Bulgarian Journal of Agricultural Science, 21 (No 2) 2015, 295–299
Agricultural Academy

EFFECTS OF HEAVY METALS FROM POLLUTED SOILS ON THE ROOTS AND NODULES FORMATION

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Abstract

KLIMEK-KOPYRA, A., A. BARAN, T. ZAJĄC and B. KULIG, 2015. Effects of heavy metals from polluted soils on the roots and nodules formation. *Bulg. J. Agric. Sci.,* 21: 295–299

Intensive development of transport in suburban areas contributes to excessive accumulation of heavy metals in cultivable soils. The negative effect of heavy metals on legumes becomes apparent in specific concentrations in the soils, hence it is not always possible to determine direct harm of environment to the growth of plants root system. The aim of the research was to evaluate the impact of soil conditions on root system weight, weight and number of root nodules in different legumes species; field pea (*Pisum sativum*) and spring vetch (*Vicia sativa*). Additionally, we assess the degree of metals accumulation in roots dry mass. Research studies have shown that an increase in heavy metals content in agricultural soils in an urbanized area results in a decrease in microbiological activity of soils, their fertility, and in consequence a decrease in plants productivity. Metals pollution of soils resulted in deterioration of biometrical parameters of the root system and deterioration of efficiency of the symbiotic system. Field pea showed little sensitivity to the presence of heavy metals in the soil. Spring vetch was more sensitive to soil pollution, developing lesser root mass. Field pea was less sensitive to the cultivation station, the effect of which was a lower level of zinc, chromium and nickel accumulation. Moreover, field pea showed high detoxification capacity, accumulating a large amount of lead in the roots.

Key words: heavy metal, root system, legumes

Introduction

Heavy metals coming from industrial districts, they accumulate in soils of agricultural use and pose a real threat to the agrosystem (Cheung and Gu, 2007; Petrova et al., 2013). Successive accumulation metals in soils causes changes in the soil environment and in soil biological activity, in consequence leading to a loss in soil fertility (Paudyal et al., 2007). Symptoms which are usually caused by the presence of high metals concentrations in soil include: a decrease or inhibition of shoots growth, a decrease in the number of leaves, and their premature ageing as well as deficits of micro-and macroelements (Wani et al., 2007). High concentrations of metals reduce the increase rate of root mass and root hair density, which was observed in, among other things, *Phaseolus vulgaris*, *Pisum sativum*, *Vicia faba, Medicago sativa* (Piechalak et al., 2002; Broos et al. 2005; Aydinalp and Marinova, 2009). A high heavy metals concentration affects a number of physiological and biochemical processes of plants, the effect of which is lower seed yield (Ahmad et al., 2008a). Hirsh et al. (1993) showed that legumes (*Triforium repens*) which are cultivated in conditions of a metal-contaminated soil have the possibility to form root nodules, but without the capacity for nitrogen fixation from the atmosphere (which is indicated by their pink color). Some heavy metals belong to microelements and, in small concentration, play a significant role in the essence of activity of many enzymes: proteinases,

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dehydrogenases and peptidases. Zinc is a metal that is required for the synthesis of carbohydrates, proteins as well as some hormones. Copper also plays a critical role in different physiological processes such as respiration, photosynthesis, carbohydrate distribution as well as seed formation (Kabata-Pendias and Pendias, 2001).

However, some metals show high toxicity to plants as a result of the increase in concentration in soil. An example of such metals is cadmium, which, although does not take part in biological processes, after being uptaken by a plant becomes more toxic to that plant or disturbs the biological processes of plants. According to Stan et al. (2011), numerous heavy metals such as copper, nickel, zinc or cadmium contribute to the inhibition of the processes of plant growth and development as well as nitrogen fixation by nodule bacteria of the *Rhizobium* genus. Taking into account the role of leguminous plants in animal and human feeding as well as the resulting benefits for the environment, more attention should be drawn to understanding to what degree heavy metals deform the root system of legumes and its biological functions. Gabara and Golaszewska (1992) observed changes in the behavior of roots which were connected with the loss of fresh and dry matter in the presence of lead in soil. Young (primary) roots penetrated the areas of soil in search for heavy metal ions. Woźny et al. (1995) and Kumar et al. (1995) establish that roots cane take up 90–95 times more lead than stems and leaves. When studying different cultivars of pea, Alzandi (2012) indicated that the root system of some pea cultivars may accumulate high doses of lead (90 mg $Pb \cdot g^{-1}$) in fresh root mass. This fact proves that field pea is a species (detoxificator organisms) with high capacities to detoxicate metals. One may assume that the detoxification capacity of legumes may have a negative effect on parameters of the formed root nodules as well as on the amount of root mass.

The aim of the research was to assess the effect of soil properties on the size of the root system and root nodules of field pea and spring vetch.

Materials and Methods

The field experiment was conducted in the years 2006-2008 at an Experimental Station in Prusy, near Kraków, located within a 7 km radius from Huta Sendzimira (Tadeusz Sendzimir Steelworks) as well as in a production field (Olszanica) situated 6 km from the Kraków-Balice Airport. Degraded chernozem (soil 1), formed from loess and classified as very good wheat complex and soil quality class I, was the soil of the experimental fields in Prusy in all years of the research. In Olszanica, the soil of the experimental fields was determined as typical brown soil (soil 2) with granulometric composition of medium soil, classified as good wheat complex and class II. The soil reaction in Prusy was neutral, with the mean value of pH_{KCl} = 6.8, and the content of available forms of phosphorus in that soil was 23.2 mg, and of in the case of potassium it was 15.4 mg · $100g^{-1}$ · In Olszanica, the content of assimilable forms of potassium in the soil reached 16.9 mg, whereas the content of assimilable forms of phosphorus reached 26.2 mg·$100g^{-1}$, and the soil reaction was neutral (pH_{KCl} = 6.8). The soil of the experimental fields in Prusy had an average content of organic matter (1.3%, on average), whereas the soils from Olszanica contained significantly larger amounts (2.0%, on average).

Parameters of the root system of pea and vetch were studied in the flowering stage, which is characterized by the maximum increase in the number of roots. 20 monoliths were collected from the studied objects from the upper (0–30 cm) soil layer. Individual samples were washed in an automated root washer (Delta-T Root Washer, type RWB/RWC). The washed roots were transferred onto absorbent paper in order to drain excess of water. Roots and nodules were then isolated, the number of root nodules was counted and their viability was determined by assigning a color (pink color for viable ones, grey for the ones with no viability). After scanning, roots and nodules of the plants were dried at a temperature of 105° in order to determine the dry matter content.

Soil samples from the pea or vetch cultivation were collected from chernozem soil (soil 1) and typical brown soil (soil 2) every year in the flowering stage of the plants. The soil was dried, and then the content of selected heavy metals was determined. In order to determine the total content of heavy metals, the soil material was digested in mixtures of HNO_3 and $HClO_3$ (3:2) acids. In the obtained filtrates the zinc content was determined using the ICP-OES method with the PerkinElmer Optima 7300 DV apparatus.

Statistical analysis was performed using ANOVA (Statistica 9.1 software). Comparison of means was performed using Tukey's HSD test, at the significance level $p \leq 0.05$.

Results and Discussion

The performed laboratory analysis indicated a considerable diversification in soil parameters and in their influence on the degree of metals accumulation in the root mass (Figure 1*a,b*, 2). The chernozem (soil-1) accumulated a higher amount of heavy metals than the brown soil (soil-2). A lower degree of pollution with cadmium, chromium, nickel, lead and zinc was observed in the brown soil. On the other hand, a growing presence of copper was found. The higher amount of Fe and Mn in the soil indicates a lower mobility of these elements connected with the demand of the plants.

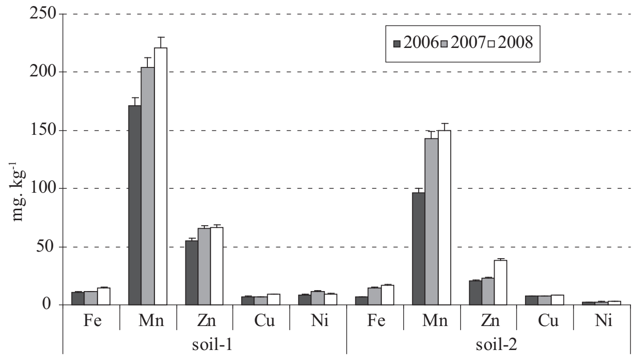

Fig. 1*a*. Accumulations of metals in different soil type

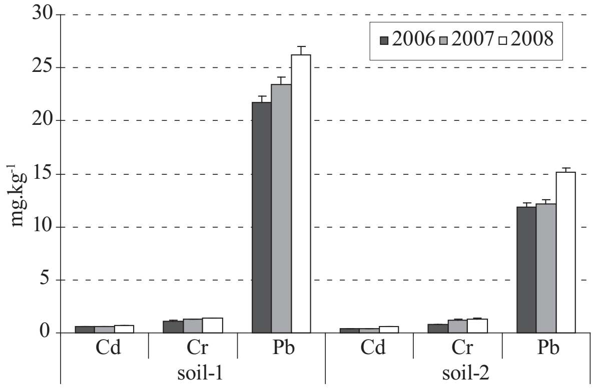

Fig. 1*b*. Accumulations of heavy metals in different soil type

The chernozem (soil-1), located in the area of the steelworks, distinguished itself by a higher lead content in comparison to the typical brown soil (soil-2). Moreover, a slight increase in the amount of lead was observed in subsequent years of the research, which may be an evidence of growing pollution of the agriculturally active soil within a close distance from the steelworks and airport.

The increase in heavy metals in the studied soils in subsequent years of the research made the metals more available to the pea and vetch plants. The degraded chernozem contained more heavy metals, the effect of which was higher accumulation of iron, manganese, nickel, chromium, cadmium and zinc in pea roots. The analyzed legumes had different degrees of heavy metal accumulation in the root system (Table 1). Spring vetch was more sensitive to soil pollution, developing lesser root mass, whereas field pea was less sensitive to the heavy metals content in soil. It finds confirmation in the research of Baran et al. (2008), based on which it was found that pea is more resistant to the phytotoxic effect of metals than spring vetch. The increase in pollution with zinc and cadmium limited the longitudinal growth of the roots to a lesser degree.

Growth inhibition of the roots, and in consequence a lesser mass of the roots and nodules of field pea and spring vetch in the chernozem were observed (Table 2, Figure 2). The root system of vetch showed significantly (*$P \leq 0.05$) higher sensitivity to the presence of heavy metals, the effect of which was a lesser increase in the amount of dry matter.

Table 2

Determination of dry root weight (RDW) and nodules dry weight (NDW) depending on experimental factors

Treatments	RDW (g)		NDW (g)	
	Pisum sativum	*Vicia sativa*	*Pisum sativum*	*Vicia sativa*
2006	0.339a	0.304 b	0.207 a	0.206a
2007	0.338a	0.276 a	0.189a	0.204a
2008	0.393b	0.362 c	0.266b	0.237b
Soil-1	0.394b	0.311a	0.219a	0.226b
Soil-2	0.318a	0.316 a	0.222a	0.205a

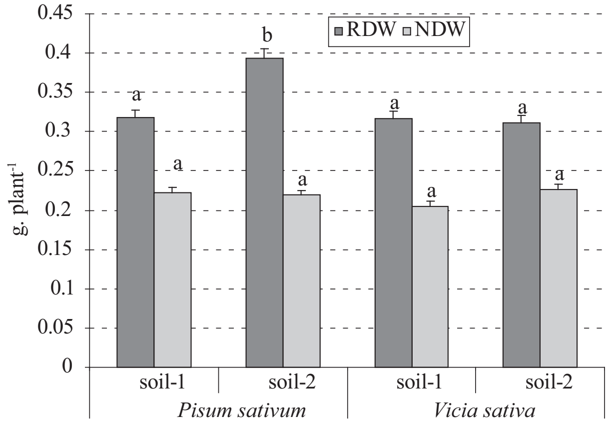

Fig. 2. Root dry weight (RDW) and nodule dry weight (NDW) of *Pisum sativum* and *Vicia sativa* depending on soil type

Table 1

Heavy metals contamination in roots of legumes, average from 2006–2008

Species	Soil type	Cd mg.kg^{-1}	Cr mg .kg^{-1}	Cu mg .kg^{-1}	Fe mg .kg^{-1}	Mn mg .kg^{-1}	Ni mg .kg^{-1}	Pb mg .kg^{-1}	Zn mg .kg^{-1}
Vicia sativa	soil-1	1.31 b	45.97 a	21.83 a	1235.73a	45.05a	22.52a	3.32a	47.99b
	soil-2	1.16 a	28.40 b	20.24 b	1107.57b	30.67a	14.77a	3.75b	37.03a
Pisum sativum	soil-1	1.44 a	41.29 a	21.36 a	1171.61a	54.73a	19.89a	6.04b	46.03b
	soil-2	0.83 a	22.73 b	21.10 a	903.41a	29.61a	11.87a	4.24a	26.97a

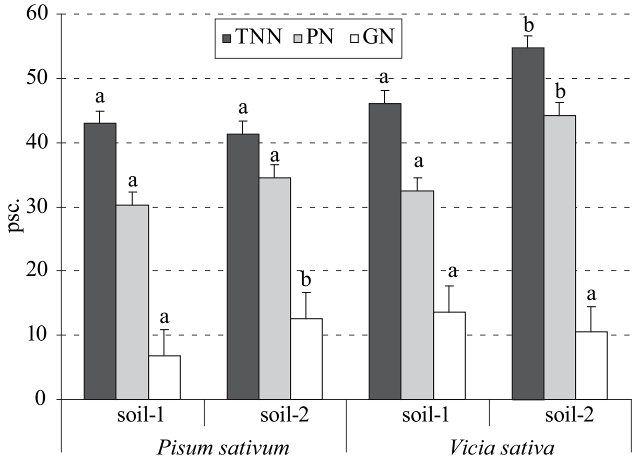

Fig. 3. Total of nodules number (TNN), number of pink nodules (NPN), number of grey nodules (NGN) in *Pisum sativum* and *Vicia sativa* roots system depending on soil type

The root nodules were less sensitive to substratum quality, the result of which was slight quantitative diversification of nodules, regardless of soil genus. Reichman (2007) states that legumes react negatively to the increase in the amount of metals in the soil, causing a reduction of the number of plant nodules; the number of lateral branches in the root system decreases, and so does the root mass. Awosanya et al. (2012) establishes that cowpea is sensitive to soil quality. The increase in soil pollution with metals led to a significant fall in the number of nodules fixated onto the roots.

The number of pea root nodules as well as their viability significantly varied (*$P \leq 0.05$) over the years and depended on soil conditions (Figure 3, Table 3). Along with the increase in the amount of heavy metals in the chernozem, a proportional increase in the number of gray nodules and an inversely proportional increase in the number of pink nodules on the pea roots were observed in subsequent years of the research. A significant, quantitative diversification in the years was obtained for the pink nodules, regardless of soil type. The number of vetch root nodules as well as their viability varied over the years and depended on soil conditions (Figure 3, Table 3). Along with the increase in metals content in the chernozem, an increase in the number of dark nodules with low activity of nitrogen fixation from the atmosphere was observed. Research of Obbard and Jones (1993) indicate low physiological sensitivity of legumes to the presence of heavy metals in soil.

Heavy metals have a negative effect on the growth, development and activity of root nodules (Stan et al., 2011). Manier et al. 2009 stated that an increase in concentration of heavy metals in soil, i.e. zinc, lead, and cadmium (300 mg Zn kg^{-1}, 130 mg Pb kg^{-1}, 2.64 mg Cd kg^{-1}) significantly decreased the number of root nodules of white clover. The Author suggests that root nodules may serve as a suitable bioindicator of the increase in the number of heavy metals in soil. Paudyal et al. (2007) confirmed the rightness of this thesis by conducting an experiment with aluminum. The nodule bacteria of the Rhizobium genus grew poorly in conditions of low soil abundance of aluminum, but an increase in aluminum dose (50 mM) stopped development of the nodules entirely.

It was observed in own research that the increasing soil pollution with heavy metals (Cd, Pb, Cr) caused a lesser increase in the amount of mass of the roots and nodules. Moreover, no inhibition of nodule development was observed, but the number of bright nodules decreased, which may suggest poorer efficiency of the symbiotic system in successive research.

Wierzbicka (1995) observed that lead ions do not affect the increase in roots mass directly but indirectly through disturbances in the process of water uptake by plants, the result of which is a reduced increase in the amount of root mass. Geebelen et al. (1999) confirmed this by studying the effect of lead on the increase in the amount of root mass in bean (*Phaseolus vulgaris*). Alzandi (2012) proved that pea can accumulate large doses of lead in fresh root mass, showing losses in increases in the number of root mass. The above-mentioned research was confirmed in own research, proving the high activity of field pea for heavy metals accumulation in dry matter of roots. Te high activity of field pea for heavy metals accumulation had an unbeneficial effect on the amount of root mass and the number of nodules. The formed nodules were smaller and exhibited lower

Table 3
Determination of total nodules (TNN) number of pink nodules (NPN), number of grey nodules (NGN) depending on experimental factors

Treatments	TNN (psc)		NNP (psc)		NNG (psc)	
	Pisum sativum	*Vicia sativa*	*Pisum sativum*	*Vicia sativa*	*Pisum sativum*	*Vicia sativa*
2006	45.7b	54.5b	36.7b	40.7b	9.0a	13.8a
2007	33.2a	41.9a	25.3a	29.3a	7.9a	12.6a
2008	47.4b	54.9b	35.1b	45.1b	12.3	9.8a
Soil-1	41.2a	54.7b	34.5a	44.2b	6.8a	10.5a
Soil-2	42.9a	46.2a	30.3a	32.5a	12.7b	13.7a

symbiotic activity, which was indicated by their gray color. Pereira et al. (2006), Younis (2007) and Noriega et al. (2007) showed that even a low cadmium content in soil hampers the productive activity of plants and disturbs the process of creating new nodules and the growth process, and also the process of root, shoot and leaf elongation. Paudyal et al. (2007) determined the effect of metals (Al, Fe and Mo) on *Rhizobia* strains isolated from nodule bacteria of two tropical legumes. Aluminum had a negative effect on the growth and development of root nodules. Iron and molybdenum showed the opposite properties; in doses of 25 μM and up to 20 μM they produced a positive effect in terms of growth in root mass and in mass of the root nodules. Hirsch et al. (1993) showed that *Rhizobium leguminosarum bv. trifolii* nodule bacteria changed as a result of long-term presence of metals in soil, the effect of which was a lost capacity for synthesis of root nodules. Another, multi-annual field research (Chaudri et al., 2000) showed a decrease in the activity of *Rhizobium leguminosarum* bv. *viciae* and *Rhizobium leguminosarum* bv. *trifolii* bacteria in agricultural soil.

Conclusions

An increase in heavy metals content in agricultural soils in an urbanized area results in a decrease in microbiological activity of soils, their fertility, and in consequence a decrease in plants productivity. Field pea (*Pisum sativum*) and spring vetch (*Vicia sativa*) showed little sensitivity to the presence of heavy metals in the soil. Metals pollution of soils will result in deterioration of biometrical parameters of the root system and deterioration of efficiency of the symbiotic system. Spring vetch (*Vicia sativa*) was more sensitive to soil pollution with heavy metals, developing lesser root mass, whereas field pea (*Pisum sativum*) distinguished itself by high capacity for lead accumulation.

The conducted research approves legumes as a good biomonitor of urban contamination, which can be successfully used for the biomonitoring of the environmental quality.

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Received June, 2, 2014; accepted for printing December, 2, 2014.