



Analysis of metal profile in soybean after cadmium–induced oxidative damage

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PEER REVIEW

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Comments

This is an interesting paper that focuses on Cd–nutrients relationship. This work also reports a detailed analysis of effects of Cd exposure and nutrient imbalance on Dw and Fw. Data here reported expand and complement those reported by Grazic *et al* (2004).
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ABSTRACT

Objective: To analyze the effect of cadmium (Cd) on soybean seedlings growth and the relationship with the distribution and concentration of macro–microelements.

Methods: The ions concentrations were determined by ICP–MS. The extraction efficiency and digestion time were optimized. Also, oxidative stress parameters were determined and related with metal content.

Results: The accumulated amount of dry matter in roots and leaves was lower in the Cd–treated group. Regression analysis showed that the exposure to Cd affected the accumulated amount of dry matter as well as the content of mineral elements in the analysis samples. In Cd treated plants, electrical conductivity increased respect to the controls, indicating that ionic permeability became altered. A strong inhibition of the chlorophylls (*chl*) biosynthesis in the Cd–treated group was also demonstrated by a decrease of *chla* and *chl b* concentration. This result was related with the observed significant decrease in the Mg uptake at the roots and leaves level.

Conclusions: The stress caused by Cd exposure, evidenced by significantly high hydrogen peroxide levels in roots and leaves after 24 h and the content of specific macro–microelements is a factor that affects the accumulation of dry matter, electrical conductivity and chlorophylls concentration.

KEYWORDS

Soybean, Macro–microelements, Dry matter, Chlorophyll, Ion leakage, Hydrogen peroxide

1. Introduction

The adverse effects of heavy metals on human health have been known for a long time^[1]. However, the exposure to heavy metals continues and is increasing in some areas due to anthropogenic activities^[2]. Heavy metals such as cadmium (Cd), copper (Cu), mercury (Hg) and lead (Pb) are toxic even at low concentrations; they are not biodegradable and have the tendency to accumulate in living organisms. It is considered that they induce the production of reactive oxygen species that may result in damage to cellular

constituents^[3].

Cd is released into the environment by power stations, heating systems, metal working industries, nickel–cadmium batteries and phosphate fertilizers^[4,5]. It is a non–essential element and it is one of the most aggressive and persistent toxic in natural environments. Several experiments have demonstrated that Cd generates morphological and physiological alterations in plants^[6,7].

Toxic heavy metals enter plant cells through transport systems involved in micronutrient uptake. In particular, Cd²⁺ uptake occurs through transmembrane carriers engaged

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in the uptake of calcium (Ca^{2+}), iron (Fe^{2+}), magnesium (Mg^{2+}), copper (Cu^{2+}) and zinc (Zn^{2+}) ions.

Cadmium uptake is affected by Ca levels because a competition for transport channels is produced. A low external Ca level enhances the uptake of Cd, which may result in toxicity for many plant species[6]. A decrease in root surface and consequently in the number of absorption sites occurs, leading to disorders at ion uptake processes[8].

At the cellular level, Cd causes membrane damage due to changes in lipid composition, disruption of electron transport, inhibition/activation of enzymes and interaction with nucleic acids among other modifications[9]. Possible mechanisms by which these disorders are generated are induction of oxidative stress and replacement of essential cofactors of many enzymes such as Zn, Fe, and manganese (Mn)[10].

Studies of the relationship between heavy metal ion toxicity and oxidative stress in plants showed that the damage was due to an enhanced production of reactive oxygen species, such as superoxide anions, hydrogen peroxide (H_2O_2) and hydroxyl radicals[6]. It has been demonstrated that Cd-stress induces the H_2O_2 production in different plant species and the activation of H_2O_2 generating enzymes.

Some toxic effects of heavy metals consist of the Ca replacement at essential sites of cell membranes and also a reduction in the uptake of K across the root plasma membranes[8,11,12]. As a consequence of the oxidative stress process, biological systems have evolved to develop a complex and effective network of defense mechanisms to face the harmful oxidative environments[13,14].

After exposure to Cd, this element is accumulated in different plant parts, preferentially in root[15] and only a small portion is transported to the aerial organs. Several metabolic activities in different cell compartments are indirectly affected.

Mineral elements are essential nutrients for the healthy growth of plants. Although many authors have investigated the antioxidative response of *Glycine max* (*G. max*) L. after Cd-induced oxidative damage, little attention has been paid to the effect produced on the mineral elements profile in the different organs[16]. The results obtained after Cd treatment can provide some references for understanding the relationship between the macro-microelement content and the accumulated dry matter. Also, the mineral profile can be related with other oxidative stress markers, such as chlorophyll content and ion leakage. These oxidative markers have also been investigated in other types of stress-induced plant damage[17].

The aim of the present work was to analyze the role of Cd in the behaviour and distribution of macroelements (K, Ca and Mg) and microelements (Cu, Mg, Zn, Mn, Cu and Fe) in different organs of *G. max*. The accumulated amount of the dry matter in root and leaf was measured. Also, the H_2O_2 concentration, ion leakage and chlorophyll content were

determined in leaves of soybean (*G. max*) after exposure to Cd. In all cases, the obtained metal content profile was related to the oxidative stress parameters. Additionally, the extraction efficiency and digestion time were optimized and thus, significantly improved as well as the sample quantity requirements. All the procedure was fully validated.

2. Materials and methods

2.1. Plant material, growing conditions and treatment

Soybean seeds (*G. max*) were sterilized with 2.5% of sodium hypochlorite before they were potted and allowed to grow under hydroponic conditions in a controlled climate room [24 ± 2 °C, 50% of relative humidity and a photoperiod of 16 h with a photon flux density of $280 \mu\text{mol m}^{-2} \text{s}^{-2}$]. The plants were watered with a Hoagland nutrient solution, and separated into two groups, control and Cd-treated seedlings. Then, they were exposed to 40 $\mu\text{mol/L}$ Cd solution prepared from anhydrous CdCl_2 at Day 10. Roots and leaves of control and Cd-supplied plants were processed and tested at 4 h, 6 h, 24 h, 72 h and 144 h. For all determinations, mean values were obtained from 5 replicates ($n=5$), each replicate was a pool composed by 3 different plants at least.

Roots and leaves were washed with ultrapure water, oven-dried at 80 °C to constant weight after physiological measurement and were ground to powder with a pestle and mortar under liquid nitrogen. For the extraction of ions, samples were mineralized by a microwave-assisted digestion as follows: 100 mg of dried biomass were placed in a 100 mL polytetrafluoroethylene reactor and after that, 5 mL of concentrated nitric acid, 2 mL of H_2O_2 and 1 mL hydrofluoric acid were added. Then the samples were digested applying different microwave powers, *i.e.* MW power was held at 250 W (5 min), 500 W (10 min). The vessels were then removed from the oven and cooled at 20 °C.

2.2. Instrumentation

The measurements were performed with a model ICP 2070 sequential spectrometer (Baird, Bedford, MA, USA). The 1 m Czerny-Turner monochromator had a holographic grating with 1800 mm^{-1} groove. An inductively coupled plasma mass spectrometer (ICP-MS), Perkin-Elmer SCIEX, ELAN DRC-e (Thornhill, Canada) was also used for metal content determination. Microwave digestion was performed with a domestic microwave oven (Philco, Ushuaia, Argentina). A Beckman coulter UV-vis spectrophotometer model DU640 with 10 mm optical path cells was used to record the absorption spectra (Beckman Coulter Inc., Fullerton, CA, USA). A pH meter (Orion Expandable Ion Analyzer, Orion Research, Cambridge, MA, USA) model EA940 with combined glass electrode was used for monitoring pH adjustments.

2.3. Analysis of Cd and macro–microelements

The production of low levels of interfering molecular ions and the attenuation of matrix effects are the two major goals in establishing multi–element screening procedures with detection by ICP–MS. Considering the complexity of the investigated samples, the procedure applied before the quantitative analysis needs to be optimized. In particular, sample treatment is a crucial step for obtaining reliable results[18].

The concentrations ($\mu\text{g/g}$ dry weight) in the dried biomass of macro–microelements were measured in roots and leaves. The ions Cd, Cu, Zn, Mn and Fe were determined by ICP–MS and Ca, Na, K and Mg were determined by inductively coupled plasma optical emission spectrometry (ICP–OES). Standard solutions were prepared by appropriate dilutions of a 1000 mg/L stock solution using 0.1 mol/L HNO_3 as diluent.

2.4. Reagents and solutions

All chemicals used in this work were of analytical reagent grade and were used without further purification. The water used in all studies was ultra–high–quality water with a maximum resistivity of $18.2 \text{ mol/L}\Omega \text{ cm}^{-1}$ obtained from a Barnstead easy pure RF compact ultrapure water system. All the plastic and glassware materials were cleaned by soaking in diluted HNO_3 and were rinsed with distilled water prior to use. All solutions were de–gassed by ultrasonication (Testlab, Argentina).

2.5. Hydrogen peroxide determination

According to Sergiev *et al.*, 250 mg of roots and leaves were homogenized with 2 mL 0.1% (w/v) trichloroacetic acid (Merck, USA). Then, the homogenate was centrifuged at 12000 r/min for 15 min, and 0.5 mL of the supernatant was added to 0.5 mL 10 mmol/L potassium phosphate buffer (pH 7.0 and 1 mL 1 mol/L KI). The absorbance was read at 390 nm. The blank was prepared in the same manner except that 1 mL of 10 mmol/L potassium phosphate buffer (pH 7.0) was used instead of the sample.

2.6. Ion leakage assay

The leaves were harvested and cut into 30 mm pieces. They were washed with ultra–high–quality water to remove surface added electrolytes and placed in Petri dishes with 15 mL of water at 25 °C for 3 h. Electrical conductivity (EC) in the bathing solution was determined (C1). The samples were heated at 80 °C for 2 h and the conductivity was read again in the bathing solution (C2). Relative ion leakage was expressed as a percentage of the total conductivity after heating at 80 °C (relative ion leakage $\% = \text{C1}/\text{C2} \times 100$).

2.7. Chlorophyll content determination

Leaves of soybean (0.5 g of fresh weight) were homogenized with 15 mL ethanol 96°. Extracts were heated in a boiling bath until complete bleaching occurred. After centrifugation, the supernatant absorbance was measured at 665, 654 and 649 nm as described previously by Gallego *et al*[6].

2.8. The ratio of dry weight to fresh weight

Fresh weights (Fw) of plant material were obtained; after, dry weights (Dw) were determined at 80 °C to constant weight and this relationship ($\text{Dw}/\text{Fw} = \text{dry matter}$) was analysis. The dry matter refers to the amount in grams of root/leaf minus the amount in grams of water contained in these samples.

2.9. Statistical analyses

Prior to statistical analysis, the normality of the data was tested by the Kolmogorov–Smirnov test. In all cases, the variances were homogeneous. Data were analysis by One–way ANOVA, and the treatment mean values were compared by the *post–hoc* Tukey–Kramer test at $P < 0.05$.

A linear regression was used with a stepwise regression method between dry matter and mineral elements (Cd, Cu, Zn, Mn, Fe, Ca, Na, K and Mg) accumulated in roots and leaves. The content of mineral element was regarded as the independent variable ($\mu\text{g/g}$ dry weights); dry matter accumulation was regarded as a dependent variable (g). Statistical analyses were performed by using the InfoStat[19].

3. Results

3.1. Cadmium assessment in soybean plants

The accumulation of Cd was significantly higher in roots than in leaves (Table 1). Cadmium levels in roots grew increasingly during the experiment but in leaves were not observed significant differences. The difference between roots and leaves became greater while increasing the time of exposure to Cd.

3.2. Macro–microelements concentrations after Cd exposure of plants

Table 1 shows the distribution of selected macro–microelements in roots and leaves of soybean seedlings treated with Cd. Attention was focused on the analysis of Cu, Zn, Mn, Fe, Ca, Na, K and Mg in roots and leaves during a 144 h period of Cd exposure. On average, in comparison with the control, Cu concentration in Cd–treated plants significantly increased only in roots at 144 h. While Zn

Table 1

Effect of Cd exposure on the accumulated amount of several elements in soybean seedlings.

Organ	Group	Time (h)	Cu	Zn	Mn	Fe	Ca	Na	K	Mg	Cd
Leaves	Control	4	0.023±0.001	0.025±0.001	0.048±0.002	2.06±0.08	2.47±0.15	2.52±0.19	1.69±0.06	170.44±9.45	0.005±0.001
		6	0.016±0.001	0.025±0.001	0.060±0.001	2.95±0.11	0.94±0.05	1.53±0.07	0.83±0.03	144.90±10.01	0.006±0.001
		24	0.035±0.001	0.028±0.001	0.042±0.001	3.06±0.09	1.29±0.08	0.52±0.02	0.62±0.03	135.35±9.88	0.007±0.001
		72	0.023±0.001	0.028±0.001	0.076±0.002	3.33±0.08	1.26±0.07	1.83±0.07	0.52±0.02	140.10±12.31	0.006±0.001
		144	0.025±0.001	0.031±0.002	0.038±0.002	1.77±0.05	1.21±0.06	2.85±0.09	0.55±0.01	133.93±8.43	0.004±0.001
		% RSD	0.21	0.31	0.33	0.28	0.17	0.45	0.28	0.29	0.31
	Treatment	4	0.023±0.002	0.026±0.001	0.048±0.001	2.17±0.09	2.70±0.11 ^{**}	2.66±0.11	1.77±0.05	154.24±11.45 [*]	0.006±0.001
		6	0.016±0.001	0.026±0.001	0.061±0.002	3.16±0.11 [*]	1.02±0.06	1.63±0.05	0.89±0.02	129.96±10.56 [*]	0.006±0.001
		24	0.035±0.002	0.031±0.002	0.042±0.001	3.25±0.11	1.41±0.03	0.54±0.06	0.66±0.01	121.93±9.48	0.008±0.001
		72	0.024±0.002	0.030±0.001	0.077±0.002	3.54±0.10 [*]	1.37±0.05	1.94±0.05	0.55±0.02	126.21±9.81	0.007±0.001
144		0.026±0.002	0.033±0.002	0.038±0.002	1.84±0.07	1.31±0.05	2.98±0.08	0.58±0.02	121.75±8.61	0.005±0.001	
	% RSD	0.22	0.29	0.32	0.25	0.20	0.38	0.32	0.29	0.30	
Roots	Control	4	0.170±0.020	0.422±0.020	0.130±0.020	3.64±0.22	1.30±0.15	0.89±0.02	1.79±0.11	28.13±3.21	0.021±0.009
		6	0.160±0.080	0.310±0.090	0.090±0.060	4.28±0.13	1.23±0.08	2.14±0.06	2.55±0.08	27.30±2.54	0.017±0.004
		24	0.220±0.030	0.680±0.110	0.160±0.080	4.97±0.32	1.15±0.11	3.61±0.07	1.73±0.12	17.88±1.17	0.021±0.002
		72	0.170±0.040	0.550±0.210	0.160±0.090	3.18±0.12	1.57±0.07	1.69±0.03	1.65±0.09	15.45±1.32	0.020±0.004
		144	0.170±0.030	1.280±0.040	0.190±0.060	3.12±0.57	1.71±0.13	1.37±0.08	1.43±0.07	15.66±1.01	0.017±0.008
		% RSD	0.22	0.32	0.31	0.27	0.22	0.37	0.34	0.32	0.31
	Treatment	4	0.220±0.070	0.940±0.070 [*]	0.110±0.090	0.77±0.11 [*]	0.38±0.07 [*]	1.18±0.23	1.65±0.06	19.28±2.23 [*]	9.870±0.980 [*]
		6	0.240±0.090	1.230±0.130 [*]	0.120±0.070	0.38±0.09 [*]	0.66±0.11 [*]	1.47±0.11 [*]	2.74±0.08	12.77±1.77 [*]	9.470±1.020 [*]
		24	0.220±0.020	1.430±0.090 [*]	0.060±0.030	0.71±0.13 [*]	0.53±0.09 [*]	2.22±0.08 [*]	0.61±0.09 [*]	15.41±3.22	15.760±1.230 [*]
		72	0.270±0.030	1.520±0.110 [*]	0.070±0.040	4.32±1.12	0.45±0.12 [*]	3.59±0.37 [*]	1.12±0.12 [*]	14.58±1.41	22.470±2.020 [*]
144		0.330±0.110 [*]	1.320±0.270	0.070±0.030	3.49±0.23	0.97±0.08 [*]	0.66±0.04 [*]	0.51±0.23 [*]	13.52±1.87	28.010±3.880 [*]	
	% RSD	0.24	0.36	0.28	0.28	0.19	0.34	0.29	0.33	0.32	

Values are expressed as mean±SD, n=5, ^{*}P<0.05. The relative standard deviation (RSD) was calculated from the peak heights obtained.

concentrations considerably increased in Cd-treated roots at 4 h, 6 h, 24 h and 72 h, Fe concentration decreased at 4 h, 6 h, 24 h in roots exposed to Cd and only increased at 72 h. However, Fe concentration in treated leaves increased at 6 h and 72 h.

The concentration of Mn decreased in root of Cd-treated plants but differences were not significant. The Ca concentration increased significantly at 4 h in leaves of Cd-treated plants and decreased during all the treatment in roots. The concentration of Mg significantly decreased in roots and leaves at 4 and 6 h. In comparison with controls, Na concentration significantly decreased in roots Cd-treated since 6 h during all the treatment, while K concentration significantly decreased since 24 h.

3.3. Effect of Cd on stress parameters and their relationship with macro-microelements

The H₂O₂ levels increased significantly in roots and leaves respect to the controls at 24 h, 72 h and 144 h of exposure to Cd. However, the H₂O₂ concentration decreased at 4 h and 6 h in both organs (Figure 1).

EC is a parameter that indicates membrane injury as a consequence of an oxidative damage. Although EC decreased in Cd-treated plants starting from 6 h, it was higher than control up to 72 h, as seen in Figure 2, showing values as 18.22%, 15.13% and 10.24% at 6 h, 24 h and 72 h respectively. Li *et al.* found similar results reporting that EC increased at first and then decreased. They concluded that the plant cell membranes were sensitive to Cd stress.

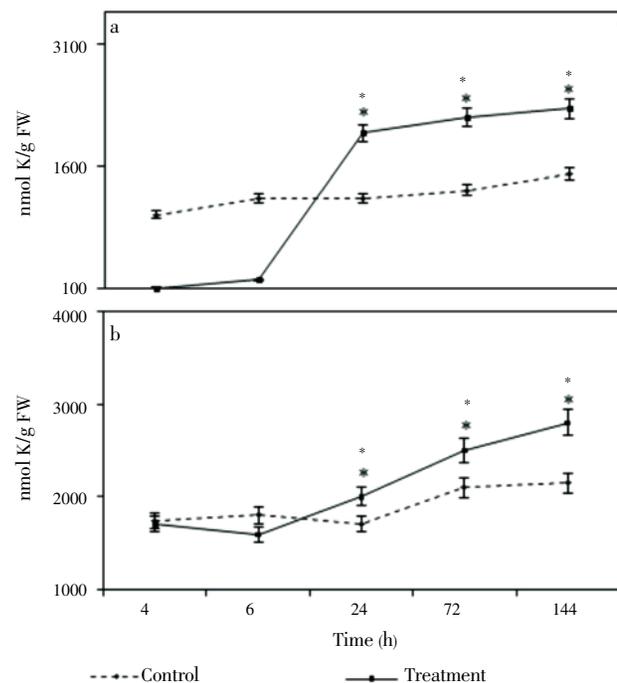


Figure 1. Effect of Cd exposure on the concentration of hydrogen peroxide of soybean seedlings.

a: leaves, b: roots. ^{*}P<0.05.

Total chlorophyll concentration is a unifying parameter for indicating the effect of oxidative stress on chlorophyll biosynthesis. However, it is also important to record changes respect to the controls in the components of chlorophyll (total *chl*=*chl*_a+*chl*_b). The reduction in *chl*_a, *chl*_b and total chlorophyll content was significantly higher in leaves at 24 h, 72 h and 144 h in Cd-treated plants (Figure 3), indicating

chloroplast damage and it is related to a significant decrease in Mg uptake at the roots and leaves level. This result demonstrated the inhibition in chlorophylls biosynthesis and therefore the toxicity of Cd in soybean seedlings.

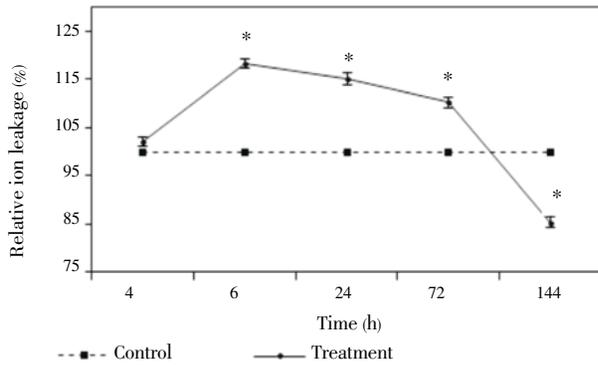


Figure 2. Effect of Cd exposure on ion leakage in leaves of soybean seedlings. * $P < 0.05$.

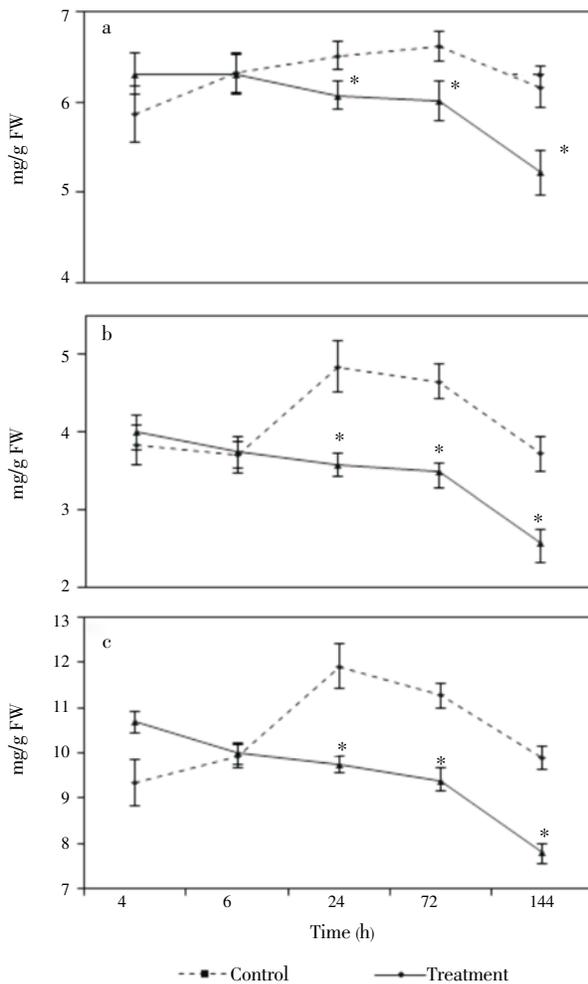


Figure 3. Effect of Cd exposure on the concentration of chlorophylls in leaves of soybean seedlings. a: *chl a*, b: *chl b*, c: total chlorophyll. * $P < 0.05$.

Figure 4 shows Fw, Dw and the relationship Dw/Fw in roots and leaves. Both, Dw and Fw significantly decreased at 144 h in roots and leaves of Cd-treated plants compared to control ones. Also, a decrease of the ratio Dw/Fw was observed only in roots at 144 h, demonstrating that the

growth of soybean is inhibited by Cd.

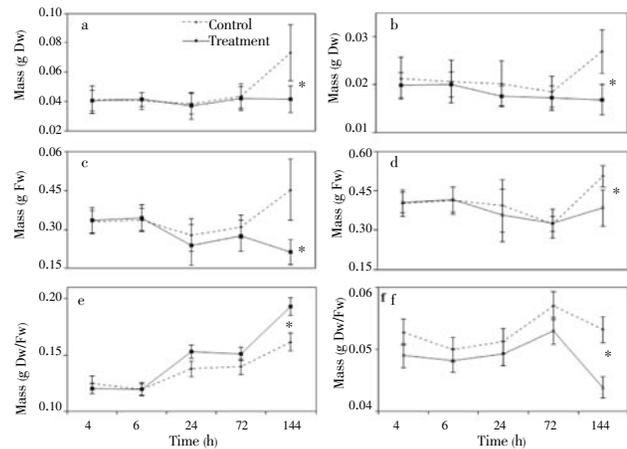


Figure 4. Effect of Cd exposure on dry (Dw) and fresh (Fw) weight in leaves and roots of soybean seedlings.

a: Dw in leaves, b: Dw in roots, c: Fw in leaves, d: Fw in roots, e: Dw/Fw in leaves, f: Dw/Fw in roots. * $P < 0.05$.

3.4. Stepwise regression analysis

Table 2 shows the result from the stepwise regression analysis. The content of Fe, Ca and Mg was only induced into the regression equation for leaves of soybean seedlings under the control conditions. The accumulation of dry matter in leaves, under the effect of Cd, was significantly correlated with the content of Na, Ca, K, Cd, Cu and Mg, showing the content of Cd was the main factor influencing the accumulation of dry matter in leaves. The stepwise regression analysis equation for roots control was correlated with the content of Zn, Mn, Mg and K and during the stress period, K and Ca were the main factors influencing the accumulation of dry matter.

Table 2

Regression analysis between the accumulation of dry matter in roots and leaves and the content of various elements in soybean seedlings.

Organ	Group	Regression equation	R^2
Leaves	Control	Dw=0.0400+0.0232 Fe-0.00615 Ca+0.000245 Mg	0.999 ^a
	Treatment	Dw=0.00741-0.00485 Na+0.00339 Ca-0.00063 K-3.11 Cd+0.0115 Cu+0.000084 Mg	0.998 ^a
Roots	Control	Dw=0.0517+0.0172 Zn-0.178 Mn+0.000198 Mg-0.00794 K	0.999 ^a
	Treatment	Dw=0.0138+0.000261 K+0.000289 Ca	0.989 ^a

^aSignificance at 5% levels.

4. Discussion

Macro-microelements are taken up from soil for the plant growing, playing very important roles in the accumulation of dry matter and in the resistance to stress. Due to its great solubility in water and high mobility in the soil-plant system, Cd is readily taken up by the root and translocated to other parts of the plant[20]. Cd causes many physiological and biochemical changes in growing plants. H₂O₂ analysis in roots and leaves evidences an altered redox state

condition which probably contributes to general stress-induced morphological changes[6]. Our experimental results indicated that Cd-treated plants showed a decrease in the amount of macro-microelements distributed in roots and leaves of soybean, revealed by a reduction of Dw and Fw. The results are in agreement with previous reports about the effects of Cd-stress on the growth of soybean and other plants[7,15].

In our work, a total digestion using HNO₃, H₂O₂ and microwave technology and metal quantification was performed by ICP-MS and ICP-OES. The digestion time was optimized and thus significantly reduced as well as the sample requirements.

Regression analysis showed that the exposure to Cd affected the content of mineral elements and the accumulation of dry matter in roots and leaves of soybean plants. There was a negative correlation between Cd, Na and K and the accumulation of dry matter in leaves treated with Cd; while Cu and Mg showed the opposite effect, favoring the accumulation of dry matter. However, a positive correlation was observed between the content in roots of major elements like Ca and K and dry matter during stress period.

The similitude between the ionic ratio of Cd and some mineral essential cofactors of many enzymes, as in the case of Ca, may lead to a large Cd uptake by the plant, when it is exposed to high levels of this metal. The relationship between Cd exposure and the concentration of macro-microelements was variable in different organs of the seedlings. Alterations in the concentration of elements were observed in soybean plants grown with exposure to Cd[21,22]. Our experimental results indicated that in the Cd-treated plants, the amount of macro-microelements distributed in roots and leaves could decrease; it is according to López-Millan *et al.*, who investigated alterations by Cd in tomatoes. The macro and microelements are required by plants to regulate the synthesis of specific substances as glutathione and others in the antioxidant system for resisting Cd-stress[23].

The primary bioindicator of Cd phytotoxicity is the strong inhibition of chlorophyll biosynthesis indicating chloroplast damage. Chlorosis symptoms were also observed. This is probably due to the inhibition of the protochlorophyllide reductase by Cd. Also, the water-splitting enzyme located on the oxidizing site of photosystem II is inhibited[24] affecting the photosynthetic electron transport.

Manios *et al.*, observed a negative effect in the ratio of *chl a* to *chl b* due to a faster hydrolysis of *chl a* compared with *chl b* when plants are under stress by heavy metals. Mobin *et al.* observed that the substitution of Mg in the chlorophyll molecule by toxic heavy metals, such as Cu, Zn, Cd or Hg, resulted in an abrupt cessation of photosynthesis.

Cd might act as an oxidation-related disturbance, including the disturbance of lipid peroxidation. The observed increase in ion leakage is related to the higher

malondialdehyde (MDA).

MDA concentration, a marker of membrane injury[25], is an indicator of lipid peroxidation and plasma membrane damage. Our results allowed confirming the increase of MDA levels in soybean after Cd exposure[26].

In conclusion, our results demonstrated that the stress caused by Cd evidence an altered redox state by alteration in H₂O₂ level. The content of specific macro-microelements is a factor that affects the accumulation of dry matter, chlorophyll level by inhibition in this biosynthesis and ionic permeability by dis-balance in concentration of ions during stress by heavy metal. Soybean seedlings require these macro-microelements to regulate the synthesis of specific substances for resisting or adapting to stress. Lower ion absorption due to a decrease in root surface occurs and consequently in the number of absorption sites, leading to disorders at ion uptake processes.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

The adverse effects of heavy metals on human health have been known for a long time. However, the exposure to heavy metals continues and is increasing in some areas due to anthropogenic activities. The current bibliography in Cd toxicity is mainly related to oxidative stress and mechanisms involved in plant response (Andersen and Küpper, 2013). Effects of Cd on nutrient balance have been less explored (Grazic *et al.*, 2004).

Research frontiers

This article analyzed the effect of cadmium (Cd) on soybean seedlings growth and the relationship with the distribution and concentration of macro-microelements.

Related reports

There is brief communication related to this work. Grazic

et al. (2004) reported variations in macro and micronutrient content in soybean plants after exposure to different concentrations of Cd.

Innovations and breakthroughs

Authors have improved a metal profile throughout different times to exposure to Cd. Authors also assayed different times of digestion.

Applications

The results of this work demonstrated that the stress caused by Cd evidence an altered redox state by alteration in H₂O₂ level which can be used and referred by other researchers.

Peer review

This is an interesting paper that focuses on Cd–nutrients relationship. This work also reports a detailed analysis of effects of Cd exposure and nutrient imbalance on Dw and Fw. Data here reported expand and complement those reported by Grazic et al (2004).

References

- [1] Lalor GC. Review of cadmium transfers from soil to humans and its health effects in the Jamaican environment. *Sci Total Environ* 2008; **400**: 162–172.
- [2] Sarma H, Deka S, Deka H, Saikia RR. Accumulation of heavy metals in selected medicinal plants. *Rev Environ Contam Toxicol* 2011; **214**: 63–86.
- [3] Barthwal J, Nair S, Kakkar P. Heavy metal accumulation in medicinal plants collected from environmentally different sites. *Biomed Environ Sci* 2008; **21**: 319–324.
- [4] Sanità di Toppi L, Gabbriellini R. Response to cadmium in higher plants. *Environ Exp Bot* 1999; **41**: 105–130.
- [5] Wagner CJ. Accumulation of cadmium in crop plants and its consequences to human health. *Adv Agric* 1993; **51**: 173–212.
- [6] Gallego SM, Pena LB, Barcia RA, Azpilicueta CE, Iannone MF, Rosales EP, et al. Unravelling cadmium toxicity and tolerance in plants: insight into regulatory mechanisms. *Environ Exp Bot* 2012; **83**: 33–46.
- [7] Finger-Teixeira A, Lucio Ferrarese Mde L, Soares AR, da Silva D, Ferrarese-Filho O. Cadmium-induced lignification restricts soybean root growth. *Ecotoxicol Environ Saf* 2010; **73**: 1959–1964.
- [8] Fodor E, Szabó-Nagy A, Erdei L. The effects of cadmium on the fluidity and H⁺-ATPase activity of plasma membrane from sunflower and wheat roots. *J Plant Physiol* 1995; **147**: 87–92.
- [9] Sandalio LM, Dalurzo HC, Gomez M, Romero-Puertas MC, del Rio LA. Cadmium-induced changes in the growth and oxidative metabolism of pea plants. *J Exp Bot* 2001; **52**: 2115–2126.
- [10] Smeets K, Cuypers A, Lambrechts A, Semane B, Hoet P, Van Laere A, et al. Induction of oxidative stress and antioxidative mechanisms in *Phaseolus vulgaris* after Cd application. *Plant Physiol Biochem* 2005; **43**: 437–444.
- [11] Geuns JM, Cuypers AJ, Michiels T, Colpaert JV, Laere A, Van Den Broeck KA, et al. Mung bean seedlings as bio-indicators for soil and water contamination by cadmium. *Sci Total Environ* 1997; **203**: 183–197.
- [12] Stroiński A, Floryszak-Wieczorek J. Effects of cadmium on the host–pathogen system. IV. influence of cadmium and *Phytophthora infestans* on membrane permeability of potato tuber. *J Plant Physiol* 1993; **142**: 575–578.
- [13] Fridovich I. The biology of oxygen radicals. *Science* 1978; **201**: 875–880.
- [14] Halliwell B, Gutteridge JM. *Free radicals in biology and medicine*. Oxford: Clarendon Press; 1999.
- [15] Molina AS, Nieves C, Chaca MV, Garibotto F, González U, Marsá SM, et al. Cadmium-induced oxidative damage and antioxidative defense mechanisms in *Vigna mungo* L. *Plant Growth Regul* 2008; **56**: 285–295.
- [16] Salazar MJ, Rodriguez JH, Leonardo Nieto G, Pignata ML. Effects of heavy metal concentrations (Cd, Zn and Pb) in agricultural soils near different emission sources on quality, accumulation and food safety in soybean [*Glycine max* (L.) Merrill]. *J Hazard Mater* 2012; **233–234**: 244–253.
- [17] Płażek A, Tatrzańska M, Maciejewski M, Kościelniak J, Gondek K, Bojarczuk J, et al. Investigation of the salt tolerance of new Polish bread and durum wheat cultivars. *Acta Physiol Plant* 2013; **35**: 2513–2523.
- [18] Wang J. On–line sample–pre–treatment schemes for trace–level determinations of metals by coupling flow injection or sequential injection with ICP–MS. *TrAC Trends Anal Chem* 2003; **22**: 836–846.
- [19] Di Rienzo JA, Casanoves F, Balzarini MG, Gonzalez L, Tablada M, Robledo CW. *InfoStat*. Argentina: National University of Córdoba; 2011.
- [20] Benavides MP, Gallego SM, Tomaro ML. Cadmium toxicity in plants. *Braz J Plant Physiol* 2005; **17**: 21–34.
- [21] Chen YX, He YF, Yang Y, Yu YL, Zheng SJ, Tian GM, et al. Effect of cadmium on nodulation and N₂–fixation of soybean in contaminated soils. *Chemosphere* 2003; **50**: 781–787.
- [22] Dražić G, Mihailović N, Stojanović Z. Cadmium toxicity: the effect on macro– and micro–nutrient contents in soybean seedlings. *Biol Plant* 2004; **48**: 605–607.
- [23] Foyer CH, Noctor G. Redox regulation in photosynthetic organisms: signaling, acclimation, and practical implications. *Antioxid Redox Signal* 2009; **11**: 861–905.
- [24] Assche FV, Clijsters H. Effects of metals on enzyme activity in plants. *Plant Cell Environ* 1990; **13**: 195–206.
- [25] Moon CH, Lee YS, Yoon TH. Variation of trace Cu, Pb and Zn in sediment and water of an urban stream resulting from domestic effluents. *Water Res* 1994; **28**: 985–991.
- [26] Munoz N, González C, Molina A, Zirulnik F, Luna CM. Cadmium-induced early changes in O₂^{•-}, H₂O₂ and antioxidative enzymes in soybean (*Glycine max* L.) leaves. *Plant Growth Regul* 2008; **56**: 159–166.