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HEAVY METAL ACCUMULATION IN *SOLANACEAE*-PLANTS GROWN AT CONTAMINATED AREA

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ABSTRACT. Crops members of the family *Solanaceae*, tomato (*Licopersicon esculentum* L.), eggplant (*Solanum melongena* L.) and pepper (*Capsicum annum L.*), were grown in industrially polluted region. The experimental plots were situated at two different distances (0.5 and 15 km) from the source of pollution—the Non-Ferrous-Metal Smelter (NFMS) near to Plovdiv. We investigated the level of soil contamination and the concentration in aerial parts of the plant with heavy metals, by taking soil and plant samples. The plant heavy metals content (roots, stems, leaves, seeds, flowers) were determined after the method of the wet mineralization. The quantitative measurements were carried out with atomic absorption spectroscopy (AAS). We found that the level of soil contamination depends of the distance from the source (NFMS) and is very high, reaching levels of 630 mg Pb kg^{-1}, 13.2 mg Cd kg^{-1}, 60.1 mg Cu kg^{-1}, 974 mg Zn kg^{-1}.
A strongly exhibited tendency towards decrease of the contents of heavy metals in these crops is observed as the distance from the NFMS increases. The crops from the family of Solanaceae are cultures that are not suitable for growing in industrially polluted regions — they remove considerable quantities of heavy metals from the soil with their roots, leaves and fruit-sets, and are potential poison for the end consumer of the nutrient net. The microbiological activity was strongly influenced by the contamination. Decreasing the contamination the microorganisms' activity goes up (at 0.5 km from the NFMS), while near to the factory the physiological groups and microbial respiration had a reduced levels.

KEY WORDS. heavy metals, tomato, eggplant, pepper, accumulation, microorganisms.

INTRODUCTION

In the last decades the contamination of soil became very extensive and dangerous problem as a consequence of industrial activities (metallurgy, chemistry, energetics, etc.). While the organic pollutants are destructible true microbial mineralization, the inorganic ones cause serious problems contaminating soils and are just transported from one to other soil properties, which appear as their final depot. The anthropogenic contamination of soils with heavy metals is a premise for environmental and health risk (Hocking, P.J. and McLaughlin, M.J., 2000). In this sense, growing crops in toxic metal polluted regions with the purpose agricultural production for ordinary use could provoke tremendous problems concerning the health and the future of our children.

The contamination of soils is the main problem, which can be partially resolved by the application of new technologies as phytoremediation. The recuperation of contaminated soil true this technology, based on the use of higher plants, has the objective to extract or immobilize the metals in soil (McGrath, 1998; Salt et al., 1998). The plant can concentrate a huge amount of elements and compounds from the environment and to mobilize different molecules in their tissues. Toxic metals are one of the main objects of phytoremediation. It is known, also, that some rhizospheric microorganisms play an important role in the phytoasimilation of microelements (Giller et al., 1998). These microorganisms can enhance the plan capabilities to absorb metals (Shilev et al., 2003). The rhizospheric bacteria stimulators of plant growth (PGPR) could be very suitable for the phytoextraction processes of metals, bettering plant growth on the polluted soils (Burd et al., 1998). On the other hand, microbial reductions to volatile or less mobile forms could be determinant for the phytoextraction.

In regions of metallurgical activities the environmental pollution has a complex influence on grown crops. While the plant roots absorb heavy metals from the soil solution, the aerosol contaminants penetrate into the plant through the leaves' tissues. The contamination depends on the source distance, the weather conditions and the size of the particles. Lead (Pb) remains on the surface as precipitate, while Zn, Cu and Cd can partially penetrate into the leaves (Kabata-Pendias and Pendias, 1992).

In Western Europe exist a huge amount of sites contaminated with metals which surface is approximately 140 000 ha (McGrath et al., 2001). In Bulgaria the arable lands contaminated with metals are approximately 19 050 ha (Tasev, 1995). In the past the basic pollutant of soil in Plovdiv region was the Non-Ferrous-Metal-Smelter (NFMS). It is clear, that the contamination is multicomponent with basic emphasis on Pb, Zn, Cu and Cd. During the period 1991-2002 the medium Pb-aerosol air concentrations in the NFMS region were between 0.5 and 5.05 $\mu g/m^3$ (Epidemiological investigations, 2003). On the other hand, *Solanaceae* plants (tomato, pepper, eggplant, etc.) are traditionally grown on that area.

Comparing the element concentrations in studied vegetables, Frieke et al. (1973) found Cu in tomatoes at 3.88–3.6 ppm and Ni in tomatoes at 0.08–0.16 ppm and in potatoes at 26.9–28.8 ppm. Mohamed et al. (2003) comparing Cd, Co, Cu, Mn, Pb, and Zn concentrations in plants of family *Solanaceae*, found that eggplant had the highest levels of Mn, Pb, and Zn, potatoes had the highest levels of Cd and Co, and tomatoes had the highest levels of Cu and Ni. The same authors described that toxic elements Cd and Pb appeared to be at low levels in tomato and pepper in two different agricultural soils which practically are not polluted (Pb – 2,5 ppm, Cd – 0,67 ppm, Zn – 14,4 ppm, Cu – 8,8 ppm). Generally, the biggest concentration of Cd into the plant tissue was observed in the leaves (Moral et al., 2002). This behaviour has been reported by other researchers of tomato plants (Wolterbeek et al., 1988; Moral et al., 1994). Kim et al. (1988) found that the ratio between leaf and fruit concentration of Cd in tomato plants grown in polluted soils was about 30–60, depending on the type of soil.

The purpose of the present study is to determine the quantities and the tissues of accumulation of Pb, Cu, Zn and Cd in three *Solanaceae* plants – tomato (*Licopersicon esculentum* L.), pepper (*Capsicum annum L.*) and eggplant (*Solanum melongena* L.), and to investigate the possibilities for growth on heavy metals polluted sites.

MATERIAL AND METHODS

Field experiment. The research was carried out during the period 2003-2004. Three *Solanaceae* crops, Bulgarian cultivars – tomato, pepper and eggplant, were grown true sowing on the industrially polluted area. The experimental plots were situated oat 0.5 km from the source of pollution, the NFMS. The field tests were set on four replications. The size of test parcel was set to 10 m^2 per each species. Tomato cultivar "Rila", pepper cultivar "Sivriya 600" and eggplant cultivar "Patladzhan 12" were the object of present research. The crops were grown after cereal forecrop. Each one of the crops was sown in the end of April with sowing density 1 g seeds per 1 m^2 and after germination was organized an interrow space of 60x30 cm. The harvesting was in the end of August. During the experiment the plants were watered regularly.

Sample preparation and digestion for metal analysis. Because of investigation of the level of pollution in the soil and the accumulation in different plant organs, soil and plant sample were taken. Soil samples were taken from 0-20 cm depth and were prepared for analysis of total metal content using partial digestion in autoclave with nitric acid 7 mol l^{-1} (HNO_3) (Karstensen et al., 1998). Soil physical and chemical properties are listed in Table 1.

The heavy metal concentration was determined in different parts of the studied crops (roots, stems, leaves and fruit-sets) using the method of wet mineralisation (Lozano-Rodríguez et al., 1995; Shilev, 2003). All tissues were carefully wash with distilled water, dried at 85 ^oC for 24 h, powdered and weighted for analysis. The digestion was made in autoclave using 100 ml bottles, 0.25g plant sample, 10 ml double distillated water, 3 ml HNO_3 (65 %) and 2 ml H_2O_2 (35 %). All the reagents for the study were provided by Sharlau Chemie S.A., Barcelona, Spain, or Merck KGaA, Darmstadt, Germany. The measurement was made with Atomic Absorption Spectroscopy.

Soil microbiological studies. Together with plant harvesting for heavy metal analysis, rhizosphere soil was taken from each one of the harvested plants for investigation on soil microorganisms. For control of microorganisms was used the same soil but not contaminated. The depth from where soil samples were taken was 0 – 20 cm. Viable microorganisms of different physiological groups were assessed, as follows: most probable number of bacteria, cellulose-decomposing aerobic bacteria, spore forming bacteria, fungi, bacteria grown on mineral nitrogen, and actinomycetes (Alef and Nannipieri, 1998; Goushterov et al., 1977). On the other hand, study of microbial respiration was made to evaluate soil microbial activity estimating the soil CO_2 content as difference between the sample (rhizosphere soil of each one of the cultivars) and control (non-contaminated soil) (Stotzky, 1965).

RESULTS AND DISCUSSION

The properties of the contaminated rhizosphere soil at distance 0.5 km from the NFMS (Table 1) have shown relatively low humus and phosphorus content. On the other hand, the soil heavy metal content (Table 2) was very high and exceeds the Bulgarian MPC. Lead soil concentration was determined at 630 mg kg^{-1} for the layer 0 – 20 cm, while the permissible limits are estimated to 80 mg kg^{-1} of soil. The results obtained for Cd, Zn and Cu were analogous. Values of 13.2 mg Cd kg^{-1} soil, 974 mg Zn kg^{-1} and 60.1 mg Cu kg^{-1} exceeds in times the permissible limits for Cd and Zn. The level of soil copper concentration was the unique which value was in the range of MPC – more than 4 times below. In the same time the Pb concentration was 8 times higher than the MPC, the concentration of Cd more than 5 times and the Zn ones almost 3 times higher.

The results for heavy metal content in the studied crops are given in Tables 3 – 5. There were observed significant differences in the accumulated values of metals in the plant tissues. The highest values of Pb were found in the leaves of eggplant, while the Pb content in the leaves of pepper and tomato was much lower (4 and 2 times). The lowest concentration of this metal was observed into the stems, particularly of tomato. In surprise, the concentrations of lead in fruit-sets were found to be significant – 81.82 mg kg^{-1} for tomato, 115.38 mg kg^{-1} for pepper and 42.1 mg kg^{-1} for the eggplant.

Cadmium is the unique metal for which some tissue concentrations were found to be higher than the soil ones. The highest concentration of Cd was observed in the roots of tomato, while the lowest one was found in the leaves of pepper. Cadmium content in fruit-sets was not significant compared with the concentrations in the rests of tissues.

On the other hand, the Zn concentrations were higher than the rest of the elements. It is clear that the Zn is not so toxic as Cd and Pb, but is more mobile. The roots of each of the studied plants had been accumulated the highest values of Zn: 321 mg kg^{-1} in tomato, 222 mg kg^{-1} in pepper and 241.55 mg kg^{-1} in the eggplant. The lowest rates were observed for the fruit-sets, accept for the pepper, where the accumulated value of Zn (190.39 mg kg^{-1}) was higher that in the stems (108 mg kg^{-1}) and leaves (94.2 mg kg^{-1}).

In the case of Cu, the accumulated values were less than soil Cu concentration. The highest Cu concentration was found in the roots for the tomato, in the fruit-sets for pepper and in the leaves for eggplant. The lowest concentrations of that metal were observed in the steams of all studied plants.

It seems that the highest values of all four heavy metals were accumulated in the roots for tomato plants, while for pepper and eggplant the highest values were found in roots and fruit-set and in leaves, respectively.

The results of the most probable number (MPN) of bacteria (Table 6) were found to be quite similar for all of the studied plant species accepts for the pepper, where the viable cells were less. On the other hand, the number of spore-forming bacteria was reduced in rhizosphere pepper and eggplant compared with the tomato. The rest of results on assessment of viable microorganisms did not show significant differences.

The microbial respiration in the soil samples taken from plant rhizosphere of each one of the studied crops shown similar results (Table 7). The rate of CO_2 was found to be higher in the rhizosphere than in non-rhizosphere samples, which is logical because of in the root zone the microbial activity is much higher, also in contaminated soil.

CONCLUSIONS

The following conclusions can be made on basis of the obtained results for heavy metal content and microbial activity.

1. The heavy metal concentrations found in the soil samples exceed in times the maximum permissible limits accept for the copper.
2. The accumulated quantities of heavy metals were concentrated in different parts for each one of the crop – in roots (tomato), in fruit-set (pepper) and in leaves (eggplant).
3. Tomato, pepper and eggplant are not crops for which is suitable to be grown in industrially polluted areas, because they accumulate significant quantities of heavy metals and can be dangerous for consumption.
4. The microbial activity was higher in the rhizosphere of tomato plants.

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Table 1. Soil properties sampled from 0-20 cm depth of rhizosphere of the studied plants.

Classification	pH (H_2O)	Humus (%)	Mineral N (mg kg^{-1})	P_2O_5 (mg kg^{-1})	K_2O (mg kg^{-1})
Contaminated soil	7.45	1.48	17.7	132.5	562

Table. 2. Soil Pb, Cd, Zn and Cu content (mg kg^{-1}). The results express the mean ± the standard error of three replications.

	Pb	Cd	Zn	Cu
Contaminated soil	630 ± 27.5	13.2 ± 0.62	974 ± 23.06	60.1 ± 2.95
MPC	80	2.5	340	260

MPC – maximum permissible limits.

Table 3. Heavy metal (Pb, Cd, Zn and Cu in mg kg^{-1} DW) content in tomato. The results express the mean of three replications. The standard error was in the range of 5 %.

	Pb	Cd	Zn	Cu
Roots	99	18.9	321	55.5
Stems	16.8	9.6	258	16.8
Leaves	88.8	15.3	219	37.5
Fruit-set	81.82	7.09	125.45	49.09

Table 4. Heavy metal (Pb, Cd, Zn and Cu in mg kg^{-1} DW) content in pepper. The results express the mean of three replications. The standard error was in the range of 5 %.

	Pb	Cd	Zn	Cu
Roots	57.6	14.4	222	42.6
Stems	27.6	15.6	108	16.8
Leaves	39.6	2.52	94.2	39.6
Fruit-set	115.38	11.54	190.39	75

Table 5. Heavy metal (Pb, Cd, Zn and Cu in mg kg^{-1} DW) content in eggplant. The results express the mean of three replications. The standard error was in the range of 5 %.

	Pb	Cd	Zn	Cu
Roots	42.3	13	241.55	34.76
Stems	23.6	12	219	18.3
Leaves	155	14.1	216	40.4
Fruit-set	42.1	8.2	145.8	39.9

Table 6. Colony forming units (c.f.u.) of different physiological groups microorganisms assessed in the rhizosphere of *Solanaceae* plants in soils of the area of NFMS.

Plant species	**Soil**	**Bacteria**				**Actinomycetes**	**Fungi**
		Spore-forming	Grown on mineral nitrogen	Cellulose decomposing	Most probable number		
Tomato	cont.	10^5	4.10^6	10^4	10^7	4.10^5	3.10^4
Pepper	cont.	$6,8\ .10^4$	$3,2\ .10^6$	10^4	$4,5.10^6$	$6,65.10^5$	$3,6.10^4$
Egg-plant	cont.	5.10^4	$5,5\ .10^6$	10^4	8.10^6	$4,5.10^5$	$3,5.10^4$

Table 7. Respiration rates of non-rhizosphere and rhizosphere soil of the *Solanaceae* plants grown in contaminated area.

	Non-rhizosphere soil	**Rhizosphere soil**		
		Tomato	**Pepper**	**Eggplant**
CO_2 production (mg kg^{-1} soil)	900	1100	1050	1010