Differential behavior of the summer cover crops in the absorption and translocation of copper

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ABSTRACT: Phytoremediation is an alternative technique used to treat copper-contaminated soils. The objective of this research was to explore the behavior of nine summer cover crops regarding the growth, absorption and translocation of copper in soils with contamination levels exceeding the Value of Prevention, with a view to selecting plants for phytoremediation programs. In the experiments the Cambisol was contaminated with copper, added in doses of 0, 100, 200, 400, 500 and 600mg kg⁻¹, in which the following nine plants were cultivated under greenhouse conditions: Canavalia ensiformis, Cajanus cajan, Dolichos lablab, Mucuna cinereum, Mucuna aterrima, Crotalaria juncea, Crotalaria spectabilis, Pennisetum glaucum and Paspalum notatum. At 90 days after sowing the plants were evaluated for dry mass of shoot and root, Cu, N, P, K, Ca, Zn, and Fe levels in the shoot and Cu in the roots. High soil Cu levels induced a decline in the phosphorus absorption by the plants. Canavalia ensiformis displayed high potential for phytoextraction as these plants could translocate high copper concentrations to the aerial plant parts, while the Mucuna cinereum and M. aterrima are indicated for the copper phytostabilization programs, due to the high copper accumulation in their root systems.

Key words: heavy metal, soil pollution, phytoremediation, phytostabilization, phytoextraction.

INTRODUCTION

The soil copper contamination is a problem that occurs in several countries (MIRMONSEF et al., 2017). In Brazil, the principal areas susceptible to copper contamination are the wastes disposed from mining, swine breeding and vineyards. Although, the areas affected by mining are comparatively small, they showed high contamination levels (PERLATTI et al., 2015). On the contrary, large areas exhibiting less copper contamination levels are observed where large quantities of liquid swine manure are frequently disposed, as food rations are high in copper (MALLMANN et al., 2014). The same is true
for vineyards due to the repeated addition of cupric fungicide (GIROTTO et al., 2016).

In southern Brazil, ANDREAZZA et al. (2013) reported high Cu contamination levels (576 mg kg$^{-1}$ extracted by HCl 0.1 mol L$^{-1}$) in the mining waste disposal regions of the Camaquã River basin. In their study, TIECHER et al. (2013) in the agricultural areas used for frequent liquid swine manure disposal quantified 29 mg kg$^{-1}$ (Cu-EDTA) in the surface sandy soil layer. According to CASALI et al. (2008) copper concentration as high as 506 mg kg$^{-1}$ extracted by HCl 0.1 mol L$^{-1}$) in the surface sandy soil layer. According to CASALI et al. (2008) copper concentration as high as 506 mg kg$^{-1}$ extracted by HCl 0.1 mol L$^{-1}$) was reported at 0 to 20 cm depth in the Humic Cambisol supporting the vineyards.

Copper is an indispensable micronutrient for all living organisms as it plays a crucial role in several physiological processes including respiration, photosynthesis and nitrogen fixation. (FEKIACOVA et al., 2015, RUSCINITTI et al., 2017). However, when copper is present in high concentrations in animals it can cause enzyme inactivation, liver diseases, muscle pain, nervous alterations, vomiting and, in severe instances of overexposure, even death (MAHER, 2018).

In plants, high copper levels induce decreased growth, chlorosis and foliar necrosis (ANJUM et al., 2015). Metal toxicity is evident in the reduced root length, biomass and production of photosynthetic pigments, as well as a decline in the absorption of essential nutrients by the plants (DE MARCO et al., 2017; XU et al., 2017). According to CAETANO et al., (2016), 28 mg kg$^{-1}$ (USEPA method) are damaging to the development of plants and other soil organisms. Decline or impairment of plant development potentiates particle transport through surface runoff and copper transference to the surface waters. Besides, the lack of an active root system permits greater percolation through the soil profile, and copper cycling in the soil-plant system is nil. Copper contamination of soil and water exposes several living organisms to these metals, with possibility of intoxication at different links in the trophic chain and impeding vital ecosystem services (CORNU et al., 2017; RUSCITTI et al., 2017).

Some plants can grow in soils containing high heavy metal concentrations. Phytoremediation is an effective alternate technique to treat such soils. This method involves employing plants to stabilize or remove an environmental contaminant (MAHAR et al., 2016). This environmental biotechnology is relatively new and present cost effective and well accepted as a natural pollution control method. Well recognized phytoremediation processes include phytodegradation, phytovolatilization, phytostimulation, rhizodegradation, phytostabilization and phytoextraction (ALI et al., 2013). While phytoremediation is a means of minimizing the migration of the heavy metal through the soil, phytostabilization is useful with plants that immobilize and/or accumulate large quantities of copper in the root system, reducing its translocation to the aerial plant organs (SANTIBÁÑEZ et al., 2008). If the aim is to eliminate the copper from the contaminated soil, phytoextraction can be employed with plants which store large quantities of copper in the aerial tissues (KUMAR et al., 1995).

In spite of the serious environmental problem caused by copper contamination and the enormous plant diversity in Brazil, only a few studies have investigated the behavior of the cover plants in terms of accumulation of dry mass, absorption and translocation of the copper to their root and aerial tissues, even though they have been used for many years and practically throughout the national territory, for the most diverse purposes. This information is essential so that suitable plants can be selected for phytoremediation programs aiming to control the high copper pollution present in several areas of the country. In this way, the objective of this research was to explore the behavior of nine summer cover crops regarding growth, absorption and translocation of copper in soils with contamination levels exceeding the Value of Prevention, with a view to select plants for phytoremediation programs.

MATERIALS AND METHODS

Soil

A Humic Cambisol without history of contamination was collected from a depth of 0 to 20 cm in EMBRAPA Uva e Vinho (Bento Gonçalves/RS, 29°09'44"S; 51°31'50"W). Soil analysis presented the following values: clay (densimeter) 500g kg$^{-1}$; organic matter (Walkley-Black) 35g kg$^{-1}$; pH (water 1: 1) 4.0; P (Mehlich-1) 3.7mg dm$^{-3}$; K (Mehlich-1) 92.0mg dm$^{-3}$; Cu (Mehlich-1) 2.9mg dm$^{-3}$; Zn (Mehlich-1) 3.3mg dm$^{-3}$; base saturation 9.0%; and aluminum saturation 60.5%.

After drying and sieving the soil, pH was corrected to 5.5 by adding calcium hydroxide and magnesium oxide in the molar ratio of 2:1 (of Ca and Mg). Soil was incubated for 60 days with 80% moisture of the field capacity. After, phosphorus and potassium were added so that their contents were elevated to a high level (CQFS RS/SC, 2016). Soil was contaminated with increasing doses of copper (0,
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100, 200, 400, 500 and 600mg kg\(^{-1}\) of soil) using an aqueous solution composed of 66.66% (m/v) copper chloride and 33.34% (m/v) copper sulfate, to achieve concentrations above 60mg kg\(^{-1}\), the Prevention Value according to CETESB (2016). Before the planting, copper levels present in the soil were determined by three different methods: Melich-1 (MELICH, 1953), 0.1mol L\(^{-1}\) HCl (TEDESCO et al., 1995) and 0.01mol L\(^{-1}\) Na\(_2\)-EDTA 1.0mol L\(^{-1}\) ammonium acetate (CHAIGNON et al., 2009). The Mehlich-1 method was performed by adding 10mL of the extractive solution (8.10mL of HCl PA + 1.40mL of H\(_2\)SO\(_4\) PA) to 1g of soil and followed by stirring for 5min. It was centrifuged 18 hours later, at 7,500g. To determine the copper using HCl, 10mL of the 0.1mol L\(^{-1}\) HCl solution was added in 40g of soil, and stirred for 30min. After 24h, 10mL of this supernatant was drawn out. To determine the Cu level using Na\(_2\)-EDTA/ ammonium acetate, 1mL of the solution of Na\(_2\)-EDTA 0.01 mol L\(^{-1}\)ammonium acetate 1.0mol L\(^{-1}\) was added at pH 7.0 to 0.25g of soil. The mixture was stirred for two hours and centrifuged for 3min at 15,000g. Supernatants from all the soil extracts the copper content was ascertained in an atomic absorption spectrometer (882nm wave length, 0.057mg L\(^{-1}\) of Cu sensitivity, flame air/acetylene).

Experimental design

The study was divided in two experiments. The first one involved 40 treatments, in a 4 x 10 factorial design, using four copper doses (0, 100, 200 and 400mg of copper per kg of soil) and nine plant genotypes, plus control soil (without cultivation). The completely randomized design was adopted with three replications, to give a total of 120 experimental units. The nine genotypes evaluated included: Canavalia ensiformis DC., Cajanus cajan L., Dolichos lablab L., Mucuna cinereum L., Mucuna aterrima L., Crotalaria juncea L., Crotalaria spectabilis Roth., Pennisetum glaucum L. and Paspalum notatum L. The Canavalia ensiformis, Mucuna cinereum and M. aterrima displayed the highest growths and were therefore, chosen for the second experiment.

The second experiment involved cultivation under doses of copper in the concentrations of 0, 400, 500 and 600mg kg\(^{-1}\) in the same soil and under the conditions identical to those of the first experiment. In the second experiment 12 treatments were distributed in a 4 x 3 factorial design, with four copper doses and three plant genotypes (Canavalia ensiformis, Mucuna cinereum and M. aterrima). The completely randomized design was adopted, with three replications, for a total of 36 experimental units.

Plant cultivation

N\(_2\)-fixing bacteria recommended for each crop were inoculated into seeds of the leguminous plants. Grasses were provided nitrogen fertilization via ammonium nitrate at the time of sowing and during two other cover applications, based on the technical recommendations (CQFS RS/SC, 2016). The 5-L capacity plastic pots were filled with 4kg of soil. Nine seeds from each plant genotype were sown in each vessel. Some plants have been removed eight days post germination, until only three plants remained per pot. Soil moisture was maintained at 80% of the field capacity by recording the daily pots weights and adding distilled water when required.

Plant analysis

Plants were harvested at 90 days post sowing, and the aerial parts were cut close to the soil level. Then the roots were manually cleaned to remove the soil and washed under water, 0.02mol L\(^{-1}\) EDTA solution and distilled water, respectively. For the dry mass determination of shoots and roots were oven dried with forced air circulation at 65°C until constant mass was achieved. To determine the concentrations of copper, phosphorus, potassium, calcium, iron and zinc in the aerial and roots tissues plant tissues, nitric-perchloric (HNO\(_3\) + HClO\(_4\)) was added to digest the dry and crushed tissue samples. As it is risky to handle the perchloric acid in the laboratory, this solution was prepared under stringent safety measures (TEDESCO et al., 1995). The copper, calcium (after addition of Sr 0.3% in 0.2M HCl), iron and zinc concentrations were determined using an atomic absorption spectrometer (GBC, 932 AA, Australia). The N content in the shoot was determined by the Kjeldahl-1 method post sulfur digestion (BREMNER; MULVANEY, 1982). The K was determined using the flame photometer (DM-62, DIGIME, Brazil) while the P was assessed through colorimetry, according to MURPHY and RILEY (1962).

Statistical analysis

The bioaccumulation factor (BF) was calculated by dividing the copper concentration in the root and the copper levels present in the soil (Mehlich-1) (LAI et al., 2010). The Translocation Index (Ti) was determined by dividing the copper concentration in the shoot by that presented in the root system.

The data on the dry mass and absorption of the chemical elements were submitted to the analysis of variance. Averages were compared using the Scott-Knott test with 5% probability of error, employing the SISVAR 5.6 program. Regression equations were confirmed by the best fit with the Table Curve.
2D for Windows v. 5.01 (SYSTAT Software Inc). Pearson’s correlations for nutrient levels and dry mass production were also estimated using STATISTICA 7 (Stat Soft, Inc., USA).

RESULTS AND DISCUSSION

Copper content in soil

The addition of copper to the soil in doses of 0, 100, 200, 400, 500 and 600mg kg⁻¹ in the form of an aqueous solution of copper chloride (66.66%) and copper sulfate (33.34%) resulted the available concentrations of 0.94; 35.79; 58.60; 166.14; 198.69 and 232.11mg kg⁻¹ of Cu extracted by Melich-1; concentrations of 1.16; 47.95; 72.29; 218.83; 260.02 and 304.67mg kg⁻¹ Cu were extracted with 0.1mol L⁻¹ HCl; and concentrations of 12.14; 95.56; 136.03; 324.23; 386.46 and 452.28mg kg⁻¹ were extracted with Na₂-EDTA 0.01mol L⁻¹/ammonium acetate 1.0mol L⁻¹. In all the three extractions, high correlation values were noted between the copper available in the soil and the accumulated copper in the aerial parts of the C. ensiformis, M. aterrima and M. cinereum (Figure 1).

Addition of increasing doses of Cu resulted in high levels of available heavy metal in the soil,
similar to those observed in several contaminated areas of Brazil (BRUNETTO et al., 2013; ANDREAZZA et al., 2013). According to PERLATTI et al., (2015) the soil available metal content are determined by extractors solutions that showed a high correlation with the heavy metal concentrations in the plant tissues. Under the experimental conditions of this study, the Mehlich-1 extractor revealed a higher correlation with the Cu levels in the plants.

Experiment 1

The dry matter yield of the aerial plant parts and root system of the nine cover crops was observed to decrease as the copper doses in the soil increased (Figure 2); however, different degrees of tolerance to the heavy metal were evident. The plants *P. glaucum*, *C. cajan*, *P. notatum*, *C. juncea*, *C. spectabilis* and *D. lablab* showed low tolerance for copper and were unsuitable for cultivation in

![Figure 2 - Production of dry mass of the shoot and root systems of nine summer cover crops (Crotalaria juncea and C. spectabilis, Canavalia ensiformis, Cajanus cajan, Dolichos lablab, Pennisetum glaucum, Mucuna cinereum, M. aterrima and Paspalum notatum) cultivated in soil contaminated with 100, 200 and 400mg kg⁻¹ of Cu in relation to the dry mass of the control, cultivated in uncontaminated soil. Bars represent the standard deviation. Averages followed by the same letter, comparing the dry mass of the aerial parts or roots of all the plants under a specific dose, do not differ among themselves by the Scott-Knott test with P<0.05.](image-url)
copper-contaminated soils. All plants revealed significantly decreased growth in response to copper doses above 100 mg kg\(^{-1}\) in the soil. Copper induces many physiological damages like stomatal closure, protein complex denaturation, and phaeophyte modifications, besides the formation of Cu-chlorophyll complexes, and inhibition of the photosystem II reaction center, causing poor plant development (BAZIHIZINA et al., 2015).

*C. ensiformis* was identified as having the highest copper tolerance at the dose of 200 mg kg\(^{-1}\) (Figure 2). These plants also exhibited the least decrease in the yield of the dry matter of shoots and roots when compared with the control. At the highest dose added, the *C. ensiformis* produced the second largest quantity of shoot dry mass. *M. aterrima* was the most copper-tolerant plant at the 400 mg kg\(^{-1}\) dose (Figure 2). It is emphasized that dry matter of the shoot and roots produced were only 30% and 27% less, respectively, than that of the control. The *M. cinereum* was distinctive for its high quantity of dry mass of the roots at the 400 mg kg\(^{-1}\) dose (Figure 2). *C. ensiformis, M. aterrima* and *M. cinereum* clearly emerged as the plants with the highest tolerance for the copper levels tested and were therefore, selected for experiment 2.

**Experiment 2**

The three plants selected showed a dramatic drop in development in response to the increasing doses of copper (Figure 3). A strong negative correlation was observed between the dry mass and Cu content in the shoot and roots of all three plants (*M. cinereum*: \(r_{\text{Cu shoot}} -0.98, r_{\text{Cu root}} -0.96, M. aterrima*: \(r_{\text{Cu shoot}} -0.91, r_{\text{Cu root}} -0.89; C. ensiformis*: \(r_{\text{Cu shoot}} -0.90, r_{\text{Cu root}} -0.95\), all having \(P <0.001\)). However, it was remarkable that for the three doses evaluated, the *C. ensiformis* displayed the highest shoot dry mass production. Again the *M. aterrima* was highlighted by root system growth at copper doses of 400 and 500 mg kg\(^{-1}\), very similar to the control.

The accumulation of copper in the tissues increased according to the dose of copper added to the soil (Table 1). *C. ensiformis* showed the highest copper levels in the shoot, about 60, 30 and 55% more than the average of the *Mucuna* spp., at the 400, 500 and 600 mg kg\(^{-1}\) doses of Cu, respectively.

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**Figure 3** - Dry mass of the aerial parts and roots produced by the *Canavalia ensiformis, Mucuna aterrima* and *M. cinereum* in soils uncontaminated and contaminated with copper at 400, 500 and 600 mg kg\(^{-1}\). Bars represent the standard deviation. Regression equations for the *M. cinereum* (1 and 4), *M. aterrima* (2 and 5) and *C. ensiformis* (3 and 6), where \(x\) is the copper dose in the soil and \(y\) the dry mass production of the plant, with the coefficient of determination significant (*) to 5% probability of error.
Mucuna spp. root systems could accumulate much higher copper levels compared with that of the C. ensiformis, for all the doses tested. This is confirmed by the Mucuna spp. bioaccumulation factor, which showed on average, ten times higher value at the 400mg kg⁻¹ dose and a three times higher value at the 600mg kg⁻¹ dose when compared to the C. ensiformis (Table 1). The M. cinereum notably absorbed 18%, 50% and 38% more copper, respectively, via the root system than did the M. aterrima, at the doses of 400, 500 and 600mg kg⁻¹ of Cu.

While the Mucuna spp. absorbed large quantities of copper through the root systems, they translocated much less to the shoot. The C. ensiformis; however, takes up less copper via the root system, but translocate more amounts to the shoot system. The translocation index (Table 1), confirmed this differential behavior where the average values for the C. ensiformis are five times higher than those seen for the Mucuna spp. in the copper-contaminated soil.

On exposure to high copper levels in the soil, the C. ensiformis, M. aterrima and M. cinereum exhibited less growth decrease when compared with the other plants. All three are leguminous. According to HAO et al., (2014), plants that can form symbiotic associations with N₂-fixing bacteria increase their ability to tolerate various stresses and grow better in metal-contaminated soils.

Independently of the Cu dose added to the soil, the C. ensiformis exhibited an increase in the shoot production, showing statistical difference from the other plants evaluated. Besides, in relation to the Mucuna spp., the C. ensiformis takes up less copper via the root system, but translocate greater amounts to the shoot. According to PUGA et al., (2015) C. ensiformis has higher transfer rate for several metals in comparison to the M. aterrima, and is; therefore, being considered as plant with potential for phytoextraction programs in copper-contaminated soils.

The C. ensiformis can accumulate high levels of copper in the aerial parts and still produce high biomass, which can likely be linked to the production of phytochelatins in the foliar tissues, and which in turn lowers the concentration of the free metal in the cytosol, restricting its solubility and reactivity (OLIVA et al., 2010). Besides, some physiological mechanisms may help to minimize the toxic reactions of the metal in the plant, such as the differential output of the antioxidative and carotenoid enzymes, metal complexation within the cell compartments (like the vacuole), increase in the yield of organic acids to complex the metal, and high expression of metal-carrying proteins.

The increase in the copper levels in the soil induced a decline in the phosphorus absorption in all three plants (Table 2). A strong negative correlation was observed between the P and Cu absorption, in the root as well as the shoot (M. cinereum: rśCu shoot = -0.91, rśCu root = -0.93, M. aterrima: rśCu shoot = -0.89, rśCu root = -0.88; C. ensiformis: rśCu shoot = -0.93, rśCu root = -0.97, all with P<0.001). The presence of excess copper in the roots reduces the branching, thickening and causes lower root development, which in turn decreases the phosphorus absorption (YRUELA et al., 2005).

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ADHIKARI et al., (2016) reported less root growth in the plants exposed to high copper doses. Electron microscopy revealed that the copper was stored as aggregates, in the vascular systems, avoiding nutrients and water absorption.

For most cases, the *C. ensiformis* absorbed remarkably higher quantities of phosphorus, iron and zinc when compared with the *Mucuna* spp. (Table 2). The other nutrients showed little or no tendency to increase or decrease the absorption in response to the increase of copper doses in the soil.

From the research of TIECHER et al. (2016) it becomes clear that certain plants can absorb zinc and iron rather than copper because these elements possess several ionic carriers in common as they have similar chemical properties (ionic radius, bivalence, etc.). Likewise, plants with higher capacity to take up phosphorus, maintain a better nutritional status. The phosphorus facilitates the complexation of heavy metals via the phosphate-metal bonds inside the cells (FERREIRA et al., 2015). The root system of the *Mucuna* spp. showed most of the heavy metal concentrated in their roots, particularly the *M. cinereum*. The root system of some plant species possess mechanisms which can decrease the Cu translocation to the shoot, like chelation to the organic acids and/or other nutrients and metal compartmentalization within the vacuoles (RODRIGUES et al., 2016). PUGA et al. (2015) reported that the *M. aterrima* has the ability to accumulate the calcium oxalate crystals in their vascular bundles, which protect the plant against excess of metals in the tissue. Therefore, these plants are suited for phytostabilization of the copper-contaminated soils as they accumulate large quantities of the heavy metal in the root system and yield a high dry mass of the root.

The *C. ensiformis*, *M. aterrima* and *M. cinereum*, all of which are legumes, showed the highest tolerance to the presence of excess copper levels. However, each of these plants shows different responses to the excess copper present in the soil. *C. ensiformis* accumulates low quantities of copper in the roots, but translocates high amounts of Cu to the aerial tissues and yet is able to produce a high aerial tissue dry mass. Thus, when the goal of phytoremediation is the copper phytoextraction, the *C. ensiformis* emerges as an alternative. However, the *Mucuna* spp. yield

| Plants         | N (g kg⁻¹) | P (g kg⁻¹) | K (g kg⁻¹) | Ca (g kg⁻¹) | Fe (mg kg⁻¹) | Zn (mg kg⁻¹) |
|----------------|------------|------------|------------|-------------|--------------|--------------|
| *M. cinereum*  | 1.64 Ab    | 1.12 Aa    | 6.93 Aa    | 1.06 Aa     | 710.28 Ab    | 67.18 Ba     |
| *M. aterrima*  | 1.32 Bc    | 1.12 Aa    | 5.93 Ba    | 1.04 Bb     | 663.22 Ab    | 81.19 Ab     |
| *C. ensiformis*| 1.12 Cc    | 1.08 Aa    | 4.60 Ca    | 0.98 Cb     | 706.58 Ac    | 94.08 Aa     |
| *M. cinereum*  | 1.52 Bb    | 0.66 Bb    | 6.48 Aa    | 1.05 Aa     | 501.12 Bc    | 66.85 Ba     |
| *M. aterrima*  | 1.78 Aa    | 031 Cb     | 3.68 Cd    | 1.07 Aa     | 476.27 Bc    | 76.52 Bb     |
| *C. ensiformis*| 1.38 Bb    | 0.94 Ab    | 4.47 Ba    | 1.03 Bb     | 1106.78 Aa   | 93.11 Aa     |
| *M. cinereum*  | 1.86 Aa    | 0.56 Bc    | 6.72 Aa    | 1.06 Aa     | 840.11 Aa    | 72.17 Ca     |
| *M. aterrima*  | 1.88 Aa    | 0.27 Cb    | 4.30 Bb    | 1.05 Aa     | 609.03 Bb    | 87.80 Bb     |
| *C. ensiformis*| 1.83 Aa    | 0.77 Ac    | 3.62 Cc    | 1.03 Ba     | 885.42 Ab    | 103.42 Aa    |
| *M. cinereum*  | 1.46 Bb    | 0.46 Bd    | 7.04 Aa    | 1.01 Bb     | 671.52 Cb    | 79.58 Ba     |
| *M. aterrima*  | 1.66 Ab    | 0.26 Cb    | 4.14 Cc    | 1.05 Ab     | 875.25 Ba    | 101.49 Aa    |
| *C. ensiformis*| 1.50 Bb    | 0.61 Ad    | 4.30 Bb    | 1.00 Bb     | 1070.84 Aa   | 104.77 Aa    |
a high dry mass of the roots and accumulate great quantities of Cu in the root system, but translocate low amounts of copper to the aerial tissues. Thus, the *Macuna* spp. appear to be better suited for copper phytostabilization. Agricultural areas may be more amenable to phytoextraction, as soil management involving fertilization, pH correction (AGBENIN, OLOJO, 2004; TIECHER ET AL., 2013; SANTANA et al., 2018) and soil preparation (CASTRO et al., 1992; MOREIRA et al., 2016) can make the metal available once more. On the contrary, in soils with very high contamination levels, plants are not easy to use as phytoremediation, and phytostabilization becomes significant as an alternative to minimize the environmental mobility of the metal.

**CONCLUSION**

*C. ensiformis* produces high quantities of dry matter and accumulate large amounts of copper in the aerial tissues, revealing their potential for use in the copper phytoextraction programs.

*M. aterrima* and *M. cinereum* produce high amounts of dry mass and accumulated high concentrations of copper in the root system, demonstrating their potential for use in the copper phytoextraction programs.

In soils contaminated with copper concentrations above the Reference Value, the absorption of the other nutrients by the plants also change; however, this effect is dependent on the genotype and the nutrient involved.

**DECLARATION OF CONFLICTING INTERESTS**

The authors have no conflict of interest to declare.

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**AUTHORS’ CONTRIBUTIONS**

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

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