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## Studies on oxidative stress caused by Cu and Zn excess in germinating seeds of Indian mustard (*Brassica juncea* L.)

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**ABSTRACT** Seeds of Indian mustard were germinated at 0, 50, 100, 200 mg/L Cu and Zn concentrations, in dark for 12, 24, 48 and 96 h, at 24±1°C. The real metal content in seeds was determined by AAS. For biochemical measurements fresh material were homogenized and the supernatant was used for all assays. The following parameters were evaluated: FRAP (ferric reducing ability of plasma), lipid peroxidation (LP), glutathione (GSH), total protein content, GST (glutathione-S-transferase), GPOX (guaiacol peroxidase) and CAT (catalase).

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**KEY WORDS**

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Essential heavy metals like copper or zinc which are often cofactors of various enzymes such as oxidases or components of photosynthetic apparatus, also might act as pollutants at higher concentrations in the environment due to industrial, municipal or agricultural activities (Prasad and Strzalka 2002; Janas et al. 2010).

Not only non-essential but essential heavy metals (e.g. Cu or Zn), mainly at higher levels, usually cause oxidative stress in plants, and consequently cell wall and membrane damage, lipid peroxidation or disorders in various metabolic processes (Prasad et al. 1999; Broadley et al. 2007; Janas et al. 2010). Since, germination is very sensitive period of development, effects of heavy metal stress are more expressed and visual than later.

Our aims were to investigate potential oxidative stress and antioxidative defense mechanisms in germinating seeds of Indian mustard (*Brassica juncea* L.) exposed to Cu or Zn excess. Although, this species is generally known to be cadmium-tolerant and hyperaccumulator, we wanted know whether copper or zinc treatment can cause similar changes as cadmium did in our former study (Szöllősi et al. 2009).

### Materials and Methods

#### Plant material and germination conditions

Seeds of Indian mustard (*Brassica juncea* L.) were germinated in sterilized Petri dishes at 0, 50, 100, 200 mg/L Cu or Zn concentrations (signed as Control, Cu50, Cu100, Cu200 and Zn50, Zn100, Zn200) with 8 replicas. The seeds were sown on the Petri dishes with 10 ml of metal solutions and were kept in dark for 12, 24, 48 and 96 h, at 24±1°C. More-

over, in order to detect heavy metal content in germinating seeds, about 0.5 g fresh plant material from each treatment was separated for drying at 30°C until at least 4 days.

#### Determination of heavy metal content in seeds

The real Cu or Zn content in Indian mustard seeds was determined by atomic absorption spectrophotometry (AAS). For detection of metal content 8 replicas were taken of each concentration and time of exposure. Values of both Cu or Zn concentration in seeds are given in µM/g dry weight (DW).

#### Preparation of samples for biochemical assays

At each metal concentration 8 replicates of 0.3 g fresh material were homogenised with phosphate buffer (0.1M K<sub>2</sub>HPO<sub>4</sub>, pH 7.6+0.1 mM EDTA) and centrifuged, then the supernatant was used for all assays.

To evaluate the antioxidant power of the homogenates FRAP method was used (ferric reducing ability of plasma; Benzie and Strain 1996). The assay was modified and applied for plant material (Varga et al. 2000; Szöllősi and Varga 2002). The total antioxidant capacity was expressed in units of µmol/ml plant extract.

The assay of malonyldialdehyde (MDA) was applied to estimate lipid peroxidation (LP; modified method of Placer et al. 1966). MDA content was determined and is expressed in units nmol/ml homogenate.

Glutathione content was measured using the method of Sedlak and Lindsay (1968). Data are expressed in µmol GSH/ml plant homogenate.

Protein content of plant homogenate was measured spectrophotometrically at 675 nm using the method of Lowry et al. (1951). These data were used to calculate the enzyme (GST, GPOX, CAT) activities.

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**Table 1.** Cu content of *Brassica juncea* seeds germinated at different concentrations. Different letters refer to significant differences at  $p < 0.05$ .

Cu conc. (mg/L)	12h	24h	48h	96h
0	0 a	0.03 a	0.06 a	0.19 a
50	1.29 ± 0.21 ab	4.16 ± 0.80 ab	4.02 ± 0.84 ab	4.19 ± 1.03 ab
100	4.67 ± 0.83 b	4.96 ± 0.69 bc	11.53 ± 2.11 b	13.75 ± 1.16 b
200	3.91 ± 1.15 b	6.71 ± 0.99 c	11.18 ± 3.65 b	16.99 ± 2.23 b

**Table 2.** Zn content of *Brassica juncea* seeds germinated at different concentrations. Different letters refer to significant differences at  $p < 0.05$ .

Zn conc. (mg/L)	12h	24h	48h	96h
0	0.31 ± 0.03 a	0.29 ± 0.05 a	0.32 ± 0.06 a	0.41 ± 0.05 a
50	2.19 ± 0.28 ab	3.09 ± 0.38 ab	3.81 ± 0.51 ab	5.41 ± 0.60 ab
100	4.00 ± 0.28 bc	6.32 ± 1.23 bc	7.01 ± 0.97 bc	7.48 ± 0.85 bc
200	7.38 ± 0.98 c	12.27 ± 1.99 c	16.27 ± 3.09 c	19.99 ± 2.83 c

### Enzyme activities

The activity of glutathione-S-transferase (GST) was determined according to Mannervik and Guthenberg (1981) and detected at 340 nm. The values were calculated as the production of 1 mmol conjugate per min and expressed as unit/mg protein.

Determination of guaiacol peroxidase (GPOX) activity was executed according to Singh et al. (2006) and that of catalase (CAT) using the method of Beers and Sizer (1952).

### Statistics

Statistical analysis of results was carried out using STATISTICA 9.0 software (ANOVA, analysis of correlation). Data are given in mean values ± standard deviation (SD; N=8)

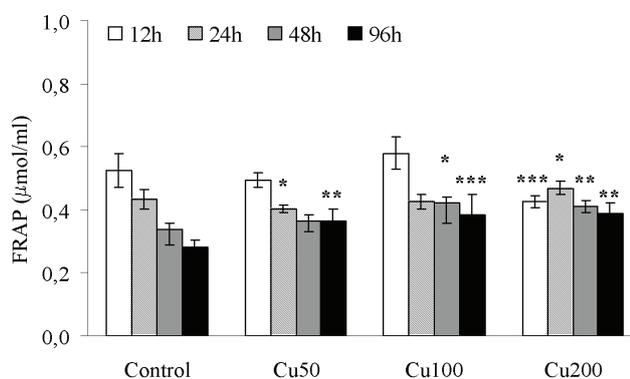
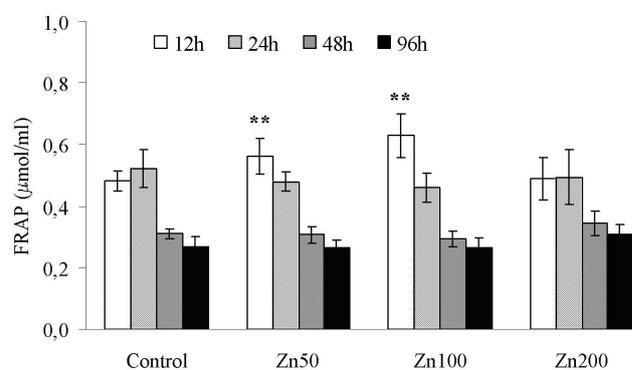
and calculated for fresh homogenate or dry weight. Level of significance was generally  $p < 0.05$ .

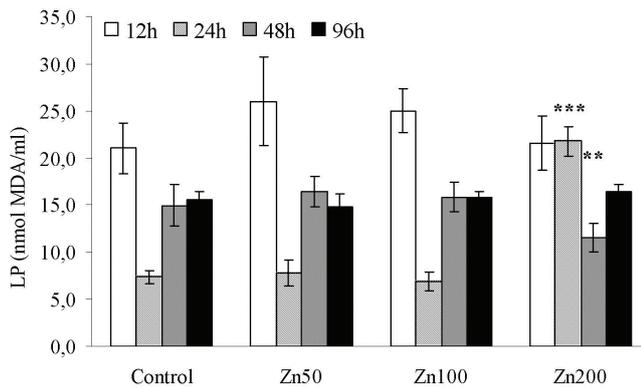
### Results and Discussion

Data analysis showed time and dose dependence in the case of both metals (Table 1 and 2), especially at higher concentrations (Spearman's  $r = 0.37$  and  $r = 0.83$ ,  $p < 0.001$  (Cu),  $r = 0.30$  and  $r = 0.93$ ,  $p < 0.001$  (Zn), similarly to Zn treated rapeseed seedlings (Wang et al. 2009).

### Antioxidant capacity and lipid peroxidation

We found the FRAP values decreased in time in the Control and Cu or Zn treated seeds (Fig. 1 and 2; Cu:  $r = -0.74$  és Zn:  $r = -0.87$ ,  $p < 0.001$ ) which might be due to the increases activ-

**Figure 1.** Total antioxidant capacity (FRAP) in *Brassica juncea* seeds germinated at different Cu concentrations. \*  $p < 0.05$ , \*\*  $p < 0.01$  and \*\*\*  $p < 0.001$  refer to significant differences between Control and treated plants.**Figure 2.** Total antioxidant capacity (FRAP) in *Brassica juncea* seeds germinated at different Zn concentrations. \*\*  $p < 0.01$  refer to significant differences between Control and treated plants.



**Figure 3.** Lipid peroxidation (LP) in *Brassica juncea* seeds germinated at different Zn concentrations. \*\*  $p < 0.01$  and \*\*\*  $p < 0.001$  refer to significant differences between Control and treated plants.

ity of the antioxidant defence system (Blokhina et al. 2003; Maksymiec 2007). GSH values showed similar tendencies (Cu:  $r = -0.78$  és Zn:  $r = -0.80$ ,  $p < 0.001$ ).

MDA level occurred to increase slightly with concentration (Cu:  $r = 0.29$ ,  $p < 0.01$  és Zn:  $r = 0.19$ ,  $p < 0.05$ , Fig. 3) applied similarly to the results of Prasad et al. (1999), while it decreased with duration (Cu:  $r = -0.22$ ,  $p < 0.05$  and Zn:  $r = -0.33$ ,  $p < 0.001$ ) which was probably the consequence of the induction of the antioxidant system.

### The activities of enzymes (GST, GPOX, CAT)

The activity of GST showed significantly positive correlation with duration in the case of Cu ( $r = 0.47$ ,  $p < 0.001$ ), while during Zn excess it rather had dose dependence ( $r = 0.46$ ,  $p < 0.001$ ) which suggests that not only one parameter affects the efficiency of this enzyme.

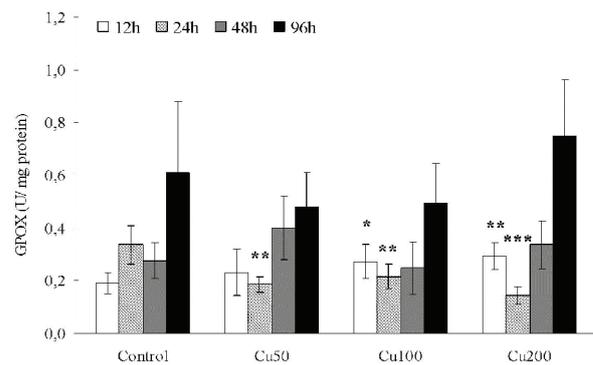
GPOX activity increased in both Control and treated seeds in time (Fig. 4, Cu:  $r = 0.58$  and Zn:  $r = 0.33$ ,  $p < 0.001$ ) according to the data of Chaoui and El Ferjani (2005).

CAT activity also increased in both Control and treated seeds in time (Cu:  $r = 0.70$  és Zn:  $r = 0.48$ ,  $p < 0.001$ ) which was in contrast with former results published about Cu treated pea seedlings (Chaoui and El Ferjani 2005).

A part of this study was presented on the 10<sup>th</sup> Congress of the Hungarian Society for Plant Biology, August 31 - September 2, 2011, Szeged, Hungary.

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**Figure 4.** The activity of guaiacol peroxidase (GPOX) in *Brassica juncea* seeds germinated at different Cu concentrations. \* $p < 0.05$ , \*\* $p < 0.01$  and \*\*\* $p < 0.001$  refer to significant differences between Control and treated plants.

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