GERMINATION ENERGY AND SEED GERMINATION - VALID PARAMETERS OF HEAVY METAL PRESENCE IN WATER

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Abstract
The use of water polluted with heavy metals for irrigation can cause phytotoxic effects and affect crop production. An important step in mitigating possible negative effects is certainly a continuous monitoring of water quality, assessment of risk for crops by biotests, right choice of test plant and selection of parameters that reliably indicate the changes in the environment. The aim of this study was to assess indicators potential of germination energy (GE) and seed germination (G) of five cultivated plants (sorghum, cabbage, sunflower, beans and buckwheat) in detection of metal (Cr, Pb and Cd) presence in water. A filter paper method according to ISTA was used. Metals were applied in series of concentrations including maxmium allowable concentrations (MAC's). Chromium significantly inhibited G and GE of sunflower seeds in treatments with 2000 μg/l, while lead caused such effects of GE and G of sorghum and sunflower in amounts exceeding 50 μg/l (two times less than MAC), and of cabbage and buckwheat at 200μg/l. Cadmium significantly inhibited GE and G of beans at 0.1 μg/l (100 fold less than MAC).The overall results suggest that GE and G can be considered as valid parameters for the detection of certain metal presence in water, in amounts exceeding MAC such as Pb (sorghum and sunflower seeds) and Cd (beans seeds).

Key words: germination energy, germination, seeds, metals, crops, phytoindicators

Introduction
Contamination of irrigation water is a pronounced problem in agricultural and industrial regions. Generally the use of such water can cause phytotoxic effects and affect crop production, due to high levels of pollutants, especially metals. To mitigate negative consequences it is necessary to carry out continuous monitoring of water quality and assess the risk to crops. An important step is certainly a right choice of test organism and selection of parameters that reliably indicate changes in water. It is familiar that some plants are very sensitive to high content of pesticides, heavy metals and organic substances in soil and water. They react with different morphological and physiological changes and are successfully used as bioindicators of water contamination, as test plants in bioassays for contamination detection and in assessment of ecotoxicological risks [1; 2; 3]. Most of the standardized phyto-toxicity tests and a number of authors point out germination as a valid parameter which indicates changes in water quality [4; 5; 6]. It has advantages such as ease of implementation and assessment, low cost and a convenience for wide range of substances and large number of samples. The aim of this study was to assess indicators potential of germination energy and seed germination of five cultivated plant species (sorghum, cabbage, sunflower, beans and buckwheat) in detection of metal (Cr, Pb and Cd) presence in water.

Materials and methods
In bioassay, the folowing test species were chosen: sorghum (*Sorghum bicolor* L.), cabbage (*Brassica oleracea* var.capitata), sunflower (*Heliantus anuus* L.), beans (*Phasolus vulgaris* L.)

and buckwheat (*Fagopyrum esculentum* Moench). The effect of water quality was evaluated according to changes in germination energy –GE and seed germination – G (%) of tested species, in bioassay which was carried out according to a standard filter paper method described by ISTA (2011), with slight modifications. Heavy metals were applied at following rates, including the MAC in [7], [8] and [9]Cr (100-4000 µg/l); Pb (1-200 µg/l) and Cd (0.01-200 µg/l).Data were analyzed using Duncan's multiple range test, for 95% confidence interval, in software SPSS version 17.0.

Results and discussions

In bioassay, seeds of test plants responded in inhibition or stimulation of GE and G, depending of the metal and applied rate. This is in accordance with [10], indicating that the sensitivity of plants to certain contaminants (e.g. heavy metals) in water depends on the concentration and the type of pollutants, but also on the development stage (germination, emergence, vegetative growth). Significant inhibition of sunflower seeds G was recorded in treatments with 2000 µg/l of chromium. However, this metal stimulated GE and G of beans seeds at rates 200-2000 µg/l (Tab. 1). Given indicates that GE and G of tested species are not valid indicators of Cr water contamination. In literature, the presented results differ. [11] showed that germination of maize and rice was inhibited with the distillery effluent containing high amounts of heavy metals (Cd, Cr, Ni and Zn). [12] reported that Cr in amount 50-250 g/l did not affect germination of sunflower seeds, but it delayed the time of germination. According to [13] Cr stimulated germination of buckwheat seeds at 40-160 ppm.

Tab. 1 Germination energy and germination of test plants depending on the applied rate of Cr

Parametar	Rate (µg/l)	Sorghum	Cabbage	Beans	Sunflower	Buckwheat
Germination energy (%)	4000	98.50 ±0.50 a	97.25 ±0.25 a	96.00 ±1.00 b	95.50 ±0.50 b	98.00 ±0.00 a
	2000	99.00 ±1.00 a	98.00 ±1.00 a	97.75 ±0.75 a	95.50 ±1.50 b	98.00 ±1.00 a
	1000	99.00 ±1.00 a	99.25 ±0.25 a	98.50 ±0.50 a	97.50 ±0.50 ab	98.00 ±1.00 a
	500*	99.50 ±0.50 a	99.00 ±0.00 a	98.75 ±0.75 a	97.50 ±0.50 ab	98.50 ±0.50 a
	200	99.50 ±0.50 a	99.75 ±0.75 a	95.75 ±0.75 a	97.25 ±0.75 ab	98.50 ±0.50 a
	100	99.75 ±0.75 a	99.50 ±0.50 a	96.50 ±0.50 ab	97.75 ±1.75 ab	98.50 ±0.50 a
	control	99.00 ±1.00 a	99.75 ±0.75 a	96.00 ±1.00 b	99.75 ±0.75 a	99.00 ±1.00 a
	F value	**0.95ns**	**5.17ns**	**6.69***	**10.61****	**0.74ns**
Germination (%)	4000	98.50 ±0.50 a	97.25 ±0.25 a	96.00 ±0.00 a	95.50 ±0.50 b	98.00 ±1.00 a
	2000	99.00 ±0.00 a	99.00 ±1.00 a	97.75 ±0.75 a	95.50 ±1.50 b	98.50 ±0.50 a
	1000	99.00 ±0.00 a	98.00 ±0.00 a	98.50 ±0.50 a	97.50 ±0.50 ab	98.50 ±0.50 a
	500*	99.50 ±0.50 a	99.25 ±0.25 a	98.75 ±0.50 a	98.00 ±0.00 a	98.50 ±0.50 a
	200	99.50 ±0.50 a	100.00 ±0.00 a	98.75 ±0.75 a	97.50 ±0.50 ab	98.50 ±1.50 a
	100	99.75 ±0.75 a	99.75 ±0.75 a	97.75 ±0.75 a	98.00 ±1.00 a	98.75 ±0.75 a
	control	99.50 ±0.50 a	100.00 ±0.00 a	97.25 ±0.25 a	99.75 ±0.75 a	99.50 ±0.50 a
	F value	**0.95ns**	**8.08ns**	**3.47ns**	**14.36****	**1.14ns**

Mean values ±SD; Values with the same letter in the column are on the same level of significance; **p<0.01;* p<0.05; ns p>0.05; Bold values in Rate column are MAC values according to the [9]*

Lead significantly inhibited GE and G of sorghum and sunflower seeds at rates exceeding 50 µg/l (2 fold less than MAC) and of cabbage at 200 µg/l. Given that MAC according to [8] is 10 µg/l and [9] is 100 µg/l, GE and G of sorghum and sunflower seeds can be considered valid parameters in Pb contamination detection (Tab. 2). [14] reported significant inhibition of sunflower seeds germination by Pb at 40 and 50 ppm in water i.e. 50 and 60 ppm in soils. [15] also point out that Pb negatively affects seeds germination.

Tab. 2 Germination energy and germination of test plants depending on the applied rate of Pb

Parametar	Rate (µg/l)	Sorghum	Cabbage	Beans	Sunflower	Buckwheat
Germination energy (%)	200	94.50 ±0.50 b	83.50 ±0.50 b	96.50 ±2.50 a	78.25 ±0.25 b	96.00 ±1.00 a
	100**	95.00 ±2.00 b	88.50 ±0.50 a	97.50 ±1.50 a	79.00 ±1.00 b	97.00 ±0.50 a
	50	95.00 ±0.00 b	91.75 ±0.75 a	97.75 ±0.75 a	78.25 ±0.25 b	97.00 ±1.00 a
	10*	96.00 ±1.00 ab	92.25 ±0.25 a	98.00 ±1.00 a	84.50 ±0.50 ab	98.50 ±0.50 a
	5	97.25 ±0.25 a	92.00 ±0.00 a	97.75 ±1.75 a	89.50 ±0.50 a	99.00 ±2.00 a
	1	98.00 ±1.00 a	92.00 ±1.00 a	98.00 ±0.00 a	88.25 ±0.25 a	98.75 ±1.75 a
	control	98.75 ±0.75 a	93.75 ±0.75 a	98.25 ±1.25 a	90.25 ±0.25 a	98.75 ±0.75 a
	F value	**4.49****	**7.06****	**0.37ns**	**9.97****	**3.13ns**
Germination (%)	200	94.50 ±0.50 b	83.50 ±0.50 b	96.50 ±2.50 a	78.25 ±0.25 b	96.00 ±1.00 a
	100**	95.00 ±0.00 ab	88.50 ±0.50 ab	97.50 ±1.50 a	79.00 ±1.00 b	97.00 ±0.50 a
	50	95.00 ±1.00 ab	92.00 ±0.00 a	97.75 ±0.75 a	78.25 ±0.25 b	97.00 ±1.00 a
	10*	96.00 ±0.00 ab	92.25 ±0.25 a	98.00 ±1.00 a	84.50 ±0.50 ab	98.50 ±0.50 a
	5	98.00 ±1.00 a	93.00 ±0.00 a	97.75 ±1.75 a	89.50 ±0.50 a	99.00 ±2.00 a
	1	98.75 ±0.75 a	93.50 ±0.50 a	98.00 ±0.00 a	88.25 ±0.25 a	98.75 ±1.75 a
	control	98.75 ±1.75 a	95.00 ±0.00 a	98.25 ±1.25 a	90.25 ±0.25 a	98.75 ±0.75 a
	F value	**6.68****	**8.64****	**0.37ns**	**9.97****	**3.13ns**

Mean values ±SD; Values with the same letter in the column are on the same level of significance; **p<0.01;* p<0.05; ns p>0.05; Bold values in Rate column are MAC values according to [8]* or [9]**.

Cadmium significantly inhibited GE and G of bean seeds at 0.1 µg/l (less than MAC), of sunflower at 100-200 µg/l and of buckwheat seeds at 200 µg/l. According to these results only GE and G of bean seeds are valid parameters in detection of Cd presence in water in amounts exceeding MAC (Tab. 3). Several authors indicate that Cd inhibits seeds germination of a number of plant species [16, 17]. [18] reported that germination of sorghum seeds is not sensitive parameter for Cd toxicity, while [19] point out germination as the most suitable for Cd toxicity detection.

Tab 3. Germination energy and germination of test plants depending on the applied rate of Cd

Parametar	Rate (µg/l)	Sorghum	Cabbage	Beans	Sunflower	Buckwheat
Germination energy (%)	200	93.00 ±2.00 a	95.00 ±2.00 a	50.00 ±1.00 d	80.00 ±1.00 c	92.00 ±2.00 b
	100	93.00 ±1.00 a	96.00 ±1.00 a	91.00 ±0.50 c	92.00 ±2.00 b	96.00 ±1.00 a
	10**	94.00 ±1.00 a	96.00 ±1.00 a	92.00 ±0.00 bc	95.00 ±1.00 ab	96.00 ±1.00 a
	1	94.00 ±0.00 a	96.00 ±1.00 a	92.00 ±2.00 bc	96.00 ±0.00 ab	97.00 ±0.50 a
	0.1	95.00 ±0.00 a	96.50 ±0.50 a	92.00 ±2.00 bc	97.00 ±1.00 ab	98.50 ±0.50 a
	0.01	95.00 ±1.00 a	96.00 ±0.00 a	97.00 ±1.00 a	99.00 ±1.00 a	99.50 ±0.50 a
	control	95.00 ±2.00 a	96.50 ±0.50 a	98.00 ±1.00 a	99.00 ±1.00 a	99.00 ±1.00 a
	F value	**1.20ns**	**0.97ns**	**146.04****	**32.71****	**14.43****
Germination (%)	200	93.00 ±2.00 a	95.00 ±1.00 a	78.00 ±0.00 c	82.00 ±0.00 d	95.00 ±2.00 b
	100	93.00 ±1.00 a	96.00 ±0.00 a	91.00 ±2.00 b	92.00 ±2.00 c	96.00 ±1.00 ab
	10**	94.00 ±1.00 a	96.00 ±1.00 a	92.00 ±2.00 b	95.00 ±1.00 b	96.00 ±1.00 ab
	1	94.00 ±0.00 a	97.00 ±1.00 a	92.00 ±2.00 b	96.00 ±0.00 ab	97.00 ±0.00 a
	0.1	95.00 ±0.00 a	97.00 ±1.00 a	92.00 ±2.00 b	97.00 ±1.00 ab	99.00 ±0.00 a
	0.01	95.00 ±1.00 a	97.00 ±2.00 a	97.50 ±1.50 a	99.00 ±1.00 a	99.00 ±1.00 a
	control	95.00 ±2.00 a	97.00 ±0.00 a	98.00 ±1.00 a	99.00 ±1.00 a	99.00 ±0.00 a
	F value	**1.20ns**	**3.25ns**	**26.85****	**27.13****	**8.68****

Mean values ±SD; Values with the same letter in column are on the same level of significance; **p<0.01;* p<0.05; ns p>0.05; Bold values in Rate column are MAC values according to [9]**

Conclusions

Based on the obtained results, Cr significantly inhibited G of sunflower seeds in treatments with 2000 µg/L but stimulated GE and G of beans at 200-2000 µg/l; Pb significantly inhibited GE and G of sorghum and sunflower at 50 µg/l (2 fold less than MAC) and higher rates, of cabbage and buckwheat at 200 µg/l; Cd significantly inhibited GE and G of beans at 0.1 µg/l (less than MAC), of sunflower at rates 100-200 μg/l and of buckwheat seeds at 200 μg/l. The overall results suggest that GE and G can be considered as valid parameters for the detection of certain metal presence in water, in amounts exceeding MAC: sorghum and sunflower seeds for Pb and beans seeds for Cd.

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