Environmental Engineering and Management Journal *September/October 2008, Vol.7, No.5, 609-615*
http://omicron.ch.tuiasi.ro/EEMJ/

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STUDIES ON TRANSFER AND BIOACCUMULATION OF HEAVY METALS FROM SOIL INTO LETTUCE

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Abstract

This paper presents the connection between the heavy metals concentration in soil and their bioaccumulation in lettuce. Lettuce is a very good bioaccumulator of heavy metals and nutrients. In general, plants manifest a certain reaction to increasing the toxic metal concentrations in soil which they are cultivated on. The differences depend on plants sensitivity, and time exposure intensity (concentration of heavy metals, cultivation length, etc.), presence or absence of nutrients and other chemical species in soil.
Lead, cadmium, zinc and cooper, in some concentrations are toxic for plants, reducing their development. Through the tests presented in this paper the transfer of heavy metals in plants cultivated on a polluted soil in the presence or absence of N, P, K nutrients, was especially followed.

Keywords: lettuce, heavy metals, bioaccumulation, fertilizer, sowing

1. Introduction

Plants as essential components of natural ecosystems and agrosystems represent the first compartment of the terrestrial food chain. Due to their capacity of toxic metals accumulating, when they grow on soils polluted with such metals, they represent a threat to the living beings which consume them. Also, their development and growth may be affected at high levels of metal concentration implying reduced cultures and economic loss.

All plants show a certain reaction to the increasing of toxic elements concentration in soil, depending upon the sensitivity of plants exposure intensity and chemical species. Some species of plants disappear from such lands, while others, on the contrary, are stimulated by these elements. On lands containing metals – some plant species (metalophytes) have developed tolerance towards metals, and others (hyperaccumulators) are characterized by the capacity to accumulate high quantities of metals in their tissues.

For example, according to some researchers cadmium shows preference, in descending order, for: spinach, carrots, rye and oak.

It has also been noticed that it accumulates mostly in lettuce, spinach, cereal, cabbage, rather than in tomatoes, corn or sweet pea (Cox, 2000).

Heavy metals such as lead, cooper, zinc, in high concentrations, are toxic for plants, preventing their proper development. Explaining the accumulation process of heavy metals in plant has led to wide research. For that purpose, species like *Agrostis* were studied, plants that grow on lands in Scotland which contain high levels of Pb, Zn, Cu and Ni. The tolerance of *Agrostis* species towards heavy metals can be explained by the fact that these plants have the capacity to rule out these metals.

From the studies performed, it was observed that a population of *Agrostis tenuis*, tolerant to copper, absorbs as much copper from nutrient mineral

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solutions as plants which are not tolerant to cooper. It has also been noticed that plants coming from a population grown on a lead rich area were tolerant for lead but not to other heavy metals (Atanasiu, 1984).

Heavy metals, through their action, disturb plant metabolism, affecting respiration, phytosynthesis, stomate opening and plant growing.

2. Phytoavailability for heavy metals in soil

Taking over the heavy metals depends on whether these appear in a shape that can be absorbed by plants. For example, lead can be heavily absorbed by particles from sediments and, thus, it is very difficult to be translocated, while cadmium ions can be directly absorbed. Also, the presence of ions that can bind metals plays a part in the metal absorption by plants.

Plants take heavy metals from soils through different reactions such as: absorption, ionic exchange, redox reactions, precipitation – dissolution, etc. As an extension to these reactions, it can be said that the solubility of trace elements depends on minerals in soil (carbonates, oxide, hydroxide etc.), soil organic matter (humic acids, fulvic acids, polysaccharides and organic acids), soil pH, redox potential, soil temperature and humidity (Tarradellas et al., 1996). From all elements in soil, only the elements which present availability are transferred in plants (Fig. 1). The amount of elements in soil solution is much lower than the amount of elements absorbed by plant. A higher rate of the bioavailable fraction is located in the solid phase. According to their mobility in soil – water system, trace elements in soil can be grouped into four compartments, as shown in Fig. 1. Metals bioavailability is presented in the first three compartments (Oros, 2001).

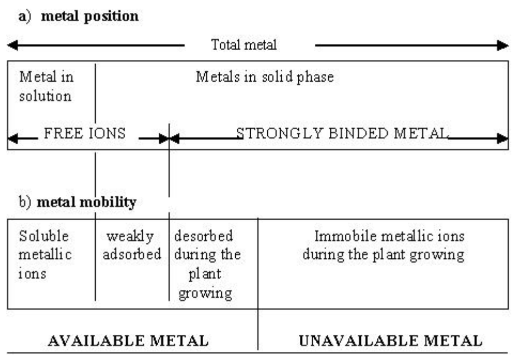

Fig. 1. Model of metal availability in soil (Tarradellas et al., 1996)

The first two compartments represent immediately available ions, ions from soil solution and weakly absorbed ions by solid phase, presenting the same mobility as soluble ions. The third compartment indicates ions bound to solid phase, but capable of passing into solution and becoming available for plants during their growing process. In the fourth compartment unavailable trace elements throughout the whole plants growing period are presented, being strongly bound to particles.

Metal absorption and accumulation in plant depend on a few soil factors, such as: pH, Eh, clay content, organic matter content, cationic exchange capacity, nutrient balance, other trace elements concentration in soil, physical and mechanical characteristics of soil, etc.

The metals availability for plants is controlled by their requests for micronutrients and their capacity to absorb and eliminate toxic elements. This availability is different, depending on plant species and their adaptation to the environment conditions. Therefore, plants can be divided into three categories: excluderators, indicators and accumulators. Herbs absorb less metal than fast growing plants such as lettuce, spinach, carrot and tobacco. When the growing takes place on the same type of soil, the cadmium accumulation in different species decreases in the following order (Oros, 2001).

Grains < Root <Vegetables < Leaf vegetables

2.1. Metals distribution in plant

Metals distribution in plants is quite heterogenous and is controlled by genetic factors, environment and toxic factors. The metal immobilization in plant roots determines the recuperation of a high proportion of metals in roots (80 – 90 %). Some species of plants can accumulate the highest quantity of absorbed metals in their high parts (tobacco accumulates more than 80 % Cd in its leaves). Linen seeds can also accumulate high concentration of Cd (Tarradellas et al., 1996).

The edible parts of vegetables like radish, cabbage, tomatoes, carrots, green beans etc. grown on sandy soils added with mud do not indicate an important accumulation of metals (Cd, Cr, Pb), while the parts that are not edible amass metals in concentrations which would not be accepted in the edible parts. The metals distribution in plant seems to be controlled by some mechanism and this suggests the existence of some boundaries and/or change in the metal chemical state (Tarradellas et al., 1996). Different plant parts contain different heavy metals quantities, the highest ones being contained in roots and leaves, and the smallest in flower buds and fruit. The analysis of the roots may indicate the degree of heavy metal accumulation in the polluted soil and offers clues on the soil pollution degree, and the analysis of leaves may even suggest the atmosphere pollution degree. If detection of metal accumulation content in plant is desired, the plant must be washed. The time and the way it should be washed depend on certain standards.

2.2. Bioconcentration and bioaccumulation of heavy metals by living organisms

Some living species have the capacity to accumulate in their organism heavy metals in concentrations much higher than these metal concentrations usually are in the environment.

This process can be defined by using two basic notions: bioconcentration and bioaccumulation (Bermond, 1998).

Bioconcentration is the direct growth of a pollutant concentration while it passes from the environment to an organism. In the case of terrestrial organism, this process takes place by the pollutant passage from soil into the plant through the radicular system or from air into the animal organism by direct inhaling. Bioaccumulation is specific for animal organisms and it manifests about direct pollutant absorption, to which accumulation by nutrition is added. These processes can be expressed by using the concentration factor (*Fc*). The concentration factor expresses the ratio between the pollutant concentration in an organism and its concentration in the biotope (Eq.1) (Bermond, 1998):

$$F_C = \frac{[Me]_{organism}}{[Me]_{biotop}} \qquad (1)$$

In a food network biocenosis inside, the bioaccumulation phenomenon can repeat when passing from a food level to another or from a prey organism to a predator organism. In this case the bioamplification phenomenon appears. This can be expressed by using the transfer factor (Ft) (Bermond, 1998).

If in an ecological pyramid a predator organism has the concentration (X_1) and its prey, on the food level n, has the concentration (X_0), the transfer from the food level [n + 1] is given by the following relation (Eq. 2):

$$F_t = \frac{[n+1]}{[n]} = \frac{X_1}{X_0} \qquad (2)$$

If the predator organism on the level (n + i) has the food level (n), which has the pollutant concentration (X_0), directly absorbs a quantity of pollutant (f_1) but also eliminates in the environment a part of the accumulated pollutant (K_1), the transfer factor from the level 0 to the level 1 will be (Eq. 3):

$$F_{t(0,1)} = \frac{a_1 f_1}{b_1 k_1} \qquad (3)$$

The equilibrium concentration for the predator organism will be expressed by (Eq. 4):

$$X_1 = \frac{a_1 f_1}{b_1 k_1} X_0 \qquad (4)$$

If this predator because in its turn is a prey for a carnivorous on a higher food level (n + 2), the transfer factor will be given by the relation (5):

$$F_{t(1,2)} = \frac{a_2 f_2}{b_2 k_2} \qquad (5)$$

The final equilibrium concentration will be (Eq.6):

$$X_2 = \frac{a_2 f_2}{b_2 k_2} \cdot \frac{a_1 f_1}{b_1 k_1} \cdot X_0 \qquad (6)$$

3. Materials

The used soil samples were taken from the land next to the three ponds of elutriation; two belonging to S.C. Remin S.A. and one to S.C. Aurul S.A. (today known as S.C. Romaltyn S.A.). The sample taking was done with an agrochemical probe for 0 – 20 cm depth.

On the 18th of March 2003, 12.00 o'clock, the following samples of alluvial soil were taken from the land between the elutriation ponds belonging to C.N. Remin S.A. and S.C. Aurul S.A. (today known as SC Romalyn S.A.):

- witness sample of alluvial soil, 0 – 20 cm depth, from land belonging to Lãpuşel village.
- agrochemical sample, from flooded soil, 0 – 20 cm depth, from the area between the three elutriation ponds.

4. Method

The taken samples were conditioned, grained in a hammer mill then screened with a screener having the mesh diameter of 2 mm. The agrochemical analyses were performed in the OSPA Maramureş Laboratory and they consisted in (Borlan et.al., 1981):

- *pH,* determined potentiometrically using a soil - water ratio of 1:2,5.
- *Mobile phosphorus* – for determination the amonium lactate acetate Egner Reihn – Domingo method was used. It is expressed in ppm.
- *SB* (base-exchange) – for determination the Kappen method was used. It is expressed in m.e. to 100 g soil.
- *Ah* (hydrolytical acidity) – determination was carried out by Kappen method. It is expressed in m.e. to 100g soil
- *Humus* – a modified Schollenberger method was used. It is expressed in (%).
- *Total nitrogen*- was determined using Kjeldalh method. It is expressed in (%).
- *Granulometry* – for determination the Kacinski method was used. It is expressed in (%).
- *Heavy metals content* was determinad by mineralization method and atomic absorption using ICP – MS (Inductively Coupled Plasma – Mass Spectroscopy).

5. Results and discussion

The agrochemical analyses were finalised on the 24th of March 2003, and on the 25th of March 2003 the seeding were done. The results are shown in Table 1.

Table 1. Agrochemical characteristics of soil

No.	*Soil Type*	*Sampling depth (cm)*	*pH*	*Humus %*	*Total nitrogen %*	*P-Al*	*P_C-AL*	*K-Al*
						ppm		
1	Aluvial soil (reference)	0 – 20	7.30	4.39	0.21	74	58.5	175
2	Aluvial soil (agrochemical sample)	0 - 20	6.75	2.96	0.14	6	5.5	265

Some characteristics could be revealed from Table 1, such as:

- soil is slightly alkaline, very rich in humus, well fed with potassium and phosphorus
- soil is slightly acid, rich in humus, very well with potassium, very badly fed with phosphorus

The heavy metal content, determined by mineralization and atomic absorption, is given in Table 2. For this experiment, eight pots of 400 mL soil taken from a land in Lăpuşel village, polluted soil taken from the land in between the three ponds, lettuce seeds – *Latuca sativa* species, Mona type were used.

Table 2. Heavy metal content in soil

Metal	*Reference sample (ppm)*	*Agrochemical sample (ppm)*
Cu	110	310
Pb	650	4570
Zn	180	1060
Cd	-	-

The seeding was carried out twice, using four samples each time. These are symbolized as follows:

1 a - simple reference sample (no fertilizers)
1 a'- simple reference sample (no fertilizers)
1 b - reference sample with fertilizers
1 b'- reference sample with fertilizers
2 a - simple agrochemical sample (no fertilizers)
2 a'- simple agrochemical sample (no fertilizers)
2 b - agrochemical sample with fertilizers
2 b'- agrochemical sample with fertilizers

In every soil pot 12 lettuce seeds was put, three in every one of the four places, and then the soil was watered every two days (Fig. 2).

Therefore, as shown in Figs. 2, 3, and Table 3, the witness soil (1 a, 1 a', 1 b, 1 b') both with fertilizers and no fertilizers creates much better condition for lettuce development than the contaminated soil with or without fertilizers (2 a, 2 a', 2 b, 2 b').

This could be due to the higher content of heavy metals from the pots with contaminated soil. The comparison made for the lettuce development on simple or with fertilized soil showed that it was a better development where nutrients on the reference soil with fertilizers had been added, thus reaching the final height of 17 cm compared to 15 cm in the pot where nutrients had not been added (Fig. 4).

In the case of the contaminated soil, the lettuce reached 15 cm on the fertilized soil, compared to 13 cm on the soil with no fertilizer.

During the twelve weeks of lettuce growth, the following were observed:

- On the first week, the plants did not spring.
- On the second week, the lettuce had a faint appearance reaching heights of 0.75 – 1 cm. There were no true leaves.

Fig. 2. Lettuce in the second week from seeding

Table 3. Weekly medium value of lettuce growth

Sample type	*Weekly medium height (cm)*											
	Week I	*Week II*	*Week III*	*Week IV*	*Week V*	*Week VI*	*Week VII*	*Week VIII*	*Week IX*	*Week X*	*Week XI*	*Week XII*
1a	0	1.00	1.75	3.50	5.50	5.75	6.25	7.75	9.00	9.50	10.00	12.50
1a’	0	0.75	1.75	2.00	3.75	4.50	5.50	7.00	9.00	11.00	13.00	15.00
1b	0	1.00	2.00	3.00	5.00	5.50	7.50	10.00	13.00	14.00	15.00	17.00
1b’	0	1.00	2.00	2.75	4.75	5.50	6.00	7.75	8.50	11.00	12.50	15.00
2a	0	1.00	2.00	3.00	3.75	4.75	5.25	5.75	6.00	6.50	7.00	8.50
2a’	0	0.75	1.75	2.50	2.75	3.50	3.75	4.50	5.00	7.00	8.50	11.50
2b	0	0.50	1.00	2.50	3.50	3.75	4.50	5.00	6.50	8.50	11.00	13.00
2b’	0	0.75	1.75	3.50	4.00	4.50	5.50	6.50	7.00	9.00	10.50	11.50

Fig. 3. Lettuce appearance in the twelveth day from seeding

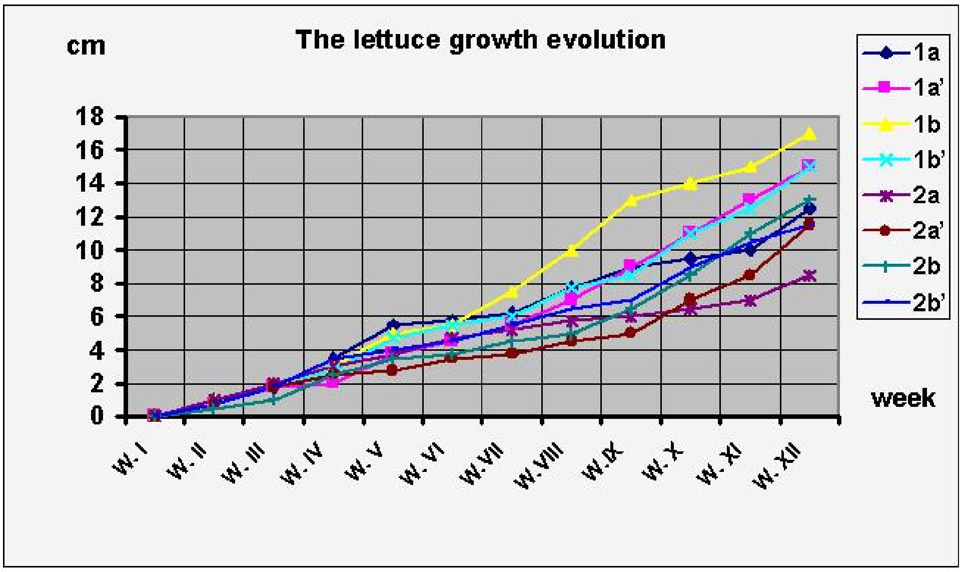

Fig. 4. Lettuce growth evolution during the twelve weeks

- On the third week, the first true leaves appeared. The soil from pots 1 b, 1 b', 2 b, 2 b' was fed with a binary N and P (27 – 13. 5 – 0) fertilizing solution, according to the Guide to fertilization planning. From this moment, these pots were watered only with nutrient solution
- The faint appearance with pale green leaves was maintained up to the end of the seventh week.
- From the eighth week, a more vigurous development of the lettuce could be noticed, the leaves colour being the specific one.
- Their continuous development was manifested progressively up to the twelveth week, when they were harvested for heavy metal content determination.
  After harvest, the lettuce from every type of pot was weighted.

The obtained results are shown in Table 4.

Table 4. Sample type and lettuce mass

No.	*Sample type*	*Lettuce mass (g)*
1	Simple reference	16.20
2	Reference with fertilizers	21.69
3	Simple agrochemical sample	11.04
4	Agrochemical sample with fertilizers	14.05

For heavy metal content determination the lettuce was dried at room temperature until it became crumbly and then it was dried again in a stove at 40^0 C for eight hours. After the plant had been ground and weighed it was mineralized with HNO_3. Heavy metal content determination in lettuce was made using spectrophotometry of atomic absorption, and the results are shown in Table 5.

Table 5. Heavy metals concentration in lettuce (mg/Kg in dry plant)

No.	*Metal type*	*Concentration in lettuce (ppm)*			
		*Witness 1**	*Witness 2**	*P1**	*P2**
1	Cu	530.93	14	7.23	4338.76
2	Pb	17.92	0	50.1	107.81
3	Zn	416	19.84	422.4	8.36
4	Cd	5.77	1.26	24.26	41.68

* reference 1 – reference without fertilizers;* reference 2 – refeerence with fertilizers;* P1 – polluted soil sample without fertilizers;* P2 –polluted soil sample with fertilizers.

The transfer factor of heavy metals from soil to plant calculated using relation (5) is shown in Table 6. According to Hygiene Norms no. 975/1998 from Official Monitor, Part I, no. 59, bis, the maximum content admitted for heavy metals in green lettuce is 0.2 mg/kg dried substance for cadmium and 0.5 mg/kg dried substance for lead. For zinc and cooper there are no established values. The exceeding of the admitted values for heavy metals in lettuce are systematized in Table 7.

Table 6. The heavy metal transfer factor from soil into plant

No.	*Metal*	*Witness 1*	*Witness 2*	*P1*	*P2*
		Transfer factor			
1	Cu	4.83	0.13	0.12	13.99
2	Pb	0.03	0.00	0.01	0.02
3	Zn	2.31	0.11	0.4	0.007

Table 7. Exceeding of the maximum admitted values for heavy metals in lettuce

No.	*Metal*	*Reference 1*	*Reference 2*	*P1*	*P2*
1	Cu	-	-	-	-
2	Pb	35.84	0.00	100.2	215.6
3	Zn	-	-	-	-
4	Cd	28.85	6.3	121.3	208.4

6. Conclusions

According to the achieved results the following conclusions could be issued:

- Cooper from reference soil was at the limit of the alert threshold for sensitive usage, and the one from the polluted samples exceeded three times this threshold.
- Lead from reference soil exceeded 6.5 times the intervention threshold for sensitive usage and 1.3 times for less sensitive usage. Regarding the soil affected by the waters from the pond, this element exceeded 45.7 times the intervention threshold for sensitive usage and 4.57 times for less sensitive usage.
- Zinc from reference soil was under the alert threshold for sensitive usage and the one from polluted soil exceeded 1.77 times for sensitive usage.
- Cadmium from reference soil was under the value of the alert threshold for sensitive usage and its content from the polluted soil exceeded 0.8 times the intervention threshold for sensitive usage.
- During the 12 weeks of lettuce growth, the following appearances were noticed:
- On the first week, the plant did not spring.
- On the second week, the lettuce had a faint appearance, reaching heights of 0.75 – 1 cm. Real leaves could not be noticed**.**
- On the third week, the first real leaves appeared. The soil from pots 1 b, 1 b', 2 b, 2 b' was nourished with binary fertilizer with N and P (27 – 13.5 – 0), according to the Guide to fertilization planning. From that moment, the plants were watered only with nutrient solution.

The weak look of the lettuce, with pale green leaves was maintained up to the end of the seventh week. From the eighth week, a more vigorous development of the lettuce was noticed, the colour of the leaves being normal. The development continued to manifest progressively up to the twelfth week, when the plants were harvested for heavy metal content determination. Also, the mass of the lettuce from the pots containing reference soil was higher than the ones of the lettuce from the pots with polluted soil. This could be due to the higher content of heavy metals from the pots with contaminated soil which, because of the bioconcentration process that affected the normal development of the lettuce.

From the data concerning the accumulation rates of heavy metals from soil to lettuce, the following results were noticed:

In the case of lettuce from the reference pots, the lettuce from the pot with no fertilizers showed a higher capacity to accumulate metals, especially cooper, cadmium and zinc. Except for zinc, the lettuce from the pots with polluted and fertilized soil presented a higher accumulation of heavy metals than the lettuce cultivated in pots with polluted but unfertilized soil.

Zinc accumulation was higher in the lettuce from the pots with unfertilized soil than in the lettuce with fertilized soil. Compared to the very high content of lead in the polluted soil, the transfer of this metal in the lettuce is quite small. This could be due to the absorption of a high quantity of lead by the clay – humic complex.

The high accumulation content of cadmium confirms the high capacity of its translocation in plant.

Opposing the high concentration of heavy metal in the lettuce to the maximum admitted values, exceeding of over 200 times result, both in lead and cadmium, in the case of the sample from the pot with polluted soil and fertilizer.

Also, exceeding of the maximum admitted concentration of over 100 times, for the same elements, was observed in the case of the lettuce from the pot with polluted soil and no fertilizers. Also, the lettuce from reference pots presented exceeding of the maximum admitted concentrations of over 28 times for lead and cadmium, in the case of the reference sample with no fertilizers and of over 6 times for cadmium in the case of the reference sample with fertilizers.

These high exceeding of the maximum admitted values for heavy metals in lettuce impose the prohibition from eating vegetable products obtained on these lands and also suggest the increased risk of contamination implied by consuming such products.

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