SZENT ISTVÁN EGYETEM

SZENT ISTVÁN UNIVERSITY

The effect of heavy metal stress in soil-plant systems

Sárközi Edit

Gödöllő

2016

PhD School

Name: Doctoral School of Horticultural Sciences

Field: Crop Sciences and Horticulture

Head of Ph.D. School: Prof. Dr. Éva Németh Zámboriné
Doctor of the Hungarian Academy of Sciences
Head of Department of Medicinal and Aromatic Plants, SZENT ISTVÁN UNIVERSITY, Faculty of Horticultural Sciences

Témavezető: †Dr.László Tőkei reader in unversity,CSc
Department of Soil Science and Water Management, SZENT ISTVÁN EGYETEM, Faculty of Horticultural Sciences

Prof. Dr. György Dénes Bisztray PhD
Institute of Viticulture and Oenology, Department of Viticulture, SZENT ISTVÁN EGYETEM, Faculty of Horticultural Sciences

Prof. Dr. János Kátai, CSc,
Institute of Agricultural Chemistry and Soil Science DEBRECENI EGYETEM, Centre for Agricultural Sciences

The applicant met the requirement of the PhD regulations of the Szent István University and the thesis is accepted for the defence process.

....

Head of Ph.D. School Supervisor

....

Supervisor

1. Background and objectives

Since the last third of the 20th century, the importance of healthy nutrition has come to the forefront, thus it has begun to observe to crop production and animal rearing to manage certified water and feed next to the mass production. Since the beginning of the 21st century the small and/or organic (bio) farming have given a key role again.

Pollutants' which might be found or detected in the environmental elements such as air, water, soil, plants, animals and humans quantitative and qualitative analysis is a series of relatively long and costly procedures. Considering the facts which were mentioned above, at the beginning the primary aim of my PhD research was to work out a soil test method that the farmers can learn also easily, so they are able to identify approximately the lead/copper/zinc content of their own soils with a simple soil test. With this method anytime, up to several times a year, or after an unexpected event (pollution, industrial accidents) the heavy metal of soils could be checked. During the studies it has been revealed that this method is very fast and cost-effective, but the results cannot be considered reliable. Besides the examination of the effectiveness of this process, my goal was to determine the adsorption capacity of lead, copper, zinc in different national soil types as well as to observe the effect of agricultural cultivation in case of the heavy metals' adsorption.

In addition to the soil column experiments I investigated the heavy metal accumulation in two plants (lettuce and grapes, which are often consumed in our country), especially in root and leaves, in order to get more information about what would be the consequences of these plants’ growing in contaminated environments.

2. Materials and methods

Four different major soil types which can be found in Hungary were examined: brown forest soil, chernozem, sand and meadow soil. Each soil type came from two sampling sites, so eight soil samples were processed. Three different soil types have been taken from the Szent István University Experimental and Research Farm, Soroksár (Ramann-type brown forest soil, meadow soil and sand with humus). Three additional soil samples (sand with humus, meadow chernozem, alluvial meadow soil) collected from cultivated areas from an agricultural Co., Tiszavasvári. Brown forest soil with lessivage came from Gödöllő and chernozem with calcareous plaque was collected from Dunaújváros. The choice of these sample sites was justified that four typical Hungarian soil types from two sample sites were examined and the difference of adsorption capacity was determined between cultivated and non-cultivated areas.

The sampling was carried out according to MSZ-08-0202. Based on standards the soil samples were collected from 0-30 cm depth. The sample preparations were done based on MSZ 21470-50. The soil samples were dried at room temperature to constant weight, and then, if it was necessary, they were sieved through 2 mm pore diameter sieve.

2.1. Soil physical examinations

These were carried out according to MSZ-08 0205-78. With two different experiments the soil texture was determined (by L% and K_A).

2.2. Soil chemical examinations

Determination of the chemical properties was carried out based on the Hungarian standards (MSZ-08 0206/2-78). Following soil analysis tests were done: pH (distilled water (DW) and KCl), total salt content, humus content and $CaCO_3$% content and hydrolytic acidity were determined as well (Magyar Szabványügyi Testület 1978b).

2.3. Soil column experiments

In this research our aim was to determine the adsorption capacity of lead, copper and zinc in laboratory.

The soil contamination experiments were carried out by amended soil columns by Mehlich (*Figure Figure* **1**).

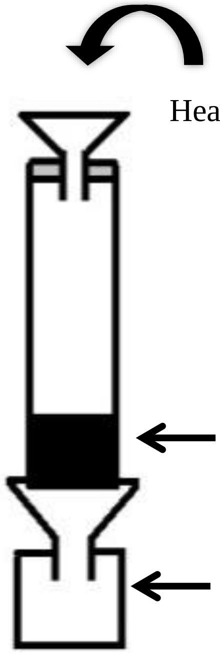

Figure 1. Soil column (own edited)

The soil column was prepared as follows: a cork was placed on top of a glass tube (length 45 cm, diameter 4,4 cm), a funnel was put in the middle of the cork. The bottom of the glass tube was tied up with mesh. 50 g pre-treated, air-dried soil samples were poured into the top of the funnel. There was also a funnel on the bottom of the soil column to guide the filtrate to the glass cup directly. The heavy metal stress experiments were performed on all eight soil samples in case of lead, copper and zinc in five different concentrations. After the contamination the heavy metal content of filtrates was determined by complexometric titration. The theoretical adsorption capacity of the soil samples were calculated from the two different concentration values ($c_{adsorbed} = c_{pollution} - c_{filtrate}$). After pollution, the soil samples were dried and Lakanen-Erviö extracts were prepared according to the Hungarian standards (MSZ 20135:1999). The lead, copper and zinc content of extracts were determined by atomic absorption spectrophotometer (AAnalyst700), which would be the real adsorption capacity. (Magyar Szabványügyi Testület 1999, Lakanen and Erviö 1971).

2.4. Studying the heavy metal accumulation effect of lettuce (*Lactuca sativa L.*) applying pot experiments

In order to monitor the heavy metal accumulation effect of lettuce breeding pots were used. The meadow chernozem soil sample was applied from Tiszavasvári. As a first step, the pots (9×9×9,5cm) were loaded by 450-500 g disturbed soil, and then the maximum water capacity (WC_{max}) was set under laboratory conditions, then the minimum water capacity (WC_{min}), to ensure same moisture state. 60-day growing period lettuce seeds were sown (King of May) into the pots. During the growing the samples were kept under open sky at the top of the K building in the Campus of Buda (May-June 2013). The samples were watered with tap water until constant weight in the first 30 days. On the 30^{th} day 1-1 sample was left in the pots, then the irrigation water was contaminated with lead, copper and zinc in corresponding, varying concentration (*Table 1*).

Table 1.: The used contamination concentration per heavy metals

Heavy metal		Concentration (mg/kg)
Lead	under limit	50
	on limit	100
	above limit	150
Copper	under limit	37,5
	on limit	75
	above limit	112,5
Zinc	under limit	100
	on limit	200
	above limit	300

In the last 30 days the samples were watered once a week with 20-20 cm^3 lead nitrate, copper sulphate and zinc sulphate in three replicates. Control samples were poured over the whole experimental period by tap water. So I worked total of 30 samples.

At the end of the experiment the plants were removed and the soil samples were dried, then Lakanen-Erviö extracts were prepared. The lead, copper and zinc content of the extracts were measured by atomic absorption spectrophotometer.

From the removed lettuces samples were taken from both the roots and leaves which were dried in an over at 105°C to constant weight. As the result of the contamination the plant samples were not developed accordance in the case of all three heavy metals, therefore three replications were homogenized in a mortar, so an average value was observed for the accumulated heavy metal content in the rot and leaves in three, different concentrations. After the homogenization the ten samples were wrecked by nitric acid/hydrogen peroxide mixture, and the lead, copper and zinc content were determined by atomic absorption spectrophotometry.

2.5. Studying the heavy metal accumulation effect of grape (*Vitis vinifera* ’Fercal’) applying pot experiments

During the grape experiment heavy metal accumulation capacity of the Fercal-type grape was also studied in the same way as in the case of lettuce. The experiment took place on the top of K Building (southern side), at the Campus of Buda, Szent István University.

The plants were planted in April 2014 into perlite-soil (50-50%) mixture (cylinder’s diameter 15 cm, its high 35 cm); the soil sample was the Ramann-type brown forest soil from Soroksár. In this medium the plant samples were until 60 days on top of the K building, while the corresponding phonological phases have not been attained, during this time the plants were irrigated with tap water. In May 2014 the samples were transplanted into Ramann-type brown forest soil. To set the humidity was carried out in the same way as I described in the case of lettuce.

After 60 days the samples were polluted with different concentrated heavy metal solutions three times per week for 30 days. The concentration of the solutions was similar to the lettuce experiment (*Table Table **1***.). 30 plant samples were measured: in three replicates, in three different concentrations, three different heavy metals (27), and three control samples.

At the end of the pollution experiment the soil samples were dried, then Lakanen-Erviö extracts were prepared to determine the lead, copper and zinc content by AAS. During the measurement of the grape samples root and leaves samples were taken from which extractions were prepared in the similar way as at the lettuce, then the heavy metal contents were measured by AAS.

3. Results

3.1. Results of soil column experiments

Table 2. summarizes the results of the soil tests and that which was the soil sample at the given soil type that could conclude more heavy metals after pollution.

Based on the results of the lead pollution Ramann-type brown forest soil concluded the least amount of lead, and then the following one is the sand with humus from Soroksár. The chernozem with calcareous plaque and the sand with humus from Tiszavasvári can adsorb the same amount of lead, and then the follow member is the brown forest soil with lessivage from Gödöllő. The two meadow soils have almost the same lead adsorption capacity. The biggest lead adsorption capacity was found in the case of alluvial meadow soil from Tiszavasvári.

In the case of copper pollution, just the same as at the lead, the least copper adsorption capacity was found in the case of Ramann-type brown forest soil. This is followed by similar adsorption values in ascending order: meadow chernozem, brown forest soil with lessivage, sand with humus from Tiszavasvári. The sand with humus from Soroksár, meadow soil and the chernozem with calcareous plaque can be treated as separated groups. The largest copper adsorption capacity was measured in the case of alluvial meadow soil from Tiszavasvári.

Table 2: Heavy metal pollution – summarizing table

Major type of soils	Soil sample	Cultivated area (x)	Major differences in soil analysis	Establishing the higher adsorption capacity after AAS		
				Lead (x)	Copper (x)	Zinc (x)
Brown forest soil	brown forest soil with lessivage (Gödöllő)	-	sand, slightly acidic, medium humus content	x	x	x
	Ramann-type brown forest soil (Soroksár)	-	sandy loam, slightly acidic, low humus content	-	-	-
Chernozem	chernozem with calcareous plaque (Dunaújváros)	-	loam, slightly alkaline, medium carbonate content, high humus content	-	x	x
	meadow chernozem (Tiszavasvári)	x	loam, acidic/slightly acidic, medium humus content	x	-	-
Sand	sand with humus (Tiszavasvári)	x	sand, slightly alkaline, slightly chalky, low humus content	-	-	-
	sand with humus (Soroksár)	-	sand, slightly alkaline, slightly chalky, low humus content (1,5 times higher humus content than at the Tiszavasvári one)	x	x	x
Meadow soil	alluvial meadow soil (Tiszavasvári)	x	loam, acidic/slightly acidic, high humus content	x	x	~
	meadow soil (Soroksár)	-	clay loam, slightly alkaline, medium carbonate content, high humus content	-	-	~

In the case of zinc pollution it can be said, that the Ramann-type brown forest soil could adsorb the least amount of zinc at 10 as well as 1 g/dm^3 concentrations, while in the case of 5 g/dm^3 the sand with humus's (Tiszavasvári) zinc adsorption capacity is the smallest. During this experiment the most zinc could be adsorbed in the case of most concentrated pollution by alluvial meadow soil from Tiszavasvári, while in the case of middle value by the chernozem with calcareous plaque (Dunaújváros), and at the smallest concentration by the meadow chernozem from Tiszavasvári.

3.2. The results of effect of heavy metal accumulation in the case of experimental plants

The following values can be determined based on Uka et al (2013) work:

- *bioconcentration factor (BCF)*, which is the ratio between the heavy metal concentration in root and soil
- *translocation factor (TF)*, which is the heavy metal ratio between in the shoots and the roots.

Knowing these values it can be determined, the plant is suitable for fitoextraction (the plant can extract the heavy metals from the soil) or for fitostabilization (it can be prevented by using plant that the heavy metals go into ground water or air from the contaminated soil): in the first case the TF and BCF values are higher than 1. In the other case BCF is higher than 1 and TF is smaller than 1 (Yoon et al 2006).

The *Enrichment Factor* (EF) shows the ratio between the heavy metal content in shoots and in soil. In the case of hiperaccumulator plants the EF and TF values are higher than1 (Ma et al 2001). By measuring *transfer coefficient* (TC = the ratio between the heavy metal content in plant and in soil) it can be said, the plant accumulates the heavy metal (TC > 1) or excludes it (TC < 1) (Uka et al 2013).

In the case of lead pollution the lettuce is suitable for fitoextraction above the limit and in the case of zinc pollution it is true on the limit and above the limit concentration, while this plant is recommended for lead fitoextraction above the limit. Knowing the transfer coefficient it can be said, the lettuce could accumulate the lead in all tested concentrations – there was a maximum value according to the limit and above the limit values, so this plant was not be able to accumulate more. Also it can be determined, that the plant sample excluded the copper and zinc more and more with the increase of concentration. These results are important for both environmental and health protection.

The grape sample behaved the same against the lead as the lettuce – the accumulation capacity is increased with the increase of concentration. It is positive from environmental health perspective; the root was able to accumulate more lead than the leaves. In the case of copper and zinc saturation was observed, but the bulk of the copper was accumulated in the roots, while the larger part of zinc was accumulated in leaves in each of the tree concentrations.

Based on my research it can be said, the grape is not suitable for lead and zinc fitoextraction and fitostabilization, while it is suitable for copper fitoextraction. It is a hiperaccumulator plant against copper. The transfer coefficient had similar values during the pollutions, so it can be said, that the grape accumulated the lead, but it was not able to absorb more lead with the increasing concentrations. It is established according to the transfer coefficient, that the grape was able to accumulate the copper and the zinc in all cases, but the TC value was decreased with the increasing concentrations, so the accumulation's inhibiting effect was increased.

4. New scientific results

1. I compared the theoretical and actual adsorption results and it can be said, that the complexometric titration cannot be considered reliable for determining lead, copper and zinc adsorption capacity in the case of tested soils.
2. I have found as new scientific result, the lead adsorption capacity is influenced in the case of chernozem and meadow soils next to the humus content by pH.
3. The organic matter content has important role in the copper adsorption next to the clay content in the case of chernozem.
4. The zinc adsorption capacity is influenced in the case of investigated meadow and chernozem soils by humus content.
5. The cultivation influenced the lead, copper and zinc adsorption in the case of meadow soil, while there was no detectable effect for adsorption in the case of chernozem and sand with humus.
6. The lead accumulation capacity of lettuce is increasing with the increasing concentrations, while it shows a saturation curve in the case of copper and zinc.
7. The lettuce can be used for fitoextraction and fitostabilization just above the limit concentration in the case of lead, while it is suitable for fitostabilization at limit and above the limit concentrations in the case

of zinc, and the grape is suitable for fitoextraction at the under the limit concentration in the case of copper.

8. The lettuce excludes the copper and zinc with the increasing concentrations that shows a decreasing accumulation capacity, which is favourable for environmental health, while the grape was able to accumulate the lead, the copper and the zinc in all cases, but it was not able to absorb more with the increasing concentrations, it excluded them.

5. Conclusions and recommendations

Based on the results a summary table was prepared (Table *Table* ***3***.) which is the soil properties in the case of all three heavy metals in the different soil types that influenced the adsorption.

Table 3. Summary table about soil properties which affected the heavy metals in the case of tested soil samples

Major soil type \ Heavy metal	**Lead**	**Copper**	**Zinc**
Brown forest soil	humus	humus	humus
Chernozem	pH	clay and humus	pH and humus
Sand	humus	humus	humus
Meadow soil	pH and humus	pH and humus	humus

It was found that the lead/copper/zinc adsorption was influenced in the case of brown forest soil and sand by humus, in the case of chernozem by pH, humus and clay content, while the pH and humus played important role in adsorption in the case of meadow soil samples. New scientific results are the role of the pH in the lead adsorption at the chernozem and meadow soils, role of the humus content in copper adsorption at chernozem samples, as well as influence factor is humus content in zinc adsorption at meadow soils and chernozems. The previous and new results can be used for any lead/copper/zinc pollution, where any intervention is required. If the pollution has small area and it does not endanger the surrounding populations, the lead/copper/zinc content of a given soil (if it has similar soil properties as I measured) can be decrease by either a pH changes (liming or fertilizing – depends on the direction), or adding humus content, thereby the chances can decrease, that these heavy metals either reach further (e.g. ground water) or plants may accumulate them.

Examining the parts of lettuce can be said, the roots were able to accumulate less heavy metal than the leaves. This is important for environmental health, because the leaf of lettuce is consumed by organisms. Analysing the grape samples can be said that the leaves were able to accumulate more in the case of lead and zinc, while this is true for root samples in the case of copper.

6. References

1. 6/2009. (IV.14.) KvVM-EüM-FVM együttes rendelet a földtani közeg és a felszín alatti vízszennyezéssel szembeni védelméhez szükséges határértékekről és a szennyezések méréséről.
2. Magyar Szabványügyi Testület. 1978b. A talajok egyes kémai tulajdonságainak vizsgálata. Budapest. MSZH Kiadói Főosztály.
3. Magyar Szabványügyi Testület. 1999. A talaj oldható tápelemtartalmának meghatározása. Budapest. Magyar Szabványügyi Testület.
4. Lakanen E. és Erviö R. 1971. A comparison of eight extractants for the determination of plant available micronutrients in soils. Acta Agralia Fennica. 123. 223-232.
5. Uka U.N., Chukwuka K.S. és Afoke C. 2013. Heavy metal accumulation by *Telfairia occidentalis* Hook f. grown on waste dumpsites in South-eastern Nigeria. Research Journal of Environmental Toxicology. 7. 1. 47-53.
6. Yoon J., Cao X., Zhou W. és Ma L.Q. 2006. Accumulation of Pb, Cu, Zn in native plants growing on contaminated Florida site. Science Total Environment. 368. 456-464.
7. Ma L.Q., Komar K.M., Tu C., Zhang W., Cai Y. és Kennelley E.D. 2001. A fern that hyperaccumulates arsenic. Nature. 409. 579.

7. Publications related to the thesis topic

Journal with impact factor

Turcsán A, Steppe K, **Sárközi E**, Erdélyi É, Missoorten M, Mees G and Mijnsbrugge KV. Early Summer Drought Stress During the First Growing Year Stimulates Extra Shoot Growth in Oak Seedlings (Quercus petraea). Frontiers in Plant Science. 2016, 7. 193. IF: 3,9

Publications in peer-reviewed journals

Sárközi E, Kardos L, Sepsi P, Ladányi M, Tőkei L. Methodological development in determination of copper content in the root zone of Hungarian soil types: preliminary results. Eur. Chem. Bull. 2012, 1(10), 418-421.

Sárközi E, Kardos L, Sepsi P, Varga Zs, Bisztray Gy, Kátai J. Ólomadszorpció-képesség vizsgálata egy csernozjom talajban és Lacutca sativa L. akkumuláló hatásának tanulmányozása tenyészedényes kísérletben. Agrártudományi közlemények. 2014, 56, 101-104.

Conference proceeding publications

Angyal Zs, **Sárközi E**, Székely D, Kardos L. Játszótéri homokozók nehézfémtartalmának vizsgálata Budapest XI. kerületében. VIII. Kárpát-medencei Környezettudományi Konferencia. Veszprém. 2012. április 18-21. 218-223. ISBN 978-963-86627-2-9

Sárközi E, Kardos L, Sepsi P, Ladányi M, Tőkei L. Preliminary results of methodological development in the determining of

lead content in the root zone of Hungarian soil types. 18th International Talent Management Student Conference on Environmental Protection and Rural Development. Szolnok. 2012. szeptember 28-29. ISBN 978-963-89339-7-3 (cd-kiadvány)

Abstracts

Sárközi E, Kardos L, Sepsi P, Bisztray Gy, Kátay J. Hazai talajtípusok réz- és ólom adszorpciós képességének meghatározása talajoszlopokon. II. Interdiszciplináris Doktorandusz Konferencia. Pécs. 2013. május 15-17.

Hermán A, **Sárközi E**, Kardos L, Sepsi P, Erőss A, Bisztray Gy, Kátai J. Rézadszorpció-képesség vizsgálata egy csernozjom talajban és a Luctuca Sativa L. akkumuláló hatásának tanulmányozása tenyészedényes kísérletekben. 21st International Student Conference on Environmental Protection and Rural Development. Szolnok. 2015. május 29-30. 23. (abstract kötet)