DEVELOPMENT OF A PLANT TEST SYSTEM FOR EVALUATION OF THE TOXICITY OF METAL CONTAMINATED SOILS. I. SENSITIVITY OF PLANT SPECIES TO HEAVY METAL STRESS

РАЗРАБОТВАНЕ НА РАСТИТЕЛЕН ТЕСТ ЗА ОЦЕНКА НА ТОКСИЧНОСТТА НА ЗАМЪРСЕНИ С ТЕЖКИ МЕТАЛИ ПОЧВИ. ЧУВСТВИТЕЛНОСТ НА РАСТИТЕЛНИ ВИДОВЕ КЪМ СТРЕС ОТ ТЕЖКИ МЕТАЛИ

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Manuscript received: February 2, 2007; Reviewed: June 19, 2007; Accepted for publication: September 3, 2007

Abstract

The sensitivity of young bean, cucumber and lettuce plants to heavy metals stress was studied at control conditions in a climatic room. The plants were grown in pots with perlite and supplied daily by half-strength Hoagland nutrient solution. The plants were treated for 8 days with different heavy metal doses (full, ½ and ¼) starting at appearance of the first true leaf (cucumber and bean) or the full development of the second leaf (lettuce). The full dose consisted 500 µM Zn, 50 µM Cd and 20 µM Cu added to the nutrient solution. Based on the measured morphological (fresh weight, leaf area, root length) and physiological parameters (photosynthetic pigments content and activity of guaiacol peroxidase in roots), the cucumber plants presented the highest sensitivity to heavy metal stress.

РЕЗЮМЕ

Проучена е чувствителността на млади фасулеви, краставични и салатни растения към стрес от тежки метали при контролирани условия. Растенията са отглеждани в съдове с перлит. В съдовете ежедневно е добавян ½ хранителен разтвор на Хогланд, като излишъкът на разтвора се оттича. Растенията са третирани в продължение на 8 дни с различни дози на комплекс от тежки метали (пълна, ½ и ¼) започвайки от появата на първия същински лист на краставичните и фасулевите растения и пълното развитие на втория лист при салатните растения. Пълната доза тежки метали включва 500 µM Zn, 50 µM Cd и 20 µM Cu, които са добавяни към хранителния разтвор. На базата на морфологични (свежа маса, листна площ, дължина на корените) и физиологични (съдържание на фотосинтетични пигменти и активност на гваякол пероксидазата в корените) параметри е установено, че краставичните растения проявяват най-висока чувствителност към стрес от тежки метали.

Key words: heavy metals, plant test system, photosynthetic pigments, peroxidase activity

INTRODUCTION

Soil contamination by heavy metals is a serious ecological problem all over the world. In Bulgaria such soils stretch over nearly 19500 ha of arable lands [4]. Due to high mobility of some metals, they may enter food chain and subsequently may provoke toxic effects on humans, animals, microorganisms and plants. Therefore, a significant effort is now addressing development of different approaches for sustainable management of metal contaminated soils.

Obviously, the heavily contaminated soils from industrial and non-urban areas should be considered differently from those of agricultural fields as they create different risk for human and environmental health. The correct risk evaluation is the first step in the concept of sustainable land management. The soil quality evaluation is included in the environmental risk assessment framework as the choice of suitable phytotechnology strongly depends on the actual soil phytotoxicity.

The commonly used physicochemical data, such as total soil metal content are not sufficiently representative for risk evaluation, as they do not directly address biological availability and metal toxicity. Thus, biological evaluation tests should complement the former analysis. For this purpose a plant test system evaluating soil metal phytotoxicity have been developed [7]. The reliability of the data obtained by the plant test has been proved in comparison with other biological tests. Presently, further optimized or new, more sensitive plants test systems, are needed for the environmental risk assessment framework.

Growth parameters are preferably used in different plant test systems. However, at low degree of metal contamination the visible symptoms, such as chlorosis, necrosis of leaf tips, etc. are less pronounced or even could be absent, although some reduction of plant quality as well as biomass inhibition persist. Furthermore, it is now well established that heavy metals are able not only to inhibit, but also to promote plant physiological activity. For example, heavy metal-induced enhanced activity of enzymes involved in plant defense against oxidative stress as well as $NAD(P)^+$ reducing enzymes [1].

The aim of this study was to develop a plant test system for evaluation of the toxicity of metal contaminated soils. The system will be performed in standard lab conditions with a sensitive plant species grown for 3-4 weeks in both contaminated and noncontaminated soil samples. The evaluation of the soil toxicity will be based on the plant responses measured by both morphological and physiological indicators. In this paper we report the first obtained data concerning the sensitivity of several tested plant species (bean, cucumber and lettuce) to complex heavy metal contamination (Zn, Cd, Cu).

MATERIALS AND METHODS

The experiments were carried out in a climatic room of the Department of Plant Physiology and Biochemistry in Agricultural University of Plovdiv, Bulgaria. The conditions maintained during the experiments were the following: light duration – 14 hours, light intensity (PAR) 250 μmol m^{-2} s^{-1}, temperature – 22 ± 2 ^{o}C and relative air humidity – 60 ± 5%. The tested plants (bean cv. Lodi, cucumber cv. Levina and lettuce cv. Jalta krasavitsa) were grown in pots filled by perlite and supplied daily by 100 ml half-strength Hoagland nutrient solution. The excess of solution was allowed to drain. At appearance of the first true leaf (cucumber and bean) and the full development of the second leaf (lettuce) were arranged an experimental design with four treatments:

- Control – plants, supplied by Hoagland solution;
- Full heavy metals dose (HM) – plants, supplied by Hoagland solution enriched with 50 μM Cd, 20 μM Cu and 500 μM Zn;
- ½ dose HM – plants, supplied by Hoagland solution enriched with ½ of the full HM dose;
- ¼ dose HM – plants, supplied by Hoagland solution enriched with ½ of the full HM dose.

The treatment of plants with heavy metals continued 8 days. The plants were harvested and fresh weight of roots and shoots, root length, plant height and leaf area (electronic area meter – NEO-3, TU-Sofia) were determined. Photosynthetic pigments content [6] and the activity of the enzyme guaiacol peroxidase GPOD [2] in the roots were measured. All data were statistically treated. The Student's t-test was used to evaluate the difference between control and other treatments within each plant species.

RESULTS AND DISCUSSION

Heavy metal-treated plants from all studied species (cucumber, bean and lettuce) were distinguished by their inhibited growth and the presence of known toxicity symptoms, such as chlorosis, turning to yellowing as well as some browning of the roots [8, 9]. The toxic effect was dose-dependant being the highest at the full heavy metals dose (Table 1). The inhibition of different morphological indicators (root length, root fresh weight, shoot fresh weight and leaf area) at that treatment varied between 38 and 53% in cucumber plants, 30 and 45% in bean plants, and 38 and 43% in lettuce plants. The observed decrease of the measured morphological parameters was statistically significant at all treatments of cucumber plants, whereas in other species it was true only at the higher heavy metals treatment. The root length was the most sensitive parameter in cucumber and bean plants. The sensitivity of root growth relies on the fact that metal-imposed root growth reduction is due to direct effects on both cell division and elongation [3].

The data presented in Table 2 showed that the applied heavy metals doses enhanced significantly the activity of the enzyme guaiacol peroxidase (GPOD) in the roots of all plant species. The strongest response was found in cucumber plants. For example, root GPOD activity at the full heavy metals dose was about 311% from that of the control value. The respective values were 129% in beans and 212% in lettuce. The strongest enhancement of GPOD was not surprising as it is well known that heavy metals may induce an oxidative stress in plant cells [8].

Photosynthetic pigments are known as a target site of the toxic heavy metals action [9]. The data presented in Table 3 showed that the applied treatment tended to diminish the content of all pigments and to some extend changed their ratios. The strongest effect was observed in Chl.a content. The decrease of Chl.a content at the full heavy metals dose was significantly different from the controls and presented 47% in cucumber, 46% in beans and 34% in lettuce plants. The decreased content of both chlorophyll and carotenoids could result from metals-induced mineral deficiency (Fe), Mg substitution in the chlorophyll molecule or oxidative damage [2, 9].

CONCLUSIONS

The results obtained in these study presented cucumber plants as the most sensitive species to the applied heavy metals stress. Both morphological and physiological indicators of this species were highly responsive to the heavy metals treatment. In addition to its high sensitivity, we considered cucumber as very suitable species for this test because of: (a) its high initial growth rate, which will allow to short test duration and (2) its high transpiration

Table 1. Biometrical parameters of both control and heavy-metals treated plants

Young plants are subjected to heavy metal stress by adding of metal ions to the root medium. Full dose treatment represents a Hoagland solution enriched with 50 µM Cd, 20 µM Cu and 500 µM Zn. L – root length, FW – fresh weight and LA – leaf area

Таблица 1. Биометрични показатели на контролните и третираните с тежки метали растения

Млади растения са подложени на метален стрес чрез добавяне на йони към кореновата среда. Пълната доза представлява Хогланд разтвор, към който са добавени 50 µM Cd, 20 µM Cu и 500 µM Zn. L – дължина на корените, FW – свежа маса и LA – листна площ

Treatments	L (cm) Roots	FW (g) Roots	FW (g) Shoots	LA (cm^2) Leaves
Cucumber plants cv. Levina				
Control	26.5 ± 3.5	2.37 ± 0.09	3.12 ± 0.13	92.7 ± 5.0
¼ dose HM	15.6 ± 5.3*	1.99 ± 0.12*	2.67 ± 0.15*	74.5 ± 2.8*
½ dose HM	13.6 ± 2.6*	1.67 ± 0.05*	2.20 ± 0.19*	64.8 ± 4.6*
Full dose HM	12.5 ± 1.6*	1.47 ± 0.02*	1.49 ± 0.11*	53.3 ± 4.9*
Bean plants cv. Lodi				
Control	33.9 ± 7.8	3.40 ± 0.25	4.02 ± 0.35	154.5 ± 8.9
¼ dose HM	28.6 ± 5.1	2.91 ± 0.73	3.35 ± 0.35	112.5 ± 4.5*
½ dose HM	25.4 ± 3.9*	2.61 ± 0.30*	3.12 ± 0.60*	96.6 ± 11.3*
Full dose HM	16.9 ± 1.5*	2.20 ± 0.15*	2.80 ± 0.25*	86.9 ± 12.0*
Lettuce plants cv. Jalta krasavitsa				
Control	23.4 ± 4.4	1.00 ± 0.20	2.70 ± 0.55	104.3 ± 15.9
¼ dose HM	17.2 ± 2.0	0.75 ± 0.15	2.00 ± 0.33	77.1± 8.7*
½ dose HM	15.3 ± 2.9*	0.68 ± 0.24*	1.82 ± 0.32*	70.1 ± 9.4*
Full dose HM	14.6 ± 2.5*	0.64 ± 0.11*	1.54 ± 0.51*	64.0 ± 10.9*

* - significantly different from the control value at p=0.05 (n=4)

Table 2 Root guaiacol peroxidase (GPOD) activity (mU / g FW) in both control and heavy metals-treated plants

Young plants are subjected to heavy metal stress by adding of metal ions to the root medium. Full dose treatment represents a Hoagland solution enriched with 50 µM Cd, 20 µM Cu and 500 µM Zn.

Таблица 2 Активност на гваякол пероксидазата (GPOD) (mU / g FW) в корените на контролни и третирани с тежки метали растения

Млади растения са подложени на метален стрес чрез добавяне на йони към кореновата среда. Пълната доза представлява Хогланд разтвор, към който са добавени 50 µM Cd, 20 µM Cu и 500 µM Zn.

Treatments	Plant species		
	Bean	Lettuce	Cucumber
Control	3550 ± 455 (100)	899 ± 128 (100)	2184 ± 153 (100)
¼ dose HM	4719 ± 467 (133)*	1614 ± 136 (180)*	4125 ± 337 (189)*
½ dose HM	4407 ± 392 (124)*	1718 ± 350 (191)*	5252 ± 395 (240)*
Full dose HM	4571 ± 347 (129)*	1908 ± 203 (212)*	6804 ± 630 (311)*

* - significantly different from the control value at p=0.05 (n=4)

Table 3 Content and ratios between photosynthetic pigments of control and heavy metals-treated plants

Young plants are subjected to heavy metal stress by adding of metal ions to the root medium. Full dose treatment represents a Hoagland solution enriched with 50 µM Cd, 20 µM Cu and 500 µM Zn.
Chl. a – chlorophyll a; Chl. b – chlorophyll b; Car - carotenoids

Таблица 3 Съдържание и отношения между фотосинтетичните пигменти в контролни и третирани с тежки метали растения

Млади растения са подложени на метален стрес чрез добавяне на йони към кореновата среда. Пълната доза представлява Хогланд разтвор, към който са добавени 50 µM Cd, 20 µM Cu и 500 µM Zn.
Chl.a – хлорофил a; Chl. b – хлорофил б; Car – каротеноиди

Treatments	Content (mg / g FW) and ratios between photosynthetic pigments				
	Chl. a	Chl. b	Car	a/b	a+b / Car
Cucumber plants cv. Levina					
Control	1.06±0.02	0.63±0.04	0.39±0.01	1.68±0.01	4.33±0.05
¼ dose HM	0.71±0.05*	0.46±0.02*	0.29±0.02	1.54±0.03	4.05±0.03*
½ dose HM	0.66±0.01*	0.44±0.03*	0.28±0.02*	1.50±0.01*	3.95±0.02*
Full dose HM	0.56±0.05*	0.40±0.00*	0.24±0.02*	1.40±0.01*	4.00±0.05*
Lettuce plants cv. Jalta krasavitsa					
Control	0.98±0.01	0.56±0.13	0.45±0.01	1.79±0.44	2.23±0.33
¼ dose HM	0.76±0.05*	0.55±0.02	0.36±0.02*	1.37±0.03*	2.28±0.05
½ dose HM	0.48±0.03*	0.35±0.04*	0.24±0.00*	1.36±0.07*	1.98±0.20
Full dose HM	0.53±0.01*	0.34±0.05*	0.26±0.01*	1.35±0.24*	1.84±0.23*
Bean plants cv. Lodi					
Control	1.34±0.12	0.78±0.07	0.48±0.10	1.70±0.01	2.98±0.08
¼ dose HM	1.15±0.16	0.87±0.22	0.47±0.10	1.34±0.16*	3.00±0.23
½ dose HM	1.01±0.12*	0.78±0.19	0.39±0.01	1.32±0.17*	3.00±0.59
Full dose HM	0.88±0.09*	0.73±0.00	0.31±0.01*	1.22±0.12*	3.22±0.03

* - significantly different from the control value at p=0.05 (n=4)

rate, which could favor the root-to-shoot heavy metals transport as well as their more general impact on plant performance. Further step in the test development will be the arrangement of cucumber bioindicator values into different metal phytotoxicity classes.

ACKNOWLEDGEMENTS

The National Science Found, financially supported these studies (project WU-AN-3/2005), is acknowledged.

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