ФИЗИОЛОГИЧНИ ПАРАМЕТРИ ПРИ МЛАДИ РАСТЕНИЯ ПАМУК, ОТГЛЕЖДАНИ НА ЗАМЪРСЕНИ С ТЕЖКИ МЕТАЛИ ПОЧВИ
PHYSIOLOGICAL PARAMETERS OF YOUNG COTTON PLANTS, GROWN ON HEAVY METAL CONTAMINATED SOILS

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Abstract

Metal contamination of the soilis a pressing ecological problem all over the world. A part of the heavy metal contaminated soils in Bulgaria are situated around the Non-ferrous Metals Smelter near Plovdiv (KCM-Plovdiv). Implementation of *adaptable agriculture* (technical crops cultivation) is recommended in this region as a way of reducing human health risk. Cotton is one of the suitable crops for that purpose; therefore the aim of our study was to evaluate the physiological state of young cotton plants, grown on heavy metal contaminated soils from this region.

Pot experiments were carried out in climatic rooms at the Department of Plant Physiology and Biochemistry. Young cotton plants (cv. *Darmi*) were grown on representative soil samples taken around KCM-Plovdiv under controlled conditions [(light intensity 250 μmol m^{-2} s^{-1} (PPFD), temperature 25°/20°C (day/night), relative air humidity 60% and photoperiod 16/8 h (light/dark)]. The experimental design included 5 treatments and each treatment was set at 4 replications (3 plants per pot). Four of the treatments represented soils containing Cd, Pb, Zn and Cu in levels exceeding the permissible soil limits, while the fifth contained uncontaminated soil sample (control). Prior to the sowing all pots were fertilized with equal amounts of ½ strength Hoagland nutrient solution.

The physiological status of the plants was evaluated three weeks after germination. The analyses included the following parameters: plant biometrics, content of heavy metals in the plant organs, leaf gas exchange, chlorophyll fluorescence, photosynthetic pigments content, etc. The obtained results showed that the physiological state of the cotton plants was differently affected by the metal contaminationof the soil. The most significant variations were reported for the plants grown in the soil ofthe highest metal content.

Key words: cotton, growth, heavy metals, photosynthesis, photosynthetic pigments.

INTRODUCTION

Pollution by heavy metals (HM) is an acute environmental issue. Heavy metals pose a potential risk for human health and the sustainable functioning of ecosystems, since in excess concentrations in the environment they can be transferred and accumulated in food chains and thus exhibit biotoxicity. Hazard HMs are mainly cadmium (Cd), arsenic (As), lead (Pb), copper (Cu), zinc (Zn), as well as the radionuclides uranium (U), cesium (137Cs) and strontium (90Sr). In our country the soils polluted with HM are about 400 000 dka. Theirsustainableuserequiresdifferentsolutions – adaptivefarming, phytoremediation, useofgenotypeswithlowaccumulationofcertainHM (Cd, As, radionuclides) intheyield, etc. For this purpose is necessary knowledge for the interactions between heavy metals and plants, the study of which requires a complex scientific approach (Vasilev i Dinev, 2012).

The sustainable use of soils contaminated with HM requires complex information about the soils in the polluted area, which also includes an assessment of their quality. In general this assessment provides information whether the contaminated soil induces signs of chronic phytotoxicity in plants grown on it, and whether cultivated plants (in case when they are grown for food) accumulate an excess of the permitted levels of the dangerous to human health HM (such as Cd). For this purpose are used bioassays, which are carried out under controlled conditions.

The use of bioassays for assessing the toxicity of soil contaminated with HM is necessary due to the fact that the total concentration of HM in the soil does not give an idea for their mobility, accessibility and biotoxicity (Adriano, 2001). The content of the HM in separate fractions of extractable soil is also not indicative enough, because there are no

equal and generally accepted methodologies in this aspect. In addition, in the majority of cases, soils are contaminated with a complex of HM and therefore their toxicity may be the result of both the action of specific metals and/or the interaction between them – synergistic, antagonistic and additive.

Bioassays can be developed on the basis of animal species, micro-organisms and plants. Historically, plant species were considered to be less sensitive to toxicants than animal ones, and that is the reason the phytotoxical database to be relatively scant. In our days it is generally accepted that the sensitivity of individual biological organisms to various toxicants is unpredictable and species specific (Smith, 1978).

The most popular plant tests for the assessment of the toxicity of the soil are relatively few in number and are based on reduction of the biomass of sensitive species and inhibition of the seed germination. Theseindicators, asarule, are not sufficiently sensitive to determine the chronic manifestations of the metal phytotoxicity. More appropriate indicators, based on functional disorders in treated plants with HM, are available from plant toxicology. Such an approach was used in the assay of Van Assche and Clijsters (1990) with bean plants (*Phaseolus vulgaris*), in which, on the basis of morphological and functional indicators (mainly enzyme activities) contaminated soils with HM were classified in phytotoxical classes. There are also herbal bioassays for assessing the toxicity of soil contaminated with HM, in which as functional indicators are used photosynthetic parameters (Koleva i dr., 2006; Vassilev et al., 2007) (gas exchange, chlorophyll fluorescence, etc.).

Since the ions of HM differ in many parameters, it can be assumed that their phytotoxical action is specific. For example, the problematic HM differ in their oxidative-reduction potential, the ability to bind to various organic ligands, their indispensability for plants, etc. All this motivates the presented work. The aim of this study was clarification of details in the phytotoxical action of the individual HM on physiological state of young cotton plantsgrown on heavy metal contaminated soils.

MATERIALS AND METHODS

The experiments were conducted in growth chambers under controlled conditions. The latter and the manner of conducting the experiments were described earlier (Vasilev i Dinev, 2012). The experimental subjects were young cotton plants (variety Darmi). Two experiments were held. The physiological state of the plants was analyzed three weeks after germination. The analyses included determination of growth parameters of the plants, leaf gas exchange, chlorophyll fluorescence, heavy metal content andamounts of plastid pigments.

The growth parameters (fresh and dry mass and leaf area) were measured with electronic area meter (NEO-2, TU, Bulgaria). The parameters of the leaf gas exchange (photosynthetic rate, transpiration intensity, stomatal conductance, intercellular concentration of CO_2 and photosynthetic capacity – photosynthetic rate in excess of CO_2 concentration and light intensity) were measured with portative photosynthetic system LCA-4 (ADC, Hoddesdon, England).Chlorophyll fluorescence parameters: zero (Fo) and maximal (Fm) and the ratio Fv/Fmin dark adapted leaves; and in steady state photosynthesis: Y–quantum yield of the converted photochemical energy, ETR–relative rate of electron transport, qp–photochemical quenching and qN–non photochemical quenching) were analyzed with a pulse amplitude modulation chlorophyll fluorometer MINI-PAM (Walz, Effeltrich, Germany). The content of photosynthetic pigments (total chlorophyll and carotenoids) were determined spectrophotometrically and calculated by Lichtenthaler & Welburn (1987). The activity of the antioxidative enzyme guaiacol peroxidase (GPOD) was measured by methods of Bergmeyer (1974). The heavy metal content was determined by atomic absorption spectrophotometer in the accredited laboratory of the Agricultural University.

The data were processed statistically using one-way ANOVA.

RESULTS AND DISCUSSION

The soils amples from the chosen defining points (5 variants in total) were identified and analyzed for total heavy metal content (HM). The results presented in table 1 show that with the exception of the defining point 5 (Airport), which was accepted for control, the contents of the HM in all other variants exceeded the established maximum permissible concentrations for the established soil reaction (pH).

The data from the biometric analysis and the values of the measurements of enzyme activity in plant rootsare presented in table 2. Despite the increased values of HM in these lected soils amples the growth parameters of the cotton plants cultivated on those soils ranged only slightly. The total mass of plants from the different variants was in the range – 1,951 - 2,217 g, which represents 96 – 108% of the mass of the control variant. The relatively greater weight of the roots (the highest recorded for variant 3) in plants grown on soil contaminated with HM compared to the control, may be due to the deposition of polyphenol compounds in their cell walls. The peroxidase activity showed a significant decrease in variant 1 (17,3) and variant 2 (21,1), which were grown on soils with the highest content of HM. A significant increase in the enzyme activity was reported for variant 4 (56,3) compared to the control plants (variant 5 (30,1)).

Table 1. Identification of the defining points and total content of heavy metals in soil samples taken from them

Defining points	GPS identification	N	E	Cu	Zn	Pb	Cd	pH
Variant 1	deg◦	42	24					
	min ‘	3	49	550	6500	6400	110	7,5
	sec″	588	332					
Variant 2	deg◦	42	24					
	min ‘	3	49	180	2825	1755	37	7,2
	sec″	125	607					
Variant 3	deg◦	42	24					
	min ‘	2	50	60	400	158	3.5	7,8
	sec″	62	650					
Variant 4	deg◦	42	24					
	min ‘	3	49	44	350	140	4.5	7,2
	sec″	554	607					
Variant 5/ Control	deg◦	42	24					
	min ‘	4	49	32	150	75	1.5	7,2
	sec″	298	534					

Table 2. Biometrical parameters and total peroxidase activity in roots of cotton plants grown on soil samples from the defining points.

Variants	Parameters			
	Root weight (g)	Plant weight (g) (% from the control)	Leaf area (cm^2 per plant)	GPOD root (U g^{-1} FW)
1	0,509b	1,951 (96)	111,1b	17,3d
2	0,494b	2,208 (108)	120,5a	21,1c
3	0,572a	2,280 (111)	126,9a	26,4b
4	0,508b	2,217 (108)	108,2b	56,3a
5	0,434c	2,038 (100)	107,7b	30,1b

Values followed by the same letter (a, b, c, d) within a column are not significantly different at P<0,05.

In table 3 are listed the values of HM in the leaves and roots of cotton plants. Itisobviousthat-despitethe relatively high values of HM in the soil, especially in variants 1 and 2, their contents in the leaves was not that dangerously high for the vital activity of the plants. The contents of Cuand Zn-werein normal values for plants, and the contents of Cd and Pb were much lower than the values indicated as phytotoxic in the literature and our previously reported results (Vassilev et al., 2011). The high values of Pb, Zn and Cd in the roots of plants, especially in variant 1, reflects the total quantity of metals adsorbed on the root surface and assimilated by the plants.

In table 4 the data from the measurements of leaf gas exchange of cotton plants are presented. Significantly reduced photosynthetic values of the plants from variant 1 and 2 were obtained. Lower transpiration, stomatal conduction and intercellular concentration of CO_2 in plants from variant 2 were also detected.

The results in table 5 show that the total chlorophyll content and the carotenoids were significantly decreased in plants grown on soil samples from variants 1 and 4. Thehighestvalue of chlorophylls and carotenoids were obtained in plants from variants 2 and 3.

In table 6 are presented the data from measurements of parameters for photosynthetic electron transport. From the results presented it could be seen that the investigated indicators had not responded equally in the different variants as a result of high concentrations of HM in the soil samples. The quantum yield and the electronic transport were the most significantly reduced in variant 4 in comparison to the control plants.

Table 3. Heavy metal content in organs of cotton plants grown on soil samples from the defining points

Variants	Organs	Heavy metal content (mg/kg^{-1})			
		Pb	Cu	Zn	Cd
1	Leaves	29,1±1,1	5,12± 0,22	55,40 ± 1,65	5,76± 0,28
2		3,28±0,25	3,04± 0,25	24,56± 0,80	1,28± 0,05
3		3,04±0,25	3,16± 0,25	25,36± 0,80	1,12± 0,05
4		6,00 ±0,80	3,05 ± 0,25	24,6± 1,65	3,72± 0,15
5		2,32± 0,20	4,80 ± 0,28	25,32± 0,80	1,32± 0,05
1	Roots	1296,8±35,0	126,16± 3,86	1087,2±32,40	425,6± 12,7
2		73,76±2,65	39,36±1,20	98,80 ±2,94	30,96± 1,00
3		44,76±1,80	66,32± 2,55	92,52± 2,76	19,04± 0,86
4		64,4± 35,0	160,4±7,5	118,4± 42,4	30,6± 9,5
5		102,1± 3,2	185,48±7,8	115,67±6,9	39,84± 1,22

Table 4. Photosynthetic parameters on cotton plants grown on soil samples from the defining points. A – net photosynthesis (µmol/m^{-2}s^{-1}), E – transpiration intensity (mmol/m^{-2}s^{-1}), gs – stomatal conductance (mol/m^{-2}s^{-1}), ci – intercellular concentration of CO_2 (vpm).

Variants	Parameters			
	A	E	gs	ci
1	5,97b	1,24b	0,06a	181,67a
2	5,05b	0,78b	0,03b	70,33b
3	8,98a	2,05a	0,08a	175,00a
4	8,80a	2,12a	0,09a	205,33a
5	8,47a	2,17a	0,08a	185,33a

Values followed by the same letter (a, b) within a column are not significantly different at P<0,05.

Table 5. Content (mg g^{-1} FW) and ratio of photosynthetic pigments of cotton plants grown on soil samples from the defining points

Variants	Parameters					
	Chl (a)	Chl (b)	Chl(a+b)	Carotenoids	a/b	a+b/carot.
1	0,76c	0,26c	1.02b	0,23c	3,00a	2,73a
2	1,67a	0,55a	2,21a	0,68a	3,03a	3,25a
3	1,67a	0,58a	2,25a	0,64a	2,89a	3,51a
4	1,01b	0,36b	1,37b	0,41b	2,79a	1,89b
5	1,43a	0,53a	1,96a	0,56a	2,69a	2,39a

Values followed by the same letter (a, b, c) within a column are not significantly different at P<0,05.

Table 6. Parameters of chlorophyll fluorescence of cotton plants grown on soil samples from thedefining points (GPS). Fv/Fm – maximal photochemical activity of PS 2, Fm''- maximal fluorescence in light adapted leaves, Y – quantum yield, ETR - electron transport (μmol$m^{-2}s^{-1}$)

Variants	Parameters			
	F_v/F_m	F_m'	Y	ETR
1	0,818a	618c	0,626c	18,8b
2	0,793b	798a	0,665a	19,1b
3	0,821a	766a	0,644b	23,1a
4	0,814a	667c	0,642b	18.0b
5	0,786a	729b	0,680a	25,5a

Values followed by the same letter (a, b, c) within a column are not significantly different at P<0,05.

Plants possess two strategies to cope with an excess of HM in the environment: (1) uptake limitation and (2) increased tolerance to excessive HM absorbed into the cell. The mechanisms of tolerance are based on (a) linking of HM with phytochelatins, metalothyoneins or other organic ligands and subsequent compartmentalization, (b)reduction of the oxidative stress induced by HM in cells and (c) active secretion of HM via trichomes. On the basis of the above mechanisms plants can tolerate high concentrations of HM untill reaching a certain critical level of metal ions into the cells. Afterthatthreshold levelappear physiological disorders, whose integrated expression trigger phytotoxic events.

At the cellular level indicators of the metal phytotoxicity are changes in the antioxidative defense system of the cell and the structural-functional state of the membranes(Vassilev et al., 2002; Stoeva and Bineva, 2003; Stoeva et al., 2005). It is assumed that there are several mechanisms through which the HM may cause manifestation of oxidative stress: (a) direct interaction of HM with SH-groups of membrane components and the active centers of enzymes; (b) inhibition of the activity of enzymes with a metal cofactor; (c) oxidizing damage to macromolecules.

When the antioxidative cell system is no table to control the oxidative-reduction potential in the normal range, occurs inhibition of the activity of enzymes and reduction of the content of non-enzymatic antioxidants. A similar situation was observed in the results obtained for plants from variant 1, under which the activity of the enzyme guaiacol peroxidase in the roots was significantly decreased (17,3 U g^{-1}) compared to the control (table 2). These results can be explained by the measured highest concentration of HM in soil (table 1) and roots (table 3). Increased enzyme activity in plants was observed only in variant 4 (56,3 Ug^{-1}FW), where the content of the HM in the roots was lower than reported in the control plants (table 2).

The uncontrolled increase in the production of free radicals and active oxygen species in the cell causes oxidation of the important macromolecules. The most common event is oxidation of un saturated fatty acids in the membrane lipids, which breaks the membrane integrity resulting in increased leakage of electrolytes in the cell environment. Multiplication of these negative effects of HM on basic physiological processes lead to different functional disorders in the plant organism.

The indicators of HM phytotoxicity on the organism level are reduced growth parameters and visual symptoms (chlorosis, necrosis, root browning, etc.). Heavy metals disrupt basic physiological processes – photosynthesis, mineral nutrition, water exchange, etc. The effects of HM on water exchange most of ten are related to a reduction of stomatal conductance and the intensity of transpiration (Stoeva et al., 2003; Stoeva and Bineva, 2003). Similar results were observed in variants 1 and 2 (table 4) in our experiments. Those effects led to inhibition of photosynthesis shown in table 4. Together with stomatal limitations, mesophyll limitations of photosynthesis are also possible (Vassilev, 2002; Vassilev et al., 2002). They are related to the biochemical processes from the Calvin cycle, reduction of the quantity of photosynthetic pigments (Stoeva et al., 2003) and disruption of photosynthetic electron transport. Photosynthetic pigments are considered to be a characteristic sign of the toxic metal effects, mainly due to the incidences of chlorosis plants subjected to HM stress. Heavy metals inhibit or completely inactivate the activity of the basic photosynthetic enzyme ribulozobisphosphate carboxylase/ oxigenase (Rubisco) and cause a number of perturbations in the electron-transport processes associated with PS1 and PS2. The photosynthetic electron transport disruptions are due to ultrastructural disorders of the thylakoid membranes, reduced levels of electron carriers, inhibition of the PS2 (photoinactivation) and a number of other negative effects.

The mentioned events,characteristic for metal phytotoxicity, could be observed in plants grown on soil contaminated by a complex of HM. Using the data obtained from the studied parameters a quick screening approach for early diagnosis of stress in the environment can be offered.

CONCLUSIONS

1. Contaminated soils with HM induced different disorders in cotton plant, whichdepend on the severity of the contamination.

2. The most significant variations were reported in plants grown in soil with a high content of heavy metals.

3. These disorders include reduction of the biomass, decrease of the photosynthetic activity and changes in the stress enzyme (guaiacol peroxidase) activity.

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