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Potential analysis of *Baccharis dracunculifolia* and *Baccharis trimera* for phytoremediation of heavy metals in copper mining tailings area, Southern Brazil

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26 **Abstract**

27 The aim of this study was to investigate the phytoremediation potential promoted by
28 *Baccharis dracunculifolia* DC. and *Baccharis trimera* (Less.) DC. in copper mining
29 tailings area, Southern Brazil. The plants were selected considering their
30 spontaneous growth on tailings. The phytoremediation indexes as a translocation
31 factor (TF), bioconcentration factor (BCF), metal extraction ratio (MER) and plant
32 effective number (PEN) were carried out. Both species showed higher concentration
33 of heavy metals in the roots in relation to the shoots. *B. trimera* showed potential for
34 phytoextraction of Zn, Cd, Cr, Pb and phytostabilization of Ba and Ni, whereas *B.*
35 *dracunculifolia* showed potential for phytoextraction Pb and phytostabilization of Cu,
36 Zn, and Ba. *B. trimera* showed higher potential in phytoremediation of the metals
37 Cu>Zn>Cr>Ni and Cd than the *B. dracunculifolia*. *B. trimera* requires a smaller
38 number of plants to remove 1 g of Cu, Zn, Cr, Pb, Ni and Cd than the *B.*
39 *dracunculifolia*. The values of PENs for Cu were close to those estimated for *B.*
40 *dracunculifolia*, but the PENs (Cu) and PENt (Cu) in *B. trimera* are much lower,
41 meaning that a smaller number of plants are required for decontamination. Both
42 species showed potential for phytoremediation of metals and grew spontaneously in
43 the tailing mining area.

44
45 **Keywords** Environmental damage, Contaminated soils, Phytoremediation, Heavy
46 metals
47
48

49 **Introduction**

50 The abundance of mineral resources in Brazil is well known. Brazil has about 207
51 actived mines, producing a wide variety of industrial minerals, including metals (Al,
52 Cu, Cr, Fe, Mg, Nb, Ni, Au, V and Zn), and it is almost 80% of its production [1].

53 Mining activity is essential for society, and economy. However, in current years, the
54 disruption of the tailings dams of Brumadinho (2019) and Mariana (2015) brought
55 worldwide prominence to Brazil due to the environmental liabilities generated [2]. It
56 was evident that the country has been facing problems regarding the proper control
57 and management of its mining tailings.

58 Mine tailings are generated after the mineral processing, and consist in a mixture
59 of rock (solid waste) composition that varies according to the type of ore [3]. The
60 generation of mine tailings can affect the quality of the environment and contributes
61 to high concentrations of heavy metals, whether in soil and/or water [4, 5].

62 Due to the high content of heavy metals, tailings pose a health risk to the
63 environment and must be chemically and physically stabilized. There is a range of
64 techniques that can be used in the remediation process of an area that contains
65 heavy metals, and phytoremediation is a technique for stabilizing mining tailings in
66 both aspects, chemical and physical [6].

67 Phytoremediation uses plants capable of removing or immobilizing organic or
68 inorganic contaminants [7]. They also have different mechanisms such as
69 phytostabilization and phytoextraction.

70 Phytoextraction is the uptake of contaminants from soil or water by plant roots and
71 their translocation to and accumulation in aboveground biomass i.e., shoots [8].
72 Plants that do not transport the metals to the shoots, but instead bind them in the root

73 or the rhizosphere, are preferred by phytostabilization [9, 10]. Both techniques are a
74 way to investigate whether a species has potential for phytoremediation.

75 The species *Gazania rigens* e *Pelargonium hortorum* that, occur naturally in a
76 copper mining tailings area in Chile, are an example of plants that showed potential
77 in the phytoremediation of heavy metals [11]. However, the species *B. trimera*
78 (carqueja) and *B. dracunculifolia* (Alecrim-of-the-field), although identified in some
79 studies in copper and gold mining areas in southern Brazil [12, 13, 14] are still poorly
80 studied for phytoremediation purposes.

81 Both species belong to the family Asteraceae. They are a rustic, perennial
82 and dioecious shrub, native to the southern and southeastern regions of Brazil [15,
83 16], and in South America [17, 18, 19, 20].

84 *B. dracunculifolia* plays an important role in the colonization and regeneration of
85 contaminated areas [21,22], being considered in Brazil as a secondary vegetation or,
86 in regeneration and advanced stages of regeneration, that is resulting from natural
87 processes of ecological succession [23]. Some studies have shown that both species
88 have the ability to accumulate heavy metals in their biomass [13, 14, 24].

89 In this context, the aim of this study was to investigate the phytoremediation
90 potential promoted by *B. dracunculifolia* and *B. trimera* in copper mining tailings area,
91 Southern Brazil.

92

93 **Materials and Methods**

94 **Study area**

95 Camaquã Mines belongs to the municipality of Caçapava do Sul, which it is located
96 in the Southern region of Brazil, state of Rio Grande do Sul (Figure 1).

97 The Camaquā Mines belongs to the Pampa Biome characterized by a vegetation
98 of the wooded steppe type [25]. The climate is temperate of the subtropical type,
99 classified as humid mesotherm and the region is located in the sub-basin of Arroyo
100 João Dias [26].

101 The soils that comprise the Camaquā Mine region are classified as A-
102 Chernozemic [27]. The Camaquā Mine region is marked by what remains of the
103 copper mining and it is located on plane levels of mining tailings where the open pit
104 (already mined area) has been filled.

105 Camaquā Mines region contain one of the largest deposits of basic copper metals
106 associated with Pb-Zn in the southern of Brazil. The exploration of the base metals
107 (mainly copper) has occurred since the 19th century [28]. The region, includes the
108 tailings of Uruguay Mine (Cu) (closed in 1996) which is the specific area of this study.
109

110 **Plant samples and elements detection**

111 Both species *B. trimera* and *B. dracunculifolia*, are colonist plants on the copper
112 mining tailings of the Uruguay mine. *B. trimera* obtained an average total dry biomass
113 of 2.4 ± 0.7 g (roots), 4.8 ± 2.8 g (shoots), and an average height of 42.0 ± 4.0 cm,
114 while *B. dracunculifolia* showed an average height of 33.5 ± 12.0 cm and dry
115 biomass of 0.9 ± 0.6 g (roots), 3.9 ± 2.2 g (shoots).

116 The plants ($n=12$) were sampled in the copper mining tailings area. The sampling
117 was performed in the summer season (2017) and the collected material was packed
118 in stored in properly identified bags and taken to laboratory. The samples of plants
119 were washed individually and divided into shoots and roots and fresh weight was
120 determined. Afterwards, the biomasses were dried in an oven at 70 °C for 48 hours
121 and ground to a homogeneous power[29]. For the digestion process, it was used a

122 3:1 concentrated nitric-perchloric acid ($\text{HNO}_3\text{-HClO}_4$) according to the methodology
123 described by Tedesco *et al.* [30]. The determination of the elements was performed
124 by ICP-OES (PerkinElmer® - version Optima™ 8300).

125

126 **Chemical analyses of tailings**

127 Sample collection of the copper mining tailings was taken from a sampling composed
128 of five replications at a depth of 0 to 20 cm, obtained mainly from the copper mining
129 tailings of the Uruguay Mine (Closed at 1998) in the region of Caçapava do Sul -RS,
130 Brazil (Figure 1).

131 The tailings samples were dried at room temperature, crushed and sieved (3 mm)
132 prior to analysis. The Physico-chemical properties of the material were determined
133 (Table 1). The pH ratio in water is 1:1.5; (w/v) adapted from the methodology in
134 Landon [31]; CEC: Cation Exchange Capacity; P, Na, Cu and Zn (extractable):
135 extracted with 0.1 M HCl; Mg (exchangeable): extracted with 1.0 M KCl; H + Al:
136 titration; sulfur (S): extracted with 500 M of calcium phosphate; organic matter (O.M)
137 by wet digestion; Cd, Ni, Cr, Pb, Mo, Ba [32]; using internal standards for control and
138 verification of procedures (with recovery between 81 and 105% for metals and Limits
139 of Detection (LD) of: Cd - 0.2; Cr and Ni - 0.4; Cu - 0.6; Pb and Zn - 2.0; ug/g.) and,
140 determined by ICP-OES.

141

142 **Metal accumulation**

143 Once determined the elements in the roots and shoots systems, as well as in the
144 tailings, it was determined the phytoremediation indexes to evaluate the plant's ability
145 to remediate the contaminated area.

146 Translocation factor (TF) and Bioconcentration factor (BCF) are parameters to
147 study the soil-to-plant transfer accumulation of heavy metals [33]. The parameters
148 evaluate the phytoextraction potential of the plants.

149 Translocation factor value is represented in according to the following Equation 1.
150 Translocation factor value greater than 1 indicates the translocation of the metal from
151 root to above-ground part (tailings in this case)[34].

152
$$TF = \frac{[\text{metal}]_{\text{shoots}}}{[\text{metal}]_{\text{roots}}} \quad (1)$$

153

154 Where, $[\text{metal}]_{\text{shoots}}$ and $[\text{metal}]_{\text{roots}}$ are the metals concentrations in dry biomass (mg
155 kg^{-1}).

156

157 The bioconcentration factor (BCF) that is used to calculate the distribution of
158 heavy metals between sediment (tailings in this case) and plant is defined by
159 Equation 2. According to Yoon *et al.* [8] and Kamari *et al.* [35], only plant species with
160 both BCF and TF greater than 1 have the potential to be used for phytoextraction.

161
$$BCF = \frac{[\text{metal}]_{\text{roots}}}{[\text{metal}]_{\text{tailings}}} \quad (2)$$

162

163 Where, $[\text{metal}]_{\text{roots}}$ is the metals concentration in the dry biomass (mg kg^{-1}) and
164 $[\text{metal}]_{\text{tailings}}$ is metals concentration in the tailings (mg kg^{-1}).

165

166 The metal extraction ratio (MER) was also determined. This index expresses the
167 capacity of accumulation of metal in the shoots of the plant in relation to that in the
168 tailings [36] (Equation3).

169
$$MER = \left(\frac{[C_{\text{plant}}] \times [M_{\text{plant}}]}{[C_{\text{tailings}}] \times [\text{zone Mrooted}]} \right) 100 \quad (3)$$

170 Where, [Cplant] is the concentration of the metal in the plant (mg kg^{-1}); [Mplant] is the
171 dry weight biomass; [Ctailings] is the concentration of the metal in the tailings (mg kg^{-1})
172 and [zone Mrooted] is the tailings volume occupied by the plant (value adopted of 1
173 kg).

174

175 To determine the number of plants required to extract 1.0 g of element or metal of
176 interest, the PENs (plant effective number) were determined, which considers the
177 biomass of the shoots of the plant and the PENt that takes into calculation the total
178 biomass of the plant (shoots and roots)[37]. Both the PENs and PENt were
179 determined considering the concentration of the target element present in the dry
180 biomass of the plants as well as the dry weight biomass. It was used following
181 equation:

182

183 $\text{PENs} = \frac{\text{level}_{\text{shoots}} \times \text{mass}_{\text{shoots}}}{1000} \quad (4)$

184

185 $\text{PENt} = \frac{\text{level}_{\text{entire plant}} \times \text{mass}_{\text{entire plant}}}{1000} \quad (5)$

186

187 The phytoremediation potential (g ha^{-1}) was estimated considering the total
188 concentration of the target metal in the plant and weight biomass. For this, the dry
189 biomass production of the plant was estimated considering on studies by Santos *et al.*
190 [38] for *B. dracunculifolia* ($3.86514 \text{ t ha}^{-1}$) and Amaral *et al.* [16] (10.3 t ha^{-1}) for *B.*
191 *trimera*, multiplied by the total concentration of each target metal in the plant (total
192 mg kg^{-1}).

193 The experimental design was completely randomized and the software Statistica®
194 version 7.0 was used. It was performed Analysis of Variance (ANOVA), and the
195 Tukey test ($p < 0.05$) was carried out when ANOVA showed significant results.

196 **Results**

197 **Nutrients uptake and heavy metal contents in biomass**

198 The behaviour of both species *B. trimera* and *B. dracunculifolia* in relation to the
199 concentration of macronutrients in the roots was similar. The *B. trimera* showed the
200 following sequence of macronutrient concentrations Ca>K>Mg>S>P, whereas in the
201 *B. dracunculifolia* Ca>K>S>Mg>P, being the sulphur concentration ($1,956.8 \text{ mg kg}^{-1}$)
202 higher than the concentration of magnesium ($1,142.1 \text{ mg kg}^{-1}$) in *B. trimera*
203 (Figure2A).

204 The concentrations of the elements in *B. trimera* shoots showed the following
205 order, Fe>Na>Al>Mn>Cu>Zn>Ni>Co, whereas in *B. dracunculifolia* the order was
206 Fe>Al>Na>Mn>Cu>Zn>Ni>Co (Figure2B). Even with an inversion between the
207 aluminum and sodium sequences between the species, the other metals followed the
208 same order in both species (Figure2B). In the roots, this elements concentration
209 followed the same order in both species *B. trimera* and *B. dracunculifolia*
210 (Fe>Al>Na>Cu>Mn>Zn>Ni>Co).

211 The concentration of Cu in shoots of *B. dracunculifolia* was almost half of the
212 concentration shown by the *B. trimera* (Figure2B). The same behaviour was occurred
213 with the concentrations of Cu in the roots, being more than twice in *B. trimera*, when
214 compared to *B. dracunculifolia* (Figure2B).

215 The concentrations of Ni in the shoots were similar between the species(Figure2B).
216 The *B. trimera* showed higher concentrations of all elements in both shoots and roots,
217 except for Zn and Ni, compared with *B. dracunculifolia* (Figure2B).

218 Both species presented similar concentrations of heavy metals in roots than in
219 shoots, except for Cd and V (Figure2C).

220

221 **Phytoremediation index**

222 The translocation factor (TF) in *B. dracunculifolia* was higher than one (TF>1) for Fe,
223 Cd, Cr, Pb and Zn. In *B. trimera*, only Pb obtained value of TF>1 (Table 2).In *B.*
224 *dracunculifolia*, the bioconcentration factors greater than one (BCF>1) were detected
225 for the metals Zn, Cd, Cr, Ni, Pb and Ba; however, the *B. trimera* showed the BCF>1
226 for Cu, Zn, Pb and Ba (Table 2).

227 Both species showed TF>1 for Pb and BCF>1 for Zn, Pb, Ba. The species *B.*
228 *dracunculifolia* showed BCF>1 and TF>1 for metals Zn, Cd, Cr, Pb and BCF>1 and
229 TF<1 only for Ba (Table 2). However, *B. trimera* showed BCF>1 and TF>1 only for
230 Pb and BCF>1 and TF<1 for the metals Cu, Zn, Ba (Table 2).

231 Both *B. dracunculifolia* and *B. trimera* exhibited high value MER index for
232 extraction of Zn and Ba metals (Table 2). Although, MER index of Cr and Pb were
233 not so high in both species, they were higher than one (MER>1). Considering the
234 copper element, only the *B. trimera* obtained MER>1 (Table 2).

235 The *B. trimera* species requires a smaller number of plants to remove 1 g of Cu,
236 Zn, Cr, Pb, Ni, Cd, for both PENs and PENT in relation to *B. dracunculifolia* (Table 2).

237 Regarding the presented results, *B. trimera* species showed a higher potential in
238 phytoremediation of Cu>Zn>Cr>Ni>Cd than *B. dracunculifolia* (Figure3). While *B.*
239 *trimera* showed potential to remove 6,039.60 gha⁻¹ of Cu, the *B. dracunculifolia* would
240 remove 992.10 g ha⁻¹, even that, it is a good potential of the *B. dracunculifolia*. The
241 specie *B. trimera* would remove more than twice as much Cd and Pb as *B.*
242 *dracunculifolia* (Figure3).

243

244 **Discussion**

245 There is an increasing search for species of plants which are able to adapt to areas
246 under extreme conditions such as copper mining tailings in the Camaquā Mines. The
247 impacted physical structure of mining tailings, the low nutrient content and high
248 concentration of heavy metals restricts the conditions for proper development of plant
249 species[14].

250 In this context the species *B. dracunculifolia* and *B. trimera* are growing
251 spontaneously under the copper mining tailings. Species of the genus *Baccharis*,
252 such as *B. trimera* and *B. dracunculifolia* naturally vegetate environments such as
253 roadsides, steep and high-altitude areas, acidic or mildly acidic soils with elevated
254 contents of Al, Ca,Mg, K and low P content [39]. The species also showed good
255 development and adaptation in altered or disturbed sites [40, 41], as it is the case
256 ofthe cooper tailings area in the Mines of Camaquā region.

257 *B. trimera* and *B. dracunculifolia* were in early stages of development over the
258 copper mining tailings area in the present study, since the *B. trimera* height ranges
259 from 0.5-1.6 m [42]. The height of *B. dracunculifolia* usually ranges from 2-4 m [17,
260 20, 43].

261 Therefore, depending on the period of development in which species are, the
262 production of dry biomass can variety. The biomass production of *B. trimera*, for
263 example, infertile soils can variety from 3.5 to 10.3 t ha⁻¹. However, under natural
264 conditions, the value ranges from 0.046 to 47.9 t ha⁻¹[16, 44, 45].

265 Some studies have shown that the production of the biomass of *B. trimera* can
266 achieve 21.89 g plant⁻¹[16]. In *B. trimera* with a mean height of 33.5 cm, the dry
267 biomass in the roots was 0.9 g plant⁻¹, and in the shoots 3.9 g plant⁻¹, whereas *B.*

268 *dracunculifolia* with a mean height of 20-30 cm, the dry biomass was 1.58 g plant⁻¹ for
269 roots and 6.14 g plant⁻¹ for shoots [16, 46, 47]. Values are much higher than those
270 found in this study.

271 The increase in the biomass production of both species would enhance their ability
272 to phytoremediation, as a copper mining tailings area in this study. The variation in
273 biomass production is related to the amount of nutrients available in the mining tailing.
274 Thus, fertilization generates an increase in the biomass production of *B. trimera*
275 (carqueja) to a certain extent [39]. In highly fertile soil, *B. trimera* does not respond to
276 mineral or organic nitrogen fertilization, meaning that *B. trimera* develops well with
277 low N, P and K contents [16], as the species in the present study.

278 Plants in mining areas in Southern Brazil are called "savanna metallophile" and
279 presents mechanisms of tolerance and resistance to heavy metals [48], as the
280 species *B. trimera* and *B. dracunculifolia* in mining tailings area. It is commonly the
281 presence of species of the family Asteraceae in areas that are in the process of
282 environmental regeneration. Melo-Júnior *et al.* [49] in a floristic survey in a degraded
283 region found more than 16 species of the Asteraceae family, among them were the *B.*
284 *dracunculifolia* and *B. crispa*.

285 Some species of the genus *Baccharis* are already known as indicative of the initial
286 stages of environmental regeneration (*B. trimera*) and indicative of primary
287 vegetation of the middle and advanced stages of regeneration (*B. dracunculifolia*)
288 according to the Brazilian legislation [23]. This was verified in this study for both
289 species, even after 24 years of passive vegetation, since 1996 that was finished the
290 activities in the mine.

291 Depending on the ability of plants to adapt under adverse conditions, the species
292 showed different phytoremediation mechanisms, capable of extracting or stabilizing

293 metals, in the soil or in their system [50], as is the case of *B. trimera* and *B.*
294 *dracunculifolia*, which showed mechanisms of phytoextraction, phytoaccumulation
295 and phytostabilization of metals (Table 2).

296 Both species seem to face difficulties in translocation of some metals to the shoots,
297 this suggests resistance mechanisms, such as the exclusion of metals [51]. Thus, the
298 transfer of the metal to the plant prevents it from accumulating this metal in its shoots
299 biomass. This can be seen in the analysis of Table 2.

300 When evaluating native plants in mining areas, Estrada *et al.* [52] found that higher
301 concentrations of Cu, Pb, and Zn in roots biomass in different species studied were
302 higher in the species of impacted areas (mining) than in other areas. This difference
303 was observed by the authors in concentrations of Pb (158 mg kg⁻¹), Cu (689 mg kg⁻¹),
304 and Zn (514 mg kg⁻¹) in *B. dactyloides*, among others. This can also be seen in the
305 species *B. trimera* and *B. dracunculifolia*, which presented high concentrations of
306 metals and their biomass (Figures 2 and 3).

307 Other studies have also demonstrated high concentration of metals in the biomass
308 of plant species growing under the area of mining tailings, for example, Menares *et al.*
309 [53] determined low concentrations of Cu, Zn, Mg and Fe in the species *B. linearis*
310 under tailings of copper mining in Chile. In contrast, both *B. dracunculifolia* and *B.*
311 *trimera* showed very high Cu and Fe concentrations in the biomass compared to
312 results obtained by Menares *et al.* [53].

313 The metals accumulated in shoots indicate that *B. dracunculifolia* is more efficient
314 in translocation of Zn, Fe, Cd, Cr and Pb than *B. trimera*, which showed high
315 efficiency only for lead. Resistance mechanisms may be involved as low absorption,
316 permeability and/or active efflux of the metals by *B. trimera*, which reflects in the low
317 translocation of these metals [54].

318 The translocation factor for Cu, Pb and Zn were higher than one for all species
319 studied by Estrada *et al.* [52] in soils contaminated with heavy metals, such as *P.*
320 *incanum*, also a native of Rio Grande do Sul (Brazil), which obtained TF=2 for lead
321 and TF=3 for Cu and Z. *B. sarotroides* growing in copper mining areas showed high
322 translocation values for the metals Cu, Cr, and Mo[55]. Translocation factors found by
323 these authors were higher than those found in *B. dracunculifolia* and *B. trimera* for
324 the same metals (Table 2). High concentration of metals in the roots may be due a
325 slow translocation considering the exposure to high concentration of metals in
326 tailings[56]. Nevertheless, the *B. dracunculifolia* has potential for phytostabilization
327 and phytoaccumulation for application in the recovery of areas contaminated with
328 toxic metals [47, 57].

329 When the metal is transferred from the soil to the biomass of the plant, the plant
330 uses mechanisms such as exudation, that send toxic compounds to isolate sites,
331 such as apoplast, in order to avoid their entry into the symplast [58]. In this way, the
332 plant restricts the absorption and translocation of the metals, demonstrating tolerance
333 to the metal. Therefore, typical symptoms of intoxication were not shown in the *B.*
334 *dracunculifolia* and *B. trimera*, since both species showed similar tolerance to
335 concentrations which were considered to be toxic by Kabata-Pendias [59] for the
336 metals Cu (20-100 mg kg⁻¹), Cr (5-30 mg kg⁻¹) and Ni (10-100 mg kg⁻¹).

337 The *B. dracunculifolia* is a species that achieves a high survival in places with low
338 nutrient concentration [22] being indicated for environmental regeneration [60, 61]
339 because it presents a good adaptation in degraded areas [62]. This was shown in the
340 study of Boechat *et al.* [13] in which *B. trimera*, in gold mining areas in Southern
341 Brazil obtained metal contents (Cu, Zn, Cd, Ni, Pb, Ba) higher than those found in
342 this study for the same species (Figures 2 and 3). Whereas, Silva *et al.* [63] showed

343 low concentration of metals (Cd, Co, Cr, Cu, Pb Zn) in the whole plant of *B. trimera*.
344 Although, *B. trimera* shows variations in terms of metals concentration in its biomass,
345 it showed TF>1 for metals Zn, Cd, Pb and BCF>1 for Cu, Zn, Cd and Ba [13],
346 demonstrating its potential in phytoremediation of metals.

347 On steel slag, the species *A. thaliana* had low biomass production, low BCF and
348 TF, but MER>1% for Cd (MER=7.7%), Cu (MER=8.1%) and Pb (MER=1.8%) [64].
349 The rate of extraction of the metal is related to the production of biomass by the plant
350 so that the more biomass promotes higher rate of extraction of the metal [36, 64].
351 Thus, both *B. trimera* and *B. dracunculifolia* presented lower extraction rates for Cu,
352 Pb, Cd compared to *A. thaliana* but presented very high rates for Zn and Ba.

353 Analyzing the phytoremediation factors in the *B. trimera* and *B. dracunculifolia*, it
354 can be seen that *B. trimera* showed a potential for phytoextraction (TF>1 and BCF>1)
355 of Zn, Cd, Cr, Pb and phytostabilization (TF<1 and BCF>1) of Ba and Ni, whereas *B.*
356 *dracunculifolia* showed potential for phytoextraction of Pb and phytostabilization of
357 Cu, Zn and Ba. In phytostabilization, plants immobilize the metals in the rhizosphere
358 while in phytoextraction they translocate the metals to the shoots [50].

359 In this context, the potential of *B. trimera* and *B. dracunculifolia* in the removal of
360 heavy metals contained in contaminated soils is higher than those found by
361 Andreazza *et al.* [65] in the species *B. pilosa* (Asteracea family). According to the
362 author, this species had characteristics of hyperaccumulating Cu. In Cu
363 contaminated soils, *B. pilosa* would need 83,001 shoots of plants for the copper
364 removal (PENs in inceptisol) and 3,536 whole plants (PENT in mollisol) also for Cu.

365 The values of PENs for Cu were close to those estimated for *B. dracunculifolia*,
366 but the PENs (Cu) and PENT (Cu) in *B. trimera* are much lower, meaning that a
367 smaller number of plants are required for decontamination (Figure3). Both species (*B.*

368 *trimera* and *B. dracunculifolia*) present a phytoremediation potential in areas
369 contaminated with heavy metals.

370 **Conclusions**

371 The results showed that *B. trimera* showed potential for phytoextraction of Zn, Cd, Cr,
372 Pb and phytostabilization of Ba and Ni, whereas *B. dracunculifolia* showed potential
373 for phytoextraction of Pb and phytostabilization of Cu, Zn, and Ba in tailings area.
374 Both species showed a high ratio of Zn and Ba metals extraction.

375 Considering the copper element, only *B. trimera* obtained MER>1, which it showed
376 the potential to remove Cu. Besides, *B. trimera* requires a smaller number of plants
377 to remove 1 g of Cu, Zn, Cr, Pb, Ni, Cd, comparing to *B. dracunculifolia*.

378 The values of PENs for copper were close to those estimated for *B. dracunculifolia*,
379 but the PENs (Cu) and PENt (Cu) in *B. trimera* are much lower, meaning that a
380 smaller number of plants are required for decontamination. It is concluded that *B.*
381 *trimera* showed a higher phytoremediation potential of Cu>Zn>Cr>Ni>Cd metals in
382 relation to *B. dracunculifolia*, although both species exhibited potential to be applied
383 in phytoremediation in tailings area.

384 The results must be viewed carefully, as they are the initial data of an in-situ
385 experiment. I consider plants to behave differently in other areas. Therefore, this
386 work opens space for new studies involving such plants.

387

388 **Authors' Contributions** TFA and RA conceptualized the study. TFA performed the
389 main experimental work, evaluated the dataset, and wrote the main text of the
390 manuscript. TFA and FAOC performed the analyses. SP, MSQ and CFD were
391 involved in proofreading of the manuscript.

392

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397

398 **Data Availability** The datasets used and/or analyzed during the current study are
399 available from the corresponding author on reasonable request.

400

401 **Compliance with Ethical Standards**

402

403 **Competing Interests** The authors declare that they have no competing interests.

404

405 **Ethics Approval and Consent to Participate** Not applicable. The manuscript does
406 not contain data collected from humans or animals.

407

408 **Consent for Publication** Not applicable. The manuscript does not contain any
409 individual person's data.

410

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621 **Table 1**Chemical-physical characteristics of copper mining tailings.

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Tailings	Texture	Value in Tailings	Ref. Value
Clay	%	13.0	41.0-60.0e*
pH	1:1	6.2	6.0-6.5d
OM	%	0.5	-
CEC	cmol _c dm ⁻³	6.7	>15.0e
Na	(mg/kg)	9.3	5000.0d
P	(mg/kg)	77.0	800.0d
S	(mg/kg)	1.2	700.0d
Mn	(mg/kg)	11.0	418.0c
Cu	(mg/kg)	259.7	7.0-11.0a
Zn	(mg/kg)	0.9	19.0-20.0a
Cd	(mg/kg)	<0.2	0.3-0.4a
Cr	(mg/kg)	15.3	16.0-21.0a
Ni	(mg/kg)	9.0	4.0-7.0a
Pb	(mg/kg)	<2.0	13.0-16.0a
Ba	(mg/kg)	0.3	≤150b
Mo	(mg/kg)	3.3	≤30b

623 CEC: Cation-exchange capacity; OM: Organic Matter;*^a[66], ^b[67], ^c[59], ^d[68], ^e[69].

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Table 2 Phytoremediation indexes by *B. dracunculifolia* and *B. trimera*.

Metals	<i>B.dracunculifolia</i>				
	TF	BCF	MER	PENs	PENT
	----%----				
Cu	0.41a*	0.77b	0.40c	8,586b	1,308b
Zn	1.12a	24.01b	21.78c	35,635b	10,666b
Fe	1.03a	nd**	nd	405b	116b
Cd	1.09a	1.14b	0.29c	21,754,045a	2,290,952a
Cr	1.08a	2.20b	1.07c	35,820b	7,512b
Ni	0.92a	1.60b	0.75c	87,946b	18,119b
Pb	1.12a	2.18b	1.31c	139,871b	47,308b
Al	0.81a	nd	nd	806b	162b
Co	0.45a	nd	nd	6,999,739b	578,942b
Ba	0.78a	603.16a	245.27b	6,349b	1,564b
V	0.86a	nd	nd	426,894b	111,162b

Metals	<i>B. trimera</i>				
	TF	BCF	MER	PENs	PENT
	----%----				
Cu	0.23a	1.83b	1.86c	3,349b	376b
Zn	0.75a	17.39b	20.97b	24,085b	6,126b
Fe	0.36a	nd	nd	315b	26b
Cd	0.23a	0.99b	0.86c	6,089,032ab	672,261b
Cr	0.91a	0.89b	1.27c	23,484b	6,735b
Ni	0.85a	0.67b	0.93c	56,821b	15,817b
Pb	1.11a	1.83b	2.79c	68,902b	21,46b
Al	0.39a	nd	nd	463b	39b
Co	0.24a	nd	nd	929,026b	103,338b
Ba	0.98a	278.00ab	380.16a	3,907b	1,051b
V	0.38a	nd	nd	214,260b	20,843b

663 TF: translocation factor; BCF: bioconcentration factor; MER: metal extraction ratio, PENs:plant
664 effective number of the shoots; PENT: total plant.

665 *Values are means and, means followed by the same letter within a column are not significantly
666 different at the 95% confidence level (Tukey's test). **nd means not determined.

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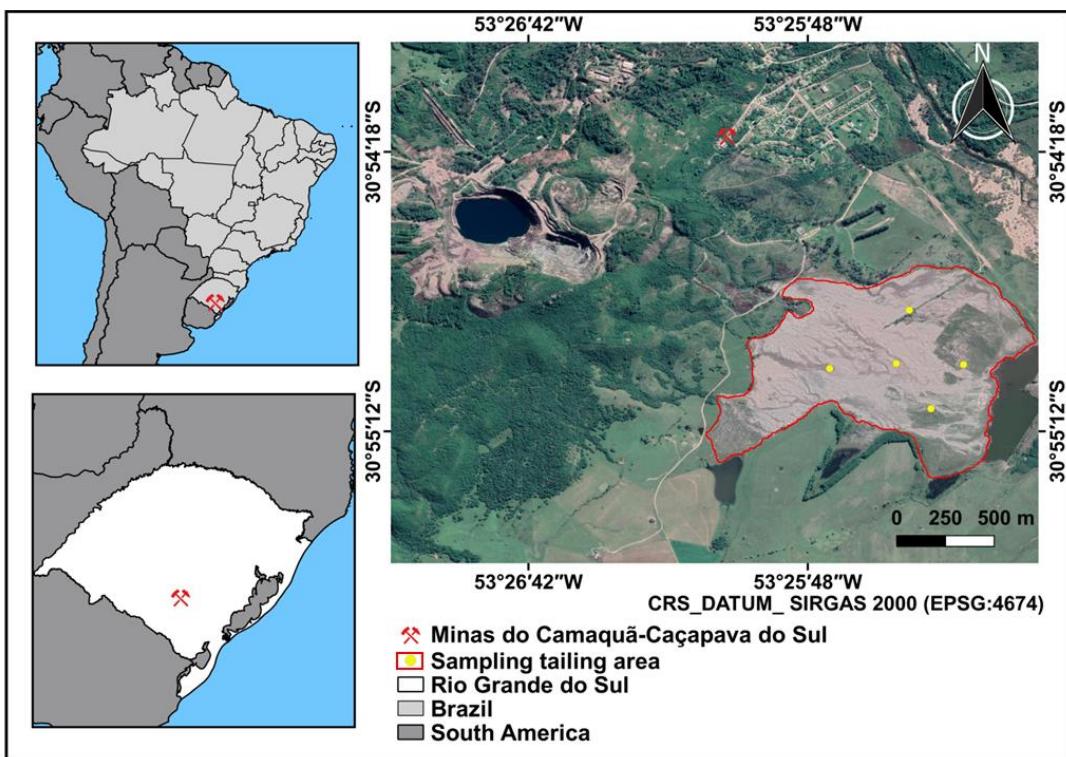


Fig 1 Location map of the copper mining tailings area, Minas do Camaquã, Southern Brazil.

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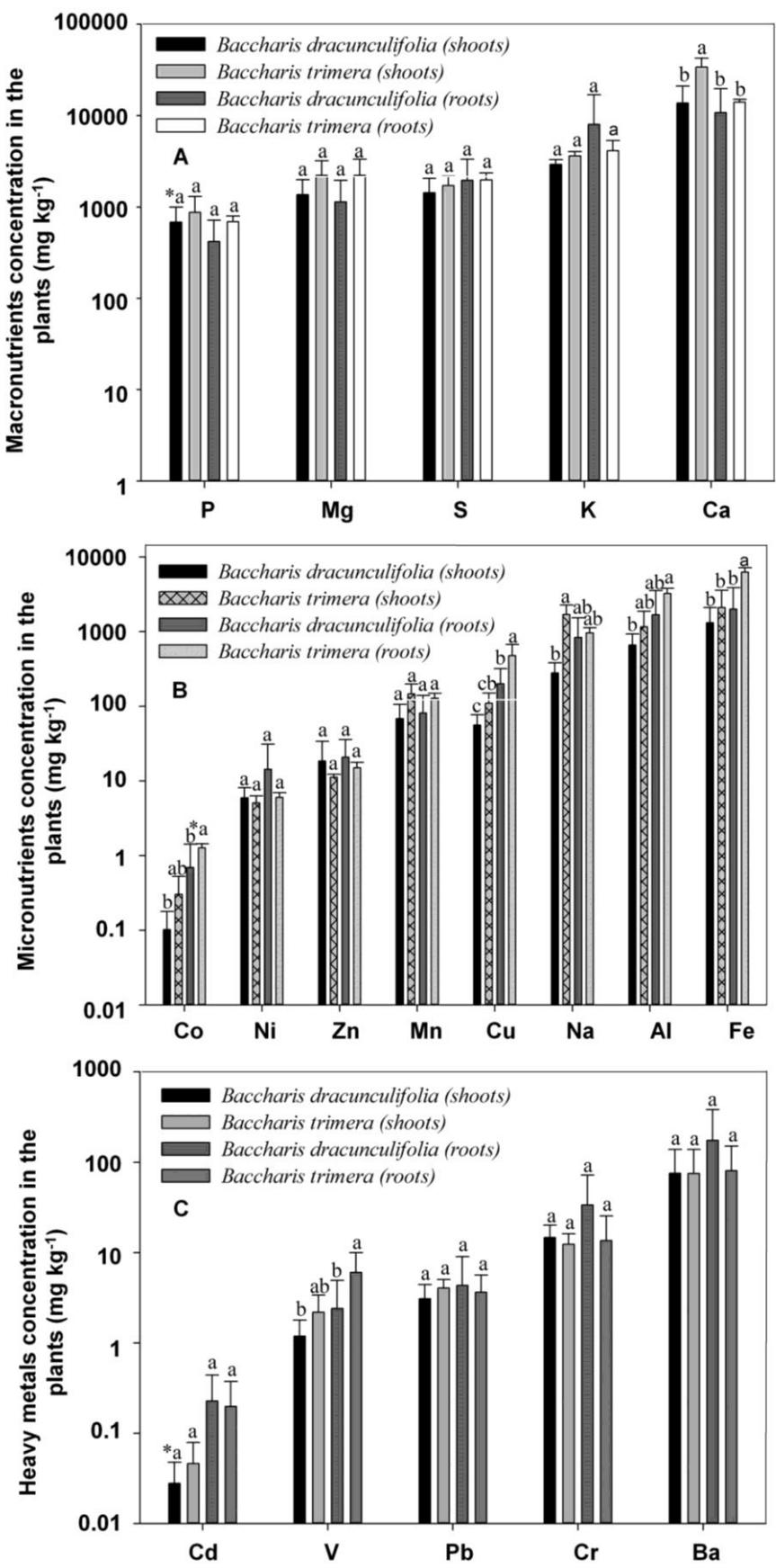


Fig 2 Macronutrients (a), micronutrients (b) and heavy metals (c) concentration in dry biomass of *B. dracunculifolia* and *B. trimera*. *Means followed by the same letter within a column are not significantly different at the 95% confidence level (Tukey's test).

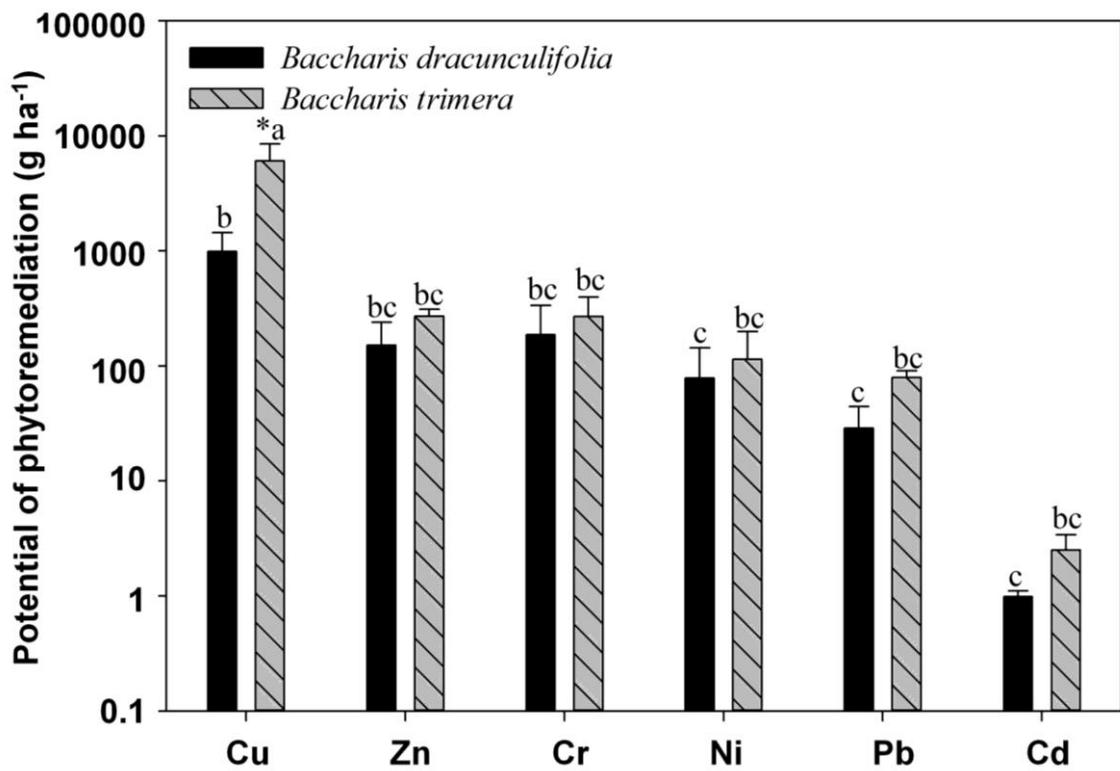


Fig3 Potential of phytoremediation of *B. dracunculifolia* and *B. trimera*. Error bars are calculations of standard error. *Means followed by the same letter within a column are not significantly different at the 95% confidence level (Tukey's test).

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Figures

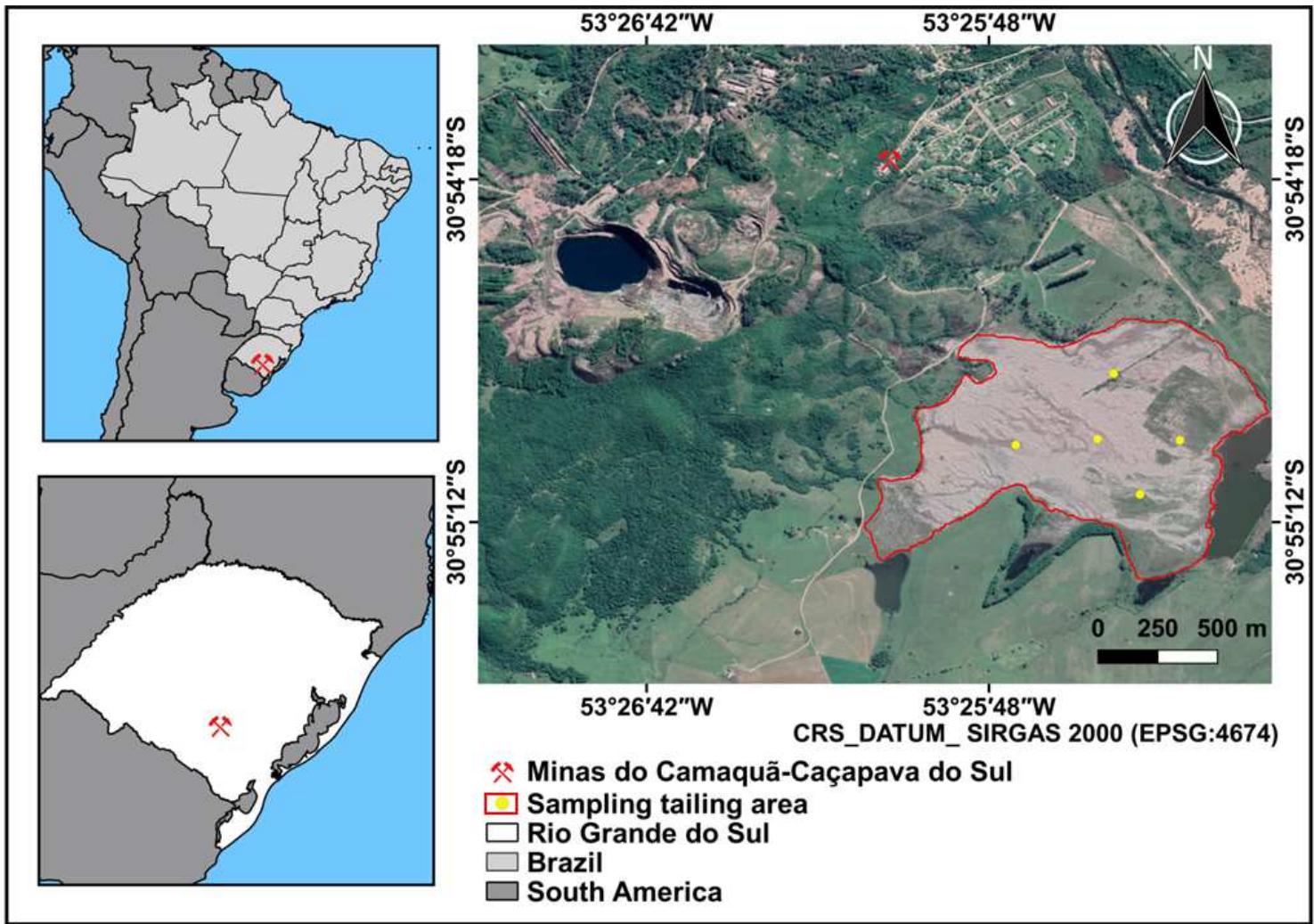


Figure 1

Location map of the copper mining tailings area, Minas do Camaquã, Southern Brazil. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

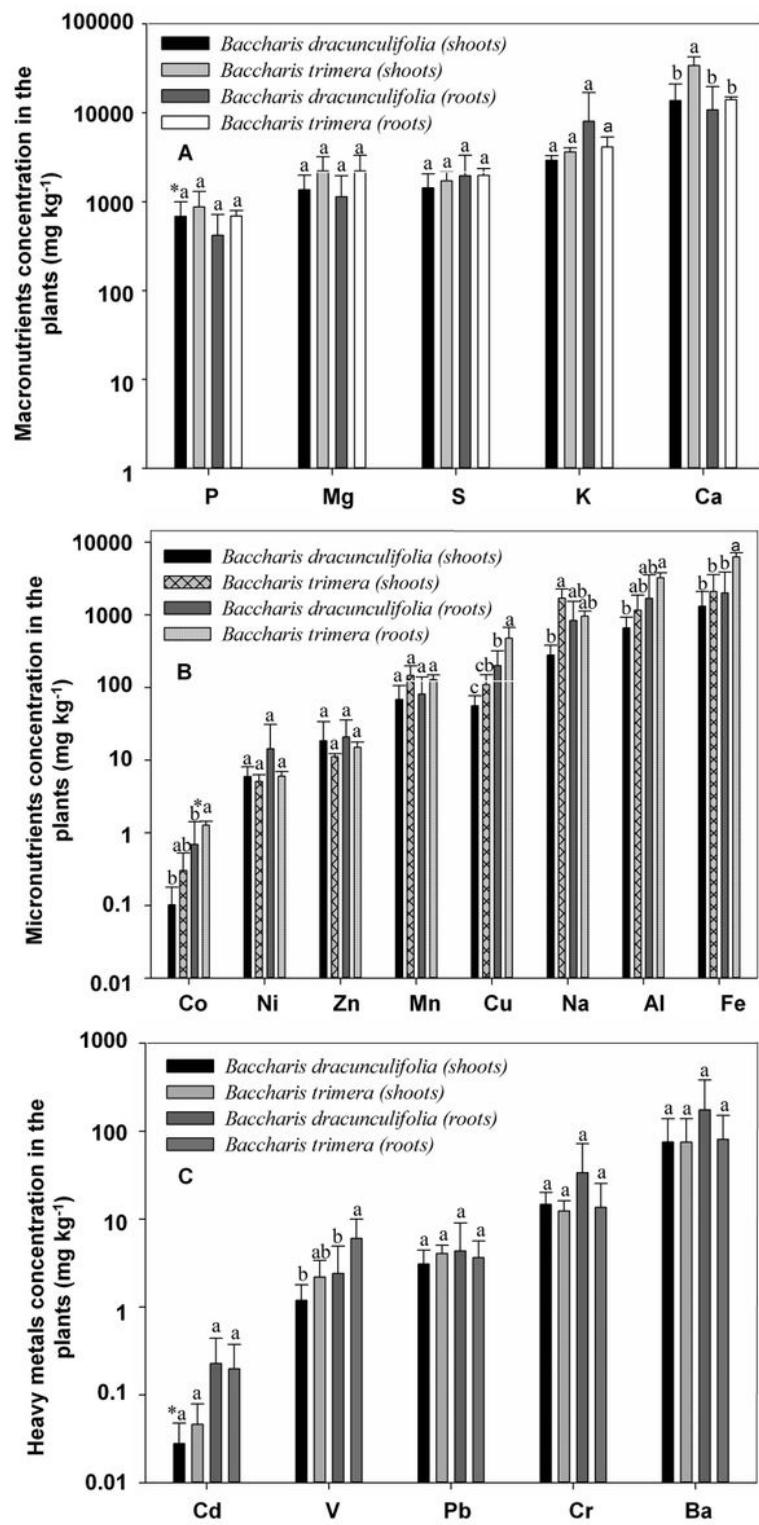


Figure 2

Macronutrients (a), micronutrients (b) and heavy metals (c) concentration in dry biomass of *B. dracunculifolia* and *B. trimera*. *Means followed by the same letter within a column are not significantly different at the 95% confidence level (Tukey's test).

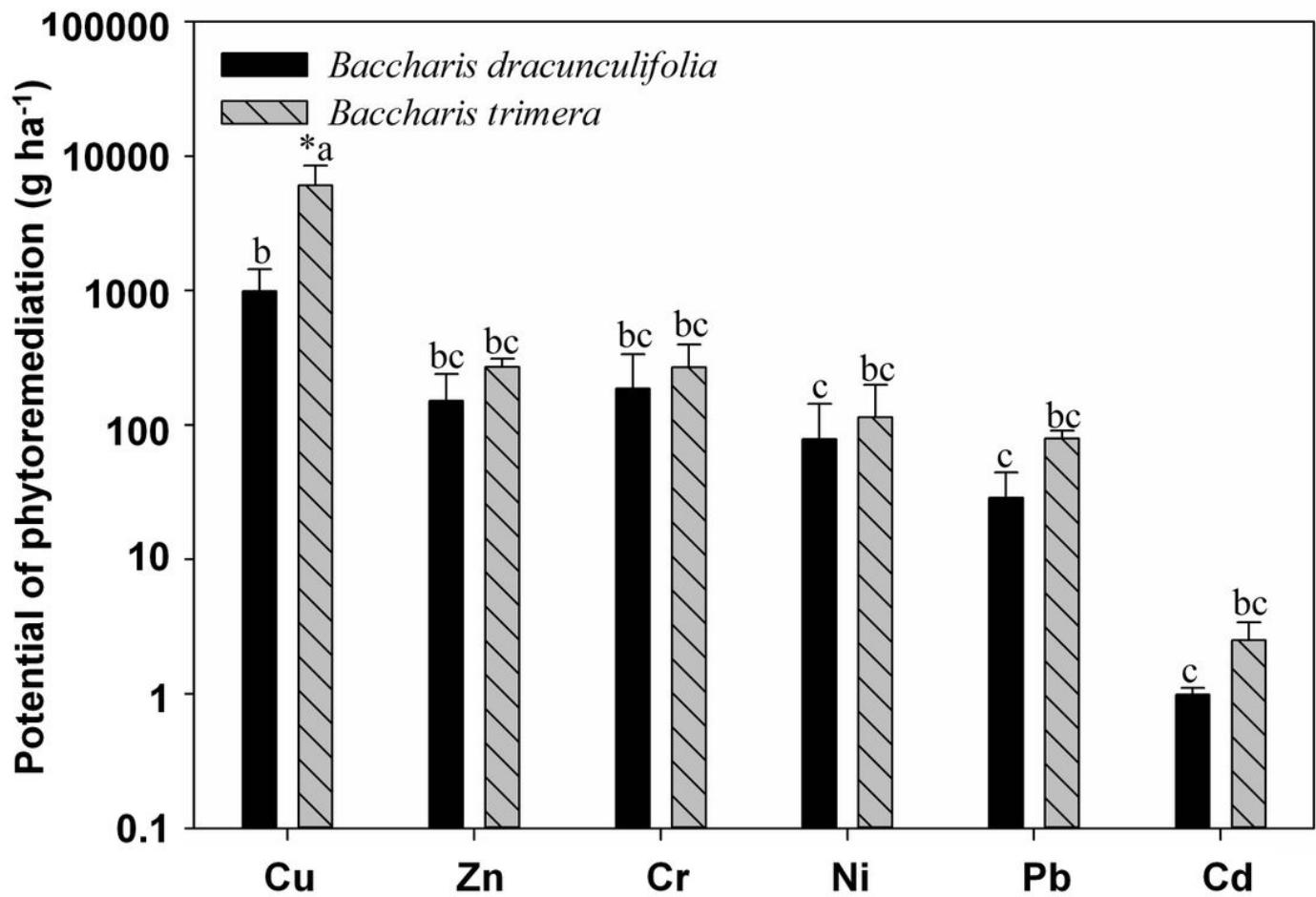


Figure 3

Potential of phytoremediation of *B. dracunculifolia* and *B. trimera*. Error bars are calculations of standard error. *Means followed by the same letter within a column are not significantly different at the 95% confidence level (Tukey's test).