GERMINATION ENERGY AND SEED GERMINATION OF CULTIVATED PLANTS AS INDICATORS OF WATER CONTAMINATION WITH NICKEL

Sonja Gvozdenac[1], Vojislava Bursić[2], Martina Mezei[2], Jelena Ovuka[1], Mladen Tatić[1], Dejan Prvulović[2]

[1]Institute of Field and Vegetable Crops, Maksima Gorkog 30, Novi Sad, Serbia
[2]University of Novi Sad, Faculty of Agriculture, Trg Dositeja Obradovića 8, Novi Sad, Serbia
sonja.gvozdenac@ifvcns.ns.ac.rs

Abstract

Heavy metal pollution of water ecosystems is currently one of the major environmental problems. Nickel (Ni^{+2}) is a metal that usually occurs in the environment at very low levels. However, its presence in water at higher concentrations is becoming more frequent, as a result of antropogenic activity. The majority of cultivated plants do not tolerate high concentrations of nickel in the environment. Therefore, its detection in water is of great importance for irrigation water risk assessment. The aim of this study was to evaluate the tolerance of sunflower, maize, buckwheat, cabbage and white mustard seeds to different levels of nickel in water and assess their potential as bioindicators of water contamination with this heavy metal. Bioassay was carried out according to a standard filter paper method, and the results were processed with statistical software SPSS 17, using Duncan's multiple range test. The effect of nickel on GE and G differed depending on plant species and the applied concentration. GE and G of sunflower seeds were significantly inhibited by Ni^{+2} when applied at MAC rate for irrigation water (100μg/l), while maize and buckwheat seeds were tolerant to MAC rates of this metal. Ni^{+2} stimulated GE and G of cabbage seeds at MAC for irrigation water (100μg/l) and G of white mustard seeds at MAC rate for II class of water (ICPDR - 50μg/l).

Key words: nickel, seed, germination, phytotoxicity, bioindicatiors

Introduction

Water pollution with heavy metals is one of the major environmental problems, because their uncontrolled release has led to accumulation in aquatic systems [1,2]. Nickel (Ni^{+2}) is a metal that occurs in the environment at very low levels. Maximal allowable amount (MAC-EQS) in surface waters according to ICPDR (The Danube River Basin District Document IC/084) is 50μg/l for II class of water and according to Regulation on allowable amounts of dangerous and harmful substances in soil and irrigation water and methods of analysis (Off. Gazette 23/94) is 100μg/l. However, due to antropogenic activities, it is more often present at higher concentrations in water bodies and often exceeds the abovementioned limits.Although Ni^{+2} is essential element in plants, the higher levels can cause phytotoxic symptoms to sensitive species. There are more than 300 taxa of Ni^{+2} hyperaccumulators, i.e. plants which accumulate more than 1000 mg Ni kg-1 of dry weight in their shoots. There are also plants called hypernickelophores, which accumulate >10,000 mg N kg-1 (*Psychotria douarrei, Geissois pruinosa, Alyssum murale*) and can be developed as a commercial crop for phytoremediation/phytomining of Ni^{+2} from metal-enriched soils [3,4]. However, the majority of cultivated plants do not tolerate high concentrations of Ni^{+2} in the environment. Thus, its detection in water is of great importance for irrigation water risk assessment. Certain plant species are highly sensitive and react with physiological and morphological changes and are successfully used as test organisms in bioassays for contamination detection [5].

The aim of this study was to evaluate the tolerance of sunflower, maize, buckwheat, cabbage and white mustard seeds, to different levels of Ni^{+2} in water and determine their potential as bioindicators of water contamination with this heavy metal.

Material and methods

Bioassay was carried out according to a standard filter paper method recommended by [6] and [7]. The toxic effects of nickel, applied at rates: 25, 50, 100, 250, 500, 750 and 1000µg/l, was assessed according to changes in germination energy - GE (%) and seed germination – G (%) of sunflower, maize, buckwheat, cabbage and white mustard.

For germination assessment, 50 sunflower and maize and 100 cabbage seeds were placed in plastic boxes on pleated filter paper, previously moistened with 25 mL of Ni^{+2} solution, while 100 seeds of buckwheat and white mustard were placed in Petri dishes, also on filter paper, but moistened with 10 mL of Ni^{+2} solution. Distilled water was the control. Seeds were incubated in dark at 25 ±2 °C and after three (white mustard) or four days (sunflower, maize, cabbage, buckwheat) GE was recorded. After seven (maize, buckwheat and white mustard) and ten (sunflower and cabbage) days G was recorded. The experiment was set in four replicates.

The values for GE and G were transformed in arcsin√% prior to statistical analysis. The results were processed with statistical software SPSS 17, using Duncan's multiple range test (F value) for confidence interval 95%.

Results

The effect of nickel on GE and G differed depending on plant species and the applied concentration. The results are presented in Table 1.

GE and G of sunflower seeds were statistically significantly inhibited by Ni^{+2} when applied at MAC rate for irrigation water (100µg/l) and at higher concentrations ($F=10.51^{**}$; 10.51^{**}, $p<0.01$, respectively). However, Ni^{+2} did not affect GE and G of maize, regardless on the applied rate and all values were on the same level of significance with the control ($F=1.85$ns; 1.85ns, $p>0.05$, respectively). Mentioned parameters of buckwheat seeds were significantly inhibited by Ni^{+2} at 750-1000µg/l ($F=12.96^{**}$; 13.44^{**}, $p<0.01$, respectively), but when applied at MAC rate, did not express effect on GE and G. Ni^{+2} stimulated GE and G of cabbage seeds at MAC for irrigation water (100 µg/l), and the difference between treatments were statistically highly significant ($F=19.12^{**}$, 19.12^{**}, $p<0.01$, respectively). G of white mustard seeds was also significantly stimulated by Ni^{+2} applied at MAC rate for irrigation water (100µg/l), while at other rates, the values of GE and G were on the same level with the control ($F=5.38^{**}$, $p<0.01$, respectively).

A number of standardized phytotoxicity tests point out germination, as a parameter that reliably indicates at changes in water quality [8, 9, 10,]. Also, according to [11] germination is fast indicative parameter for phytotoxicity, which was proven in this work for seeds of sunflower, buckwheat, cabbage and white mustard, because G of mentioned seeds was influenced by Ni^{+2}. Nickel is toxic to most plant species, because it is affecting amylase, protease and ribonuclease activity, causing the inhibition of seed germination and growth of many crops [12]. It has been reported to affect the digestion and mobilization of food reserves like proteins and carbohydrates in germinating seeds. [13] confirmed that high concentrations of this metal can cause changes in seed metabolic activity and inhibit germination, which was proven in the case of sunflower and buckwheat seeds in this work. According to [14], Ni^{+2} in amount of 60 mg/l reduces the physiological parameters and the inhibition is stronger with the increase of concentration. Ni^{+2} toxic effects on seeds are a result of disturbed activity of amylase, protease and ribonuclease, which is manifested as a delaying of seed germination [15]. According to [16], low concentration (10 and 20 mg L-1) of nickel significantly promoted seed germination and improved early seedling growth, while higher levels (40, 20 and 60 mg L-1) caused a significant inhibition in germination and resulted in a considerable delay to achieve 50% germination.

Table 1. The effect of Ni^{+2} on germination energy and seed germination of cultivated plants

Plant species	**Ni (µg/l)**	**Germination energy (%)**		**Germination (%)**	
		%	arcsin√%	%	arcsin√%
Sunflower	1000	92.0	73.6 ±0.30 c	92.0	73.6 ±0.30 c
	750	90.0	71.6 ±2.00 c	90.0	71.6 ±2.00 c
	500	96.0	78.6 ±2.00 ab	96.0	78.6 ±2.00 ab
	250	96.0	78.5 ±0.00 ab	96.0	78.5 ±0.00 ab
	100[2]	93.0	74.7 ±0.70 bc	93.0	74.7 ±0.70 bc
	50[1]	98.0	82.0 ±1.00 a	98.0	82.0 ±1.00 a
	25	98.0	82.0 ±0.00 a	98.0	82.0 ±0.00 a
	control	96.0	78.5 ±0.00 ab	96.0	78.5 ±0.00 ab
	F value		**10.51****		**10.51****
Maize	1000	100	90.0 ±0.00 a	100	90.0 ±0.00 a
	750	100	90.0 ±0.00 a	100	90.0 ±0.00 a
	500	99.5	88.0 ±1.00 a	99.5	88.0 ±1.00 a
	250	99.5	88.0 ±1.00 a	99.5	88.0 ±1.00 a
	100[2]	100	90.0 ±0.00 a	100	90.0 ±0.00 a
	50[1]	99.5	88.0 ±1.00 a	99.5	88.0 ±1.00 a
	25	99.5	88.0 ±1.00 a	99.5	88.0 ±1.00 a
	control	98.0	83.1 ±1.00 a	98.0	83.1 ±1.00 a
	F value		**1.85ns**		**1.85ns**
Buckwheat	1000	86	68.1 ±0.00 b	86.0	68.1 ±0.00 b
	750	88.5	70.7 ±1.50 b	88.5	70.7 ±1.50 b
	500	94	75.9 ±0.00 ab	95.0	77.1 ±0.00 ab
	250	98	82.0 ±0.00 a	98.0	82.0 ±0.00 a
	100[2]	98.5	83.1 ±0.50 a	98.5	83.1 ±0.50 a
	50[1]	98	82.0 ±1.00 a	98.0	82.0 ±1.00 a
	25	98.5	83.1 ±0.50 a	98.5	83.1 ±0.50 a
	control	98.5	83.1 ±0.00 a	99.0	85.9 ±0.00 a
	F value		**12.96****		**13.44****
Cabbage	1000	78.0	62.0 ±1.00 c	78.0	62.0 ±1.00 c
	750	98.7	84.5 ±0.70 a	98.7	84.5 ±0.70 a
	500	98.0	82.0 ±0.00 a	98.0	82.0 ±0.00 a
	250	98.7	84.5 ±1.60 a	98.7	84.5 ±1.70 a
	100[2]	98.0	82.0 ±0.00 a	98.0	82.0 ±0.00 a
	50[1]	94.0	75.9 ±0.00 b	94.0	75.9 ±0.00 b
	25	93.5	75.4 ±0.50 b	93.5	75.4 ±0.50 b
	control	94.5	76.4 ±0.50 b	94.5	76.4 ±0.50 b
	F value		**19.12****		**19.12****
White mustard	1000	71.0	57.4 ±3.00 a	75.0	60.0 ±2.00 b
	750	73.0	58.7 ±2.20 a	76.7	61.3 ±2.10 b
	500	75.0	60.0 ±1.30 a	76.7	61.3 ±2.30 b
	250	74.0	59.3 ±1.70 a	79.0	62.7 ±0.70 b
	100[2]	80.0	63.4 ±1.50 a	84.0	66.4 ±1.00 a
	50[1]	72.0	58.0 ±2.50 a	77.7	61.4 ±2.50 b
	25	72.0	58.0 ±4.00 a	75.0	60.0 ±1.20 b
	control	73.0	58.7 ±2.70 a	76.7	61.3 ±1.60 b
	F value		**2.34ns**		**5.38****

F test ±SD; values with the same letter in the column are on the same level of significance for the confidence interval 95%; **p<0.01;* p<0.05; ns p>0.05, [1]-MAC for II class of water (ICPDR, 2004); [2]-MAC in irrigation water (Regulation, Off. Gazette RS 23/94)

The stimulating effect that Ni^{+2} expressed on cabbage and sunflower seeds is in accordance with [16, 17]. These authors report that, when applied at 10 and 20 mg/l, Ni^{+2} significantly stimulated germination of different seeds.

However, the results of [2] indicate that the seed germination is a physiological parameter that is rarely affected by heavy metals, including Ni^{+2}. This was proven for maize seeds in this work.

Conclusion

Germination energy and germination of sunflower seeds were significantly inhibited by Ni^{+2} at maximum allowable rate, stipulated by several Regulations, which indicates at good potential of this plant species and mentioned parameters in detection of water pollution with Ni^{+2}. Maize was very tolerant to high levels of in Ni^{+2} in water and does not have potential to be used as an indicator of water pollution with this heavy metal.

Acknowledgement

This work is a part of project III43005, financed by the Ministry of Education and Science of the Republic of Serbia.

References

[1]M. Prica, B. Dalmacija, M. Dalmacija, J. Agbaba, D. Krčmar, J. Tricković, E. Karlovic (2010): Changes in metal availability during sediment oxidation and the correlation with immobilization potential. Ecotoxicology and Environmental Safety, 73(6): 1370-1377.

[2]M. Aycicek, M. Ince, M. Yaman (2008): Effects of cadmium on germination, early seedling growth and metal content of cotton (*Gossypium hirsutum* L.), International Journal of Science and Thechnology, 3(1): 1-11.

[3] R. Tappero, E. Peltier, M. Grafe, K. Heidel, M. Ginder-Vogel, K. Livi, M. Rivers, M. Marcus, R. Chaney, D. Sparks (2007): Hyperaccumulator *Alyssum murale* relies on a different metal storage mechanism for cobalt than for nickel. New Phytologist, 175(4): 641-654.

[4] C. Grison, V.Escande, E.Petit, L.Garoux, C.Boulanger, C.Grison (2013): *Psychotria douarrei* and *Geissois pruinosa*, novel resources for the plant-based catalytic chemistry. RSC Adv., 44(3): 22340-22345.

[5] G.T. Ankley, D.A. Benoit, R.A. Hoke, E.N. Leonard, C.W. West (1993): Development and evaluation of test methods for benthic invertebrates and sediments: Effects of flow rate and feeding on water quality and exposure conditions, Archives of Environmental Contamination and Toxicology, 25(1): 12-19.

[6] ISTA (International Seed T Association: International Rules for Seed Testing (2013).

[7] Regulations on the quality of seed of agricultural plants (Official gazette, RS 34/2013).

[8] OECD Guideline for Testing of Chemicals 208 (1984): Terrestrial Plants, Growth Test

[9] US EPA, OPPTS Ecological Effect Guideline, 850 Series, (1996).

[10] AFNOR X31-203/ISO 11269-1 (1993): Détermination des effets des pollutants sur la flore du sol: Méthode de mesurage de l'inhibition de la croissance des racines.

[11] X. Wang, C. Sun, Sh.Gao, L. Wang, H. Shokui (2001): Validation of germination rate and root elongation as indicator to assess phytotoxicity with *Cucumis sativus*. Chemosphere, 44, 1711-1721.

[12] M. Smiri (2011): Effect of cadmium on germination, growth, redox and oxidative properties in *Pisum sativum* seeds, Journal of Environmental Chemistry and Ecotoxicology, 3(3): 52-59.

[13] R.M. Khan, M.M. Khan (2010): Effect of varying concentration of nickel and cobalt on the plant growth and yield chickpea. Australian Journal of Basic and Applied Science, 4(6): 1036-1046.

[14] M.Y. Ashraf, R. Sadiq, M. Hussain, M. Ashraf, M.S.A. Ahmad (2011): Toxic effect of nickel (Ni) on growth and metabolism in germinating seeds of sunflower (*Helianthus annuus* L.). Biol. Trace Elem. Res., 143: 1695-1703.

[15] S.K. Sethy, S. Ghosh (2013): Effect of heavy metals on germination of seeds. J. Nat. Sc. Biol. Med. 4: 272-275.

[16] S.A. Ahmad, M. Hussain, M. Ashraf, R.I. Ahmad, M.A. Ashraf (2009): Effect of nickel on seed germinability of some elite sunflower (*Helianthus annuus* L.) cultivars. Muhammad Pak. J. Bot., 41(4): 1871-1882.

[17] M.S. Ahmad, M. Ashraf, M., Hussain (2010): Phytotoxic effects of nickel on yield and concentration of macro-and micro-nutrients in sunflower (*Helianthus annuus* L.). Achenes. J. Hazard. Mater., 10: 234-240.