

## CU AND CD TOLERANCE OF BARLEY AND WHITE MUSTARD: POTENTIAL INDICATORS OF WATER CONTAMINATION WITH THESE HEAVY METALS

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**Abstract:** Environmental pollution with heavy metals is one of the major current problems, because their uncontrolled release has already led to accumulation in water and sediment. Some heavy metals are essential for plant development like Cu while others like Cd are not. However, both can cause phyto-toxic effects when present at high levels in the environment. Maximal allowable amounts (MAC) according to valid Directives are: in surface and underground waters of II and III class 5 µg/l Cu, 0.45-0.6 µg/l Cd and in irrigation water 100 µg/l Cu, 10 µg/l Cd. However, the content of Cu and Cd in watercourses often exceeds the abovementioned limits. Therefore, its detection in water is significant for water risk assessment, and the roll of plants as bioindicators is gaining in importance. The aim of this study was to evaluate the tolerance of barley (*Horedum vulgare* L.) and white mustard (*Sinapis alba* L.) to different levels of Cu and Cd in water, as potential bioindicators of water pollution with these heavy metals. A standard filter paper method (ISTA Regulations book, 2011) was used. The toxic effects of Cu (0.025-100 µg/l) and Cd (0.01- 200µg/l) were assessed on physiological (germination) and morphological (root and shoot length and fresh and dry weights) traits of test species. The results were processed with Duncan's multiple range test. Plant responses to metals were species and concentration dependant. Cu and Cd did not affect germination of barley seeds, regardless on concentration. However, Cu inhibited seedlings root growth and fresh weight at 0.025 µg/l, dry weight, shoot growth and fresh shoot weight at 2 µg/l. Cd inhibited only root and shoot growth of barley seedlings at 100 µg/l. Germination of white mustard seeds was significantly reduced in treatments with 5 µg/l Cu and 1 µg/l Cd. Root growth was inhibited at 2, 50 and 100 µg/l Cu, while the presence of 1 µg/l Cu in water significantly reduced fresh root weight. Cd at 200 µg/l inhibited root length of white mustard seedlings, while shoot length was stimulated at >10 µg/l Cd. Results indicate at barley and white mustard sensitivity towards Cu, when present in water in amounts lower than MACs. However, Cd in amounts lower than MACs had no effect on most of the observed traits of tested plants.

**Keywords:** Cu, Cd, barely, white mustard, metal tolerance, bioindicators

### INTRODUCTION

Heavy metal pollution of water is one of major current environmental problems. Their uncontrolled release has already led to accumulation in water and sediment, due to which they present a threat for aquatic organisms, negatively affect plant production when present in water for irrigation and indirectly, after incorporation in food chains, can cause human health problems (AYCICEK et al., 2008; PRICA et al., 2010).

Some metals, such as copper (Cu), are essential for plant metabolic activity because they catalyze a number of physiological processes. Cu is involved in photosynthetic and respiratory electron transport chains and presents a cofactor in prosthetic group in biosynthesis (HARRISON et al., 1990, KASTORI, 1998). When present at lower concentrations in medium it stimulates root and shoot growth and biomass (JIANG et al., 2001; CHHOTU and FULEKAR, 2008; MANIVASAGAPERUMAL et al., 2011). However, at higher concentrations, Cu causes phyto-toxic effects like growth inhibition, reduction of photosynthetic activity and chlorophyll content, total protein content, increase of proline and

ABA, mitosis disorder, cell elongation inhibition, damages cell walls and integrity of plasma membrane etc. (JIANG et al., 2001; ZENGIN and MUNZUROGLU, 2004; ALAOUI - SOSSE et al., 2004; ZENGIN and KIRBAG, 2007; SINGH et al., 2007). Nevertheless, plants have the ability to absorb and accumulate metals whose role in metabolic processes has not been yet determined, such as cadmium (Cd) (KASTORI, 1997). Cadmium (Cd) presents an increasing international concern because it is very persistent in the environment, extremely toxic to plants and animals and is easily absorbed by plants and transported to upper parts, thus presents a risk for consumers (SMIRI et al., 2011). A long-lasting activity of Cu or Cd may have indirect effect on photosynthesis, disturb water uptake and water relations in plant tissues (BURZYŃSKI and ŻUREK, 2007). Main sources of Cu and Cd are agriculture production, namely the use fungicides, mineral and organic fertilizers and sewage waste waters (KASTORI, 1993).

The evaluation of metal content in water is a part of routine monitoring of water quality and relies on chemical methods, while risk assessment is based on maximal allowable concentrations-MAC, stipulated by national and/or European Directives. In this work, several directives were consulted, aiming to assess the compliance of established limits (MACs) with the results on bioavailability and effects on plants. MAC for Cu and Cd in water differ depending on its purpose:

- in irrigation water 100 µg/L Cu and 10 µg/L Cd (Regulation on permissible amounts of hazardous and harmful substances in soil and water for irrigation, and methods for their analysis (Official gazette RS 23/94)
- in irrigation water 5.0 µg/L Cd (EC Directive 98/83),
- in surface water of II class 40 µg/L Cu and 1µg/L Cd (ICPDR -The Danube River Basin District Document IC/084)
- in surface water of II class 100 µg/L Cu (Regulation on limit values for pollutants in surface and ground waters and sediments, Official gazette, 50/12) and
- in surface waters of II and III class 0.45-0.6 µg/L Cd (Directive 2008/105/EC of the European Parliament (2008) Official Journal of the European Union, 348/84-348/96)
  - 10.0 µg/L Cd (FAO)

However, the content of Cu and Cd in watercourses often exceeds the abovementioned limits. Therefore, their detection in water is of great importance for water risk assessment and evaluation of bioavailability. During recent years, studies on toxic effects of heavy metals, especially Cd, on crop plants are receiving considerable attention, but also the role of plants as bioindicators of water pollution is gaining in importance. Certain plant species are highly sensitive and respond in physiological and morphological changes, thus are successfully used as test organisms in bioassays (ANKLEY et al., 1993).

The aim of this study was to evaluate the tolerance of barley and white mustard to different levels of Cu and Cd in water, and to determine their potential as bioindicators of water pollution with these metals.

#### **MATERIAL AND METHODS**

The toxic effects of Cu (0.025; 0.05; 0.1; 0.25; 0.5; 1; 2; 5; 10; 50 and 100 µg/L) and Cd (0.01; 0.1; 1; 10; 100 and 200µg/L) were assessed on physiological (germination) and morphological (seedlings root and shoot length, fresh and dry weight) traits of barley (*Horedum vulgare* L.), cultivar Novosadski 525 and white mustard (*Sinapis alba* L.) cultivar Torpedo. Bioassay was carried out according to a standard filter paper method recommended by ISTA Regulations book (International rules for seed testing, 2011) with slight

modifications. White mustard seeds (100 per replication) were placed in Petri dishes (Ø15 cm) on filter paper moistened with 10 ml of test solution. Maize seeds (50 per replication) were placed in plastic boxes (21x15cm) on pleated filter paper moistened with 30 ml of solution. Dishes and boxes were incubated in dark at 25±2 °C for three day (white mustard) i.e. four days (maize). After that period, germination energy was assessed and 10 seedlings per replicate were separated and placed on filter paper lanes (18x30cm) previously moistened with 30ml of test solution. Lanes were rolled up and put in PVC bags and in thermostat together with Petri dishes and boxes. After seven days following assessments were made: germination (%), length of roots and shoots of seedlings from rolls (cm), fresh and dry weights (g) of roots and shoots. The experiment was set in four replicates.

The results were processed with statistical software SPSS 17, using Duncan's multiple range test (F value) for confidence interval 95%.

## RESULTS AND DISCUSSION

The effect of heavy metals on plants is specific, complexed and depends on a number of factors: species, variety, pheno-phase, type of metal, concentrations, exposure etc. (BOGDANOVIĆ et al., 1997). This fact was proved in our work since different effects (inhibitory or stimulating) were achieved, depending on species, tested metal and applied rates. When interpreting these results, one should have in mind the different properties of seed coat i.e. permeability for metal ions, caused by differences in seed morphology between species (WIERZBICKA and OBIDZINSKA, 1998).

### Cu effect on barely and white mustard

The effects of Cu on physiological and morphological traits of barely and white mustard are presented in Tab.1 and 2.

**Barley.** Germination of barely seeds was not affected by Cu, regardless on the applied rate (F=0.79ns, p>0.05). This is in accordance with results presented by MAHMOOD et al. (2005) for *Zea mays* and MULLER et al. (2001) for *Typha latifolia*. However, the opposite results for maize were reported by TOMULESCU et al. (2007).

Root length of seedlings was significantly inhibited at 0.025 µg/L Cu i.e. values lower than MAC, and the reduction of root growth was more prominent with the increase of Cu levels (F=17.32\*\*, p<0.01). Root fresh weight was also significantly reduced at 0.025 µg/L Cu, while the strongest inhibition of both fresh and dry weight was recorded in treatments with 5-100 µg/L Cu (F=31.48\*\*, F=9.12\*\*, p<0.01, respectively). A number of literature data report inhibitory effect of high concentrations of Cu on root and shoot growth indicating at stronger underground parts inhibition compared to upper parts (CHEN et al., 2000; FARGAŠOVA, 2001; MULLER et al. 2001 SINGH et al., MAHMOOD et al. 2005; 2007; MUNZUROGLU and ZENGIN, 2006). But, according to LI et al. (2005) even at low concentrations Cu inhibited growth of *Arabidopsis thaliana* seedlings.

A strong inhibitory effect of Cu on shoot growth was recorded already in treatment with 2µg/L Cu i.e. value lower than MAC, and in all other treatments (F=8.17\*\*, p<0.01). Fresh shoot weight was highly significantly reduced at 2-100 µg/L Cu (F=8.11\*\*, p<0.01), while dry weight was not affected by this metal, regardless on the applied rates (F=0.89ns, p>0.05). AIT ALI et al. (2004) report the reduction in dry root and shoot weight of *Hordeum vulgare* under Cu stress, with root being more sensitive than shoot, which was also proved in this work. CHHOTU and FULEKAR (2008) determined that dry root and shoot weight of *Medicago sativa* increased at lower doses of Cu, while at higher rates (40 and 50 ppm) they

were reduced. According to SINGH et al. (2007) Cu at 5 and 100 mg/L reduced dry roots weight of *Triticum aestivum*.

Barely seedlings responded in morphological changes to Cu when applied at rates lower than MACs. Therefore this species can be considered a good indicator of water pollution with Cu. However, when interpreting the results of samples from actual sites, the possible interactions of water constituents and the total chemistry of sample should not be overlooked.

Tabel 1

Cu effects on physiological and morphological traits of barley

Treatme nt ( $\mu\text{g/l}$ Cu)	Trait						
	Germination (%)	Root length (cm)	Root fresh weight (g)	Root dry weight (g)	Shoot length (cm)	Shoot fresh weight (g)	Shoot dry weight (g)
100	98.50 $\pm$ 0.58 a	7.21 $\pm$ 0.56 f	0.13 $\pm$ 0.06 c	0.05 $\pm$ 0.04 bc	10.11 $\pm$ 0.51 cd	1.03 $\pm$ 0.11 cd	0.09 $\pm$ 0.01 a
50	99.75 $\pm$ 0.50 a	8.23 $\pm$ 0.99 ef	0.16 $\pm$ 0.05 c	0.05 $\pm$ 0.05 bc	10.78 $\pm$ 0.72 bc	1.13 $\pm$ 0.07 bcd	0.11 $\pm$ 0.00 a
10	99.25 $\pm$ 0.96 a	9.14 $\pm$ 1.13 d	0.17 $\pm$ 0.07 c	0.05 $\pm$ 0.02 bc	9.78 $\pm$ 0.38 cd	0.97 $\pm$ 0.07 cd	0.10 $\pm$ 0.01 a
5	98.25 $\pm$ 0.96 a	9.21 $\pm$ 0.69 de	0.14 $\pm$ 0.04 c	0.04 $\pm$ 0.02 bc	9.25 $\pm$ 0.37 d	0.87 $\pm$ 0.05 de	0.09 $\pm$ 0.01 a
2	98.75 $\pm$ 2.50 a	10.35 $\pm$ 1.65 cd	0.36 $\pm$ 0.17 bc	0.05 $\pm$ 0.02 bc	10.55 $\pm$ 2.44 bc	1.14 $\pm$ 0.12 bcd	0.09 $\pm$ 0.01 a
1	99.50 $\pm$ 0.58 a	11.34 $\pm$ 0.63 bc	0.53 $\pm$ 0.08 b	0.07 $\pm$ 0.01 abc	13.25 $\pm$ 1.42 a	1.21 $\pm$ 0.09 ab	0.10 $\pm$ 0.01 a
0.5	99.50 $\pm$ 0.58 a	11.62 $\pm$ 0.60 bc	0.54 $\pm$ 0.03 b	0.07 $\pm$ 0.01 abc	12.87 $\pm$ 0.62 ab	1.23 $\pm$ 0.10 ab	0.10 $\pm$ 0.01 a
0.25	98.75 $\pm$ 0.96 a	12.09 $\pm$ 0.39 b	0.55 $\pm$ 0.05 b	0.07 $\pm$ 0.00 abc	12.38 $\pm$ 0.60 ab	1.19 $\pm$ 0.05 ab	0.09 $\pm$ 0.00 a
0.1	99.00 $\pm$ 0.82 a	11.29 $\pm$ 0.70 bc	0.55 $\pm$ 0.07 b	0.07 $\pm$ 0.01 abc	12.18 $\pm$ 0.51 ab	1.20 $\pm$ 0.07 ab	0.10 $\pm$ 0.01 a
0.05	99.00 $\pm$ 1.41 a	11.33 $\pm$ 0.45 bc	0.46 $\pm$ 0.08 b	0.07 $\pm$ 0.01 abc	12.10 $\pm$ 0.29 ab	1.27 $\pm$ 0.03 ab	0.09 $\pm$ 0.01 a
0.025	99.25 $\pm$ 0.96 a	12.30 $\pm$ 0.80 b	0.49 $\pm$ 0.11 b	0.07 $\pm$ 0.01 abc	12.89 $\pm$ 0.77 ab	1.28 $\pm$ 0.11 ab	0.09 $\pm$ 0.01 a
control	99.75 $\pm$ 0.50 a	14.12 $\pm$ 0.46 a	0.72 $\pm$ 0.05 a	0.07 $\pm$ 0.00 a	13.32 $\pm$ 0.44 a	1.37 $\pm$ 0.10 a	0.09 $\pm$ 0.01 a
<b>F value</b>	<b>0.79ns</b>	<b>17.32**</b>	<b>31.48**</b>	<b>9.12**</b>	<b>8.17**</b>	<b>8.11**</b>	<b>0.89ns</b>

**White mustard.** Germination of white mustard was highly significantly inhibited in treatments with 5 to 100  $\mu\text{g/L}$  Cu (MAC values and higher), compared to the control ( $F=5.18^{**}$ ,  $p<0.01$ ). This is in accordance with reports of several authors indicating at inhibition of seeds germination by heavy metals. SINGH et al. (2007) noted inhibition of wheat germination under Cu, but also a slight stimulating effect at 5mg/L. According to CHHOTU and FULEKAR (2008) germination of *Medicago sativa* seed was inhibited at 40 and 50 ppm Cu, while at 5 and 10 ppm Cu it was stimulated. The reduction of seed germination when treated with Cu was also recorded for *Oryza sativa*, *Hordeum vulgare*, *Triticum aestivum* and *Lycopersicum esculentum*, (PERALTA et al., 2001; HAMID et al., 2001; AIT ALI et al., 2003; ASHAN et al., 2007; SINGH et al., 2007). As reported by GUPTA and MUKHERJI (1977) even very low concentrations of Cu stopped germination of rice seeds completely, while according to AYAZ and KADIOGLU (1997), higher concentrations of copper, mercury and cadmium decreased the germination of lentil seeds gradually.

In treatments with 2, 50 and 100 µg/L Cu, root length of white mustard seedlings was inhibited ( $F=3.45^{**}$ ,  $p<0.01$ ), but a slight stimulation was recorded in treatments with 5 and 10 µg/L Cu. Fresh root weight was significantly inhibited at rates exceeding 1 µg/L Cu ( $F=6.72^{**}$ ,  $p<0.01$ ), while dry weight was not under the influence of Cu, regardless on the applied rates ( $F=3.40^*$ ,  $p<0.05$ ). Results obtained in this work are in consistence with JIANG et al. (2001) indicating that *Zea mays* root growth was stimulated by Cu at  $10^{-5}M$ , while high concentrations ( $10^{-4}$  and  $10^{-2}$ ) inhibited it. Also CHHOTU and FULEKAR (2008) determined that lower doses of Cu (5 and 10 ppm) stimulated root and shoot growth of *Medicago sativa*, while higher (20, 40 and 50 ppm) inhibited these traits.

Cu did not affect shoot length of white mustard seedlings, regardless on the applied rates ( $F=2.24^*$ ,  $p<0.05$ ), but significant reduction of shoots fresh weight was recorded in treatments with 0.1-100 µg/L Cu ( $F=4.12^{**}$ ,  $p<0.01$ ), while dry weight was also not affected by this metal. ( $F=0.63ns$ ,  $p>0.05$ ).

White mustard responded in both physiological and morphological changes to Cu presence in water even at rates lower than MAC thus can be considered a good indicator of water pollution with Cu.

Tabel 2

Cu effects on physiological and morphological traits of white mustard

Treatment (µg/l Cu)	Trait						
	Germination (%)	Root length (cm)	Root fresh weight (g)	Root dry weight (g)	Shoot length (cm)	Shoot fresh weight (g)	Shoot dry weight (g)
100	75.50 ± 5.44 c	3.01 ± 0.28 c	0.16 ± 0.03 de	0.02 ± 0.01 ab	4.39 ± 0.38 a	0.003 ± 0.01 c	0.003 ± 0.01 a
50	75.75 ± 2.21 c	3.07 ± 1.05 c	0.15 ± 0.06 de	0.02 ± 0.03 ab	3.28 ± 0.87 b	0.004 ± 0.02 c	0.003 ± 0.01 a
10	81.00 ± 3.16 bc	5.06 ± 1.30 a	0.17 ± 0.06 de	0.02 ± 0.03 ab	4.15 ± 0.91 ab	0.006 ± 0.01 c	0.004 ± 0.01 a
5	81.25 ± 1.25 bc	5.21 ± 1.28 a	0.12 ± 0.02 e	0.02 ± 0.02 ab	3.89 ± 0.82 ab	0.005 ± 0.03 c	0.005 ± 0.03 a
2	83.75 ± 5.12 ab	3.19 ± 1.09 c	0.07 ± 0.08 e	0.02 ± 0.04 ab	4.02 ± 0.36 ab	0.004 ± 0.02 c	0.003 ± 0.01 a
1	84.25 ± 2.87 ab	3.67 ± 0.44 bc	0.09 ± 0.06 e	0.01 ± 0.07 b	3.31 ± 0.40 b	0.004 ± 0.03 c	0.006 ± 0.01 a
0.5	85.50 ± 2.38 ab	3.46 ± 1.20 bc	0.25 ± 0.04 bcd	0.02 ± 0.01 ab	3.70 ± 0.71 ab	0.006 ± 0.01 c	0.003 ± 0.01 a
0.25	85.25 ± 3.09 ab	3.42 ± 0.50 bc	0.25 ± 0.09 bcd	0.01 ± 0.04 b	3.62 ± 0.50 ab	0.009 ± 0.01 bc	0.002 ± 0.01 a
0.1	84.25 ± 3.86 ab	3.38 ± 0.52 bc	0.26 ± 0.08 abcd	0.01 ± 0.02 b	3.80 ± 0.27 ab	0.009 ± 0.01 bc	0.002 ± 0.02 a
0.05	87.75 ± 6.18 a	3.92 ± 1.15 b	0.35 ± 0.10 ab	0.02 ± 0.04 ab	3.43 ± 0.77 b	0.03 ± 0.01 a	0.003 ± 0.01 a
0.025	88.00 ± 2.94 a	4.04 ± 1.64 ab	0.30 ± 0.07 abc	0.02 ± 0.03 ab	3.00 ± 0.57 b	0.03 ± 0.01 a	0.003 ± 0.01 a
control	88.50 ± 4.04 a	4.39 ± 0.71 ab	0.28 ± 0.09 abc	0.02 ± 0.02 ab	3.75 ± 0.49 ab	0.03 ± 0.02 a	0.002 ± 0.05 a
<b>F value</b>	<b>5.18**</b>	<b>3.45**</b>	<b>6.72**</b>	<b>3.40*</b>	<b>2.24*</b>	<b>4.12**</b>	<b>0.63ns</b>

### Cd effects on barely and white mustard

The effects of Cd on physiological and morphological traits of barely and white mustard are presented in Tab. 3 and 4.

**Barley.** Germination of barely seeds was not under the influence of different Cd levels (F=0.12ns, p>0.05), which is partially in accordance with the results of several authors (AYCICEK et al., 2008, SMIRI, 2011) confirming that this physiological trait is rarely affected by heavy metals, including Cd.

Root length of barely seedlings was highly significantly inhibited by Cd at 100 and 200µg/L, but those values exceed all mentioned MAC more than 100fold (F=12.45\*\*, p<0.01). Fresh and dry weights of roots were not affected by Cd regardless on the applied rates (F=1.24ns, 0.44ns, p>0.05). Findings of MUNZUROGLU and ZENGIN (2006) show that Cd inhibited barley seedlings root growth, but at much lower rates (>5.46 µg/L Cd) than in our work and even ceased it completely at 3mM (16.38 µg/L).

Shoot length of barely seedlings was, like roots, significantly inhibited by Cd at 100 and 200µg/L (F=6.61\*, p>0.01), while fresh and dry weights were not affected by Cd, regardless on the applied rates (F=1.47ns, 1.88ns, p>0.05). Previous research of THAMAYANTHI et al. (2011) showed that when present in soil in amounts higher than 10 mg/kg, Cd significantly inhibited root and shoot growth.

Based on the obtained results, it can be concluded that barely is very tolerant to high levels of Cd in water and does not have the potential to be used as an indicator of water pollution with this heavy metal, because it responded to Cd presence in water at rates exceeding MACs.

Tabel 3

Cd effects on physiological and morphological traits of barley

Treatment (µg/l Cd)	Trait						
	Germination (%)	Root length (cm)	Root fresh weight (g)	Root dry weight (g)	Shoot length (cm)	Shoot fresh weight (g)	Shoot dry weight (g)
200	98.50 ±0.58 a	6.75 ±1.40 b	0.51 ±0.44 a	0.07 ±0.02 a	11.31 ±0.77 b	0.87 ±0.39 a	0.08 ±0.01 a
100	98.75 ±0.96 a	8.33 ±0.44 b	0.54 ±0.10 a	0.07 ±0.01 a	11.89 ±1.02 b	1.09 ±0.12 a	0.11 ±0.01 a
10	98.25 ±1.71 a	11.05 ±2.01 a	0.56 ±0.08 a	0.06 ±0.00 a	14.35 ±0.30 a	1.22 ±0.06 a	0.10 ±0.01 a
1	98.50 ±1.29 a	11.33 ±1.66 a	0.75 ±0.59 a	0.07 ±0.03 a	13.17 ±0.19 a	0.79 ±0.40 a	0.08 ±0.01 a
0.1	98.50 ±0.58 a	12.50 ±1.66 a	0.65 ±0.11 a	0.07 ±0.00 a	13.35 ±0.54 a	1.11 ±0.03 a	0.08 ±0.02 a
0.01	98.75 ±0.96 a	12.84 ±0.45 a	0.66 ±0.42 a	0.08 ±0.01 a	13.46 ±1.69 a	0.79 ±0.51 a	0.08 ±0.02 a
control	98.75 ±1.26 a	13.24 ±1.21 a	0.83 ±0.43 a	0.08 ±0.01 a	14.03 ±0.47 a	0.79 ±0.51 a	0.08 ±0.02 a
<b>F value</b>	<b>0.12ns</b>	<b>12.45**</b>	<b>1.24ns</b>	<b>0.44ns</b>	<b>6.61**</b>	<b>1.47ns</b>	<b>1.88ns</b>

**White mustard.** Germination of white mustard seeds was inhibited by Cd at 1 µg/L (F=3.00\*, p>0.01), value exceeding MACs. MUNZUROGLU and ZENGIN (2006) have reached the similar conclusions indicating that germination was decreased proportionally with increasing concentrations of Cd ranging from 0.5mM-8.5mM (2.73 µg/L-46.41 µg/L).

At 200 µg/L Cd significantly inhibited root length of white mustard (F=9.14\*, p>0.01), while root fresh and dry weight were not under the influence of Cd, regardless on the applied rate (F=1.83ns, 1.26ns, p>0.05). According to AYCICEK (2008) Cd applications in irrigation water decreased dry root weight of cotton plants.

Shoot growth was significantly stimulated in treatments ranging from 10-200 µg/L Cd compared to the control (F=5.39\*\*, p<0.01), while fresh and dry shoot weights were not under the influence of this metal (F=0.51ns, 1.72ns, p>0.05).

The overall results indicate that physiological trait-germination of white mustard seeds can be considered as valid indicator of water pollution with Cd. However, regarding the morphological traits, this species has poor indicators potential in detection of water pollution with Cd, since it responded to Cd presence in water at rates exceeding MAC.

Tabel 4

Cd effects on physiological and morphological traits of white mustard

Treatment (µg/l Cd)	Trait						
	Germination (%)	Root length (cm)	Root fresh weight (g)	Root dry weight (g)	Shoot length (cm)	Shoot fresh weight (g)	Shoot dry weight (g)
200	75.00 ±6.98 b	3.55 ±0.77 c	0.35 ±0.12 ab	0.03 ±0.00 a	5.21 ±0.46 a	0.01 ±0.00 a	0.01 ±0.00 a
100	77.75 ±3.40 b	5.26 ±0.87 b	0.31 ±0.13 ab	0.02 ±0.00 a	5.31 ±0.21 a	0.01 ±0.01 a	0.01 ±0.00 a
10	77.00 ±5.53 b	5.72 ±0.66 b	0.43 ±0.07 a	0.03 ±0.01 a	5.37 ±0.35 a	0.02 ±0.00 a	0.02 ±0.00 a
1	79.00 ±7.11 b	6.87 ±0.33 a	0.27 ±0.11 b	0.03 ±0.01 a	4.46 ±0.42 cd	0.02 ±0.02 a	0.01 ±0.00 a
0.1	84.25 ±7.99 ab	6.21 ±0.54 ab	0.35 ±0.09 ab	0.02 ±0.00 a	4.35 ±0.42 cd	0.02 ±0.02 a	0.01 ±0.02 a
0.01	88.00 ±3.65 a	6.09 ±0.75 ab	0.43 ±0.10 a	0.03 ±0.01 a	3.87 ±0.86 d	0.01 ±0.00 a	0.01 ±0.00 a
control	88.50 ±5.32 a	5.32 ±0.80 b	0.41 ±0.07 ab	0.03 ±0.00 a	4.55 ±0.30 bcd	0.01 ±0.00 a	0.01 ±0.00 a
<b>F value</b>	<b>3.00*</b>	<b>9.14**</b>	<b>1.83ns</b>	<b>1.26ns</b>	<b>5.39**</b>	<b>0.51ns</b>	<b>1.72ns</b>

### CONCLUSION

The biological effect of Cu and Cd differed depending on plants species, tested metal and applied rates.

Barely and white mustard expressed good indicators potential for detection of Cu presence in water at rates lower than MACs.

Barely was very tolerant to Cd in water at rates exceeding MAC, thus is not good indicator of water pollution with this heavy metal.

Physiological trait i.e. germination of white mustard seeds is valid indicator of water pollution with Cd, while morphological traits are poor indicators of water pollution with this heavy metal.

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