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Embryo growth alteration and oxidative stress responses in germinating Cucurbita pepo seeds exposed to cadmium and copper toxicity

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Abstract

To assess the impact of heavy metals (cadmium, copper) on the germination, metabolism and growth of zucchini seedlings (*Cucurbita pepo* L.), a test was conducted 'in vitro' on the germination and growth of zucchini seedlings, subjected to two concentrations (100 and 200 µM) of $CdCl_2$ and $CuCl_2$. The germination parameters, as well as the biochemical and phytochemical properties of the embryonic axes of the seedlings, were studied. The obtained results showed that the seed vigor index (SVI) of zucchini seeds recorded a significant and remarkable decrease under the effect of Cd. A significant decrease was also noted in the length and the dry weight of the embryonic axes of the young seedlings, which differed according to the type of metal. No significant increases in malondialdehyde (MDA) and H_2O_2 content, or in catalase (CAT) activity were noted at the tested concentrations of the Cd and Cu salt, but very significant decreases in superoxide dismutase (SOD) activity of embryonic axis tissues were observed. Glutathione S-transferase (GST) activity exhibited a significant increase at 200 µM $CdCl_2$ whereas the glutathione (GSH) content decreased with increasing metal concentrations (Cd, Cu) of the medium. An increase in total phenol content and antioxidant activity was recorded at 200 µM of $CuCl_2$. Based on the obtained results, we can conclude that heavy metals (Cd, Cu) reduce the germination efficiency of zucchini seeds and cause metabolic alterations of embryonic tissue cells, and the response of zucchini seedlings to metal stress depends on the type of metal and its concentration.

Introduction

For several decades, the increase in human activities (industrial waste, urban transport, excessive use of fertilizers in the agricultural sector, etc.) have been the cause of environmental pollution by heavy metals (Appenroth 2010). The accumulation of heavy metals in ecosystems has led, over the years, to the deterioration of the environment quality, the decline of forests and the reduction of agricultural productivity (Wahid 2006).

In plants, if metals are often essential for the development of biological processes (Oligo-elements), many of them can prove to be contaminants for various forms of life when their concentration exceeds a certain threshold. Indeed, several studies have confirmed that metal ions alter the biological functions of plants (Hasan et al. 2017; Sharma and Dietz 2009; Sharma and Agrawal 2005). Metal ions such as Cd^{2+} and Cu^{2+} have toxic effects on seed germination by altering the structure of certain proteins, inhibiting their enzymatic activity (Yruela 2009), inducing oxidative stress (ROS), and disrupting the processes of cellular oxidation-reduction metabolism of seedling tissues (Shahid et al. 2014; Sharma and Dietz 2009).

Faced with these stressful situations, plants mobilize antioxidant defense systems, of an enzymatic nature (catalase, superoxide dismutase, peroxidase, glutathione reductase, glutathione S-transferase, glutathione peroxidase, etc.), or non-enzymatic (vitamins C and E, glutathione, phenols, etc…) involving different compounds that have an affinity for free radicals (Sytar et al. 2013; Wei et al. 2020).

Germination and early seedling growth are the most sensitive physiological stages of a plant, and they are regulated by several hormonal interactions, as well as environmental factors (Miransari and Smith 2014). In addition, these stages are more sensitive to metal pollution (Liu et al. 2012). The effect of heavy metals on crop plants differs from one species to another, and *C pepo* species has been used as a biological model by several authors (Asadi Aghbolaghi et al. 2022; Stratu et al. 2015; Valivand et al. 2019), to test the toxicity of metallic elements in the early stages of plant development.

Zucchini (*Cucurbita pepo* L.) belongs to the Cucurbitaceae family; it is grown all over the world for food supply (Paris 2016). Its economic and medical importance is increasingly apparent for its nutrients and richness in bioactive compounds, including phenols, flavonoids, vitamins, amino acids, carbohydrates, and minerals (Ayyildiz et al. 2019; Kopczyńska et al. 2021). In addition, it is characterized by high protein content (Badu et al. 2020).

The objective of this work is to study the effect of cadmium (Cd) and copper (Cu) on the germination and growth of zucchini seedlings (*Cucurbita pepo* L.) and to evaluate some biochemical and phytochemical responses of the embryonic axes under the effect of metallic stress (Cd, Cu) in the medium.

Material And Methods

Seed preparation

In the laboratory, zucchini seeds (*Cucurbita pepo* L.), Quarantaine variety, were thoroughly washed with tap water to eliminate the residues of the active ingredient (thirame) used for the treatment of the seed, then they were disinfected by soaking in sodium hypochlorite (NaClO 12°) diluted to 10%, for 10 min, then rinsed abundantly with distilled water in order to eliminate traces of NaClO. The initial state (ungerminated seeds) was obtained by swelling the seeds in distilled water for 30 min at 5°C (Murray et al. 1979).

Germination test and metallic treatments

After 30 minutes of soaking in distilled water, zucchini seeds, taken at random, were germinated in plastic dishes (15 cm in diameter x 2 cm high), sterilized and lined with filter paper, at a rate of 50 seeds/dishes. The used metals were cadmium and copper in the form of salts: cadmium chloride ($CdCl_2$) and copper chloride ($CuCl_2$). Zucchini seeds were moistened in distilled water (for the control) or treated with metallic solutions at two concentrations, 100 and 200 µM (15 to 20 ml/dishes/day), and 3 repetitions were carried out for each treatment.

The germination test was performed in an incubator, under average temperature conditions of 27 ± 1°C and in total darkness for 8 days.

Studied parameters and performed measurements

Germination criteria

- The average germination rate (GP) is expressed as the maximum percentage of germinated seeds after 8 days out of the total number of sown seeds (Kandil et al. 2012). A seed is considered to have germinated when its radicle is at least 2 mm in length (Krishnasamy and Seshu 1990).

- The seed vigor index (SVI,) is determined according to the formula described by Abdul-Baki and Anderson (1973): SVI = (GP x EAL) / 100, where, EAL: total length of the embryonic axes (radicle + hypocotyl), and GP: Average germination rate.

Lengths and dry weights of embryonic axes of *C. pepo* seedlings

For eight (8) day old zucchini seedlings, the length of the embryonic axes EAL (cm) was measured for 20 samples of each treatment, using a millimeter paper. The dry weight of the embryonic axes EAW (mg) was determined, after drying the samples at 70°C for 48 hours, using an analytical balance.

Heavy metal phytotoxicity rate on the growth of the embryonic axes was calculated according to the formulas below described by Wierzbicka et al. (2015):

$$Phytotoxicity(\%) = ((EAL_{control} - EAL_{treatment}) / EAL_{control}) \times 100$$

$$Phytotoxicity(\%) = ((EAW_{control} - EAW_{treatment}) / EAW_{control}) \times 100$$

Biochemical parameters of the embryonic axes of *C pepo* seedlings

Preparation of tissue homogenate

The enzymatic extract of the embryonic axes' tissues (hypocotyl + radicle) of young zucchini seedlings was obtained by the method of Loggini et al. (1999). Briefly, fresh samples (1 g) were ground in a mortar, with 5 ml of phosphate buffer, after centrifugation at 4°C for 15 min at 12.000 g, the obtained supernatant was used as an extract for the determination of the various enzymatic activities.

Biochemical parameters

The biochemical assays were evaluated by measuring the variation of oxidative stress markers. The determination of hydrogen peroxide (H_2O_2) was carried out according to the method of (Velikova et al. 2000); malondialdehyde (MDA) was assayed according to the method of Draper and Hadley (1990) based on the colorimetric measurement of the reaction between thiobarbituric acid (TBA) and MDA. The catalase activity (CAT) was assessed according to the method of Regoli and Principato (1995); the superoxide dismutase (SOD) activity was assayed according to the method of (Beauchamp and Fridovich 1971) by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT). The glutathione S-transferase (GST) activity was determined according to the method of Habig et al. (1974), based on the conjugation reaction between GST and a substrate, CDNB (1-chloro 2, 4 dinitrobenzene) in the presence of a cofactor, glutathione (GSH). The reduced glutathione (GSH) content was quantified according to the method of Weckbecker and Cory (1988).

Phytochemical analyzes of the embryonic axes of *C. pepo* seedlings

Extraction of the embryonic axis tissues

Embryonic tissue samples of young zucchini seedlings (1 g of dry matter) were weighed and 20 ml of concentrated methanol were added in hermetic flasks. After agitation, the mixtures were placed in the dark at room temperature for 48 hours and then paper-filtered under a vacuum suction system. The obtained filtrate was kept at ambient temperature and protected from light for 48 hours. The obtained dry residues were then stored in the refrigerator at 4°C until measurement.

Total polyphenol content (TPC) assay

Determination of total polyphenol content was carried out according to the Folin-Ciocalteu method in an alkaline medium (Scalbert et al. 1989). Gallic acid was used as the standard, with results expressed in micrograms of gallic acid equivalent per mg of dry matter (µg GAE/mg DM).

Total flavonoid content (TFC) assay

The total flavonoids were assayed by the colorimetric method of Dewanto et al. (2002), and the content is expressed in µg of quercetin equivalent per mg of dry matter (mg QE/mg DM).

Antioxidant activity assay (DPPH test)

The antioxidant effect of extracts on the DPPH radical was measured by the colorimetric method (Blois 1958). Absorbance was measured at 517 nm using a UV-visible spectrophotometer. The radical scavenging percentage I(%) was calculated according to the following equation: $I\ (\%) = [(A0 - Ai) / A0] \times 100$, where A0: absorbance of the control (DPPH solution without extract), and Ai: absorbance in the presence of extract.

Statistical analysis

Results are expressed as the mean ± standard error (SE), and data comparisons were made by Fisher's test (LSD), for $p < 0.05$, using Minitab data analysis software (version 16.0).

Results

The germination rate of zucchini seeds (C. pepo L.)

The obtained results after 8 days of germination (Fig. 01A) showed a non-significant decrease ($p = 0.313$) in the average germination rates of zucchini seeds under the influence of the applied concentrations of the metal solutions ($CuCl_2$ and $CdCl_2$). The highest germination rate (93.33%) was recorded in control seeds, while the lowest germination rate (86.67%) was noted at the concentration of 200 µM of the cadmium salt ($CuCl_2$), i.e., 7.14%, less than the control seeds. While the lowest decrease rate (1.43%) was

recorded at the concentration of 100 µM of copper chloride ($CuCl_2$) for which a germination rate of 92% was obtained.

Seed Vigor Index (SVI)

The obtained results (Fig. 01B) show a significant decrease (P = 0.001) in the seed vigor index (SVI) under the effect of the used concentrations of the cadmium and copper salts ($CdCl_2$ and $CuCl_2$). The highest SVI (1092.47) was recorded in the control seeds, while the minimum value of this index (382.6) was noted under the effect of $CdCl_2$ at the concentration of 200 µM, with a decrease of the order of 64.98%. The lowest reduction rate (37.68%) was noted at the concentration of 100 µM $CuCl_2$ where the SVI value was of the order of 680.8.

Length (EAL) and dry biomass (EAW) of embryonic axes of zucchini seedlings (C. pepo **L.)**

The results (Table 1) show a very highly significant decrease (p = 0.000) in the average length and average dry weight of the embryonic axes of zucchini seedlings under the influence of metallic stress in the medium ($CdCl_2$ and $CuCl_2$).

Table 1
Effects of heavy metals (Cu, Cd) on total length (EAL), dry weight (EAW), phytotoxicity rate (%) of the embryonic axes of zucchini seedlings (*Cucurbita pepo* L.), after 8 days of germination. dH_2O : distilled water.

Metal Solution	Treatment	EAL (cm)	*Phytotoxicity* (%)	EAW (mg)	*Phytotoxicity* (%)
Control	dH_2O	11.71 ± 0.31^{a}	-	22.79 ± 1.05^{a}	-
$CdCl_2$	100 µM	6.90 ± 0.33^{bc}	43.98 ± 3.77^{bc}	17.32 ± 0.53^{b}	26.47 ± 3.64^{b}
	200 µM	4.42 ± 0.27^{d}	63.02 ± 2.61^{a}	16.32 ± 0.62^{bc}	31.36 ± 4.09^{ab}
$CuCl_2$	100 µM	7.40 ± 0.40^{b}	38.93 ± 4.25^{c}	15.63 ± 0.67^{bc}	33.72 ± 3.80^{ab}
	200 µM	6.07 ± 0.21^{c}	49.63 ± 2.54^{b}	14.20 ± 1.02^{c}	40.29 ± 5.10^{a}
P. value, and significance level		0.000***	0.000***	0.000***	0.141^{NS}

Data are presented as the means ± SE (n = 20). Values in the same column not sharing the same letter are significantly different at $p \leq 0.05$ by Fisher's LSD test. ***(Extremely significant), ^{NS}(not significant)

The highest embryonic axis length (EAL) (11.71 cm) was noted in the control (untreated seedlings) while the lowest value (4.42 cm) was recorded at the concentration of 200 µM of $CdCl_2$ with a maximum phytotoxicity of 63.02% (Table 1). A minimal toxicity of 38.93% was recorded at 100 µM of $CuCl_2$, with a length (EAL) of 7.4 cm.

The highest embryonic axis dry weight (EAW) (22.79 mg) was recorded in zucchini seedlings treated with distilled water (control) whereas the lowest value (14.2 mg) was noted at 200 µM of $CuCl_2$, with maximum phytotoxicity of 40.29% (Table 1). The lowest toxic effect (26.47%) was noted at 100 µM $CdCl_2$ with an EAW of 17.32 mg.

Biochemical parameters of the embryonic axes of C. pepo **seedlings**

Hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) content

The results (Fig. 2A, B) show that for the embryonic axes tissues of young zucchini seedlings, the applied concentrations of the metal solutions ($CdCl_2$ and $CuCl_2$) cause a non-significant increase (P = 0.144 and P = 0.107) in the hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) content, respectively, compared to control seedlings. The highest H_2O_2 content (0.0037 µM/g FM) and the highest MDA content (8.83 nM/g FM) were noted at 200 µM of $CuCl_2$.

Enzymatic and non-enzymatic antioxidants content

The obtained results (Fig. 2C) show that the measurement of the enzymatic activity of catalase (CAT) of the embryonic axes tissues of the zucchini seedlings (*C. pepo* L.) revealed a non-significant decrease (p = 0.147) under the effect of metallic stress. The highest value of CAT activity (137.4 IU/g protein) was observed at the concentration of 100 µM $CuCl_2$, while the lowest value (68.39 IU/g protein) was recorded at 100 µM of $CdCl_2$, against 175.3 IU/g proteins in the control.

On the other hand, the measured superoxide dismutase (SOD) activity for the embryonic tissues of zucchini seedlings, subjected to metallic stresses (Fig. 2D) exhibited a highly significant decrease (p = 0.002) compared to the control seedlings and the lowest value (192.7 IU/g protein) was noted at 200 µM of CdCl2. On the other hand, the glutathione S-transferase (GST) activity of the embryonic tissues (Fig. 2E) showed a non-significant increase (p = 0.209) under the effect of metal stresses, compared to the control. The highest value of GST activity (1.008 nM/min/mg protein) was recorded at 200 µM of CdCl2, while the lowest GST activity (0.404 nM/min/mg protein) was noted at 100 µM of CuCl2, against 0.346 nM/min/mg proteins in the control seedlings.

For the glutathione (GSH) content of the embryonic axes of young zucchini seedlings (Fig. 2F), the results revealed a non-significant decrease (P = 0.452) under the effect of $CdCl_2$ and CuCl2 salts, compared to the control seedlings and the minimum GSH content (5.66 µM/mg protein) was recorded at 100 µM $CuCl_2$.

Total polyphenol content (TPC), total flavonoid content (TFC) and antioxidant capacity (I (%)) of the embryonic axes of C. pepo **seedlings**

The results (Table 2) reveal a very highly significant effect (p = 0.000) of the used heavy metal salts ($CdCl_2$ and $CuCl_2$) on the total phenols (TPC) and total flavonoids (TFC) content of embryonic axis tissues.

Table 2
Total phenol content (TPC), total flavonoid content (TFC)) and antioxidant activity (*I (%)*) of the embryonic axes of zucchini seedlings (*Cucurbita pepo* L.), exposed to different concentrations of heavy metals salts ($CdCl_2$ and $CuCl_2$), after 8 days of germination. dH_2O: distilled water.

Metal Solution	Treatment (µM)	TPC (µg AGE mg^{-1} DW)	TFC (µg QE mg^{-1} DW)	DPPH *I (%)*
Control	dH_2O	10.87 ± 0.76^{c}	1.05 ± 0.44^{c}	45.23 ± 5.37^{bc}
$CdCl_2$	100 (µM)	38.49 ± 5.95^{b}	1.85 ± 1.39^{c}	49.39 ± 4.45^{abc}
	200 (µM)	5.54 ± 0.94^{c}	2.88 ± 0.07^{c}	38.28 ± 6.47^{c}
$CuCl_2$	100 (µM)	10.34 ± 2.24^{c}	10.72 ± 1.39^{b}	58.74 ± 8.53^{ab}
	200 (µM)	70.02 ± 0.24^{a}	19.66 ± 0.38^{a}	64.44 ± 2.82^{a}
P. value, and significance level		0.000***	0.000***	0.044 *

Data are presented as the means ± SE (n = 3). Values in the same column not sharing the same letter are significantly different at $p \leq 0.05$ by Fisher's LSD test. ***(Extremely significant), *(Significant).

For TPC content in the embryonic axes, we recorded a variable effect depending on the nature and the concentration of the metal to which the seedlings were subjected. For $CdCl_2$, an increase in TPC at the concentration of 100 µM (38.49 µg GAE mg^{-1} DW) and a decrease (5.54 µg GAE mg^{-1} DW) at 200 µM were noted, compared to the control seedlings (10.87 µg GAE mg^{-1} DW). Whereas for $CuCl_2$, an average more or less similar to that of the control (10.34 µg GAE mg^{-1} DW) was recorded at the concentration of 100 µM and a remarkable increase in TPC was obtained at 200 µm (70.02 µg GAE mg^{-1} DW).

The flavonoid content of seedlings subjected to metal stress recorded a proportional increase, dependent on the used metal salts ($CdCl_2$ and $CuCl_2$) concentration. The highest values were obtained for $CuCl_2$ with a content of 19.66 µg QE mg^{-1} DM at a concentration of 200 µM and 10.72 µg QE mg^{-1} DM at a concentration of 100 µM, against 1.05 µg QE mg^{-1} DM in control seedlings. Seedlings subjected to $CdCl_2$ recorded lower flavonoid contents compared to those subjected to $CuCl_2$, with values in the order of 1.85 µg QE mg^{-1} DM and 2.88 µg QE mg^{-1} DM, respectively at concentrations of 100 µM and 200 µM.

The results of the DPPH free radical inhibition rates (I(%)) of the embryonic tissue extracts (Table 2) show overall that this antioxidant activity increases significantly under the effect of metallic stresses. This is

particularly so for $CuCl_2$ where a mean maximum inhibition rate (64.44%) was noted at a concentration of 200 µM. However, a lower inhibition rate (38.28%) than that of the control seedlings was recorded in the zucchini seedlings exposed to a concentration of 200 µM of $CdCl_2$.

Discussion

Regarding the germination criteria, the obtained results show that there was no significant effect of the metal salts ($CdCl_2$ and $CuCl_2$) on the germination rate of zucchini seeds, within the limits of the applied concentrations (100 and 200 µM). While the seed vigor index (SVI) showed a significant decrease with increasing metal stress in the medium. Similar results were obtained by Di Salvatore et al. (2008), who did not record significant differences in the germination rates of different plant species (radish, broccoli, lettuce, and tomato) exposed to concentrations of 0 to 1024 µM of Cd, Cu, Pb, and Ni. In addition, the concentration of 2 mM of $AlCl_3$ has no effect on the seeds germination of barley (*Hordeum vulgare* L.) (Tamas et al. 2003). On the other hand, the growth of the radicle decreased significantly in the third day of germination, and its growth was completely inhibited on the fourth day.

The phytotoxic effect of cadmium (Cd) and copper (Cu) on seed germination and seedling development has been reported by several authors in different plant species, including zucchini *C pepo* L. (Labidi et al. 2021), Atriplex *A halimus* (Bankaji et al. 2017), mung bean *P aureus* Roxb (Patel and Ramana Rao 2009) and rice *O sativa* (Ahsan et al. 2007).

Recently, Asadi Aghbolaghi et al. (2022) concluded that *C pepo* seeds have relative tolerance to the toxic effects of cadmium (200 mg/l) at the early stage of germination, where depletion in protein and lipid of endosperm reserves increased, while seed vigor and the activity of antioxidant enzymes such as CAT, SOD, and POX were significantly reduced in germinated zucchini seeds.

The decrease in the zucchini seed vigor index (SVI) under the influence of environmental metal stress is mainly due to the phytotoxic effect of metal ions (Cd^{2+}, Cu^{2+}) on the enzymatic activity of amylases, proteases, and ribonucleases (Sethy and Ghosh 2013), which results in the inhibition of the seed reserves degradation, alters the various biological processes of germination and thus inhibits the growth of the seedling (Seneviratne et al. 2019).

Moreover, the phytotoxic effect of heavy metals (Cd, Cu) on the growth of zucchini seedlings was also reported in the work of Munzuroglu and Geckil (2002) and Labidi et al. (2021), who showed that the dry weights of embryonic axes (hypocotyl + radicle) were more affected by copper salt ($CuCl_2$) whereas the elongation of young zucchini seedlings was more affected by cadmium salt ($CdCl_2$). The accumulation of Cd^{2+} ions in the developing embryonic tissues exerts a phytotoxic effect, particularly at the radicle cells level, and leads to the inhibition of its growth (Kalai et al. 2014; Rucińska-Sobkowiak 2016).

The toxic effects of heavy metals on the seed's metabolic efficiency and consequently on the kinetics of reserves degradation cause a remarkable inhibition of the depletion of endospermic nutrients such as

soluble sugars and amino acids, which are essential for the development of young seedlings (Mihoub et al. 2005; Solanki et al. 2011). On the other hand, the high toxicity of Cd^{+2} and Cu^{+2} metal ions inside plant tissues can be attributed to the production of oxidizing radicals, which inhibits cell division and/or elongation, thereby reducing young seedling growth (Datta et al. 2011; Seregin and Kozhevnikova 2006).

Analyzes of oxidative stress parameters revealed an increase in the lipid peroxidation activity (H_2O_2 and MDA content), and the generation of oxidative processes in the embryonic tissues of zucchini seedlings under the effect of metal treatments (Cd, Cu), which is in agreement with the results of (Labidi et al. 2021) on the same species (*C. pepo* L.), and with those of Jaouani et al. (2016) on pea and other reports on many botanical species under metal stress (Schützendübel and Polle 2002; Lavid et al. 2001; Hu et al. 2007).

Therefore, the formed and accumulated ROS during heavy metal-induced stress react with the polyunsaturated fatty acid to form the lipid radicals and reactive aldehydes, which cause a decrease in membrane fluidity and the alteration of membrane proteins (Gill and Tuteja 2010). It has been proven that metal-stress leads to the production of malondialdehyde (MDA), following an increase in the polyunsaturated fatty acids content, resulting from the degradation of cell membranes, which is the first damaged site by heavy metals (Chaoui et al. 1997; Aravind and Prasad 2003; Dazy et al. 2009).

On the other hand, increase in MDA content may be due to the inefficiency of the antioxidant system and a disorder of the oxidant/antioxidant balance in favor of ROS (Atamer et al. 2008); this is based on the concept of oxidative stress.

However, analysis of the results relating to variation of enzymatic and non-enzymatic antioxidants showed a decrease in the catalase activity (CAT) of the zucchini seedling tissues under the influence of heavy metals (Cd, Cu) in the medium, and the most important catalase activity of the embryonic axes is associated with higher concentrations of H_2O_2 (Moussa 2004). Similar results were reported by Labidi et al. (2021) on zucchini and Sandalio et al. (2001) on pea.

According to Del Río et al. (2006), the catalase activity recorded in the embryonic axes of unstressed zucchini seedlings (control) can be explained by the concentration of this enzyme in the peroxisome, and its role in the elimination of the formed hydrogen peroxide, as a result of glycolate oxidase. On the other hand, the decrease in CAT activity in embryonic axes tissues under the effect of metallic treatments of Cd and Cu can be interpreted either by the inhibition of the enzyme by the metallic element or by elimination of ROS in the tissues of zucchini seedlings (Moussa 2004).

Our results show that the variations in the SOD activity are similar to those of CAT. The decrease in SOD activity in zucchini seedlings in response to metal-stress (Cd, Cu) was also reported by Labidi et al. (2021) on zucchini (*C. pepo* L.), Sandalio et al. (2001) on pea (*Pisum sativum* L.) and Mobin and Khan (2007) on mustard (*Brassica juncea* L.).

Glutathione S-transferase (GST) plays a key role in the detoxification mechanism of heavy metals and reactive oxygen species (ROS), as well as the regulation of redox balance (Konings and Penninga 1985; Siritantikorn et al. 2007). This enzyme is defined as a biomarker of heavy metal contamination (Cantú-Medellín et al. 2009). GST isoenzymes of the Tau class (GSTU) protect cells against metal toxicity by catalyzing the conjugation of glutathione (GSH) with metal ions for subsequent vacuolar sequestration (Dixon et al. 2002; Moons 2003).

Kilili et al. (2004) showed that GSTs (LeGSTUs) play a primary role in the defense against oxidative stress, where they transcriptionally control the enhancement of the antioxidant response of organisms under stress conditions, they are able to 'inhibit induced cell death and protect against oxidation of membrane lipids under the influence of H_2O_2 radicals.

It should also be noted that, according to Edwards et al. (2000), in breeding programs for *Triticum* species, intense GST expression was linked to resistance to environmental stressors and increased yield in wheat. Additionally, Roxas et al. (1997) confirmed that high expression of the BI-GST enzyme (Tau class homolog) improves resistance to salt stress and cold stress in tobacco seedlings.

The obtained results also show that the variations in the GST activity are similar to those of MDA. The increase in GST activity, in response to metallic stresses, corroborates with the results obtained by Labidi et al. (2021) on zucchini, Jaouani et al. (2016) on pea and Hu et al. (2007) on rice in response to Cadmium. Similarly, Halušková et al. (2009) showed that barley subjected to heavy metals (Cd, Pb, Cu, Hg, Co and Zn) exhibits an increase in GST activity. Indeed, Zhang and Ge (2008) also reported the inhibition of some GST isoenzymes in cadmium-exposed rice seedlings, while some GST isoenzymes were present in control (untreated) seedlings.

Based on these data and on the results of the work carried out by Benhamdi et al. (2014), it is assumed that some GST isoenzymes become inactive and it is only those of the tau class that will be expressed and their activity increases with increasing metallic stress in the environment. This explains the ascending GST activity under the effect of the Cd and Cu salts concentrations, used in this study.

Regarding Glutathione, our results are in agreement with those reported by Ducruix et al. (2006), Nagalakshmi and Prasad (2001) and Gallego et al. (1996), who showed that the glutathione (GSH) content decreases in response to stress-induced by different metal concentrations (Cd, Cu, Fe, Zn, etc.). This decrease in GSH can be explained by the involvement of this compound in detoxification reactions of ROS and metal ions, or by the inhibition of the glutathione synthetase (GS) activity under the effect of the metal (Hossain et al. 2012), and the rapid reduction of oxidized glutathione (GSSG) by the glutathione reductase enzyme (GR) under the effect of metal stress (Sytar et al. 2013). This directly affects the GSH/GSSG redox potential, generating the redox signal in cells exposed to stress (Nocito et al. 2006).

The results relating to the phytochemical parameters, namely the total polyphenols (TPC) and flavonoids (TFC) content and the antioxidant activity (I(%)) of the embryonic axes tissues of zucchini seedlings, reveal that these parameters are induced proportionally and significantly, under the effect of high

concentration of copper (200 µM) in the medium, contrary to the case of cadmium (Cd). This confirms the results obtained by Kısa et al. (2016) in maize (*Zea mays* L) and by Badiaa et al. (2020) in root tissues of tomato plants (*Lycopersicum lycopersicum* L.) under copper metal stress. The authors report that the increased cellular content of phenolic metabolites sustains the plant under these stressful conditions. Kısa et al. (2016) reveal that phenolic compounds act as metal chelators and potent scavengers of ROS and are able to inhibit the enzymes that produce them. Flavonoids can accumulate both outside and inside cell membranes, and act to maintain membrane integrity by blocking the entry of toxic elements into cells (Agati et al. 2012).

On the other hand, the decrease in total phenols and the regression of the antioxidant activity of the embryonic axes tissues under the influence of 200 µM of cadmium ($CdCl_2$) can be attributed, according to Kısa et al. (2016), to the decrease in the activity of key enzymes linked to the biosynthesis of phenolic compounds. This is in agreement with the results of Okem et al. (2015) on *Drimia elata* which showed that cadmium ($CdNO_3$) at high concentrations (10 mg/l) significantly reduces the biosynthesis of secondary metabolites (phenols and flavonoids). Cadmium also causes oxidative stress and induces the production of ROS, by modifying the cellular content of enzymatic and non-enzymatic antioxidants (Jawad Hassan et al. 2020). Indeed, the toxicity of Cd^{2+} on the cellular metabolism of plants causes inhibitory effects on the neoformation of cellular ultrastructures (El Rasafı et al. 2022).

Conclusion

Copper and cadmium are heavy metals found in nature, and their presence, even at low concentrations, can negatively affect plant growth and development. The results in this study enable the conclusion that cadmium (Cd) and copper (Cu) have harmful and variable effects on the germination capacity and vigor of zucchini seeds (*C. pepo* L.), resulting in a reduction in the growth of the embryonic axes of young seedlings. The harmful effect of these heavy metals on young seedlings is linked to the homeostatic imbalance inducing oxidative stress, by elevation of the radical species levels, and by depletion of enzymatic and non-enzymatic antioxidants, in response to exposure to metal ions.

Declarations

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Author contribution

Smail Acila: Conceptualization, Data curation, Formal analysis, Methodology, Investigation, Software, Writing-original draft, review and editing. Dr. Samir Derouiche and Dr. Nora Allioui: Conceptualization,

Data curation, Methodology, Project administration, Visualization and Validation, develop the idea and reviewed the manuscript. All authors read and approved the final manuscript.

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Figures

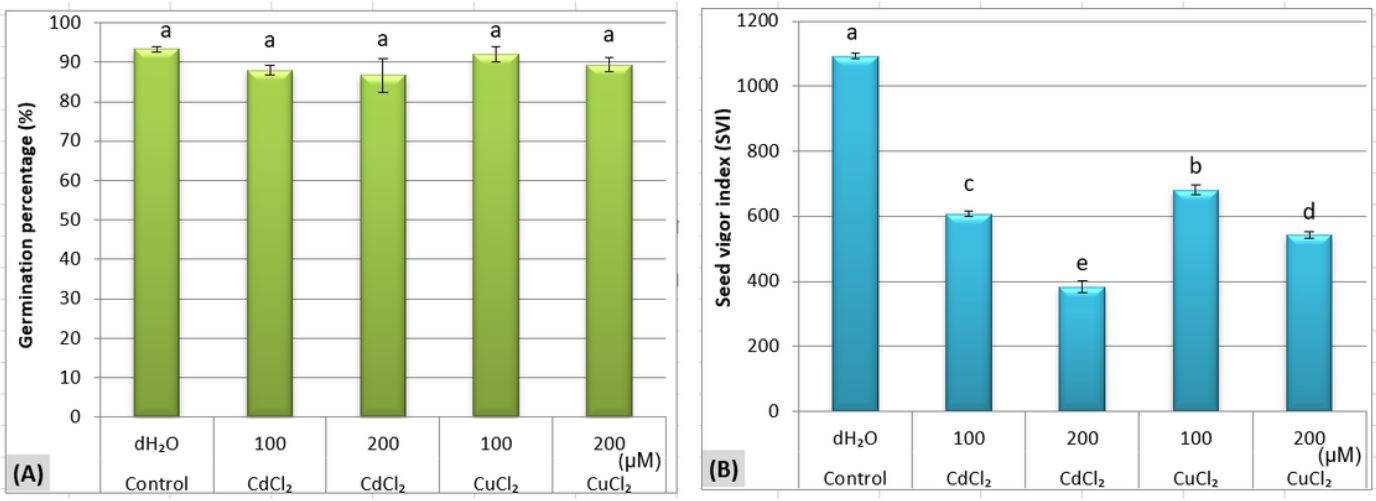

Figure 1

Average seed germination rate (GP) (A) and seed vigor index (SVI) (B) of zucchini seeds (*Cucurbita pepo* L.), after 8 days of germination as a function of the concentrations of $CdCl_2$ and $CuCl_2$. dH_2O: distilled water. Data are presented as the means ± SE (n=3 independent experiments). Means not sharing the same letter are significantly different at $p \leq 0.05$ by Fisher's LSD test.

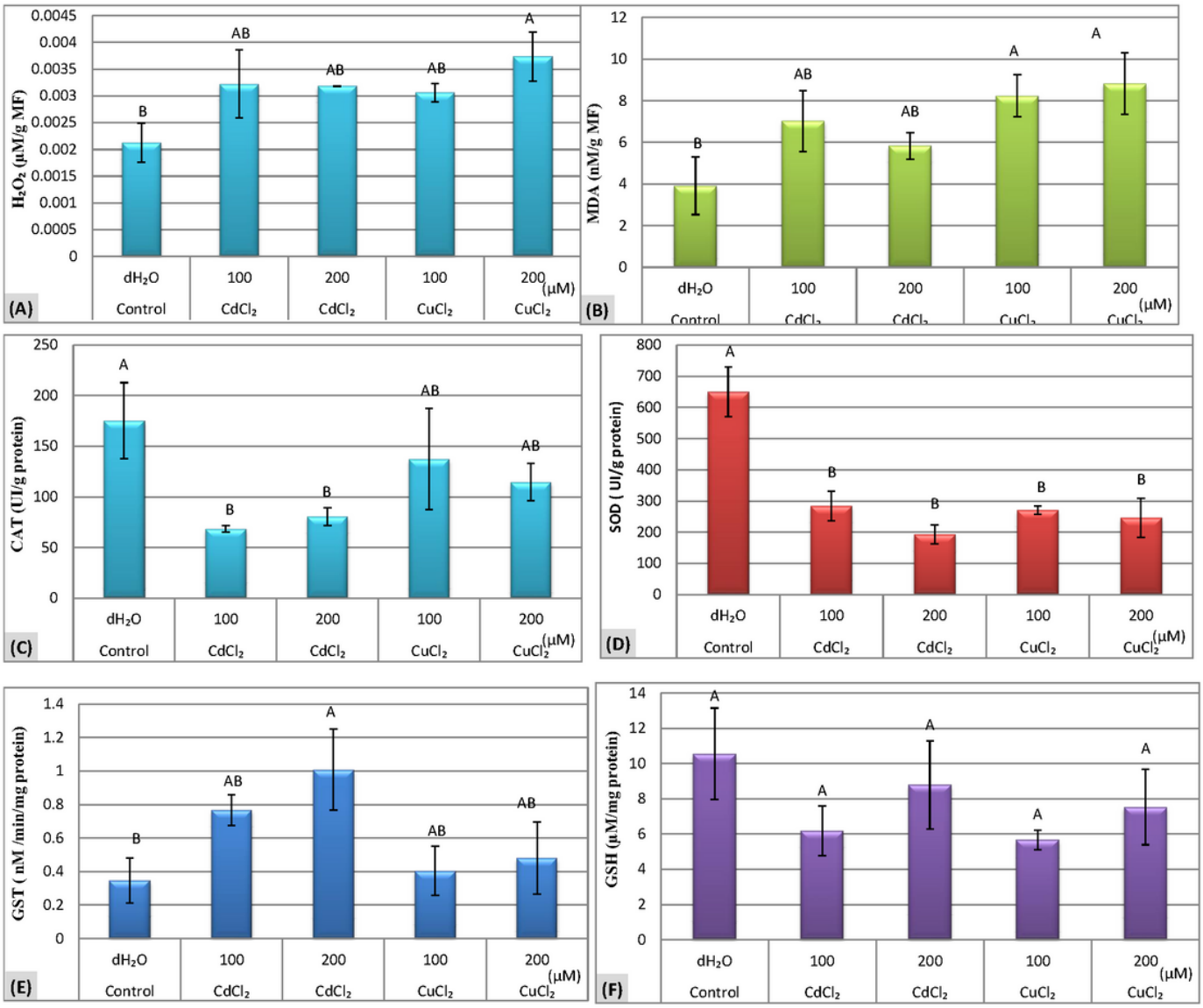

Figure 2

Hydrogen peroxide (H_2O_2) (A), malondialdehyde (MDA) (B), Catalase activity (CAT) (C), superoxide dismutase activity (SOD) (D), glutathione S-transferase activity (GST) (E) and glutathione content (GSH) (F) of the embryonic axes of zucchini seedlings (*Cucurbita pepo* L.), after 8 days of germination as a function of the concentrations of $CdCl_2$ and $CuCl_2$. dH_2O: distilled water. Data are presented as the means ± SE (n = 3). Means not sharing the same letter are significantly different at $p \leq 0.05$ by Fisher's LSD test.