

Evolution of the absorption of heavy metals in function of nutrients

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Abstract: The condition of an eco-system greatly depends on the different biotic and abiotic factors. The area along the river “Tisza” is highly imperiled by random appearance of heavy metal pollutants originated from mining accidents or other sources. Heavy metals are dangerous because of the bioaccumulation and they could be toxic or poisonous even at low concentration. The aim of our research was to test the reaction of a simplified soil-plant pot experiment, consists of garden cress (*Lepidium sativum*) for metal pollution close to the sanitary limiting value.

Garden cress is considered one of the most important agricultural vegetables and its short reaction time for various treatments makes this plant ideal object of eco-toxicological tests. Based on the results it is realistic assumption to expect decrease the above-mentioned flexibility character. Garden cress (*Lepidium Sativum*) was chosen as a test plant to simulate the accessible pollutant uptake. Additionally, *Lepidium Sativum* is a possible carrier of heavy metals in food chain, since it is a many-sided green vegetable consumed by humans and animals as well. The present study is undertaken to examine the level of accumulation as it is modified by a plant, if the plant growing up under other conditions. It appears how for each factor as in mobilizing heavy metals, the plant laboratory water will affect the special nutrient solution or soil pollution. Responsive changes are relevant for all of the different conditions relevant for mobile heavy metals. The accumulation levels may undergo variations in function of them. In the present study, such change is characterized by the cress garden.

Keywords: heavy metals, Cd and Cu accumulation, garden cress environmental risk, heavy metals

Introduction

The heavy metal pollution is one of the leading issues of nowadays. Heavy metal pollution can cause enormous changes in the flora and fauna community decreasing of resilience capacity of the soil, although the estimation of its danger level alters according to loadability of the carrier medium.

Garden cress (*Lepidium sativum*) was chosen as a test plant to simulate the accessible pollutant uptake (Alvarenga 2008);(Herwijnen 2008). Additionally *L. sativum* is a possible carrier of heavy metals in food chain, since it is a many-sided green vegetable consumed by human beings and animals (Terbe 2000).

Nowadays alarming amount of pollution enters into the environment due to anthropogenic activities. Human health is directly endangered when these pollutants get into the food chain. Because of the environmental pollution luckily there is an increased attention on the potentially toxic elements and on the dangers related to heavy metals (Mileston 1994, Vermes

1994). Detailed and intensive examinations are imperative in order to avoid extended and extensive contaminations (Kádár 1995). Wide range of technologies are available to remediate the contaminated soils however, most of these methods are costly and non aesthetic solutions (Yoon et al. 2006).

The most polluting heavy metals include cadmium (Cd), copper (Cu), lead (Pb) and zinc (Zn), while iron (Fe) and manganese (Mn) can be contaminating in large amount (Fomes et al 2009, Filep 2002).

The metals and their compounds make a significant impact on most of the biological processes. The metals can be broken down biologically, accumulate in living organisms, toxic compounds in the subsequent biochemical reactions may occur over (Kádár and Morvai 2007; Smolinsk and Cedzyska 2007).

If large amounts of toxic materials get into the soil, the adsorption and precipitation reactions become dominant. Acidification of the soil in turn significantly increases the quantity of

mobile ions, the concentration of the metal ion in solution. Acidification is specially dangerous in already polluted areas, because the insoluble state of heavy metal compounds can cause serious environmental damage to the soil when they are mobilized (Yoon et al. 2006).

According to our knowledge the essential elements for the plants can be divided into two groups, those that are required in relatively large quantities (macro-elements H, C, N, O, Mg, P, S, K and Ca), and those which are needed only in a small amount (micronutrients: C, Cl, V, Mn, Fe, Cu, Zn and Mo). However, as the experimental techniques are getting more and more precise, the number of microelements that are important for the plants will continue to grow (Györi 1998, Luo and Rimmer 1995).

Materials and methods

Sampling

During earlier investigations it was found that those samples that had Cu and Zn concentrations exceeding the pollution limits or approaching them. Into these soil samples were planted cress seeds and after 14 days the Cu and the Zn concentration were determined in the roots, stems and leaves with FAAS method. The physical parameters of the plants (e.g. root length, stem length, leaf surface) were determined in each case. In the experiment was placed 2 cm sample of the cress seeds were sowed into 50 ml beakers.

Table 1. The composition of ten times concentration of nitrogen-free Hoagland solution and content of Arnon-type solution

Composition of ten times concentration of nitrogen-free Hoagland solution			Content of Arnon-type solution	
Chemical	Concentration		Chemical	Concentration
	g dm ⁻³	mmol/dm ⁻³		
FeSO ₄ · 7 H ₂ O	0.05	0.18	H ₃ BO ₃	1.43
CaCl ₂	3.05	27.5	MnCl ₂	1.42
MgSO ₄ · 7 H ₂ O	2.46	10	CuSO ₄ · 5 H ₂ O	0.04
Na ₂ – EDTA	0.06	0.161	Na ₂ MoO ₄ · 2 H ₂ O	0.059
Arnon-type solution*			ZnSO ₄ · 7 H ₂ O	0.11
KH ₂ PO ₄ **				

* - Before filling the stock solution up to 1 cm³, 20 cm³ of Arnon-type solution was added to the flask.

** - From the potassium-dihydrogen phosphate we create a separated stock solution (111,62 dm⁻³), which was supplemented with trace elements, we only add it to the nitrogen-free Hoagland stock solution while diluting.

During the experiment, the soaked seeds were kept wet with distilled water.

The contamination happened with 0.56 mg Cu²⁺, and 1.965 mg Zn²⁺. The experiment happened in containers, that were specially designed for the rearing of plants occurred. Into the containers were sprayed 150-180 pieces of cress seeds by water. Previously these were soaked in distilled water (for 10-20 minutes) and then they were placed them on the web that layed in the containers. The cups had a 4 cm diameter and 2-3 cm thick. After the plants were contaminated, the liquid in the pot to the roots was always refilled until the net.

Besides the above mentioned container cress was planted into petri dishes, too. The contamination occurred the same level, 0.56 mg and 1.965 mg Cu²⁺ Zn²⁺ solution. Within the petri dishes 6 grams of cress was groved. This is 15-20 times more than the plants mentioned at paragraph 2. The Petri dishes had 15 cm of diameter, lined with filter paper. The amount of seeds that was spread onto the surface was 6 g. The plants were kept wet by distilled water after the pollution. They were treated for three days.

Preparation of the Hoagland solution

The medium was prepared on the day of use: 100 cm³ Hoagland stock solution was diluted to 1 dm³, while KH₂PO₄ to 5 cm³ of the stock solution was added. The pH is could be set to approximately 6 with adding 1 mol dm⁻³ NaOH (approx. 0.6 cm³).

Monitoring of soil pollution

Sampling: 50 soil and 4 control samples were collected from 0-30 cm (A) and 70-100 cm (B) depth along Szabolcsveresmart settlement, which frequently called Rétközi reservoir (located in Szabolcs – Szatmár – Bereg county, GPS coordinates: N: 48.29340° E: 22.03357°). Samples were taken according to the sampling network based on standards no. MSz 21470-50 (Msz. 2007).

After transport to the laboratory, samples were left to dry by exposing to the air. In order to remove the bigger stones and remnants of roots, the samples were sifted through a 2 mm sieve.

Dry weight content and pH determination:

Determination of dry weight content and pH were made according to standards no. MSz-08-0205-1978; MSz-08-0206/2-1987 (Msz. 1978, Msz. 1987).

Phytoextraction experiment

Phytoextraction processes with *L. sativum* in soil were conducted under laboratory condition.

General treatment of the seeds:

4 containers were filled the soil derived from Rétközi reservoir (see on Figure 1.: 17-A; 20-A; T-A; T-B). Three replicated were used in all variants. The soil type is Eutric Cambisol (WRB reference). 4 soil samples (T-1 to T4) with outstanding value of concentration (compared

to the average value) were used. The bio cross seeds, produced by BIOrganic Ltd, were scattered over the soil. Small amount of soil was sprinkled lightly over them, just to cover. A spray bottle was used to keep the seeds evenly moist. They sprouted after 2-3 days. 0.2 g (138 pieces) seeds were used for one experiment. The seedling occurred at room temperature (between 18-23 °C). At the end of 14th day, the full-grown plants were removed and carefully washed off. The redundant water was done away and every plant was separated into roots, stems and leaves. The parts of the plants were digested as it is described below.

Microwave digestion

Sample preparation:

Soil: at first samples were sifted through a 0.2 mm sieve. 0.5 g dry matter soil was taken into each teflon bomb. Then 5 cm³ 65% HNO₃ and 2 cm³ 30 % H₂O₂ were added before starting the digestion program.

Plant: The preparation of plant samples happened the same way, but different quantity of acids were added: 6 cm³ 65% HNO₃, 1 cm³ 30 % H₂O₂.

Digestion:

Samples were digested by a MILESTONE 1200 Mega Microwave Digester (see the digestion program in Table 3. – (Terbe 2000)).

Determination of heavy metal content:

After the procedure described above the

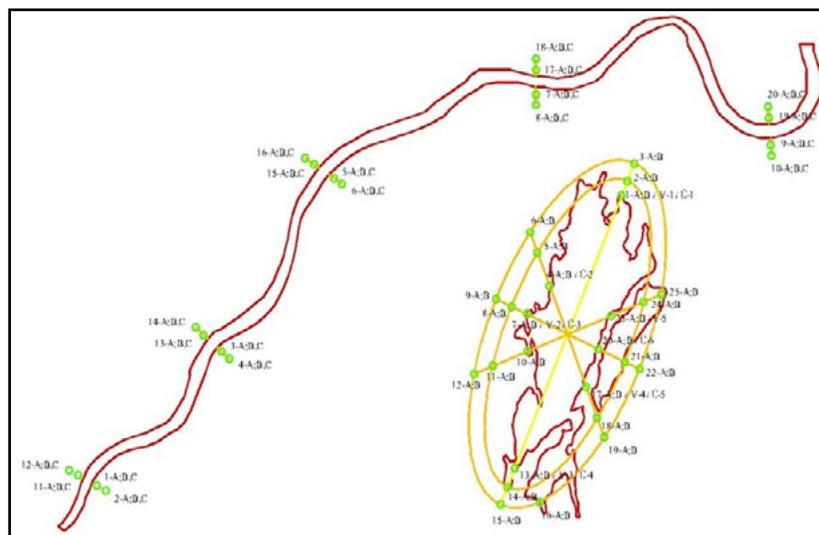


Figure 1. The “Rétközi” lake sampling points, which shows the location of the sampling grid patterns.

Table 2. Digestion program

Steps	Time [min]	Process [Watt]
	Soil / Plant	Soil / Plant
1.	5 / 2	Digesting, 250 / 250
2.	2 / 2	Aeration
3.	5 / 6	Digesting, 400 / 250
4.	5 / 5	Digesting, 250 / 400
5.	7 / 5	Digesting, 700 / 600
6.	5 / 5	Aeration

following elements were measured by ICP-OES and FAAS: Al, Ca, Cr, Cu, Fe, Ni, Mg, Mn, Pb, Zn in soil and Zn, Cu in garden cress.

Monitoring of the soil pollution

It became clear after completing the variance analysis (ANOVA one-way test) that the heavy metal content of the soil samples significantly depends on the place of sampling. Only one soil sample's Cu concentration (sample id.: 22-A, see on Figure 1.) $84,8 \text{ mg kg}^{-1}$ was higher than the limit value (75 mg kg^{-1}) based on the Governmental Regulation 6/2009. (IV. 17.) KVVMEÜM-FVM. In the case of other measured elements neither of them exceed the limit values after summarizing all the results. These results will be used as reference base of a long term monitoring system. The reason 22-A was not taken into account is that the seed sprouting was inhibited at that degree of pollution causing the insufficient quantity of plant dry matter for the analysis.

Other parameters

Soil tests should be taken into properties of soils, because the soil plays an important role in the biosphere of the transformation of materials, environmental protection (Györi 1998). Therefore, it is necessary to identify and take account of the different properties of the

soil. These parameters were performed (Table 2) on the basis of Györi (1998).

Phytoextraction experiment

It has to be taken into consideration, that soil parameters have notable effect on these experiments: metals, heavy metals, cations are not so mobile in carbonaceous soil (Kádár and Morvai 2007). The transfer coefficient (T %) was calculated, too (Formes et al. 2009). Although the organic matter content is determinant regarding the heavy metal concentration of the soil solution, straight correlation cannot be demonstrated by adsorptional experiments. The reason for this is pH affects the creation of metalorganic complexes and there is a strict correlation between pH and the degree of absorption. Another important factor beside this is that the structural diversity of the mould material, which is not mapped completely yet, cannot be regarded as a uniform material. Their absorption and cation-exchanging characteristics are different and depend on pH (Luo and Rimmer 1995).

It can be concluded that in almost every case the heavy metal concentration in the roots were significantly higher than in the other plant parts (see on Figures 2,3). It is favorable for the consumers because heavy metals accumulate only in a small proportion in leaves and stalks of the garden cress.

ATI UNICAM 939 AAS was used for determination of heavy metal concentration.

Results and Discussion

Evaluating the CU concentration

The soil samples for the enrichment of heavy

Table 3. Physical and physico-chemical characteristic and composition (dry weight basis) of studied soils

	The soil*					Concentrations (mg/kg in dry matter)			
	T-1	T-2	T-3	T-4		T-1	T-2	T-3	T-4
SP* (K_A)	30	28	55	44	Zn	165.6	32.2	189.9	42.3
CaCO ₃ (%)	1	1	1	1	Cd	23.0	10.8	41.4	14.7
Humus (%)	2.2	2.3	2.3	2.3	Cr	2.8	4.0	0.9	3.6
C/N ratio	16.7	12.4	15.0	14.4	Al	1630.7	3519.3	1031.5	2411.2
pH (H ₂ O)	5.70	6.20	7.12	5.97	Pb	3.4	2.0	1.4	1.6
pH (KCl)	5.69	6.19	7.13	5.89	Ni	1.9	2.2	0.6	1.9
DM* (%)	98	99	99	97					

* Values of the polluted sediment sample in the „Tisza” (T-1 to T-4);

SP = soil plasticity (K_A). DM = dry matter content

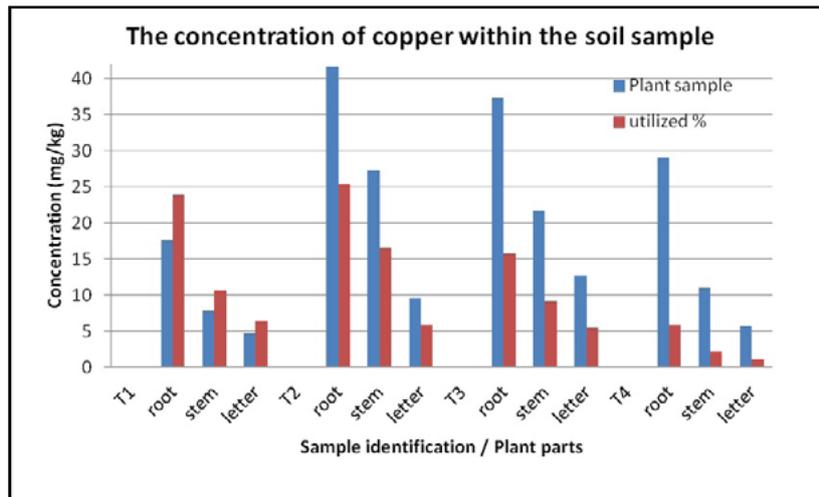


Figure 2. The concentration of copper in the soil pattern increased in plant.

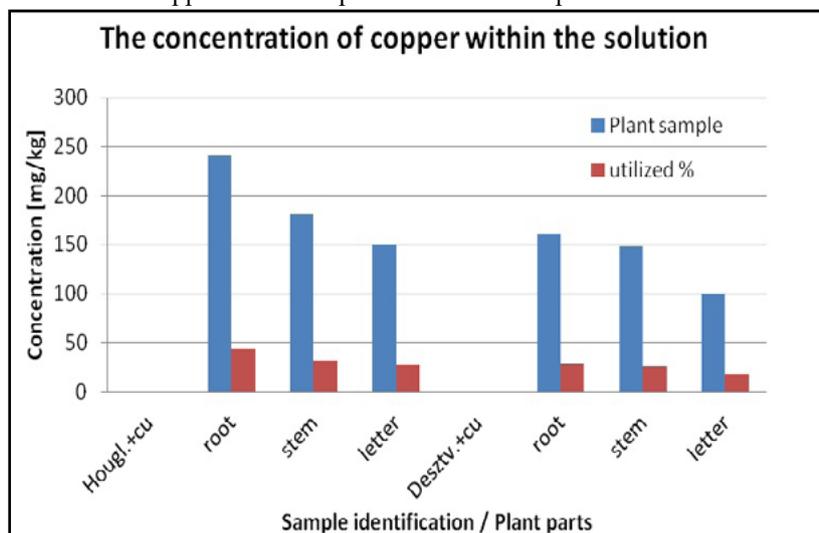


Figure 3. The concentration of copper in the solution pattern increased in plant.

metals in the accumulation of plants 20 mg / kg concentration limit varies. The higher the C / N ratio of soil, and the acid-base sensitivity of the soils neutral pH (5.6) produces the utilization value of around 25%. Declining C / N ratio and the effect of increased metal concentrations to around 5% decrease in utilization. The parts of the plant does not determine the extent of the accumulation. The root part of the largest accumulation, while the smallest part of the letter.

The liquid phase accumulation of heavy metals in plant samples grown Cu concentration ratio of deficit to alter the accumulation of plant parts. The parts of the plant does not determine the extent of the accumulation. The root portion of the maximum accumulation, but is similar to the degree of drying sections. The soil grown

plants stems the accumulation of Cu accumulates at higher rates.

The medium plant samples grown heavy metal accumulation does not alter the rate of accumulation of deficits Zn concentration in plant parts. The parts of the plant does not determine the extent of the accumulation. The same plants grown in soil accumulation rate takes place. The utilization rate minimal however, shows a utilization of around 1%. The pollution-free medium, this value of high C / N and the neutral pH plants grown in soil samples is identical.

Evaluating the Zn concentration

The soil samples for the enrichment of heavy metals in the accumulation of plants 10 mg / kg concentration limit varies. The higher the C/N

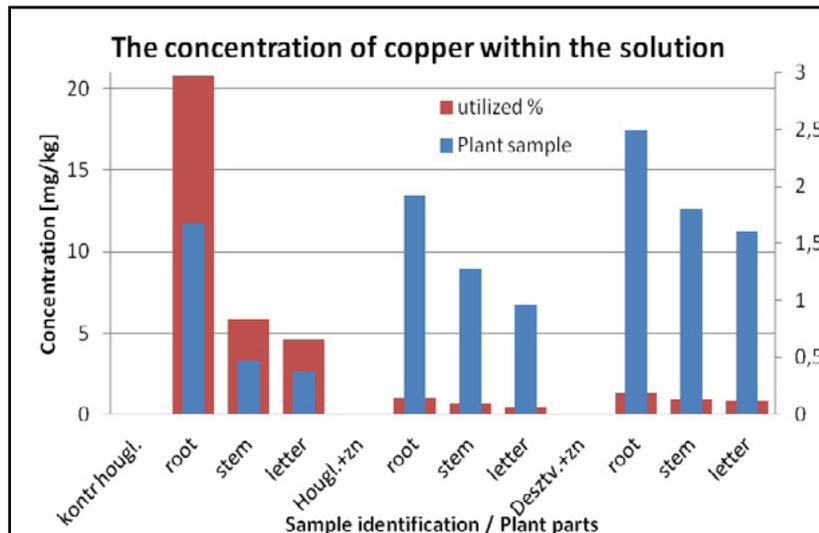


Figure 4. The utilize percentage of copper in the solution

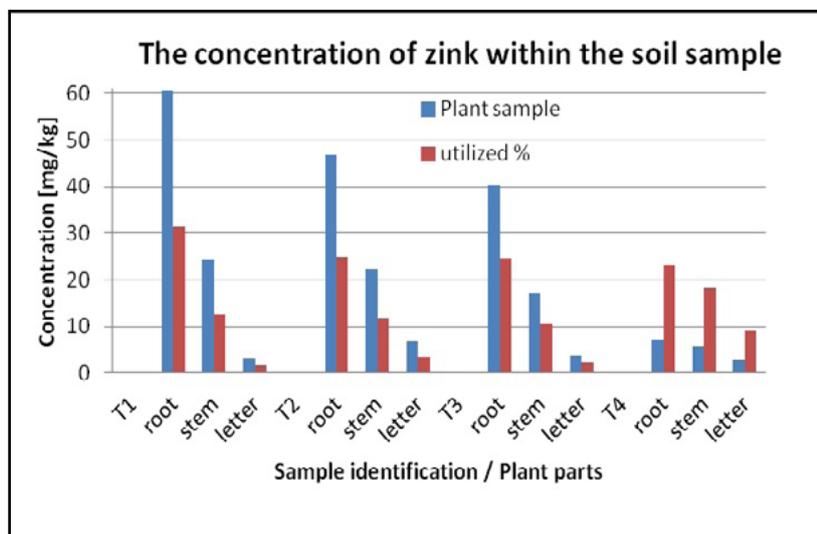


Figure 5. The concentration of zink within the soil sample

ratio of soil, and the acid-base sensitivity of the soils neutral pH (5.6) produces a value of around 32% utilization. Declining C / N ratio and the effect of increasing the metal concentration decreases utilization at around 21%. The parts of the plant does not determine the extent of the accumulation. The root part of the largest accumulation, while the smallest part of the letter.

The medium plant samples grown changing the ratio of Cu concentration of heavy metal accumulation in plant parts into deficit accumulation. The extent of the parts of the plant concentrates the accumulation of plant root and stem area. The utilization ratio of the control sample shows a high utilization of 20%. Contrary, the utilization is less than 5% of the

value of the distilled water and the contaminated medium.

The liquid phase plant samples grown heavy metal accumulation rate of accumulation of deficits Zn concentration in plant parts shifts. The extent of the parts of the plant accumulation shifts in the utilization rate of the same proportion. The root and stem part of the largest accumulation, but the minimum leaf sections. Accumulation in soil grown plants showed only a result of the control medium

Conclusions

The medium and the biological functions of plants grown in soil do not differ from each other.

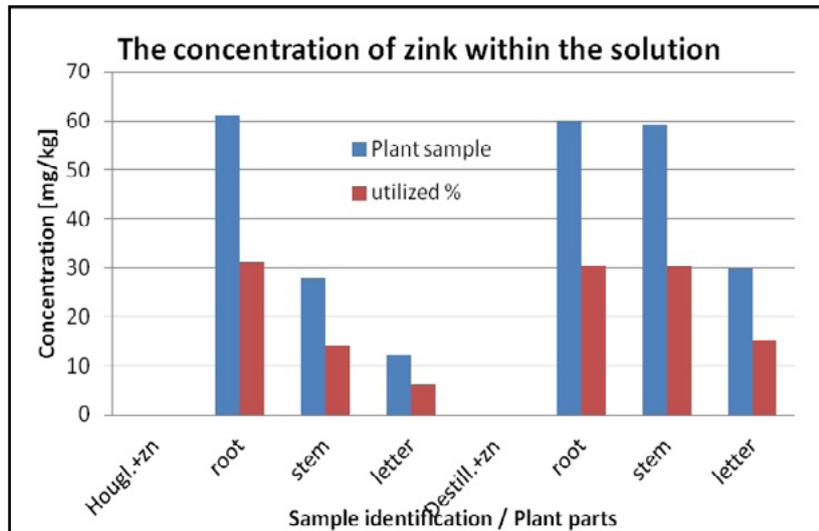


Figure 6. The concentration of zink within the solution

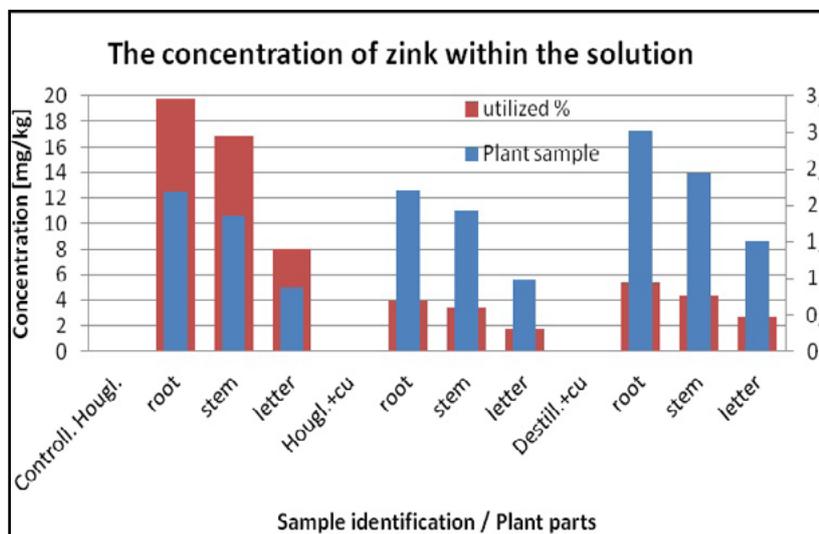


Figure 7. The utilize percentage of zink in the solution

The plants grown in different medium heavy metal contamination in soil more sensitive to the pest picture. The nutrient composition of the plants can positively influence the accumulation ability. The plant parts may be increased from the root and shank portions of the rate of accumulation and uptake rate of the contamination. In contrast to soil, the plant nutrient solution less sensitive to degree of contamination. Seedlings thus a higher ability to remove impurities of the contaminated medium. This investigation is in accordance with other authors's results of garden cress test, which show the highest accumulation

were happened in the root (Smolinks 2007). The test area can also specify the location of the akumulation for determining cress, but the concentration of pollutants. Case of both the heavy metal concentration results in a higher accumulation of the root.

The case of minor concentrations of the same extent in all parts of the plant. Since it is proved the garden cress was able to absorb and translocate heavy metals, it creates an opportunity to apply it in fitoremediation processes. The heavy metal removal from the ecosystem via melioration in all case increases its resilience.

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