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RESEARCH ARTICLE

Response of *Cajanus cajan* to excess copper in the soil: tolerance and biomass production

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Abstract Soil contamination by excess heavy metals or trace elements is a global concern, as these elements are highly bioaccumulated in living organisms, migrating throughout the food chain, and causing health problems. Sustainable technologies, using plants, have been increasingly studied and used to contain, reduce, or extract these elements from the soil. In this sense, it is essential to identify plant species that tolerate certain elements, present high biomass production and are resistant to adverse soil conditions. For this reason, we evaluated the biomass production and tolerance of *Cajanus cajan* in response to different concentrations of copper (30, 60, 120, and 240 mg/dm^3, in addition to the control treatment) in the soil, as well as the effect of this metal on photosynthetic pigments and gas exchange. *C. cajan* was sown in soil previously contaminated with copper sulfate and cultivated in a greenhouse for 60 days after emergence. *C. cajan* is copper tolerant, approximately 88% copper is accumulated in the roots and therefore there is low copper translocation to the shoot, consequently, the chlorophyll content, the net photosynthesis rate, carbon assimilation, dry biomass, the root system development, and nodulation were not affected by copper. *C. cajan* can be explored in strategies to improve soil conditions and is a promising species in soil phytoremediation studies.

Keywords Soil contamination · Trace-elements · Heavy metals · Pigeon pea · Copper accumulation · Tolerance index

Introduction

Trace elements are high-density metal elements (Pb, Cd, Zn, Cu, Hg, and Al), that accumulate in living organisms, occur naturally in the environment, and are potentially toxic to plants, depending on their concentration in the soil and water (Souza et al. 2018; Yang et al. 2020; Nworie and Lin 2021). Copper (Cu) is a trace element and is required by plants as a micronutrient, participating as a component of several enzymes that catalyze the oxidation–reduction reactions in the cytoplasm, chloroplasts, and mitochondria, by participating in mitochondrial respiration, photosynthetic electron transport, cell wall metabolism, responses to oxidative stress and protein synthesis (Shabbir et al. 2020). Human actions frequently have a negative impact on the environment (Mohajel Kazemi et al. 2020), such as inadequate agricultural practices and industrial activities that degrade the environment, contaminating the soil and water bodies with metals, with consequent bioaccumulation of these elements in the food chain (Mohajel Kazemi et al. 2020; Trentin et al. 2022).

One of the ways Cu enters the environment is through the use of organic residues as fertilizers and the application of fungicides based on Cu sulfate. The fungicides (Bordeaux mixture, Ca $(OH)_2 + CuSO_4$) are used for the control and prevention of fungal diseases in fruit, and vegetable

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production (Meier et al. 2017; Rehman et al. 2019). Swine manure and cattle manure contains amounts of Cu and it has been reported that the massive application of these manures is responsible for increasing Cu in the soil, as in China, where 76% of Cu in agricultural soils comes from cattle manure (Peng et al. 2019) and in some states in Brazil (Rio Grande do Sul and Santa Catarina) a lot of swine manure has been used (Girotto et al. 2010).

In the European Union, a limit of 100.00 mg/kg^1 Cu in agricultural soils was considered, with lower and upper reference values set at 150.00 mg/kg^1 and 200.00 mg/kg^1 Cu, respectively (Tóth et al. 2011). Concern about Cu contamination of soil has decreased in Europe (Panel 2016; Huygens et al. 2020), however, in many countries in Asia, China, northern Vietnam, and Latin America, soil Cu contamination is still widespread and very worrying (Peng et al. 2019; Dao et al. 2021), including in Brazil (Rosa-Couto et al. 2015; Perlatti et al. 2015, 2021; Fernandes et al. 2018; De Conti et al. 2018).

High levels of Cu (779.00 mg/kg) in the soil have been reported to trigger toxicity in alfalfa plants, *Medicago sativa* L. (Fabaceae), with the consequent decrease in leaf pigments, biomass and even plant mortality (Wang et al. 2022), which can further aggravate environmental damage by accelerating soil degradation, due to lack of vegetation/natural regeneration of area if not mitigated (Närhi et al. 2012). Thus, metals comprise an important group of contaminants of great interest for studies on environmental preservation and human health, since they can alter the entire balance of an ecosystem and cause serious neurological disorders (Souza et al. 2018; Vardhan et al. 2019).

An alternative way to decrease soil contamination by Cu is phytoremediation, which consists of a set of phytotechnologies used to recover soils contaminated by metals (Gautam and Agrawal 2019; Shah and Daverey 2020). In this phytotechnology, plants are used to recover soils contaminated by metals, which will be sequestered, metabolized, or immobilized by the plants structures and then decreasing the environmental impact with low financial cost (Shah and Daverey 2020).

The choice of plant species for the phytoremediation process is fundamental, requiring the identification of plants with good development in contaminated environments and with a high level of tolerance and adaptation to local growing conditions (Saleem et al. 2020), in addition they must show growth fast, high biomass production, easy propagation, easy cultivation and harvest, be widely distributed and have a highly branched root system (Zhang et al. 2010; Ali et al. 2013; Sipos et al. 2013). Therefore, some cover crops are interesting and have a high potential for application as phytoremediation agents.

Cajanus cajan, known as pigeon pea, is an important nitrogen fixing legume with desired traits highlighted above. It is one of the most relevant food legume crops in the tropics and subtropics, is the second most consumed food and about 91.00% of world production is traded by India, also cultivated in sub-Saharan Africa (Adjei-Nsiah 2012; Yadu et al. 2017; Kaushik et al. 2022). This cereal crop reached, in 2020, approximately 4.50 million tons of world production (Byatarayappa et al. 2021). It is also widely used as a green manure in various agricultural systems, due to its nitrogen fixing capacity and a catalyst for ecological restoration (Vanaja et al. 2015). It is also drought-resistant, growing in poor soils, it has wide adaptability, due to the grain maturation time (Kaushik et al. 2022). Based on this information, *Cajanus cajan* can be grown in a variety of environments and cropping systems, and it is also very suitable for crop rotation (Choudhary and Singh 2011).

Some authors have studied the effect of heavy metals like zinc and nickel; cadmium and lead; chromium and oily oil sludge (POS) in *Cajanus cajan* and its potential as a phytoremediator agent (Garg and Aggarwal 2011, 2012; Jerez and Romero 2016; Allamin et al. 2020). However, data on the tolerance of *C. cajan* L. Millsp, cultivar IAPAR 43-Aratã to potentially toxic elements such as copper are insufficient; thus, the evaluation of its tolerance and potential for Cu phytoremediation in soil is crucial to foster future literature and remediation projects. For this reason, our study evaluated the biomass production and tolerance of *C. cajan* L. Millsp, cultivar IAPAR 43-Aratã in response to different concentrations of copper in the soil, as well as the effect of this metal on photosynthetic pigments and gas exchange.

Material and methods

Soil characterization

The soil used in this study for the cultivation of *C. cajan* was obtained from FEPE (FEIS-UNESP Teaching, Research and Extension Farm), characterized as Latosols (oxisols). Two composite samples, each with 10 sub-samples, from the soil were taken for chemical characterization (Raij et al. 2001), and the following characteristics were scored: acidity and base saturation: pH 4.20 and V% 23.00; sum of SB-bases = 9.50 mmol$_c$/dm^3; Ca^{2+} = 4.00 mmol$_c$/dm^3; K$^+$ = 0.50 mmol$_c$/dm^3; Mg^{2+} = 5.00 mmol$_c$/dm^3; potential acidity (H$^+$ + Al^{3+}) = 31.00 mmol$_c$/dm^3; organic matter = 14.00 g/dm^3; cation exchange capacity—CTC = 40.50 mmol$_c$/dm^3; Al^{3+} = 10.00 mmol$_c$/dm^3; P-resin = 1.00 mg/dm^3; S-SO$_4$ = 5.00 mg/dm^3; B = 0.21 mg/dm^3; Cu^{2+} = 0.90 mg/dm^3; Fe^{2+} = 16.00 mg/dm^3; Mn^{2+} = 6.90 mg/dm^3; Zn^{2+} = 0.10 mg/dm^3.

The experiment was conducted at UNESP/Campus of Ilha Solteira-SP, Brazil, located at 20° 43′ 09″ south latitude and 51° 33′ 79″ west longitude, with an altitude of around 335 m, in the greenhouse of the Laboratory of Plant Metabolism Physiology, at a controlled temperature of 27 °C for 60 days, between January and February 2018, under natural light and temperature conditions. The temperature and relative humidity conditions were: 26.40 °C average temperature; 32.70 °C maximum temperature; 22.00 °C minimum temperature and 83.00% average relative humidity.

Treatment for Cu stress

A stock solution of copper sulfate pentahydrate was prepared according to the concentrations: 30.00, 60.00, 120.00, and 240.00 mg Cu/dm^3. For soil contamination/treatment, each soil unit, duly identified by treatment and corresponding repetition, received (single application) 20.00 ml of stock solution, was individually homogenized, and remained at rest in a closed plastic bag for 10 days. In the repetitions of the control treatment, 20.00 ml of distilled water was added. No soil conditioner, nitrogen or phosphate fertilizer was added.

After the stabilization period, soil samples were collected at each repetition of treatments to assess the availability of Cu in the soil (Raij et al. 2001). The available Cu levels in the soil per treatment were in control = 0.50 mg/dm^3; 30.00 = 39.80 mg/dm^3; 60.00 = 85.50 mg/dm^3; 120.00 = 106.20 mg/dm^3 and 240.00 = 247.20 mg/dm^3 (Table 1).

Experimental design

It was composed of five treatments: one control (0.00) and four doses of copper (30.00, 60.00, 120.00, and 240.00 mg/dm^3). Each treatment contained five replications (5×5), that is, five pots of 2.00 dm^3 of soil (being 1.00 dm^3 of sand and 1.00 dm^3 of soil), totaling 25.00 pots or experimental units in a completely randomized design in the greenhouse ($n = 25$). Each vessel was 12.50 cm high, 17.00 cm in upper diameter, and 12.00 cm in lower diameter.

Table 1 Cu availability in the soil Latosols (oxisols) before and after the cultivation of *Cajanus cajan*

Cu treatments	Before	After
	mg/dm^3	
Control	0.50	0.90
30.00 mg/dm^3	39.80	41.40
60.00 mg/dm^3	85.50	69.10
120.00 mg/dm^3	102.20	140.50
240.00 mg/dm^3	247.20	274.00

Plant materials

Our research group has studied some species of leguminous, nitrogen-fixing, of wide vegetation cover (*Crotalaria juncea, Canavalia ensiformis*) in response to increasing concentrations of potentially toxic elements in the soil. *Cajanus cajan* has been cultivated under potentially toxic concentrations of Cd, Pb, Zn, Cr, and Ni, to assess its tolerance to these elements (Garg and Aggarwal 2011; Garg and Kaur 2012; Garg and Singh 2018; Minari et al. 2020; Souza et al. 2020). There are no published data related to the tolerance of this species to copper, therefore, the object of this study is *Cajanus cajan* L. Millsp, cultivar IAPAR 43-Aratã, obtained commercially (BRSEEDS®).

The *C. cajan* seeds were surface-sterilized for 15 min in 10% sodium hypochlorite solution (v/v—commercial solution), and after remaining in distilled water for one hour for soaking, sowing was performed. The seeds of *Cajanus cajan* L. Millsp, cultivar IAPAR 43-Aratã, were sown directly in the contaminated soil. Five seeds were added to each pot, and after the emergence of the seedlings, thinning was performed, leaving only two plants in each pot. One plant was used for in vivo evaluation of gas exchange and quantification of pigments, and the other was used for evaluation of quantification of Cu by dry biomass produced.

Photosynthetic measurements

The leaf gas exchange measurement parameters were performed at 60 days of cultivation, in the vegetative stage (before the collection of the experiment), in the morning, between 8:00 and 12:00 h, using the second or third leaf of the apex caulinar. The conditions established for the measurements were 1000 μmol m^{-2}/s of photosynthetic active radiation (PAR), provided by LED lamps.

The assessments include the net photosynthesis rate—A (μmol CO_2 m^{-2}/s); transpiration rate—E (mmol H_2O m^{-2}/s); stomatal conductance—gs (mol H_2O m^{-2}/s); internal CO_2 concentration (Ci, μmol/mol); external concentration of CO_2 (Ca, μmol/mol); ratio between internal and external concentrations of CO_2—Ci/Ca; the instantaneous water use efficiency—WUE (μmol CO_2/mmol H_2O) was obtained by the ratio between A and E (WUE = A/E), and the carbon use efficiency (CUE) = A/Ci (mol C of biomass/mol C fixed) were analyzed by means of Infra-Red Gas Analyser (IRGA), using the ADC BioScientific Ltd, LC-Pro model.

Chlorophyll estimation

The plants were collected in the vegetative stage, after 61 days of cultivation. The leaves were collected, and the levels of chlorophyll *a* (Chl *a*), *b* (Chl *b*) and total chlorophyll content (TChl) were quantified, sequentially, (Hiscox and Israelstam 1979), using DMSO (Dimethylsulfoxide) as an extracting agent. The foliar tissue was cut into thin strips (1 mm) until 50.00 mg was obtained. The material was incubated in 7.00 mL of DMSO (or 25.00 mg + 3.50 ml DMSO), in the dark in a water bath (65° C/30 min.). After cooling, a spectrophotometer reading with wavelengths of 645 nm and 663 nm was performed and the concentrations were calculated according to the equations: $C_a = (12.70 \times A_{663})$—$(2.69 \times A_{645})$; $C_b = (22.90 \times A_{645})$—$(4.68 \times A_{663})$; $C_a + C_b = (20.20 \times A_{646}) + (8.02 \times A_{663})$.

Biomass and estimation of Cu contents in plant tissues

The nodules were separated from the roots, washed in distilled water, weighed (g) and counted. The roots were washed in running water to remove excess soil and then in deionized water. The aerial part and root material were placed in paper bags and dried in a forced circulation oven at 60 °C for 72 h. The dry mass (g) of the root system—RDW, of the aerial part—SDW (leaves and stems), and of the total biomass—TBDW (root + aerial part) were obtained.

Afterwards the materials were ground in a Wiley-type mill (10 mesh sieve) for further digestion with nitric acid (HNO_3) and perchloric acid ($HClO_4$) and determination of the concentration (mg/kg^1) of copper in the root system-RMC, shoot-SMC and total biomass—SBMC, using atomic absorption spectrophotometry (Malavolta et al. 1997). The detection points for the analytical determination of Cu were 0.00; 0.25; 0.50; 0.75; 1.00; 1.25 ppm. The data obtained were used for calculations of metal accumulation in the remaining dry matter and transfer factor.

After the cultivation and collection of *C. cajan*, composite soil samples from each treatment were subjected to chemical analysis of Cu availability (Raij et al. 2001), again, in order to compare the available Cu contents in the soil and verify if there was or not a reduction in the metal content in the soil (Table 1).

Phytoremediation potential

To determine the phytoremediation potential of *C. cajan* some calculations were performed: tolerance index (TI) and transfer factor (TF) (Kabata-Pendias and Pendias 2001). Tolerance index: $TI = TBDW_{\text{total biomass}}$ treatment/$TBDW_{\text{total biomass}}$ control treatment; transfer factor to shoot: $STF = SMC_{\text{copper in the shoot system}}/SBMC_{\text{total biomass}}$ and transfer factor to root: $RTF = RMC_{\text{copper in the root system}}/SBMC_{\text{total biomass}}$ (Bomfim et al. 2021).

Cu accumulation (μg/organ) was calculated using dry weight and Cu concentration data in the respective organs: root and shoot. RAC, SAC and TAC are accumulation of Cu in the root, shoot and total biomass (μg/organ) respectively. Accumulation of Cu in the root: $RAC = (RDW_{\text{dry mass of the root system}} * RMC_{\text{copper in the root system}})$ and accumulation of Cu in the shoot: $SAC = (SDW * SMC_{\text{copper in the shoot system}})$ in milligram/dry organ weight (mg/organ) (Bomfim et al. 2021). The Cu translocation index (TI%) was calculated according to Rahman et al. (2013), TI (%) = (SAC/TAC) *100.

Statistical analysis

The data answered the hypothesis of normality and homogeneity of variance by the Shapiro–Wilk tests, respectively, at 5% probability. Therefore, they were subjected to analysis of variance (ANOVA) by the F test at 5% probability ($n = 25$). With significance in the F test, the comparison of means was performed through the tests of Scott-Knott (Scott and Knott 1974). A graph of correlation networks was performed containing the Pearson correlation (r) between the variables: Chl *a;* Chl *b*; TChl; $SDW_{\text{dry mass of the shoot}}$; $RDW_{\text{dry mass of the root system}}$; $TBDW_{\text{total biomass}}$; $SAC_{\text{accumulation of Cu in the shoot}}$; $RAC_{\text{accumulation of Cu in the root}}$; $TAC_{\text{accumulation of Cu in the total biomass}}$; $TI\%_{\text{ translocation index}}$; $RT\%_{\text{transfer factor to root}}$; $TI_{\text{Tolerance index}}$. The data were analyzed using the R software (Core Team 2019).

Results and discussion

Physiological performance

The concentration of Cu in the soil solution varies between 10^{-6} and 10^{-9} M; as it is a micronutrient, plants reduce and solubilize this mineral to absorb enough Cu for proper growth (Marschner 2011). Common Cu levels in plant tissue range between 2.00 and 20.00 μg/g^1 of dry matter (DM) for most species (Kabata-Pendias 2011; Shabbir et al. 2020; Farid et al. 2021). *C. cajan* shoots presented on average, 23.66 mg/kg DM of Cu between the treatments from 30.00 to 240.00 mg/dm^3 of soil, sampling was done in 60 days after sowing. Concentrations between 20.00 and 100.00 mg/kg^1 in the dry matter of the shoot are considered toxic for some species such as legumes, cereals, citrus, among others (Kabata-Pendias 2011; Shabbir et al. 2020). Although the Cu content in *C. cajan* shoots is higher than previously reported, there were no visual symptoms of toxicity such as chlorosis in the leaves, and chlorosis is one of the primary signs of Cu toxicity (Reckova et al. 2019).

Cu is an essential element for higher plants with a fundamental role in photosynthesis (Marques et al. 2018; Zeng et al. 2019), is a constituent of the main electron donor in PSI, and of the enzymes involved in the scavenging of superoxide radicals from plants. Plant growth inhibition is a result of the toxic effects of Cu on physiological and metabolic processes, with photosynthesis (CO_2 fixation) being the most sensitive (Dai et al. 2016; Chandrasekhar and Ray 2017), by interfering with the pigment composition and the chloroplast structure, causing a reduction in the net photosynthetic rate, limiting the activity of RUBISCO and inhibiting the photosynthetic electron transport chain (Mir et al. 2021).

The content of chlorophylls *a*, *b* and total chlorophylls of *C. cajan* differed statistically among doses with the control treatment: chlorophyll content was higher in treatments 30.00; 60.00; 120.00 and 240.00 mg/dm^3 of Cu in soil, compared to the control (Fig. 1A). Unlike *C. cajan,* in soybean (Gomes et al. 2021), the total chlorophyll content presented a reduction with the addition of Cu to the soil (above 50.00 mg/kg^1); in lentil plants (Hossain et al. 2020), there was a decrease in chlorophylls *a*, *b* and carotenoids at a high concentration of Cu (3.00 mM).

Other studies showed the negative effect of Cu on the levels of photosynthetic pigments. In *Phragmites australis* leaves (Wu et al. 2021), chlorophyll *a*, *b* and total chlorophyll decreased with increasing concentration of Cu in solution (above 5.00 mg/l^1). In maize plants (cultivars SC 122 and SC 10) stressed with 100.00 µM of Cu there was a decrease in total chlorophyll contents (Aly and Mohamed 2012), as well as in *Spinacia oleracea* (Gong et al. 2021) at concentrations above 700.00 mg/kg^1. At a concentration of 800.00 µM of Cu (Giannakoula et al. 2021), it inhibited the biosynthesis of chlorophyll and carotenoids in *Citrus aurantium* L. and delayed the incorporation of these pigments into the photosynthetic machinery.

Chlorophyll concentration is a very useful indicator of heavy metal toxicity to calculate higher critical concentrations of elements in plant tissues (Giannakoula et al. 2021). The reduction in photosynthetic pigment concentration under Cu stress can be thought of as a plant-specific response to metal stress, which resulted in the degradation of chlorophylls and inhibition of photosynthesis (Giannakoula et al. 2021). However, there was no such reduction in chlorophylls and photosynthesis in *C. cajan* L. Millsp, cultivar IAPAR 43-Aratã plants, indicating a mechanism for tolerance to excess Cu.

Cu toxicity resulted in a reduction in the net photosynthetic rate in *Citrus aurantium* L., above 800.00 µM Cu

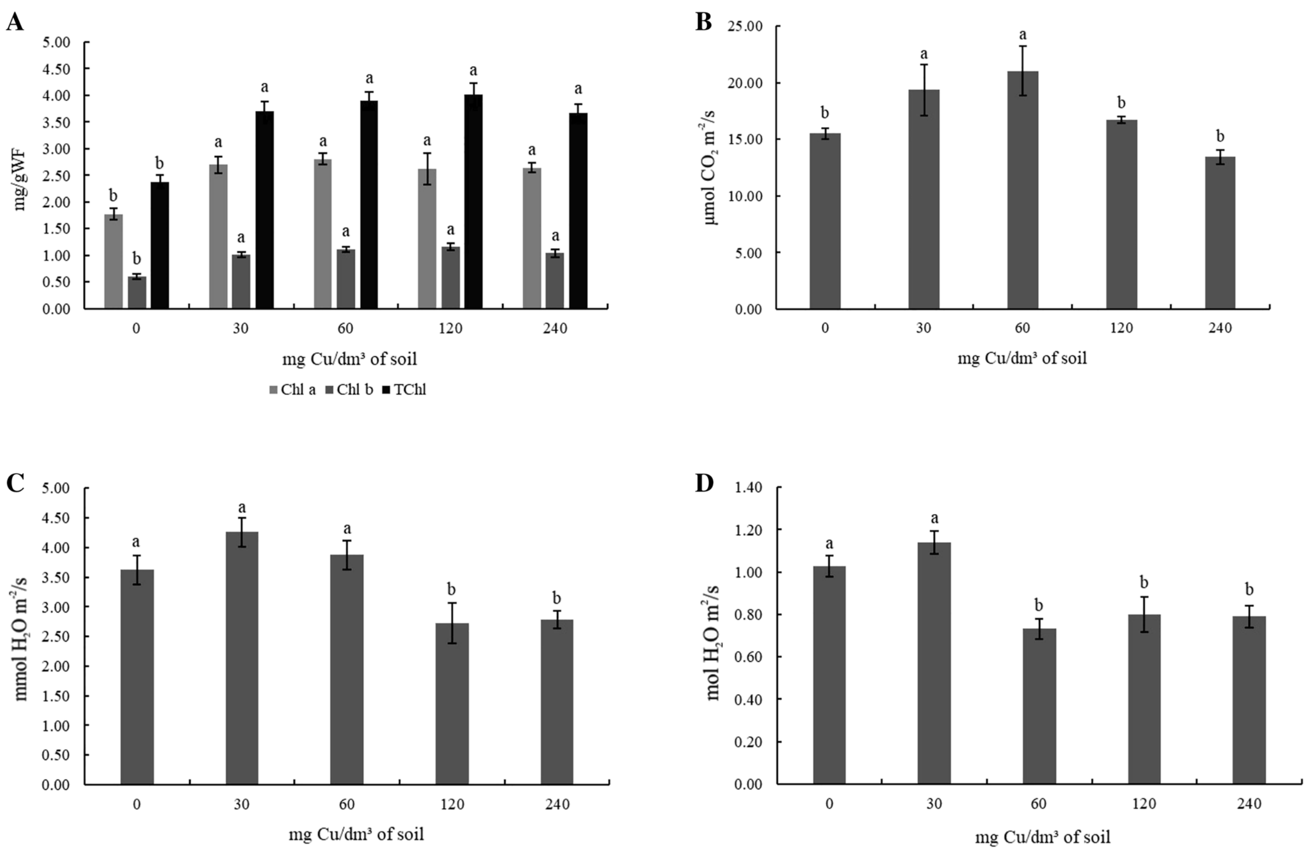

Fig. 1 Spectral parameters of *C. cajan* (at 60 days of cultivation, vegetative stage) cultivated in a Latosols with four copper concentrations: 30.00, 60.00, 120.00 and 240.00 mg Cu/dm^3 of soil, in addition to the control treatment, in a greenhouse. **A**: quantification of Chlorophylls—Chl *a*, Chl *b* and total—TChl (mg/g of fresh mass); **B**: net photosynthesis rate—A (µmol CO_2 m^{-2}/s); **C**: transpiration rate—E (mmol H_2O m^{-2}/s); **D**: stomatal conductance—gs, (mol H_2O m^{-2}/s). Different letters indicate statistical difference between treatments of the same variable, according to the Scott Knott test, at 5%. Same letters indicate that there was no statistically significant difference ($n = 25$)

(Giannakoula et al. 2021), stomatal conductance, internal CO_2 concentration, transpiration rate, in addition to A, (above 700.00 mg/kg^1) in *Spinacia oleracea*, spinach (Gong et al. 2021). Other authors have observed the same response in photosynthetic pigment content, photosynthesis, and transpiration rates (Ding et al. 2017; Ambrosini et al. 2018; Marques et al. 2018).

In soybean plants (Gomes et al. 2021), the net photosynthetic rate significantly reduced with the increase of Cu in the soil (above 50.00 mg/kg^1); in *C. cajan* (Fig. 1B) there was a reduction of the net photosynthetic rate in the treatments of 120.00 and 240.00 mg/dm^3 of Cu in the soil, compared to the low concentrations (30.00 and 60.00 mg/dm^3), but not compared to the control treatment. Therefore, we can infer that Cu was not harmful to the net photosynthesis rate in *C. cajan.*

The stomatal conductance and the transpiration rate reduced under concentrations above 50.00 and 70.00 mg/kg^1 of Cu in soybeans (Gomes et al. 2021), respectively, similar to our results (Fig. 1C, D). The ratio between internal and external concentrations of CO_2 of soybean (Gomes et al. 2021), increased at low concentrations, followed by a reduction with increasing levels of Cu in the soil (above 70.00 mg/kg^1 of Cu). However, internal, and external CO_2 concentration; ratio between internal and external concentrations of CO_2; the instantaneous water use efficiency, and the carbon use efficiency did not differ between Cu treatments in *C. cajan* plants.

Growth and nodulation

Copper in excessive concentrations in the soil disturbs nutrient homeostasis by interacting with mineral nutrient oxides such as aluminum (Al), iron (Fe), manganese (Mn), and with organic matter, altering nutrient absorption and significantly decreasing productivity and plant yield (Mir et al. 2021).

A useful method to assess the occurrence of abiotic stresses in plants is to analyze the growth and development of plants (Giannakoula et al. 2021). At high concentrations and in bioavailable forms in the soil solution, Cu can also alter plant tissues at the biochemical and physical-biological level, which can cause considerable changes in the productive potential of crops (Vardhan et al. 2019). Studies show that low concentrations of Cu in the soil benefit fertilization and stimulate soybean growth, leading to increased grain yield (Moreira and Moraes 2019). However, high concentrations of Cu, or above the tolerated limit (60.00 mg/kg^1), affected soybean growth and yield (Gomes et al. 2021).

The total dry biomass of *C. cajan,* after 60 days of cultivation, in the vegetative stage (Fig. 2C), was higher in the treatments with Cu (30.00, 60.00 and 240.00 mg/dm^3) compared to the control treatment, that is, the concentrations of Cu had a beneficial effect on *C.cajan*, as mentioned above in terms of chlorophylls and photosynthetic rate, unlike the results found for soybean (Gomes et al. 2021); cucumber (Vinit-Dunand et al. 2002); tomato (Zhang et al. 2010) and spinach (Gong et al. 2021).

Root growth and development have been observed to be more affected than aboveground growth at high Cu concentrations, such as in soybean (Gomes et al. 2021), *Citrus aurantium* L (Giannakoula et al. 2021), *Hymenaea courbaril* (Marques et al. 2018) and other plants exposed to other metals (Srivastava et al. 2011; Pérez-Chaca et al. 2014). In relation to *C. cajan*, Cu concentrations did not affect the growth and biomass of the root system (Fig. 2C). As for shoot biomass, there are differences among the treatments, but the Cu treatments had higher masses than the control treatment (Fig. 2C). Therefore, Cu treatments, from 30.00 to 240.00 mg/dm^3, were beneficial to the development of *C. cajan*, probably due to the functions of Cu as a micronutrient in plant metabolism (Yruela 2013).

Tortosa et al. (2020) reported that soybean (*Glycine max* L. Merr., cv. Williams) exposed to concentrations of 40.00, 60.00 and 100.00 µM of Cu triggered a reduction in plant biomass and nodulation capacity, at concentrations lower than 20.00 µM of Cu, the number and mass of nodules increased, however, it reduced in concentrations greater than 40.00 µM of Cu. Based on these results, the authors claim that high concentrations of Cu trigger the induction of oxidative stress in plants (Tortosa et al. 2020).

Nodule number and fresh mass were significantly different between treatments (Fig. 2A, B); however, nodulation and biological nitrogen fixation were not affected by soil copper doses, as opposed to Tortosa et al. (2020). The treatment with 30.00 mg/dm^3 of Cu in the soil obtained a higher number and mass of nodules (Fig. 2A, B), compared to the other treatments and the control. The fresh mass of nodules in the copper treatments was higher than in the control treatment (Fig. 2B), indicating that excess copper did not negatively affect nodulation.

The legume-rhizobia symbiosis improves the growth of alfalfa (*Medicago sativa*) plants exposed to copper, increasing nutrition and plant biomass, as well as increasing the uptake of Cu by plants (Duan et al. 2019), due to the biological fixation of nitrogen, and its effect on the solubility and bioavailability of the metal in soils (Pajuelo et al. 2011). Our *C. cajan* plant biomass and nodulation results are consistent with those of Duan et al. (2019), which reinforces the defense strategies of this species in tolerating Cu concentrations in the soil.

The absence of vegetation increases the impact of raindrops on the soil surface and, consequently, its degradation, as well as the transport of soil particles by surface runoff, enhancing the transfer of Cu to surface waters and its percolation in the soil profile (Shrestha and Lal 2011). The excess of Cu induces toxicity to the root system, in most plants, by negatively

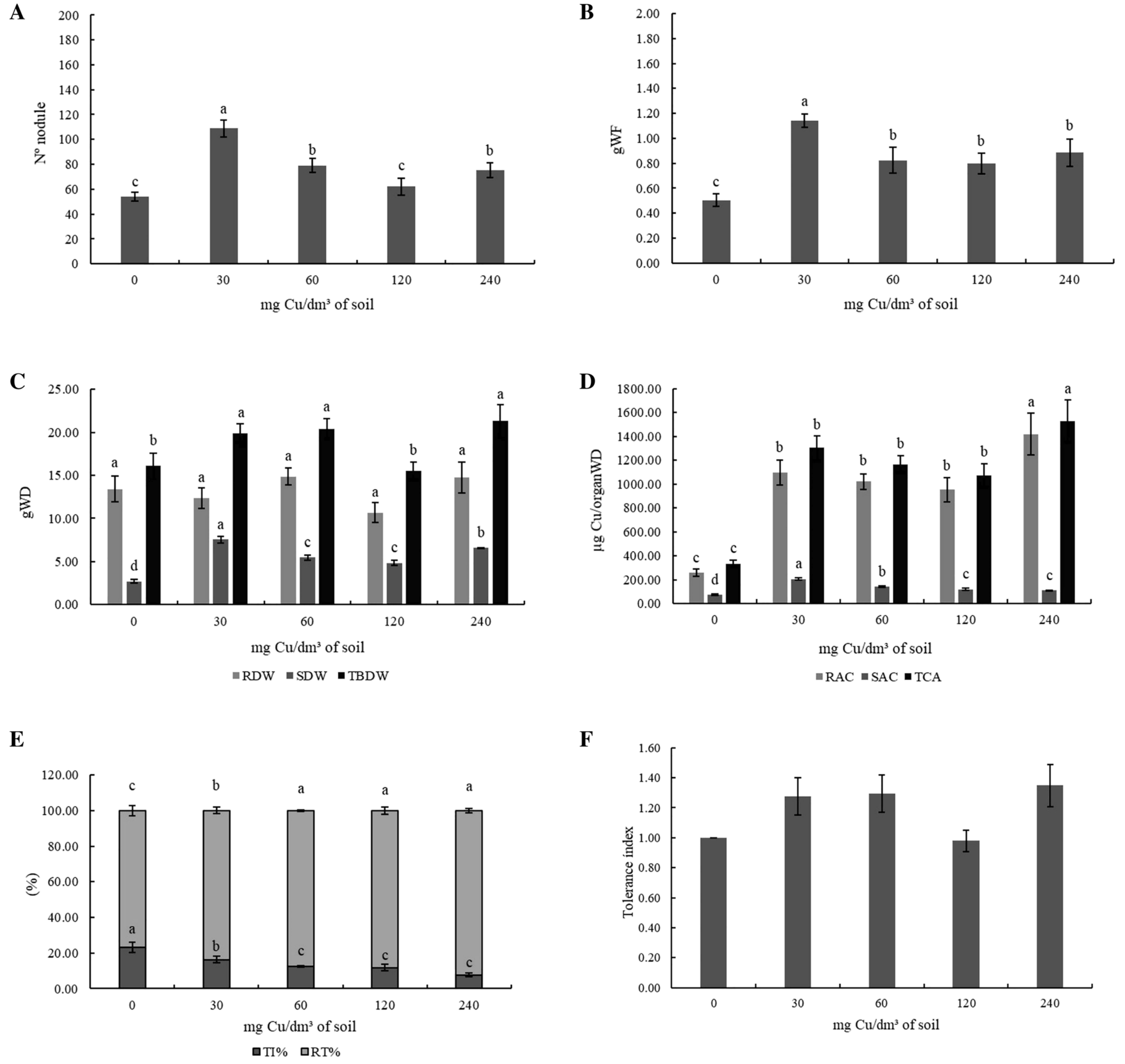

Fig. 2 Spectral parameters of *C. cajan* (at 60 days of cultivation, vegetative stage) cultivated in a Latosols with four copper concentrations: 30.00, 60.00, 120.00 and 240.00 mg Cu/dm^3 of soil, in addition to the control treatment, in a greenhouse. **A**: number of nodules; **B**: fresh mass of nodules (g); **C**: dry shoot biomass (g)—SDW, root—RDW and total plant biomass—TBDW; **D**: copper accumulation (µg/organ) in shoot—SAC, in root—RAC and total copper accumulation—TCA; **E**: copper translocation rates, translocation to shoot—TI% and translocation to root—RT%; **F**: tolerance index—TI. Different letters indicate statistical difference between treatments of the same variable, according to the Scott Knott test, at 5%. Same letters indicate that there was no statistically significant difference ($n=25$)

affecting the assimilation of water and nutrients (Mir et al. 2021), in addition, the reduction in the development of the root system or its absence, results in a decrease in the exploration of the soil by the roots, does not allow the absorption and cycling of Cu in the soil–plant system (Shrestha and Lal 2011).

The development of the root system and nodulation of *C. cajan* was not harmed, therefore, this species can be explored in strategies to improve soil conditions, since *C. cajan* is a nitrogen-fixing legume. According to our results (nodulation, pigments, gas exchange, and biomass), Cu at low concentrations (30.00 to 60.00 mg/dm^3) suggests a beneficial supplementation effect for plants (Moreira and Moraes 2019), whereas higher concentrations can be deleterious to most plants, but the results show that *C. cajan*, responds positively to concentrations of up to 240.00 mg/dm^3 of Cu in the soil.

Cu uptake and accumulation

Accumulation of metals within the tissue is an efficient strategy of most heavy metal resistant plants (Covre et al. 2020), including efflux pumps, sequestration in cells and in intracellular compartments, binding of heavy metals in cells, and production of strong ligands such as phytochelatins (Giannakoula et al. 2021). Under high Cu concentrations, most plants restrict the highest percentage of Cu accumulation in the roots, thus preventing Cu transport/translocation to the shoot as a defense mechanism (Cambrollé et al. 2015; Oustriere et al. 2016; De Conti et al. 2019; Covre et al. 2020).

As soil Cu concentrations increased, the translocation (TI) of Cu to the shoot decreased (Fig. 2E). We can notice that the translocation of the metal to shoot is low, about 12.02%, without considering the control treatment, so most of the Cu, about 87.98%, absorbed by the plants was retained or accumulated in the roots (Fig. 2E). In addition to restricting the translocation of metals to leaves, plants have several protection mechanisms against stress caused by heavy metals. Such mechanisms include compartmentalization and subcellular sequestration, effectively concentrating the metal in the plant's root system (De Conti et al. 2018, 2019).

The increase in Cu in the soil increased the accumulation of Cu in the roots of *C. cajan* (Fig. 2D), *Phragmites australis* (Wu et al. 2021), apples (Wan et al. 2019), as well as observed in other studies (De Conti et al. 2018; Ju et al. 2019). The accumulation of copper in the roots was about 10 times greater than the accumulation of copper in the shoot of *C. cajan* plants, in vegetative stage (Fig. 2D). Considering the treatments from 30.00 to 240.00 mg/dm^3 of Cu in the soil, the greatest accumulation of copper occurred in the roots of plants from the 240.00 mg Cu/dm^3 treatment, as well as in the total biomass, followed by treatments 30.00, 60.00 and 120.00 mg/dm^3 of Cu in the soil (Fig. 2D).

Previous studies suggest that low translocation of Cu to the shoot and high accumulation in the root are the important tolerance mechanisms of plants (Zhou et al. 2017), as in the case of *C. cajan*, whose pigment contents, photosynthetic activity, biomass production, nodulation were not impaired by the excess Cu. In addition to these indicators, the tolerance index of *C. cajan* to Cu was calculated, and as in Fig. 2F, *C. cajan* was highly tolerant of the evaluated Cu doses. In addition to Cu accumulation in the root indicating tolerance, this accumulation may be beneficial to prevent leaching and bioaccumulation of toxic metals in the food chain, including affecting human health (Chen et al. 2018).

Biomass and Cu uptake-correlation

The correlation network plot containing Pearson's correlations between the variables shows us some correlations between chlorophylls, the biomass produced, Cu accumulation, and the indices of phytoremediation potential (Fig. 3). The TI-tolerance of *C. cajan* to Cu is dependent (ratio +) on the total dry biomass-TBD. Chlorophyll contents (Chl *a*, *b* and TChl) are strongly dependent on the translocation-RT% and accumulation-RAC of Cu in the root, since the more Cu accumulates in the root, the less it is translocated to the leaves, thus avoiding damage to the photosynthetic apparatus of plants, similar to that reported by Covre et al (2020). The total accumulation of Cu-TAC is dependent on the translocation-RT% and accumulation-RAC of Cu in the root.

Thus, the more biomass, pigments, Cu translocation, and accumulation in the root, the lower the Cu translocation to the shoot-TI% (inversely proportional ratio), as we

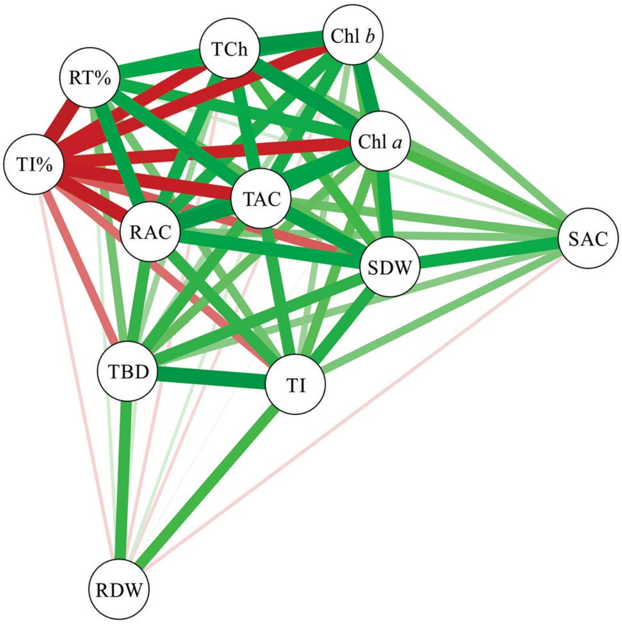

Fig. 3 Correlation networks of *C. cajan* (at 60 days of cultivation, vegetative stage) cultivated in a Latosols with four copper concentrations: 30.00, 60.00, 120.00 and 240.00 mg Cu/dm^3 of soil, in addition to the control treatment, in a greenhouse ($n = 25$). Chlorophylls (mg/g fresh mass): Chl *a*, Chl *b* and total-TChl; (g) dry shoot biomass—SDW, root—RDW and total plant biomass—TBDW; accumulation of copper (µg/organ) in the shoot—SAC, in the root—RAC and the total accumulation of copper—TAC; copper translocation rates, translocation to shoot—TI% and translocation to root—RT%; tolerance index—TI. Green color: positive correlation; red and pink color: negative correlation; thickness and color intensity of the line: intensity of the relationship, the thicker the line and the darker the color (green and dark red), the stronger the relationship between the variables; narrower line and weak color (light green and pink), the relationship between the variables is weak (color figure online)

can see in the strong negative relationships between TI% and RT%, RAC, TAC, Chl *a*, *b* and TChl.

Conclusion

Cajanus cajan L. Millsp, cultivar IAPAR 43-Aratã, is tolerant to concentrations of up to 240.00 mg/dm^3 of Cu in the soil; the nodulation; chlorophyll content; carbon assimilation and fixation, and consequent biomass production (root system and total biomass), were not affected by the doses of copper in the soil; the cultivation time was short, approximately 60 days, therefore, the potential for biomass production and Cu accumulation in its tissues was low, but it does not exclude the potential that *C. cajan* has to remediate areas with excess Cu in the soil. This cultivar can be indicated for the reduction of copper toxicity levels in the soil, based on the biomass production/growth and copper removal potential at the concentrations (from 30.00 to 240.00 mg/dm^3) and cultivation conditions studied. In addition, it can be used to improve soil quality through root development and biological nitrogen fixation. Its phytostabilizer potential must be investigated and explored based on these results.

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Declarations

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

References

Adjei-Nsiah S (2012) Role of pigeon pea cultivation on soil fertility and farming system sustainability in Ghana. Int J Agron. https://doi.org/10.1155/2012/702506

Ali H, Khan E, Sajad MA (2013) Phytoremediation of heavy metals—concepts and applications. Chemosphere 91(7):869–881. https://doi.org/10.1016/j.chemosphere.2013.01.075

Allamin IA, Halmi MIE, Yasid NA, Ahmad SA, Abdullah SRS, Shukor Y (2020) Rhizodegradation of petroleum oily sludge-contaminated soil using *Cajanus cajan* increases the diversity of soil microbial community. Sci Rep. https://doi.org/10.1038/s41598-020-60668-1

Aly AA, Mohamed AA (2012) The impact of copper ion on growth, thiol compounds and lipid peroxidation in two maize cultivars ('*Zea mays*' L.) grown "in vitro." South Cross J. https://doi.org/10.3316/informit.361413352729057

Ambrosini VG, Rosa DJ, de Melo GWB, Zalamena J, Cella C, Simão DG et al (2018) High copper content in vineyard soils promotes modifications in photosynthetic parameters and morphological changes in the root system of 'Red Niagara' plantlets. Plant Physiol Biochem 128:89–98

Bomfim NCP, Aguilar JV, de Paiva WdS, de Souza LA, Justino GC, Faria GA et al (2021) Iron phytostabilization by *Leucaena leucocephala*. S Afr J Bot. https://doi.org/10.1016/j.sajb.2021.01.013

Byatarayappa G, Guna V, Venkatesh K, Reddy N, Nagaraju N, Nagaraju K (2021) Superior cycle stability performance of a symmetric coin cell fabricated using KOH activated bio-char derived from agricultural waste–*Cajanus cajan* stems. J Environ Chem Eng 9(6):106525. https://doi.org/10.1016/j.jece.2021.106525

Cambrollé J, García JL, Figueroa ME, Cantos M (2015) Evaluating wild grapevine tolerance to copper toxicity. Chemosphere. https://doi.org/10.1016/j.chemosphere.2014.06.044

Chandrasekhar C, Ray JG (2017) Copper accumulation, localization and antioxidant response in *Eclipta alba* L. in relation to quantitative variation of the metal in soil. Acta Physiol Plant 39(9):205. https://doi.org/10.1007/s11738-017-2508-4

Chen Z, Liu X, Ai Y, Chen J, Luo X, Chen J et al (2018) Effects and mechanisms of revegetation modes on cadmium and lead pollution in artificial soil on railway rock-cut slopes. Sci Total Environ. https://doi.org/10.1016/j.scitotenv.2018.06.380

Choudhary AK, Singh D (2011) Screening of pigeon pea genotypes for nutrient uptake efficiency under aluminium toxicity. Physiol Mol Biol Plants 17(2):145–152. https://doi.org/10.1007/s12298-011-0057-7

Covre WP, Pereira WVdS, Gonçalves DAM, Teixeira OMM, Amarante CBD, Fernandes AR (2020) Phytoremediation potential of *Khaya ivorensis* and *Cedrela fissilis* in copper contaminated soil. J Environ Manag. https://doi.org/10.1016/j.jenvman.2020.110733

Dai H, Xu Y, Zhao L, Shan C (2016) Alleviation of copper toxicity on chloroplast antioxidant capacity and photosystem II photochemistry of wheat by hydrogen sulfide. Braz J Bot 39(2):787–793. https://doi.org/10.1007/s40415-016-0250-6

Dao TT, Tran TTT, Nguyen AM, Nguyen LN, Pham PTM, Tsubota T et al (2021) Fungicide application can intensify clay aggregation and exacerbate copper accumulation in citrus soils. Environ Pollut. https://doi.org/10.1016/j.envpol.2021.117703

De Conti L, Ceretta CA, Tiecher TL, da Silva LOS, Tassinari A, Somavilla LM et al (2018) Growth and chemical changes in the rhizosphere of black oat (*Avena strigosa*) grown in soils contaminated with copper. Ecotoxicol Environ Saf. https://doi.org/10.1016/j.ecoenv.2018.07.045

De Conti L, Ceretta CA, Melo GWB, Tiecher TL, Silva LOS, Garlet LP et al (2019) Intercropping of young grapevines with native grasses for phytoremediation of Cu-contaminated soils. Chemosphere. https://doi.org/10.1016/j.chemosphere.2018.10.134

Ding S, Ma C, Shi W, Liu W, Lu Y, Liu Q et al (2017) Exogenous glutathione enhances cadmium accumulation and alleviates its toxicity in *Populus canescens*. Tree Physiol 37(12):1697–1712

Duan C, Razavi BS, Shen G, Cui Y, Ju W, Li S et al (2019) Deciphering the rhizobium inoculation effect on spatial distribution of phosphatase activity in the rhizosphere of alfalfa under copper stress. Soil Biol Biochem. https://doi.org/10.1016/j.soilbio.2019.107574

Farid M, Farooq MA, Fatima A, Abubakar M, Ali S, Raza N et al (2021) Copper-induced responses in different plant species. In: Hasanuzzaman M (ed) Approaches to the remediation of inorganic pollutants. Springer, Singapore, pp 259–280

Fernandes AR, Souza ESd, de Souza Braz AM, Birani SM, Alleoni LRF (2018) Quality reference values and background concentrations of potentially toxic elements in soils from the Eastern Amazon. Braz J Geochem Explor. https://doi.org/10.1016/j.gexplo.2018.04.012

Garg N, Aggarwal N (2011) Effects of interactions between cadmium and lead on growth, nitrogen fixation, phytochelatin, and glutathione production in mycorrhizal *Cajanus cajan* (L.) Millsp. J Plant Growth Regul 30(3):286–300. https://doi.org/10.1007/s00344-010-9191-7

Garg N, Aggarwal N (2012) Effect of mycorrhizal inoculations on heavy metal uptake and stress alleviation of *Cajanus cajan* (L.) Millsp. genotypes grown in cadmium and lead contaminated soils. Plant Growth Regul 66(1):9–26. https://doi.org/10.1007/s10725-011-9624-8

Garg N, Singh S (2018) Arbuscular mycorrhiza Rhizophagus irregularis and silicon modulate growth, proline biosynthesis and yield in *Cajanus cajan* L. Millsp. (pigeon pea) genotypes under cadmium and zinc stress. J Plant Growth Regul 37(1):46–63

Gautam M, Agrawal M (2019) Identification of metal tolerant plant species for sustainable phytomanagement of abandoned red mud dumps. Appl Geochem. https://doi.org/10.1016/j.apgeochem.2019.03.020

Giannakoula A, Therios I, Chatzissavvidis C (2021) Effect of lead and copper on photosynthetic apparatus in citrus (*Citrus aurantium* L.) plants. The role of antioxidants in oxidative damage as a response to heavy metal stress. Plants. https://doi.org/10.3390/plants10010155

Girotto E, Ceretta CA, Brunetto G, Santos DRd, Silva LSd, Lourenzi CR et al (2010) Copper and zinc forms and accumulation in soil after successive pig slurry applications. Rev Bras De Ciênc Do Solo 34:955–965. https://doi.org/10.1590/S0100-06832010000300037

Gomes DG, Lopes-Oliveira PJ, Debiasi TV, da Cunha LS, Oliveira HC (2021) Regression models to stratify the copper toxicity responses and tolerance mechanisms of *Glycine max* (L.) Merr. plants. Planta 253(2):43. https://doi.org/10.1007/s00425-021-03573-9

Gong Q, Li Z-h, Wang L, Zhou J-y, Kang Q, Niu D-d (2021) Gibberellic acid application on biomass, oxidative stress response, and photosynthesis in spinach (*Spinacia oleracea* L.) seedlings under copper stress. Environ Sci Pollut Res 28(38):53594–53604. https://doi.org/10.1007/s11356-021-13745-5

Hiscox J, Israelstam G (1979) A method for the extraction of chlorophyll from leaf tissue without maceration. Can J Bot 57(12):1332–1334

Hossain MS, Abdelrahman M, Tran CD, Nguyen KH, Chu HD, Watanabe Y et al (2020) Insights into acetate-mediated copper homeostasis and antioxidant defense in lentil under excessive copper stress. Environ Pollut 258:113544

Huygens D, Orveillon G, Lugato E, Tavazzi S, Comero S, Jones A, et al. (2020) Technical proposals for the safe use of processed manure above the threshold established for nitrate vulnerable zones by the nitrates directive (91/676/EEC). Technical proposals for the safe use of processed manure above the threshold established for nitrate vulnerable zones by the nitrates directive (91/676/EEC), https://data.europa.eu/doi/https://doi.org/10.2760/984729

Jerez Ch JA, Romero RM (2016) Evaluation of *Cajanus cajan* (pigeon pea) for phytoremediation of landfill leachate containing chromium and lead. Int J Phytorem 18(11):1122–1127. https://doi.org/10.1080/15226514.2016.1186592

Ju W, Liu L, Fang L, Cui Y, Duan C, Wu H (2019) Impact of co-inoculation with plant-growth-promoting rhizobacteria and rhizobium on the biochemical responses of alfalfa-soil system in copper contaminated soil. Ecotoxicol Environ Saf. https://doi.org/10.1016/j.ecoenv.2018.10.016

Kabata-Pendias A (2011) Elements of group 10 (previously part of group VIII). Trace elements in soils and plants, 4th edn. CRC Press, New York, pp 237–251

Kabata-Pendias A, Pendias H (2001) Trace elements in soil and plants, 3rd edn. CRC Press, Boca Raton

Kaushik S, Sharma P, Kaur G, Singh AK, Al-Misned FA, Shafik HM et al (2022) Seed priming with methyl jasmonate mitigates copper and cadmium toxicity by modifying biochemical attributes and antioxidants in *Cajanus cajan*. Saudi J Biol Sci 29(2):721–729. https://doi.org/10.1016/j.sjbs.2021.12.014

Malavolta E (1997) Avaliação do estado nutricional das plantas: princípios e aplicações/Eurípedes Malavolta, Godofredo Cesar Vitti, Sebastião Alberto de Oliveira. 2. ed., ver. e atual. Piracicaba: Potafos

Marques DM, Veroneze Júnior V, da Silva AB, Mantovani JR, Magalhães PC, de Souza TC (2018) Copper toxicity on photosynthetic responses and root morphology of *Hymenaea courbaril* L. (Caesalpinioideae). Water Air Soil Pollut. https://doi.org/10.1007/s11270-018-3769-2

Marschner H (2011) Marschner's mineral nutrition of higher plants. Academic press, Cambridge

Meier S, Curaqueo G, Khan N, Bolan N, Rilling J, Vidal C et al (2017) Effects of biochar on copper immobilization and soil microbial communities in a metal-contaminated soil. J Soils Sediments 17(5):1237–1250

Minari GD, Saran LM, Lima Constancio MT, Correia da Silva R, Rosalen DL, José de Melo W et al (2020) Bioremediation potential of new cadmium, chromium, and nickel-resistant bacteria isolated from tropical agricultural soil. Ecotoxicol Environ Saf. https://doi.org/10.1016/j.ecoenv.2020.111038

Mir AR, Pichtel J, Hayat S (2021) Copper: uptake, toxicity and tolerance in plants and management of Cu-contaminated soil. Biometals 34(4):737–759. https://doi.org/10.1007/s10534-021-00306-z

Mohajel Kazemi E, Kolahi M, Yazdi M, Goldson-Barnaby A (2020) Anatomic features, tolerance index, secondary metabolites and protein content of chickpea (*Cicer arietinum*) seedlings under cadmium induction and identification of PCS and FC genes. Physiol Mol Biol Plants 26(8):1551–1568. https://doi.org/10.1007/s12298-020-00804-3

Moreira A, Moraes LAC, Schroth G (2019) Copper fertilization in soybean–wheat intercropping under no–till management. Soil Tillage Res. https://doi.org/10.1016/j.still.2019.06.001

Närhi P, Räisänen ML, Sutinen M-L, Sutinen R (2012) Effect of tailings on wetland vegetation in Rautuvaara, a former iron–copper mining area in northern Finland. J Geochem Explor 116–117:60–65. https://doi.org/10.1016/j.gexplo.2012.03.005

Nworie OE, Lin C (2021) Seasonal variation in tissue-borne heavy Metal(loid)s in herbaceous plants growing in contaminated soils developed from industrial wastes of industrial revolution age. Environ Adv. https://doi.org/10.1016/j.envadv.2021.100113

Oustriere N, Marchand L, Galland W, Gabbon L, Lottier N, Motelica M et al (2016) Influence of biochars, compost and iron grit, alone and in combination, on copper solubility and phytotoxicity in a Cu-contaminated soil from a wood preservation site. Sci Total Environ 566–567:816–825. https://doi.org/10.1016/j.scitotenv.2016.05.091

Pajuelo E, Rodríguez-Llorente ID, Lafuente A, Caviedes MÁ (2011) Legume–rhizobium symbioses as a tool for bioremediation of heavy metal polluted soils. Biomanag Metal Contam Soils Springer. https://doi.org/10.1007/978-94-007-1914-9_4

Panel EF (2016) Scientific opinion on the revision of the currently authorised maximum copper content in complete feed. EFSA J 14(8):4563

Peng H, Chen Y, Weng L, Ma J, Ma Y, Li Y et al (2019) Comparisons of heavy metal input inventory in agricultural soils in North and South China: a review. Sci Total Environ 660:776–786

Pérez Chaca MV, Vigliocco A, Reinoso H, Molina A, Abdala G, Zirulnik F et al (2014) Effects of cadmium stress on growth, anatomy and hormone contents in *Glycine max* (L.) Merr. Acta Physiol Plant 36(10):2815–2826. https://doi.org/10.1007/s11738-014-1656-z

Perlatti F, Ferreira TO, Romero RE, Costa MCG, Otero XL (2015) Copper accumulation and changes in soil physical–chemical properties promoted by native plants in an abandoned mine site in northeastern Brazil: implications for restoration of mine sites. Ecol Eng. https://doi.org/10.1016/j.ecoleng.2015.04.085

Perlatti F, Martins EP, de Oliveira DP, Ruiz F, Asensio V, Rezende CF et al (2021) Copper release from waste rocks in an abandoned mine (NE, Brazil) and its impacts on ecosystem environmental quality. Chemosphere. https://doi.org/10.1016/j.chemosphere.2020.127843

R CORE TEAM (2019). R: A language and environment for statistical computing. R Foundation for statistical computing, Vienna, Austria. https://www.R-project.org/

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(5):e62941. https://doi.org/10.1371/journal.pone.0062941

Reckova S, Tuma J, Dobrev P, Vankova R (2019) Influence of copper on hormone content and selected morphological, physiological and biochemical parameters of hydroponically grown *Zea mays* plants. Plant Growth Regul 89(2):191–201

Rehman M, Maqbool Z, Peng D, Liu L (2019) Morpho-physiological traits, antioxidant capacity and phytoextraction of copper by ramie (*Boehmeria nivea* L.) grown as fodder in copper-contaminated soil. Environ Sci Pollut Res 26(6):5851–5861. https://doi.org/10.1007/s11356-018-4015-6

Rosa Couto R, Benedet L, Comin JJ, Filho PB, Martins SR, Gatiboni LC et al (2015) Accumulation of copper and zinc fractions in vineyard soil in the mid-western region of Santa Catarina. Brazil Environ Earth Sci 73(10):6379–6386. https://doi.org/10.1007/s12665-014-3861-x

Saleem MH, Fahad S, Khan SU, Ahmar S, Ullah Khan MH, Rehman M et al (2020) Morpho-physiological traits, gaseous exchange attributes, and phytoremediation potential of jute (*Corchorus capsularis* L.) grown in different concentrations of copper-contaminated soil. Ecotoxicol Environ Saf 189:109915. https://doi.org/10.1016/j.ecoenv.2019.109915

Scott AJ, Knott M (1974) A cluster analysis method for grouping means in the analysis of variance. Biometrics 30(3):507–512. https://doi.org/10.2307/2529204

Shabbir Z, Sardar A, Shabbir A, Abbas G, Shamshad S, Khalid S et al (2020) Copper uptake, essentiality, toxicity, detoxification and risk assessment in soil-plant environment. Chemosphere. https://doi.org/10.1016/j.chemosphere.2020.127436

Shah V, Daverey A (2020) Phytoremediation: A multidisciplinary approach to clean up heavy metal contaminated soil. Environ Technol Innov. https://doi.org/10.1016/j.eti.2020.100774

Shrestha RK, Lal R (2011) Changes in physical and chemical properties of soil after surface mining and reclamation. Geoderma 161(3):168–176. https://doi.org/10.1016/j.geoderma.2010.12.015

Sipos G, Solti Á, Czech V, Vashegyi I, Tóth B, Cseh E et al (2013) Heavy metal accumulation and tolerance of energy grass (*Elymus elongatus* subsp. *ponticus* cv. Szarvasi-1) grown in hydroponic culture. Plant Physiol Biochem 68:96–103. https://doi.org/10.1016/j.plaphy.2013.04.006

Souza SCR, Souza LA, Schiavinato MA, de Oliveira Silva FM, de Andrade SAL (2020) Zinc toxicity in seedlings of three trees from the Fabaceae associated with arbuscular mycorrhizal fungi. Ecotoxicol Environ Saf. https://doi.org/10.1016/j.ecoenv.2020.110450

Souza L, Camargos L, Carvalho M (2018) Toxic metal phytoremediation using high biomass non-hyperaccumulator crops: new possibilities for bioenergy resources 1a ed. Nova Publisher

Srivastava R, Khan R, Manzoor N, Uzzafar M (2011) Responses of cadmium exposures on growth, physio-biochemical characteristics and the antioxidative defence system of soybean (*Glycine max* L.). J Phytol 3:20–25

Tortosa G, Pacheco PJ, Hidalgo-García A, Granados A, Delgado A, Mesa S et al (2020) Copper modulates nitrous oxide emissions from soybean root nodules. Environ Exp Bot. https://doi.org/10.1016/j.envexpbot.2020.104262

Tóth SZ, Nagy V, Puthur JT, Kovács L, Garab G (2011) The physiological role of ascorbate as photosystem ii electron donor: protection against photoinactivation in heat-stressed leaves. Plant Physiol 156(1):382–392. https://doi.org/10.1104/pp.110.171918

Trentin E, Cesco S, Pii Y, Valentinuzzi F, Celletti S, Feil SB et al (2022) Plant species and pH dependent responses to copper toxicity. Environ Exp Bot. https://doi.org/10.1016/j.envexpbot.2022.104791

van Raij B, Andrande JC, Cantarella H, Guaggio JA (2001) Análise química para avaliação da fertilidade de solos tropicais, 1st edn. Campinas, Instituto Agronômico, p 285

Vanaja M, Maheswari M, Sathish P, Vagheera P, Jyothi Lakshmi N, Vijay Kumar G et al (2015) Genotypic variability in physiological, biomass and yield response to drought stress in pigeon pea. Physiol Mol Biol Plants 21(4):541–549. https://doi.org/10.1007/s12298-015-0324-0

Vardhan KH, Kumar PS, Panda RC (2019) A review on heavy metal pollution, toxicity and remedial measures: current trends and future perspectives. J Mol Liq. https://doi.org/10.1016/j.molliq.2019.111197

Vinit-Dunand F, Epron D, Alaoui-Sossé B, Badot P-M (2002) Effects of copper on growth and on photosynthesis of mature and expanding leaves in cucumber plants. Plant Sci 163(1):53–58. https://doi.org/10.1016/S0168-9452(02)00060-2

Wan H, Du J, He J, Lyu D, Li H (2019) Copper accumulation, subcellular partitioning and physiological and molecular responses in relation to different copper tolerance in apple rootstocks. Tree Physiol 39(7):1215–1234

Wang X, Fernandes de Souza M, Mench MJ, Li H, Ok YS, Tack FMG et al (2022) Cu phytoextraction and biomass utilization as essential trace element feed supplements for livestock. Environ Pollut. https://doi.org/10.1016/j.envpol.2021.118627

Wu J, Hu J, Wang L, Zhao L, Ma F (2021) Responses of *Phragmites australis* to copper stress: a combined analysis of plant morphology, physiology and proteomics. Plant Biol 23(2):351–362. https://doi.org/10.1111/plb.13175

Yadu B, Chandrakar V, Meena RK, Keshavkant S (2017) Glycine betaine reduces oxidative injury and enhances fluoride stress tolerance via improving antioxidant enzymes, proline and genomic template stability in *Cajanus cajan* L. S Afr J Bot. https://doi.org/10.1016/j.sajb.2017.03.023

Yang J, Holbach A, Wilhelms A, Krieg J, Qin Y, Zheng B et al (2020) Identifying spatio-temporal dynamics of trace metals in shallow eutrophic lakes on the basis of a case study in Lake Taihu. China Environ Pollut. https://doi.org/10.1016/j.envpol.2020.114802

Yruela I (2013) Transition metals in plant photosynthesis. Metallomics 5(9):1090–1109. https://doi.org/10.1039/c3mt00086a

Zeng Q, Ling Q, Wu J, Yang Z, Liu R, Qi Y (2019) Excess copper-induced changes in antioxidative enzyme activity, mineral nutrient uptake and translocation in sugarcane seedlings. Bull Environ Contam Toxicol 103(6):834–840. https://doi.org/10.1007/s00128-019-02735-6

Zhang X, Xia H, Li Z, Zhuang P, Gao B (2010) Potential of four forage grasses in remediation of Cd and Zn contaminated soils. Bioresour Technol 101(6):2063–2066. https://doi.org/10.1016/j.biortech.2009.11.065

Zhou C, Huang M, Ren H, Yu J, Wu J, Ma X (2017) Bioaccumulation and detoxification mechanisms for lead uptake identified in *Rhus chinensis* Mill. seedlings. Ecotoxicol Environ Safety 142:59–68. https://doi.org/10.1016/j.ecoenv.2017.03.052