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RESEARCH ARTICLE

Characterization of biomass sorghum for copper phytoremediation: photosynthetic response and possibility as a bioenergy feedstock from contaminated land

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Abstract In order to decrease the concentration of toxic metals in contaminated lands, phytoextraction can be suitable considering the use of plant species with high potential for biomass production, such as biomass sorghum (*Sorghum bicolor* L.). We assessed a biomass sorghum (BRS716) potential as a copper phytoextractor as well as the physiological stability under this stressful condition. A completely randomized experimental design was used for a greenhouse experiment in which sorghum plants were submitted to a range of Cu^{2+} concentrations: 2.3, 10.9, 19.6, 30.5, 37.6 and 55.6 mg dm^{-3}. The plant growth was not affected by increasing Cu^{2+} concentrations, suggesting that this species is tolerant to copper. There was a decrease in photosynthetic rate according to the increase in Cu^{2+} concentration, but not at a level that could disturb plant metabolism and eventual death. The values obtained for transfer index ranged from 0.62 to 0.11 which evidenced the restriction of Cu^{2+} transport to the aerial parts. The more Cu^{2+} available in soil, the smaller the amount of Cu^{2+} transported to aerial parts of sorghum. So, our results show that biomass sorghum has potential to be used for Cu^{2+} phytoextraction in concentration of up to 20 mg dm^{-3}. Also, in heavily Cu^{2+} polluted sites, it can be used to produce biomass for bioenergy purpose, promoting a low rate of Cu^{2+} extraction.

Keywords Bioenergy · Environmental protection · Toxic metals · Plant production · Clean technology · Photosynthesis

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Introduction

Population growth, urbanization, economic and technological development have been causing changes in production systems and waste generation, in both quantity and quality. It is an imminent threat to ecosystems and human health due to complex composition and hazardousness of waste generation. These wastes can reach soil and water that are essential natural resources for the survival of all living beings on the planet (Thyberg and Tonjes 2016). In recent decades, human activities have led to increase in pollution, contamination and degradation of natural resources. The main pollutant action is the inadequate disposal of solid waste and the excessive use of chemicals, since much of the waste is not disposed off in a proper way (Subramani et al. 2014) and this contributes to the increase of trace elements in the environment.

The trace elements, some of them known as heavy metals, are naturally found in soil as a product of parent rock weathering (Bolan et al. 2014; Mehr et al. 2017). However, intense anthropogenic activities have increased heavy metals concentration in soil, resulting in a risk of pollution and contamination. One of the main environmental problems of metals in excessive concentrations in soil is degradation of the area, generally reaching the flora, fauna, surface water, and groundwater, resulting in damage to plants and other living organisms, thus altering natural cycles, biochemical processes and causing health problems (Mehr et al. 2017). A problem associated with the toxicity of heavy metals is that they interact with a large range of molecules and that are not degraded, making them highly bioaccumulated in living organisms (Palma et al. 2015; Carvalho et al. 2018).

The main heavy metals naturally found in soils are: Cadmium (Cd), Copper (Cu), Lead (Pb), Zinc (Zn), Chromium (Cr), Nickel (Ni), Barium (Ba), Cobalt (Co) and Mercury (Hg) (Kabata-Pendias 2004; Nogueira et al. 2018). Some of these elements, in small amounts, are vital for biological functions in living beings. However, the increase in their concentration make them toxic. Other metals, which are not essential to living organisms, even in small quantities, are harmful because they can interact with biomolecules and produce stable complexes that may result in metabolic disturbances, culminating in plant growth inhibition and death (Stankovic et al. 2014).

Among the metals mentioned, copper (Cu^{2+}) is an essential metallic element for plants that participates in carbohydrates, nitrogen, lignin and chlorophyll metabolism (Broadley et al. 2012). This metal can be generally found at high concentrations in sites of vineyards, due to Bordeaux mix application, and in mining areas whose disposals are not well managed, resulting in pollution (Andreazza et al. 2011). When copper concentration in soils is above the limit tolerated by plants, it can cause foliar necrosis and decrease overall growth and development of plants (Peñarrubia et al. 2015). However, some plant species can tolerate higher concentration of some toxic metal elements through physiological or/and biochemical processes (Antoniadis et al. 2017). These processes can be used for phytoremediation.

An interesting strategy for phytoremediation is the use of plant species with high biomass production potential, such as biomass sorghum. This sorghum variety can reach 15 to 20 t ha^{-1} in each cycle and is burnt to generate energy in factories/industries. The species presents high energy yield per hectare and has a short cycle, with the capacity to produce large amount of biomass (Rodrigues Castro et al. 2015). This may present a great opportunity to generate bioenergy material in unproductive lands, which can generate income from unused land. This approach is very well demonstrated in the work of Evangelou et al. (2012). However, besides the notable relevance of this approach, the studies concerning sorghum tolerance to heavy metals considered the sweet, forage or grain sorghum varieties (Jia et al. 2017; Tian et al. 2015; Soudek et al. 2014). Thus, there is a lack of research exploring biomass sorghum varieties for heavy metal/copper tolerance and its full potential as a phytoremediator crop. Therefore, we aimed to evaluate the physiological response and the potential of biomass sorghum (cultivar BRS 716) as a copper phytoremediator (Cu^{2+}).

Materials and methods

Plant material, growth conditions and experimental design

For this experiment, biomass sorghum (BRS 716) was used, kindly provided by Dr. Rafael Augusto Costa Parrella from EMBRAPA Maize and Sorghum. The experiment was carried out in a greenhouse at the Plant Tissue Culture Laboratory at Instituto Federal Goiano - Campus Rio Verde, Rio Verde - GO. Plants were allowed to grow for nearly 50 days under natural sunlight in the greenhouse and were monitored every day for water demand in order to avoid drought stress. The temperature inside the greenhouse was monitored and an average of 31° C was recorded. The experimental design was completely randomized containing 6 copper concentration applied in soil: 0, 30, 60, 90, 120 and 150 mg dm^{-3} to obtain nearly 10, 20, 30, 40 and 50 mg dm^{-3} of bioavailable Cu^{2+} (Fig. 1), besides the natural Cu^{2+} content in soil, which was 2.3 mg dm^{-3}. Each treatment was composed of 5 biological repetition totalizing 30 experimental units.

Soil preparation and analysis

The collected soil was homogenized in a concrete mixer, sieved in 2 mm mesh and left to dry at the environmental temperature. Afterwards, a bulk sampling was performed for chemical characterization and textural analysis. These results are shown in Table 1.

After soil drying, a volume of 2 dm^3 was packed in 3 litres plastic bags in order to carry out the artificial copper contamination. The artificial contamination was carried out

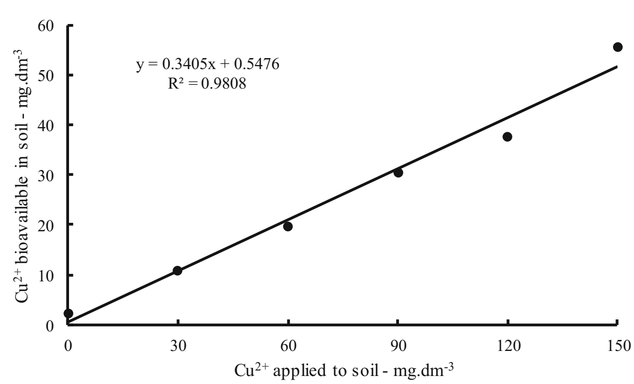

Fig. 1 Correlation between Cu^{2+} applied and Cu^{2+} bioavailable in soil after addition of Cu^{2+} solution in soil. Soil Cu^{2+} bioavailable (mg dm^{-3}): 2.3, 10.9, 19.6, 30.5, 37.6 and 55.6

Table 1 Chemical and textural soil features of the soil before any artificially soil contamination with copper

Soil parameters	
pH	4.9
O.M. (g dm^{-3})	23
Al ($mmol_c$ dm^{-3})	1
H + Al ($mmol_c$ dm^{-3})	42
SB ($mmol_c$ dm^{-3})	39.2
V %	48
m %	2
C.E.C ($mmol_c$ dm^{-3})	81.2
Ca/CTC %	20
Mg/CTC %	20
P (mg dm^{-3})	6
K ($mmol_c$ dm^{-3})	7.2
Mg ($mmol_c$ dm^{-3})	16
Ca ($mmol_c$ dm^{-3})	16
S–SO_4^{-2} (mg dm^{-3})	11
Cu (mg dm^{-3})	1.8
Fe (mg dm^{-3})	14
Zn (mg dm^{-3})	0.3
Mn (mg dm^{-3})	10.1
B (mg dm^{-3})	0.15
Clay (%)	57.8
Sand (%)	26
Silt (%)	16.2
Density (g cm^{-3})	1.5

using $CuCl_2 \cdot 2H_2O$ (copper chloride dihydrate) from a 12 g L^{-1} stock solution of free Cu^{2+} and dilutions were conducted to reach each desired copper concentration in a final volume of 400 mL of solution per plastic bag. Afterward, the soil was kept incubated in a greenhouse for 15 days. After incubation, the soil was homogenized and transferred to plastic seed bags. Then, 10 biomass sorghum seeds were planted in each pot. After 15 days, some plants were taken out to keep only 2 plants per pot. Soil determination of free available Cu^{2+} was carried out 15 days after incubation following a DTPA extractor according to Lindsay and Norvell (1978) and determination by flame atomic absorption spectrometer (FAAS).

Plant nutrition

To avoid mineral nutrition deficiency, the following nutrients and amounts were supplied: 74 mg of ammonium (NH_4^+), 400 mg of phosphate ($H_2PO_4^-$), 10 mg of sulphate (SO_4^{2-}), 7 mg of zinc (Zn^{2+}) and 2 mg of boric acid (H_3BO_3). All these nutrients were applied directly to the soil in a final volume of 50 mL of nutrient solution.

Plant analysis

Plant Growth parameters

To determine the growth response of plants subjected to copper stress, the following growth parameter measurements were performed: shoot length (S.L., cm); leaf area (L.A., cm^2); number of leaves (N.L., units); root length (R.L., cm); root volume (R.V., cm^3); shoot dry mass (S.D.M., g); root dry mass (R.D.M., g); total biomass (T.B., g); ratio between shoot dry mass and total biomass (D.S.M/T.B.); ratio between root dry mass and total biomass (R.D.M./B.T.) and ratio between leaf area and shoot dry mass (L.A./S.D.M., cm^2 g^{-1}).

Photosynthetic measurements

To determine photosynthetic response of plants under copper stress, a gas exchange analysis was performed using a portable infrared gas analyzer (IRGA, LI 6800xt, Li-Cor, Nebraska, USA, with a PAR of 1000 µmol m^{-2} s^{-1}).

The following gas exchange analysis were performed: transpiration rate (E, mmol H_2O m^{-2} s^{-1}); net photosynthesis (A, µmol CO_2 m^{-2} s^{-1}); water use efficiency (WUE = A/E); internal CO_2 concentration (Ci, µmol mol^{-1}); external CO_2 concentration (Ca, µmol mol^{-1}); ratio between internal and external concentrations of CO_2 (Ci/Ca) and stomatal conductance (gs, mol H_2O m^{-2} s^{-1}).

Copper determination in shoots

To determine copper concentration in plant tissues, the dried shoots or roots were milled. 250 mg of milled plant material was digested in 3 mL of HNO_3: $HClO_4$ acidic solution (5:1 v/v) for 12 h. Thereafter, the digester block temperature was raised to 50° C every 30 min until it reached 200° C and completed digestion of plant material. The resulting clear extract was adjusted to 25 mL with milli-Q water which was then used to detect copper on a flame atomic absorption spectrometer (FAAS).

Phytoremediation potential determination

We determined the phytoremediation potential for biomass sorghum by using Tolerance Index (Tol.I.: BM_{treat}/$BM_{control}$) and Translocation Index (T.I.: Cu_cS/Cu_cWP) according to Rahman et al. (2013) and Sauerbeck (1991). BM_{treat}: total biomass of treatment; $BM_{control}$: total biomass of control; Cu_cS: copper content in shoots; Cu_cWP: copper content in whole plant.

Statistical analysis

Statistical analysis was performed using SISVAR software (Ferreira 2011), in which data were submitted to analysis of variance and regression analysis at 5% of significance.

Results

Sorghum photosynthetic responses to increasing copper concentrations in soil

The net photosynthesis (*A*) was affected according to the copper concentration increase in the soil; this variable was most affected in 55.6 mg dm^{-3} treatment, in which we found a value of 15.57 for *A* which represents 67% of *A* for control plants (Table 2). However, other features such as transpiration rate (*E*), stomatal conductance (*gs*), internal CO_2 concentration (*Ci*), instant carboxylation efficiency (*A/Ci*) as well as its ratio and water use efficiency (*WUE*) were not affected under the different concentrations of copper (Table 2).

Biomass sorghum growth and phytoremediation potential under copper stress

We confirmed the increase in copper availability according to our treatments (Table 1) and sorghum growth was not affected by the increasing Cu^{2+} concentrations in soil (Table 3). Carbon partitioning was nearly 67% SDW and 33% RDW from control to 37.6 mg dm^{-3} treatments, changing to nearly 50% SDW and 50% RDW in the 55.6 mg dm^{-3} treatment (Fig. 2).

Copper concentration and content showed distinct patterns between shoot and root. In shoots, concentration and content decreased from 19.6 mg dm^{-3} treatment, while for roots, copper concentration and content tended to increase according to the increased copper in the soil (Table 4). Consequently, the copper translocation index clearly decreased as copper concentration increased in the soil, ranging from 0.58 to 0.11 for 10.9 and 55.6 mg dm^{-3} treatments, respectively (Table 4). Additionally, we observed an increase in the copper tolerance index according to the increase in copper in the soil, this ranged from 1.0 to 1.57 from control to the highest copper concentration (Table 4).

Discussion

Leaf gas exchanges are stable in BRS-716 biomass sorghum under copper stress

Copper is essential for photosynthesis as well as for mitochondrial activities since it is a component of cupric protein plastocyanin in photosynthetic process and it is also a component of the complex IV in the respiration chain in mitochondria (Merchant and Dreyfuss 1998). For this reason, it is clear that this element is important to the biological process. However, it is frequently reported that metal excess can disrupt any of the processes mentioned above (Vernay et al. 2007; González-Mendoza et al. 2013; Panda et al. 2015) and the way that an excess of metals can exert their toxicity is by direct interaction with non-target proteins or molecules, as well as by indirectly promoting

Table 2 Gas exchanges features of biomass sorghum BRS-716 grown in soil with increasing concentrations of copper: transpiration rate (*E*, mmol H_2O m^{-2} s^{-1}), net photosynthesis (*A*, µmol CO_2 m^{-2} s^{-1}), water use efficiency (W.U.E.), stomatal conductance (*gsw*, mol H_2O m^{-2} s^{-1}), external CO_2 concentration (Ca, µmol mol^{-1}), internal CO_2 concentration (*Ci*, µmol mol^{-1}), internal and external CO_2 ratio (Ci/Ca)

Cu^{2+} treatments	E	A	W.U.E	gsw	Ca	Ci	Ci/Ca	A/Ci
2.3	2.0 ± 0.0	21.01 ± 1.5	10271.22 ± 288.1	0.113 ± 0.01	400.03 ± 0.01	87.98 ± 10.0	0.22 ± 0.01	0.25 ± 0.03
10.9	2.0 ± 0.0	18.61 ± 2.2	10,299.59 ± 183.4	0.100 ± 0.01	400.01 ± 0.01	86.97 ± 7.6	0.22 ± 0.01	0.21 ± 0.02
19.6	1.0 ± 0.0	14.03 ± 1.3	10360.61 ± 214.4	0.073 ± 0.01	400.00 ± 0.01	76.80 ± 13.0	0.19 ± 0.01	0.19 ± 0.02
30.5	2.0 ± 0.0	16.60 ± 1.2	9597.21 ± 473.7	0.099 ± 0.01	399.95 ± 0.01	110.91 ± 17.2	0.28 ± 0.01	0.16 ± 0.02
37.6	2.0 ± 0.0	17.95 ± 0.6	10,195.35 ± 272.3	0.095 ± 0.01	400.04 ± 0.01	84.97 ± 10.0	0.21 ± 0.01	0.22 ± 0.04
55.6	2.0 ± 0.0	15.57 ± 1.1	10,242.20 ± 437.7	0.084 ± 0.01	399.98 ± 0.01	85.60 ± 15.3	0.21 ± 0.01	0.20 ± 0.03
Significance	ns	*	ns	ns	ns	ns	ns	ns
Regression	–	x	–	–	–	–	–	–

*$p < 0.05$. n = 5

x Significant linear regression

ns non-significant regression, $p > 0.05$

Table 3 Growth aspects of biomass sorghum BRS 716 grown in soil with increasing concentrations of copper: shoot length (S.L. cm); leaf area (L.A. cm^2); number of leaves (N.L. units); root length (R.L. cm); root volume (R.V. cm^3); shoot dry mass (S.D.M. g); root dry mass (R.D.M. g); total biomass (T.B. g); specific leaf area (S.L.A. $cm^2\ g^{-1}$)

Cu^{2+} treatments	S.L.	L.A.	N.L.	R.L.	R.V.	S.D.M.	R.D.M.	T.B.	S.L.A.
2.3	20.80 ± 1.5	566.58 ± 96.8	5.60 ± 0.2	46.40 ± 4.3	15.40 ± 2.2	3.42 ± 0.8	1.52 ± 0.3	4.95 ± 1.0	202.55 ± 46.7
10.9	20.90 ± 0.6	376.86 ± 39.4	5.60 ± 0.2	48.20 ± 5.3	16.80 ± 2.5	3.36 ± 0.4	1.70 ± 0.2	5.06 ± 0.6	115.35 ± 10.4
19.6	20.70 ± 0.6	476.51 ± 49.3	5.80 ± 0.2	40.60 ± 7.1	16.40 ± 2.2	3.80 ± 0.5	1.81 ± 0.3	5.61 ± 0.7	130.66 ± 12.2
30.5	19.60 ± 1.0	481.00 ± 88.1	5.80 ± 0.2	32.00 ± 2.5	14.40 ± 2.4	3.64 ± 0.8	1.98 ± 0.4	5.62 ± 0.7	137.77 ± 9.5
37.6	19.26 ± 1.1	506.23 ± 42.4	5.80 ± 0.2	32.20 ± 2.7	18.40 ± 4.3	3.07 ± 0.4	1.97 ± 0.4	5.04 ± 0.9	174.73 ± 18.3
55.6	18.92 ± 0.7	457.09 ± 62.1	5.00 ± 0.3	37.80 ± 2.9	22.60 ± 5.1	2.93 ± 0.4	2.97 ± 0.8	5.89 ± 1.0	161.24 ± 19.2
Significance	ns	ns	ns	ns	ns	ns	ns	ns	ns
Regression	–	–	–	–	–	–	–	–	–

ns non-significant regression, $p > 0.05$. n = 5

an excess of reactive oxygen species (ROS) production (Souza et al. 2017).

For a phytoremediation purpose, it is interesting that photosynthesis shows stability during the stress event when plants are able to keep their growth under unfavourable conditions such as metal toxicity. In this way, one of our findings in this work was gas exchange stability in biomass sorghum under copper stress (Table 2). Despite the fact that net carbon assimilation (*A*) was slightly affected, all other gas exchange features were not affected. Based on these results, we considered that photosynthetic activity is stable under copper toxicity in biomass sorghum as well as that which has been reported for elephant grass (Liu et al. 2009). These authors inferred that photosystem apparatus was not severely damaged by copper stress. Additionally, it seems to be a specific feature of each plant species based on the same work of Liu et al. (2009), who found that copper negatively affected photosynthesis in two other grass crops, *Vetiveria zizanioides* and *Phragmites australis*.

Photosynthetic activity decrease could not be explained by copper effect on sorghum plants growing under the higher doses, once copper transportation to aerial parts was decreased and its concentration increased in soil (Table 4). Photochemical approach has been considered in the work of Sharma et al. (2017) who found that copper was able to disturb photochemical process at 5 mM copper concentration. Effects on photochemical process directly impacts carbon fixation since these two processes are biochemically linked to each other. However, although this kind of response can explain the decrease in photosynthesis, it does not seem to apply to our findings because copper concentration in leaves did not reach phytotoxic amounts. According to Krämer (2010), the critical toxicity amount for copper ranges from 20 to 30 mg kg^{-1} DW and our finding for copper concentration in higher doses treatments is below the referenced concentrations for phytotoxicity cited above. The intriguing fact is that under non-toxic copper concentration in soil, in the second and third treatments, the carbon fixation was shown to be higher than for other treatments where copper concentrations were higher. A good explanation for this can be related to sorghum tolerance and an efficient use of higher amounts of copper in leaf tissues (Table 4) because these two copper concentration is above the phytotoxicity limit considered by Krämer (2010).

It is well known that the photosynthetic apparatus is versatile, which means that plants can coordinate the work flow and stress response in order to keep all photosynthetic machinery working properly (Ashraf and Harris 2013). In this sense, based on *E*, *gs* and *Ci* values, we strongly believe that when net CO_2 assimilation has been decreased temporally, as an adaptation to the stress, then higher values of *A* would be recovered. This behaviour has already

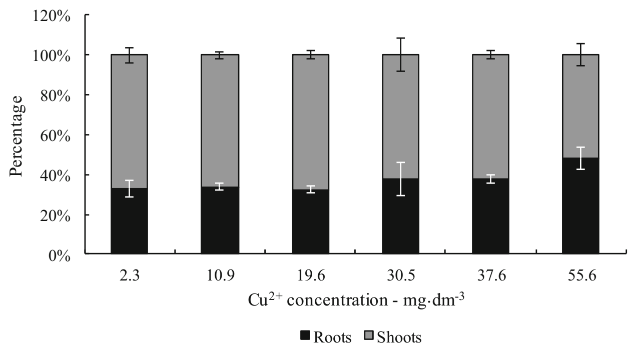

Fig. 2 Carbon partitioning between shoot and root in biomass sorghum grown in soil with increasing copper concentrations. n = 5

been reported for other abiotic stresses such as elevated temperatures in maize (Crafts-Brandner and Salvucci 2000) and, therefore, it may also be applied to the condition of copper stress in sorghum. Furthermore, considering the normal *A* rate recovering, the biomass sorghum capacity for phytoremediation can be strongly considered as it will be able to produce high amounts of biomass even in a stressful environment and can then be suitable for bioenergy feedstock production in copper polluted areas.

The BRS-716 biomass sorghum suitability for copper phytoremediation

A great concern has been raised about phytoremediation process feasibility, mostly due to the cost, further plant material disposal, and economic interest. In this way, some researches began to consider the use of high biomass crops as phytoremediators after the growth season, when above ground plant materials can be mechanically harvested and then delivered to factories as feedstock for burning and energy generation. This approach has been well presented in a review of Evangelou et al. (2012). Considering this trend, some high biomass producing crops have been assessed for metals tolerance. However, there has been little research into biomass sorghum production in contaminated land for bioenergy purposes.

The feasibility for using BRS-716 biomass sorghum for copper phytoremediation could be determined based on its high tolerance to copper that was found in our experiments (Table 2, 3). Generally, copper tends to be highly toxic to several organisms (Sipos et al. 2013), however there are few reports considering copper toxicity in high biomass producing plants for bioenergy purpose. Li et al. (2014) showed that *Arundo donax* and *Miscanthus sacchariflorus*, two energy grasses, were tolerant to high zinc and chromium concentrations such as 2000 and 1000 mg kg^{-1}, respectively, for each metal. In this sense, although there is no evidence for sorghum tolerance to copper, our findings allow us to suggest that BRS-716 biomass sorghum can be an extremely interesting energy crop for phytoremediation of copper contaminated land because we found no effect in growth, as well as high values of phytoremediation indicators (Tables 2, 4). Specially, with regard to copper, the energy grass *Elymus elongatus* showed extreme sensitivity to this metal at a low concentration of 10 µM in nutrient solution. In this experiment this plant showed low values of stomatal conductance as well as decreased root and shoot growth, which lead the authors to conclude that this crop is not suitable for copper phytoremediation (Sipos et al. 2013). Thus, we can confirm that any plant considered for a phytoremediation purpose must be assessed for different metals toxicity, tolerance, and extraction potential.

Other sorghum materials have already been tried for metals other than copper, and a high level of tolerance for

Table 4 Phytoremediation features of biomass sorghum BRS 716 grown in soil with increasing concentrations of copper: copper concentration in shoot (C.C.S.—mg kg^{-1}); copper content in shoot (C.C.S.*—µg $plant^{-1}$); copper concentration in roots (C.C.R.—mg kg^{-1}); copper content in roots (C.C.R.*—µg $plant^{-1}$); copper concentration in whole plant (C.C.W.P.—mg kg^{-1}); copper content in whole plant (C.C.W.P.*—µg $plant^{-1}$); translocation index (T.I); Cu tolerance index (Cu-Tol.I)

Cu^{2+} treatments	C.C.S.	C.C.S.*	C.C.R.	C.C.R.*	C.C.W.P.	C.C.W.P.*	T.I	Cu-Tol.I
2.3	19 ± 4.3	69.17 ± 23.7	44 ± 7.6	67.45 ± 16.7	91 ± 19	136.62 ± 37.8	0.46 ± 0.07	1.00 ± 0.0
10.9	68 ± 17.8	151.51 ± 22.5	69 ± 4.2	115.31 ± 11.9	205 ± 36	266.81 ± 29.9	0.58 ± 0.02	1.06 ± 0.08
19.6	36 ± 11.2	116.26 ± 13.8	81 ± 4.0	147.39 ± 26.3	153 ± 31	263.64 ± 26.5	0.45 ± 0.05	1.02 ± 0.07
30.5	18 ± 1.2	68.04 ± 17.6	173 ± 15.1	360.11 ± 89.2	418 ± 182	428.15 ± 82.9	0.20 ± 0.08	1.21 ± 0.28
37.6	18 ± 0.7	55.13 ± 9.5	165 ± 13.2	312.09 ± 55.4	294 ± 58	367.22 ± 63.2	0.15 ± 0.02	1.20 ± 0.11
55.6	18 ± 2.4	50.79 ± 6.4	171 ± 12.6	502.13 ± 129.0	378 ± 62	552.92 ± 132.3	0.11 ± 0.02	1.57 ± 0.29
Significance	*	*	*	*	*	*	*	ns
Regression	x	x	x	x	x	x	x	–

*$p < 0.05$. n = 5

x Significant linear regression

ns non-significant regression, $p > 0.05$

chromium, cadmium and zinc have been found (Bonfranceschi et al. 2009; Soudek et al. 2014; Jia et al. 2016). The tolerance level can define how much the crop can grow in stressful conditions and then we can predict the contaminant extraction capability. In our experiment, we found a higher tolerance index for sorghum under the highest copper concentration, 1.57 (Table 4) which we related to the decrease in copper transfer to the aerial parts (Table 4). According to these results, we could conclude that sorghum kept copper accumulation in the roots, increased carbon allocation to root production and then prevented copper accumulation in the aerial part. We noticed that response from 19.6 mg dm^{-3} treatment to the highest copper concentration treatment (Table 4) and all these features can be considered very relevant to a phytoremediation program of soil restoration, once sorghum cultivation contributes to improve several aspects of soil characteristics such as the increase of organic matter and stimulation of microbial activity, which leads to an improvement in soil quality, as reported by Gomez-Sagasti et al. (2016).

Copper uptake and transportation is tightly regulated by the COPT transporters and is expressed in different plant organs from root to pollen germination (Yruela 2005). However, plants possess other copper transporters that are related to homeostasis within cells that control the threshold between toxicity and functionality (Grotz and Guerinot 2006; Krämer et al. 2007). In this sense, little research has considered copper accumulation in energetic crops. We found that copper is mainly retained in roots of biomass sorghum without negative effects to roots and aerial parts development and we can therefore consider this crop as a good option for copper phytoremediation of contaminated land as also reported by Li et al. (2016). These authors inoculated a sorghum cultivar with a copper tolerant strain endophytic bacterium, *Enterobacter* sp. K3-2, and then showed the great potential of sorghum for phytoremediation. Additionally, these authors also showed that copper is mainly retained in roots, as is also reported by Toler et al. (2005) and by Jia et al. (2017) except for cadmium. Subsequently we may consider copper accumulation in roots as a common feature for sorghum plants.

This is an interesting feature because we can note important advantages from this behaviour: firstly, copper can be phytoestabilized in soil by the root system, thus preventing copper spread in the soil; secondly, as biomass sorghum showed tolerant to copper, its large amount of biomass can be harvested for bioenergy purposes; and finally, the cycle can be repeated until copper contamination decreases to an acceptable concentration. Additionally, the BRS-716 biomass sorghum has already been assessed for production in cultivated land and Rodrigues Castro et al. (2015) showed that it can produce up to 37 t ha^{-1} of dry matter with more than 4000 kcal kg^{-1} of dry mass, which has a great energetic potential.

Considering 37 t ha^{-1} of dry mass produced for sorghum BRS716 (Rodrigues Castro et al. 2015), the tolerance index and the amount of copper accumulated in shoots (Table 4), we can assume that in the lowest shoot copper accumulation found in harvestable parts (i.e. 18 mg kg^{-1} dry weight—Table 4), a single crop cycle would extract 660 g of copper from soil in a hectare, which is a considerable amount of copper extraction. This is due to the high biomass production linked with the high level of tolerance of this plant species whose growth is not affected by toxic levels of copper tested. This approach has been explored so that phytoremediation could operate independently of hyperaccumulator plants (Rabêlo et al. 2018; Souza et al. 2018) because these plants, although very specialized in accumulating high amounts of toxic metals, they produce such little amount of biomass. This is not desired for contaminant removal in phytoremediation (Rascio and Navari-Izzo 2011). Therefore, the approach with high biomass plant species engages with the possibility to generate economic revenues from plant material produced in contaminated lands (Fässler et al. 2010; Evangelou et al. 2015).

Finally, other supporting data for our finding is related to copper toxicity threshold. As reported by Krämer (2010), the critical copper toxicity concentration in living tissues is from 20 to 30 ppm, which means that any organism may experience toxic effects of this metal in such conditions. Our data shows that the copper retainment in root does contribute to decreased copper accumulation in aerial parts, which allowed sorghum plants to grow vigorously as being suited for phytoremediation and subsequent use as feedstock for bioethanol production, as reported by Tian et al. 2015.

Conclusions

Biomass sorghum BRS-716 is tolerant to excess copper in soil and can be used for the phytoremediation of contaminated land. This is therefore an interesting approach to generating economic values from contaminated land that is not used for food production.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Appendix: Complementary statistical data

This table displays statistical elements that showed significance in results for net photosynthesis (*A*, μmol CO_2 m^{-2} s^{-1}); copper concentration in shoot (C.C.S.—mg kg^{-1}); copper content in shoot (C.C.S.*—μg $plant^{-1}$); copper concentration in roots (C.C.R.—mg kg^{-1}); copper content in roots (C.C.R.*—μg $plant^{-1}$); copper concentration in whole plant (C.C.W.P.—mg kg^{-1}); copper content in whole plant (C.C.W.P.*—μg $plant^{-1}$) and translocation index (T.I).

	Variable	Regression model	Equations	R^2	ANOVA	
					F	*p*
Table 2	*A*	Linear	y = − 0.0253x + 19.19	0.337	3.01	0.03
Table 4	C.C.S.	Linear	y = − 0.1662x + 41.97	0.215	5.20	0.002
Table 4	C.C.S.*	Linear	y = − 0.6575x + 148.97	0.263	3.39	0.018
Table 4	C.C.R.	Linear	y = 0.9691x + 44.38	0.852	31.92	0.000
Table 4	C.C.R.*	Linear	y = 2.8347x + 38.14	0.894	5.90	0.001
Table 4	C.C.W.P.	Linear	y = 1.8763x +116.27	0.666	3.11	0.026
Table 4	C.C.W.P.*	Linear	y = 2.1771x + 187.11	0.742	3.02	0.029
Table 4	T.I.	Linear	y = − 0.0032x + 0.58	0.788	14.69	0.000

References

Andreazza R, Bortolon L, Pieniz S, Giacometti M, Roehrs DD, Lambais MR, Camargo FO (2011) Potential phytoextraction and phytostabilization of perennial peanut on copper-contaminated vineyard soils and copper mining waste. Biol Trace Elem Res 143:1729–1739

Antoniadis V, Golia EE, Shaheen SM, Rinklebe J (2017) Bioavailability and health risk assessment of potentially toxic elements in Thriasio Plain, near Athens, Greece. Environ Geochem Health 39:319–330

Ashraf M, Harris PJC (2013) Photosynthesis under stressful environments: an overview. Photosynthetica 51:163–190

Bolan N, Kunhikrishnan A, Thangarajan R, Kumpiene J, Parque J, Makino T, Kirkham MB, ScheckeL K (2014) Remediation of heavy metal(loid)s contaminated soils—to mobilize or to immobilize? J Hazard Mater 266:141–166

Bonfranceschi AB, Flocco CG, Donati ER (2009) Study of the heavy metal phytoextraction capacity of two forage species growing in an hydroponic environment. J Hazard Mater 165:366–371

Broadley M, Brown P, Cakmak I, Rengel Z, Zhao F (2012) Function of nutrients: micronutrients. In: Maschner P (ed) Marschner's mineral nutrition of higher plants, 4th ed. Academic Press

Carvalho MEA, Piotto FA, Nogueira ML, Gomes-junior FG, Maria H, Pescarin C, Pizzaia D, Azevedo RA (2018) Cadmium exposure triggers genotype-dependent changes in seed vigor and germination of tomato offspring. Protoplasma 255:989–999

Crafts-Brandner SJ, Salvucci ME (2000) Rubisco activase constrains the photosynthetic potential of leaves at high temperature and CO_2. Proc Natl Acad Sci USA 97:13430–13435

Evangelou MWH, Conesa HM, Robinson BH, Schulin R (2012) Biomass production on trace element-contaminated land: a review. Environ Eng Sci 29:823–839

Evangelou MWH, Papazoglou EG, Robinson BH, Schulin R (2015) Phytomanagement: phytoremediation and the production of biomass for economic revenue on contaminated land. In: Ansari AA, Gill SS, Gill R, Lanza GR, Newman L (eds) Phytoremediation: management of environmental contaminants. Springer, Switzerland, pp 115–132

Fässler E, Robinson BH, Stauffer W, Gupta SK, Papritz A, Schulin R (2010) Phytomanagement of metal-contaminated agricultural land using sunflower, maize and tobacco. Agric Ecosyst Environ 136:49–58

Ferreira DF (2011) Sisvar: a computer statistical analysis system. Cien Agrotec 35:1039–1042

Gomez-Sagasti MT, Epelde L, Alkorta I, Garbisu C (2016) Reflections on soil contamination research from a biologist point of view. Appl Soil Ecol 105:207–210

González-Mendoza D, Espadas y Gil F, Escoboza-Garcia F, Santamaría JM, Zapata-Perez O (2013) Copper stress on photosynthesis of black mangle (*Avicennia germinans*). An Acad Bras Cienc 85:665–670

Grotz N, Guerinot ML (2006) Molecular aspects of Cu, Fe and Zn homeostasis in plants. Biochim Biophys Acta Mol Cell Res 1763:595–608

Jia W, Lv S, Feng J, Li J, Li Y, Li S (2016) Morphophysiological characteristic analysis demonstrated the potential of sweet sorghum (*Sorghum bicolor* (L.) Moench) in the phytoremediation of cadmium-contaminated soils. Environ Sci Pollut Res 23:18823–18831

Jia W, Miao F, Lv S, Feng J, Zhou S, Zhang X, Wang D, Li S, Li Y (2017) Identification for the capability of Cd-tolerance, accumulation and translocation of 96 sorghum genotypes. Ecotoxicol Environ Saf 145:391–397

Kabata-Pendias A (2004) Soil-plant transfer of trace elements—an environmental issue. Geoderma 122:143–149

Krämer U (2010) Metal hyperaccumulation in plants. Annu Rev Plant Biol 61:517–534

Krämer U, Talke IN, Hanikenne M (2007) Transition metal transport. FEBS Lett 581:2263–2272

Li C, Xiao B, Wang QH, Yao SH, Wu JY (2014) Phytoremediation of Zn- and Cr-contaminated soil using two promising energy grasses. Water Air Soil Pollut 225:2027–2039

Li Y, Wang Q, Wang L, He LY, Sheng XF (2016) Increased growth and root Cu accumulation of *Sorghum sudanense* by endophytic *Enterobacter* sp. K3-2: implications for *Sorghum sudanense* biomass production and phytostabilization. Ecotoxicol Environ Saf 124:163–168

Lindsay WL, Norvell WA (1978) Development of a DTPA soil test for zinc, iron, manganese, and copper. Soil Sci Soc Am J 42:421–428

Liu X, Shen Y, Lou L, Ding C, Cai Q (2009) Copper tolerance of the biomass crops elephant grass (*Pennisetum purpureum* Schumach), vetiver grass (*Vetiveria zizanioides*) and the upland reed (*Phragmites australis*) in soil culture. Biotechnol Adv 27:633–640

Mehr MR, Keshavarzi B, Moore F, Sharifi R, Lahijanzadeh A, Kermani M (2017) Distribution, source identification and health risk assessment of soil heavy metals in urban areas of Isfahan province, Iran. J Afr Earth Sci 132:16–26

Merchant S, Dreyfuss BW (1998) Posttranslational assembly of photosynthetic metalloproteins. Annu Rev Plant Physiol Plant Mol Biol 49:25–51

Nogueira TAR, Abreu-Junior CH, Alleoni LRF, He Z, Soares MR, Santos Vieira C, Lessa LGF, Capra GF (2018) Background concentrations and quality reference values for some potentially toxic elements in soils of São Paulo State, Brazil. J Environ Manag 221:10–19

Palma P, Ledo L, Alvarenga P (2015) Assessment of trace element pollution and its environmental risk to freshwater sediments influenced by anthropogenic contributions: the case study of Alqueva reservoir (Guadiana Basin). CATENA 128:174–184

Panda P, Sahoo L, Panda SK (2015) Heavy metal and metalloid stress in plants: the genomics perspective. In: Chakraborty U, Chakraborty B (eds) Abiotic stresses in crop plants. CABI

Peñarrubia L, Romero P, Carrió-Seguí A, Andrés-Bordería A, Moreno J, Sanz A (2015) Temporal aspects of copper homeostasis and its crosstalk with hormones. Front Plant Sci 6:1–18

Rabêlo FHS, Borgo L, Lavres J (2018) The use of forage grasses for the phytoremediation of heavy metals: plant tolerance mechanisms, classifications, and new prospects. In: Matichenkov V (ed) Phytoremediation: methods, management and assessment. Nova Science Publishers, New York, pp 59–102

Rahman MM, Azirun SM, Boyce AN (2013) Enhanced accumulation of copper and lead in Amaranth (*Amaranthus paniculatus*), Indian Mustard (*Brassica juncea*) and Sunflower (*Helianthus annuus*). PLoS ONE 8:e62941

Rascio N, Navari-Izzo F (2011) Heavy metal hyperaccumulating plants: how and why do they do it? And what makes them so interesting? Plant Sci 180:169–181

Rodrigues Castro FM, Bruzi AT, Rodrigues Nunes JA, Costa Parrella RA, Romeiro Lombardi GM, Brant Albuquerque CJ, Lopes M (2015) Agronomic and energetic potential of biomass sorghum genotypes. Am J Plant Sci 6:1862–1873

Sauerbeck DR (1991) Plant element and soil properties governing uptake and availability of heavy metals derived from sewage sludge. Water Air Soil Pollut 57:227–237

Sharma P, Sirhindi G, Singh AK, Kaur H, Mushtaq R (2017) Consequences of copper treatment on pigeon pea photosynthesis, osmolytes and antioxidants defense. Physiol Mol Biol Plants 23:809–816

Sipos G, Solti A, Checo V, Vashegyi I, Tóth B, Cseh E, Fodor F (2013) Heavy metal accumulation and tolerance of energy grass (*Elymus elongates* subsp. *Ponticus* cv. *Szarvasi* -1) grown in hydroponic culture. Plant Physiol Biochem 68:96–103

Soudek P, Petrová Š, Vaňková R, Song J, Vaněk T (2014) Accumulation of heavy metals using *Sorghum* sp. Chemosphere 104:15–24

Souza LA, Monteiro CC, Carvalho RF, Gratão PL, Azevedo RA (2017) Dealing with abiotic stresses: an integrative view of how phytohormones control abiotic stress-induced oxidative stress. Theor Exp Plant Physiol 29:109–127

Souza LA, Camargos LS, Carvalho MEA (2018) Toxic metal phytoremediation using high biomass non-hyperaccumulator crops: new possibilities for bioenergy resources. In: Matichenkov V (ed) Phytoremediation: methods, management and assessment. Nova Science Publishers, New York, pp 1–26

Stankovic S, Kalaba P, Stankovic AR (2014) Biota as toxic metal indicators. Environ Chem Lett 12:63–84

Subramani T, Florence HR, Kavitha M (2014) Climate change energy and decentralized solid waste management. Int J Eng Res Appl 4:205–216

Thyberg KL, Tonjes DJ (2016) Drivers of food waste and their implications for sustainable policy development. Resour Conserv Recycl 106:110–123

Tian YL, Zhang HY, Guo C, Wei XF (2015) Morphological responses, biomass yield, and bioenergy potential of sweet sorghum cultivated in cadmium-contaminated soil for biofuel. Int J Green Energy 12:577–584

Toler HD, Morton JB, Cumming JR (2005) Growth and metal accumulation of mycorrhizal sorghum exposed to elevated copper and zinc. Water Air Soil Pollut 164:155–172

Vernay P, Gauthier-Moussard C, Hitmi A (2007) Interaction of bioaccumulation of heavy metal chromium with water relation, mineral nutrition and photosynthesis in developed leaves of *Lolium perenne* L. Chemosphere 68:1563–1575

Yruela I (2005) Copper in plants. Braz J Plant Physiol 17:145–156