreting the results related to Pb-BAC and Pb-TF. Thus, we do not want to conclude on the

213 capacity of chicory to phytostabilize and phytoextract Pb. However, it is worth mentioning that
214 chicories were described in other studies as Pb-phytoextractor (Vamelari et al., 2010) and Pb-
215 accumulator (Baker et al., 1981).

216 As reported in Sêkara et al. (2005), Mandzhieva et al. (2016) and Zeng et al. (2019), the data for
217 Cd distribution suggest that chicories are characteristic of good accumulators since BAC and TF were
218 higher than 1. These two indicators increased over the growth period to reach values higher than 4 and
219 8, respectively corroborating the decrease of the BCF values over the same period. Unfortunately, due
220 to the size of containers, the growth of plants was limited between 156 days and 211 days, potentially
221 leading to a bias in the data collected. This result is even more pertinent as 60% of the total Cd-
222 concentration was found in the acido-soluble fraction of the fourth-step sequential extraction
223 procedure (Pelfrêne et al., 2015). Thus, by removing the shoots of chicory from the field, this plant
224 should be able to be used for the removal of the bioavailable pool of Cd from the soil. Although Cd
225 and Zn have similar chemical properties, the Zn-BAC and Zn-BCF were lower than 1 from the first
226 harvest to the last one. However, Zn-BAC decreases whereas Zn-BCF increases over the growth
227 period meaning that Zn-TF increases. As shown in Table 1, this indicator increases in time up to 156
228 days and was higher than 3 at 211 days meaning that chicories are able to extract Zn from soil.
229 Recently, Wolf and Paulino (2020) pointed out the potential toxicity effects of Zn when chicories were
230 produced on Zn-contaminated soils. In fact, the authors spiked their soils with zinc sulfate to obtain
231 concentrations of 1200 mg Zn kg⁻¹ and 2400 mg Zn kg⁻¹. They highlighted a decrease of leaf
232 biomasses 45 days after seedling and chlorosis effects. In the current study, no visual symptoms were
233 noticed.

234

235 ***3.3. Plant uptake and foresight***

236 The total harvested-plant biomass was expressed as leaf and root fresh-biomass distribution
237 (Figure 2). As reported on it, the absolute mass distribution of these two plant-parts depends on
238 cultivars. Leaf and root biomasses were in the same order of magnitude for the first two cultivars
239 whereas they were different for the last three cultivars. For those, biomasses of roots ranged from
240 58.6% to 59.5% and so, were higher than in leaves. Based on the total mass of sampled roots and

241 leaves, exported metals from the soil to the chicories were calculated and presented in [Table 2](#). The
242 total metal uptake in leaves and roots were slightly dependent on cultivars and were highly metal-
243 dependent. The ascending order of metals mass in the leaves of chicories was Zn>Cd>Pb whereas it
244 was Zn>Pb>Cd in roots. Whatever the results, the data highlighted that if chicories are produced on
245 contaminated soils, collections of leaf are required to avoid the recycling of leaf-bound MTE before
246 the root harvest. As shown in [Figure 3](#), chlorophyll contents in the reference (R), and the cultivars C3
247 and C5 were statistically the same. This result corroborates with the fact that the exported MTE by
248 these two cultivars were the lowest. On the contrary, statistical differences were highlighted between
249 the chlorophyll contents in R and the cultivars C1, C2 and C4, for which the exported MTE were the
250 greatest. This means that higher the exported MTE is, lower the concentration of chlorophyll in the
251 leaves of chicory is. Indeed, among the survival mechanisms of plants are MTE-translocation to leaves
252 and decreasing chlorophyll ([Uzu et al., 2010](#)). Moreover, the decrease of chlorophyll may be linked to
253 the senescence ([Wojciechowska et al., 2018](#)).

254 Although there is no statistical difference in the concentration of flavonols between the reference
255 and the five cultivars studied, it is worth mentioning the relationship between the results obtained with
256 chlorophyll and anthocyanins. No statistical difference was highlighted between anthocyanins in R and
257 C3 and C5, whereas anthocyanin concentrations in R were statistically different from those in C1, C2
258 and C4. As shown in [Figure 3](#), concentrations of anthocyanin were the highest in these three cultivars.
259 This corroborates with previous studies in which it was well established that one of the role of
260 anthocyanin is the protection of plants from several abiotic and biotic stress ([Chalker-Scott,](#)
261 [1999](#); [Ahmed et al., 2014](#)), which may provide them a better adaptation to contamination level. Acting
262 as antioxidant, anthocyanins are able to bind with MTE reducing consequently their toxicity ([Dai et](#)
263 [al., 2012](#); [Akbari et al., 2013](#)). It is worth mentioning that the formation of anthocyanin-MTE
264 complexes is pH-dependent since it affects the protonation/deprotonation of anthocyanins and
265 consequently their reactivity towards heavy metals.

266 As for chlorophyll concentrations, the NBI indices ([Figure 3](#)) were the lowest for C1, C2 and C4
267 and statistically different from that of R. In addition, no statistical difference was highlighted between
268 the reference and the cultivars C3 and C5. Defined as an indicator of C:N allocation changes ([Cerovic](#)

269 [et al., 2012](#)), the NBI could be a simple valuable tool for preventing the rate of senescence of chicory
270 leaves in addition to the chlorophyll measure, and so the premature leaf fall of chicories produced in
271 contaminated soils. This highlight has been suggested in several studies about miscanthus ([Mos et al.,](#)
272 [2013](#); [Rusinowski et al., 2019](#)).

273 **Despite** the short time-experimentation (211 days) and the limited-size of the containers, the
274 exported metal from soil ranged from 0.83 mg to 1.59 mg Pb, 1.44 to 2.03 mg Cd and 6.94 to 10.87
275 mg Zn. Based on the dataset, a remediation factor (RF) was calculated according to the definition
276 described in [Sabir et al. \(2015\)](#). **Briefly, this factor was defined as the ratio of the mass of metal in the**
277 **set of plants cultivated in each container to soil as follows** (Eq. 4):

$$278 \quad RF = \frac{MTE \text{ concentration in plant} \times \text{plant dry biomass}}{MTE \text{ concentration in soil} \times \text{mass of air-dried contaminated soil}} \times 100 \text{ (eq 4)}$$

279 It was clearly demonstrated that the mechanism uptake of MTE depended on several parameters
280 like the root zone, the environmental conditions, the soil parameters, the plant species, the speciation
281 of MTE ([Tangahu et al., 2011](#)) and so, the bioavailability of MTE. Thus, two remediation factor
282 indexes, named RF_{LMWOAs} and RF_{CaCl_2} were calculated considering the extractable MTE concentrations
283 measured in the mixture of organic acids (LMWOAs) and in the 0.01 M calcium chloride solution.
284 The results are summarized in [Table 3](#). Although it was demonstrated that Pb, Cd and Zn were poorly
285 phytoavailable using miscanthus as plant model ([Nsanganwimana et al., 2015; 2021](#)), the RF-Cd
286 values were in the range 0.67-0.95 %, depending on the cultivars. Even if the maturity of chicories has
287 not been reached, this result is interesting since the RF values tend to increase with time ([Saraswat and](#)
288 [Rai, 2011](#)). According to our knowledge, no study reported similar data using chicory cultivars. In
289 contrast, some RF-Cd values were reported in literature using various plant models in
290 anthropogenically or artificially contaminated soils ([Hu et al., 2014](#); [Chhajro et al., 2018](#); [Moameri](#)
291 [and Khalaki, 2019](#)). [Chhairo et al. \(2018\)](#) revealed RF-Cd values of 0.39 without any amendment and
292 up to 1 using chelators (EDTA, NTA, citrate) and castor bean (*Ricinus communis*) whereas Rf-Cd
293 values below 0.7 was highlighted whatever the treatments (EDTA, EGTA, citrate; [Sun et al., 2011](#); [Hu](#)
294 [et al., 2014](#)). However, RF-Cd values between 1.0 and 4.0 using *Secale montanum* and *Leucaena*
295 *leucocephala* were reported under a phytoextraction context ([Saraswast and Rai, 2009, 2011](#); [Moameri](#)

296 and Khalaki, 2019) As shown in Table 3, the RF-Pb values are very low, from 0.006 to 0.01. They are
297 in the same order of magnitude as those reported in Moameri and Khalaki, (2019) and Sun et al.
298 (2011). Nevertheless, depending on the ability of shrub and tree species to phytoextract Pb, some
299 authors highlighted medium and high RF-Pb values (from 0.5 to 13.5; Aman et al., 2018; Singh et al.,
300 2020). The RF-Zn values were ten-fold higher than those of RF-Pb although the total concentrations of
301 these MTE in the soil studied were quite similar. Even if similar RF-Zn values were reported in Sun et
302 al. (2001) as those obtained in the current study, it is worth noting that RF-Zn values are between 0.3
303 to 2.1, depending on plant species and soil characteristics (Aman et al., 2018; Sarawat and Rai, 2011;
304 Sabir et al., 2015).

305 In the Gentle soil Remediation Options (GRO) the common goal is to decrease the labile pool of
306 MTE because it represents the most available pool of MTE. This pool is a part of the total metal
307 concentration and is commonly named bioavailable. In this concept, phytoextraction and
308 phytoremediation are both strategies that can be used to achieve this objective if this plant-based soil-
309 management is integrated in the bioeconomy concept. From these global considerations, we defined
310 the RF_{LMWOAs} and RF_{CaCl_2} described above. Values of these two remediation factors were higher than
311 those of RF since there were defined from the extractable-MTE concentrations. As indicated in Table
312 3, the $RF_{LMWOAs-Cd}$ is higher than 4 meaning that chicories can be used as phytoextractor for
313 bioavailable-Cd in long run. This result correlated with the conclusion described previously from the
314 BAC, BCF and TF values. In contrast, the results remain less evident for Pb and Zn since their
315 respective RF_{LMWOAs} values were generally below 1. Anyway, chicories are able to decrease the pool
316 of the bioavailable-MTE in short time as shown by the RF_{CaCl_2} values for the three MTE studied.

317 Although the growth period was limited in time due to the size of containers, this new approach
318 and the whole of results obtained from container-experiments should provide new insights into the
319 potential of chicories in lowering the environmental risks after a large-scale chicory production on
320 contaminated agricultural soils. However, it is worth mentioning that further studies need to be
321 conduct due to (i) the restriction of the root size in the current study and not for field studies and (ii) a
322 potential lateral and vertical gradient of metal(loid)s contamination levels in real contaminated sites.

323

324

325 **4. Conclusion**

326 This study, carried out over a period of 211 days, was conducted to determine the concentration
327 and time distribution of Cd, Pb and Zn in five cultivars of chicory which have been produced on an
328 agricultural contaminated soil. Leaves and roots analysis at 4 time points during experimentation (at
329 74, 105, 156 and 211 days, respectively) revealed that the Cd and Zn concentration in plants organs at
330 the end of the experiments were higher in shoots than in roots (up to 7-fold and 3.4-fold higher,
331 respectively) whatever the cultivars were, while no significant differences were observed for Pb.
332 Indeed, Cd-BAC and Cd-TF increase during the growing period to reach values above 4 and 7,
333 highlighting the ability of chicory to reduce the Cd content of the soil. This reasoning is also valid for
334 Zn, but to a lesser extent since the Zn-TF reaches values higher than 3 at 211 days. The Zn-BAC
335 values are lower than 1 from the first harvest to the last one. In addition, MTE concentrations in shoots
336 have been related to the content of chlorophyll, anthocyanins and nitrogen balance index. It appears
337 that an increase of the MTE concentration in the leave induces i) an increase of the anthocyanin
338 content, which may provide to the chicory a better adaptation to contamination level ii) a decrease of
339 the content of chlorophyll and nitrogen balance index, which could be related to the senescence
340 phenomenon. Finally, the phytoextracting power of chicory has been discussed regarding the
341 bioavailable part of the total MTE concentration and has been identified as a good phytoextractor,
342 particularly for Cd. These news results thus highlight the interest of harvesting the aerial parts of
343 chicory thus limiting the deposition of MTE after large-scale chicory production on contaminated
344 agricultural soils.

345

346 **Declaration of Competing Interest**

347 The authors declare that they have no known competing financial interests or personal relationships
348 that could have appeared to influence the work reported in this paper.

349

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355

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544

Table 1 Mean values of bioaccumulation factor (BAC), bioconcentration factor (BCF) and translocation factor (TF)

Cultivar	Time (days)	BAC			BCF			TF		
		Pb	Cd	Zn	Pb	Cd	Zn	Pb	Cd	Zn
C1	74	0.01	2.06	0.15	0.13	1.05	0.16	0.11	1.97	0.95
	105	0.02	2.14	0.13	0.02	0.44	0.05	0.81	4.91	2.54
	156	0.01	2.45	0.17	0.01	0.39	0.03	1.44	6.31	5.37
	211	0.01	3.29	0.21	0.01	0.57	0.05	0.89	5.80	4.47
C2	74	0.02	1.93	0.15	0.17	1.09	0.20	0.10	1.77	0.75
	105	0.03	1.77	0.11	0.07	0.44	0.04	0.42	4.06	2.62
	156	0.02	2.89	0.15	0.01	0.33	0.04	3.36	8.70	4.22
	211	0.01	3.22	0.20	0.01	0.62	0.05	1.04	5.17	3.63
C3	74	-	-	-	-	-	-	-	-	-
	105	0.02	2.03	0.12	0.05	0.60	0.09	0.45	3.38	1.40
	156	0.02	2.28	0.14	0.01	0.31	0.03	2.48	7.45	4.10
	211	0.02	2.55	0.16	0.01	0.56	0.05	1.34	4.57	3.33
C4	74	0.04	1.90	0.14	0.22	1.15	0.18	0.16	1.65	0.77
	105	0.02	2.23	0.11	0.03	0.57	0.07	0.66	3.90	1.73
	156	0.01	2.73	0.15	0.00	0.09	0.05	3.53	28.82	2.90
	211	0.01	4.69	0.20	0.01	0.70	0.06	0.89	6.65	3.38
C5	74	0.01	2.06	0.14	0.13	1.14	0.18	0.10	1.81	0.78
	105	0.01	1.57	0.11	0.03	0.50	0.06	0.44	3.12	1.90
	156	0.02	2.26	0.13	0.01	0.30	0.03	2.02	7.49	4.05
	211	0.01	3.58	0.15	0.01	0.49	0.05	0.86	7.38	2.97

Table 2 Leaf accumulation and root uptake of MTE by chicories

cultivar	MTE accumulation in leaves			MTE uptake by roots			MTE uptake by chicories		
	Cd (mg)	Pb (mg)	Zn (mg)	Cd (mg)	Pb (mg)	Zn (mg)	Cd (mg)	Pb (mg)	Zn (mg)
C1	1.18	0.28	6.23	0.41	0.56	2.72	1.59	0.83	8.96
C2	1.42	0.33	6.65	0.51	0.51	4.21	1.93	0.84	10.87
C3	1.02	0.54	4.94	0.57	1.05	3.91	1.58	1.59	8.86
C4	1.44	0.39	5.04	0.59	0.67	4.51	2.03	1.06	9.56
C5	1.01	0.30	3.40	0.43	0.93	3.54	1.44	1.23	6.94

Table 3 Remediation factors (RF)

cultivar	RF			RF _{LMWOAs}			RF _{CaCl₂}		
	Cd	Pb	Zn	Cd	Pb	Zn	Cd	Pb	Zn
C1	0.74	0.006	0.05	4.42	0.79	0.26	28.4	119.2	80.5
C2	0.90	0.006	0.06	5.37	0.79	0.32	34.5	119.6	97.7
C3	0.74	0.010	0.05	4.40	1.50	0.26	28.3	227.0	79.6
C4	0.95	0.007	0.06	5.65	1.00	0.28	36.3	152.0	85.9
C5	0.67	0.008	0.04	4.00	1.16	0.20	25.7	175.8	62.4

Fig. 1. Pb, Cd and Zn accumulation (A,B,C) in the roots and shoots depending on the growing time and cultivars

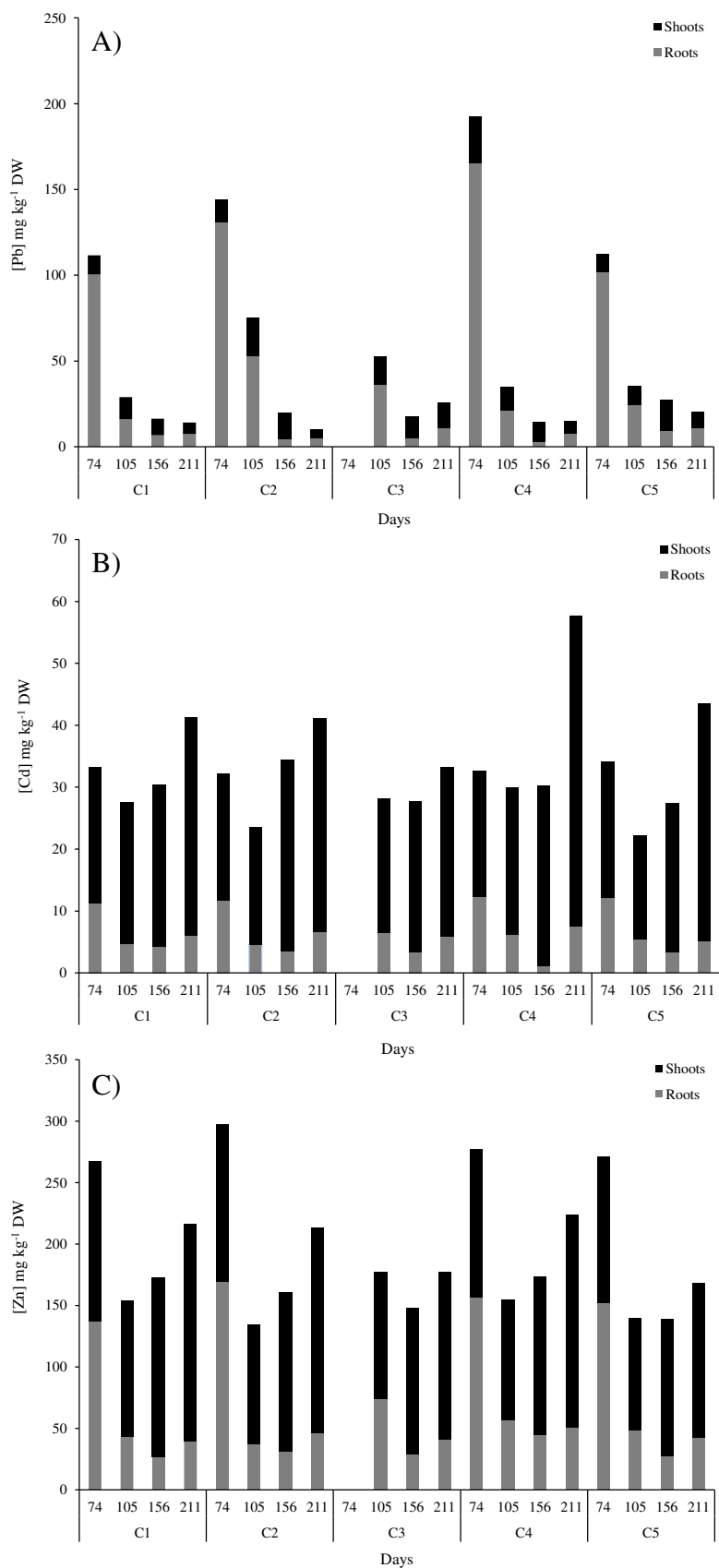


Fig. 2. Distribution (%) of leaf and root biomass for the five cultivars studied

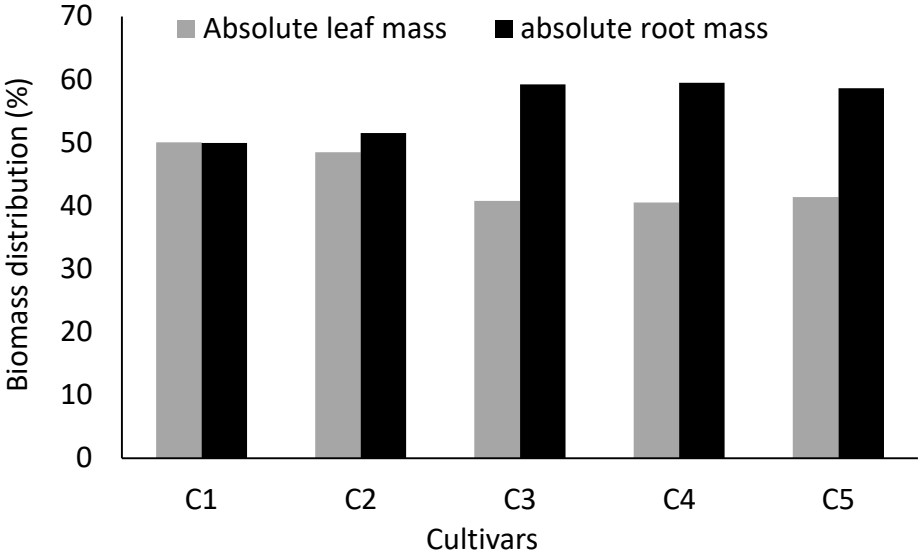
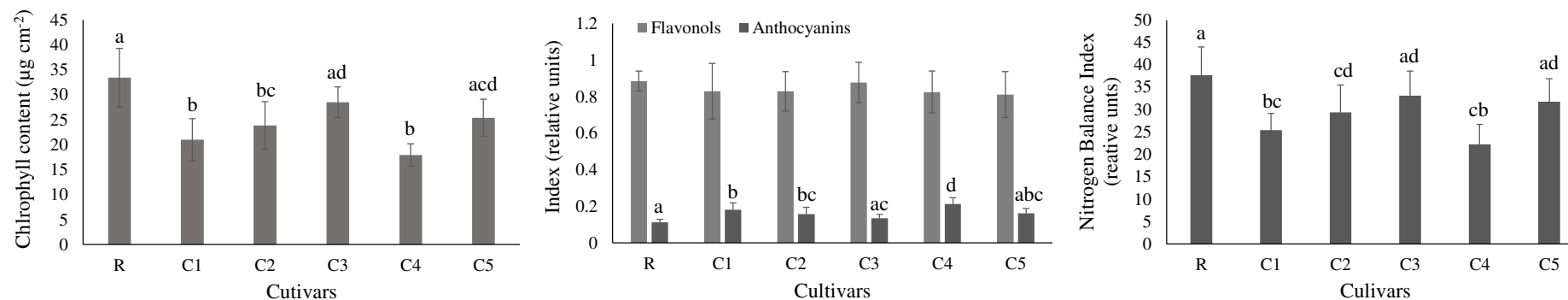


Fig. 3. Chlorophyll content, Flavonols index, Anthocyanins index and Nitrogen Balance Index measured in leaves of chicories (reference and cultivars)



Letters (a, b, c, d, e) refer to significant differences ($p < 0.05$) between reference and the five cultivars studied. Values are means \pm standard deviation ($n=20$).

Root chicory as selective MTE phytoextractor and high root-leaf translocation of Cd

