Eurasian J Soil Sci 2016, 5 (3) 249 - 254

Eurasian Journal of Soil Science

Journal homepage : http://fesss.org/eurasian_journal_of_soil_science.asp

Evaluation of heavy metal complex phytotoxicity

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Article Info

Received : 26.12.2015
Accepted : 07.03.2016

Abstract

The experimental data dealing with the effect of heavy metals contained in the technogenic contaminated soils on plant objects under controlled conditions was discussed. The aim of this work is to define the quantitative indicators of copper and zinc potential phytotoxicity, namely germination energy, simultaneous germination and duration of the test plants. It was found that the activity of the test plant growth is linked with copper and zinc complex action. Joint effect of copper and zinc is manifested both in inhibition of lettuce growth and determined, above all, by the nature contamination, soil properties and biological specificity of the test plants.

Keywords: Phytotoxicity, copper, zinc, biological test culture.

Introduction

Electroplating enterprise waste, called galvanic slime (GS), has a negative impact on atmosphere, hydrosphere and soil cover. As a result, such waste influences plants and animal organisms state (Kasimov, 2011; Baiseitova and Sartayeva, 2014; Black et.al., 1999). The migration of chemical elements, contained in stockpiled GS, is the result of physical and chemical processes under the influence of climatic and weather factors. The contamination, precipitation and infiltration through a layer of waste may spread into adjacent soil areas. While estimating ecotoxicological effects, combined effects of technological waste should be studied. It is very important to estimate the most effective phytotoxic waste using biological methods of analysis. These methods allow to find in addition to the general non-specific effects on biotest and to determine some specific reactions of individual chemicals or groups of substances (Olkhovich and Musienko, 2005).

Most of researchers paid attention to the problem of copper and zinc effect on different plants (Baiseitova and Sartayeva, 2014; Black et al., 1999; Olkhovich and Musienko, 2005; Grodzinsky et al., 2006; Hubachov, 2010; Mayachkina and Chugunov, 2009). But in spite of numerous studies, the problem of their translocation into plants remains open to discussion (Gruzdev, 2010; Gladkov, 2010). The research is rather difficult because of different soil conditions and various types of soil sensitivity. In this connection, it seems appropriate to conduct the study for specific areas which are most inclined to heavy metal contamination under homogeneous soil and climatic conditions (Grodzinsky et al., 2006; Hubachov, 2010).

The **e**xperiments studying the influence of different pollutants on plants in a controlled environment allows to solve many problems: a) to find out the causes of different plant resistance and the tendency to adapt to toxicants; b) to reveal the influence of the most important factors; c) to find the lethal dose of pollutant, etc. (Grodzinsky et al., 2006; Hubachov, 2010). Special test plants having the greatest sensitivity to various

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e-ISSN: 2147-4249 DOI: http://dx.doi.org/10.18393/ejss.2016.3.249-254

ecotoxicants were usually used for biological indication of the total soil pollution. These investigations have shown that the sensitivity of biological method may be compared with the instrumental one and sometimes even exceeds it (Olkhovich and Musienko, 2005; Mayachkina and Chugunov, 2009). In this regard, the aim of this study was to determine the potential phytotoxicity of galvanic production waste using the example of copper-zinc GS model. Cu and Zn metals were selected because they possess the greatest concentrations of GS in heavy industry and are the metals of high-class risk.

The aim of this study was to determine the effect of heavy metals, contained in technogenic contaminated soil on plants under controlled conditions. According to our aim the effect of Cu and Zn on the indexes of seed germination, initial growth and biomass of test plant were modeled and studied. In accordance with our aim the following tasks were put: to create an experimental model in vitro and to study lettuce (Odessa curly hair) its growth beginning and its sprout biomass.

Material and Methods

The determination of soil toxicity degree was performed by bioassay method (Olkhovich and Musienko, 2005). This method is the study of test object reaction to the pollutants action and allows getting an integral evaluation of phytotoxicity degree. Lettuce seeds (Odessa curly hair) were taken as a test object and the duration of seed germination and their energy in different GS contaminated soil layers were taken as a measure of toxicity.

The laboratory experimental model was devised to study copper-zinc GS phytotoxicity. Preliminarily contaminated by GS soil samples such as haplic arenosol, mollic gleyic fluvisol, gleyic chernozem, calcic voronic chernozem were used in the experiment. The top layers of soil were selected from the following depths: 0-5, 10-15, 20-25, 50-75, 100 cm. The selected soil was dried to constant weight in the open air. The air-dry soil 1500 g was put into plastic pots of 15 cm high and 9 cm in diameter. 15 seeds were placed in each vessel, pre-soaked in water for a day at a depth of 1 cm. A constant temperature 20 °C was maintained during the process of germination.

The duration and simultaneous germination energy were used to evaluate the effect of Cu and Zn in soil on seeds (Ubugunov and Dorzhonova, 2010; FR.1.39.2006.02264, 2009). Under germination is meant the number of seeds germinated for 7 days and expressed as a percentage to total number of seeds taken for germination, and under germination energy is meant the number of seeds germinated for the first 3 days of germination percentage to the total number of seeds taken for germination. Daily records of sprouted seeds were also used for a more precise characterization of germination speed. Therefore, the simultaneous germination energy was determined by the formula

$$\mathrm{D} = \frac{\mathrm{P}}{\mathrm{A}}, \quad (1)$$

where D – simultaneous germination (average percentage of seeds germinated for the first day); P – percentage of complete germination; A – the number germination days.

The duration of germination is calculated:

$$C = \frac{(a \cdot 1) + (b \cdot 2) + (d \cdot 3) + \ldots}{(a + b + d + \ldots)}, \quad (2)$$

where *C* – is the average duration of one seed a day; *a* – a number of seeds germinated for one day; *b* – the number of seeds germinated for two days; *d* – the number of seeds germinated for three days, etc.

In addition to germination indicators, the growth rate was determined by seeds which characterize the viability of the plant best of all. The value of the control index (L_0) and experienced (L_{ex}) seeds were calculated as the mean arithmetic (L_{meam}) from the data totality about the length of vegetative part or the seedlings roots (FR 1.39.2006.02264, 2009).

$$L_{cp} = \frac{\sum L_i}{n}, \quad (3)$$

where L_i – the maximum length of the vegetative part or the root of each seedling; ∑ – the sum; *n* – the total number of experimental seedlings.

To evaluate the significance of differences between the experimental variants the whole experimental process was performed in triplicate.

Results and Discussion

Comparative data (Table 1) of Cu and Zn limited concentration* (LC) (LC (Cu) = 3 mg/kg; LC (Zn) = 23 mg/kg) in test soils were described in paper (Datsenko and Svashenko, 2015).

The result of Table 1 allow to formulate the following data:

– all Cu layers have the low concentration level;

– the concentration level for Zn was defined by soil character and it changes from very great in the upper layers to critical concentration in the lower ones (Damage determination from soil concentration by chemical substances).

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Table 1. The index of the harmfulness of Cu and Zn in the contaminated soils in comparison with their limiting concentration in usual soils

Elements	The index exceeded hazard control samples	The index exceeded hazard in the respective layer (cm) of soil				
		0-5	10-15	20-25	50-75	100
Haplic arenosol						
Cu	0,75 LC	7.18 LC	1.36 LC	1.68 LC	1.68 LC	1.97 LC
Zn	0,28 LC	21.65 LC	12.10 LC	10.80 LC	3.93 LC	0.65 LC
Mollic gleyic fluvisol						
Cu	0,57 LC	7.70 LC	1.10 LC	0.50 LC	0.60 LC	0.60 LC
Zn	0,08 LC	33.50 LC	23.35 LC	7.90 LC	0.74 LC	0.15 LC
Gleyic chernozem						
Cu	0,89 LC	3.29 LC	1.84 LC	1.69 LC	1.09 LC	1.81 LC
Zn	0,17 LC	114.60 LC	32.65 LC	2.53 LC	0.30 LC	0.26 LC
Calcic voronic chernozem						
Cu	7,10 LC	36.57 LC	5.54 LC	5.75 LC	4.87 LC	7.08 LC
Zn	3,12 LC	229.30 LC	7.53 LC	1.59 LC	2.39 LC	1.73 LC

* LC – normative – is the quantity of unhealthy substances in surrounding components (water, air, soil). At constant contacts or at the influence of definite period they do not practically influence the human health and do not give rise to unhappy consequences for human posterity. Such normative is determined by law and is recommended to competent offices.

The analysis of morphometric parameters in lettuce shoots (Figure 1) grown in all soil tests showed that both inhibition and stimulating of the growth in the vegetative part take place. Similar effects were also observed for the growth of the root system.

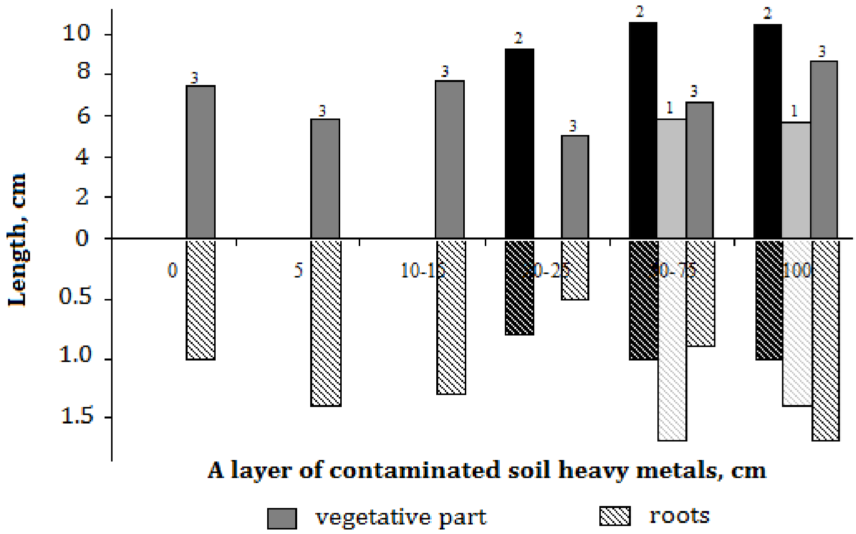

Figure 1. The length of lettuce sprouts (30 days cultivation) depending on the contaminated soil layer of heavy metals: 1 – mollic gleyic fluvisol; 2 – gleyic chernozem; 3 – typical heavy black earth

Haplic arenosol soil is characterized by a significant inhibition of test culture growth. The lettuce seeds spring up only in the lower layers of the soil under test (20-25, 50-75, 100 cm) and all germs were killed in 20 days. However, the biotesting studies of the rest test soils showed some stimulating development and test culture growth. Almost all layers showed a significant stimulation for calcic voronic chernozem soils. The shoot growth occurred throughout the whole growing period (30 days). The length change of both root and vegetative part of seedlings which depends on hazard index exceeding (LC) metal-toxicants shows the absence of unfavorable phytotoxic action. The average length of vegetative part and root system (L_{meam}) is the same and in some layers even greater than analogous indices in the control sample (L_0).

However, the analogy is not so much expressed and the stimulating effect is observed in the lower soil layers (20-25, 50-75, 100 cm) in mollic gleyic fluvisol and gleyic chernozem. The test soils impacted positively on the above-ground part and lettuce sprouts of root system showed good development. The aerial length in the gleyic chernozem is equal to 10 cm and 5.7 cm in the mollic gleyic fluvisol loam correspondingly. The root system development is equal to 1 cm in the gleyic chernozem and 1.5 cm in the mollic gleyic fluvisol (Figure 1). Significant seed sensitivity to copper and zinc was seen in all experimental samples. According to the results obtained, the reducing of metal concentration in the layers (50-75, 100 cm) decreases the metal toxic action. It is vividly expressed in the testimony of vigor (Figure 2). It is seen in the figure that it is 2.3-9 % in the haplic arenosol soils; 11-27 % in the mollic gleyic fluvisol; 42-47 % in the gleyic chernozem and 40-49 % in a typical heavy black earth.

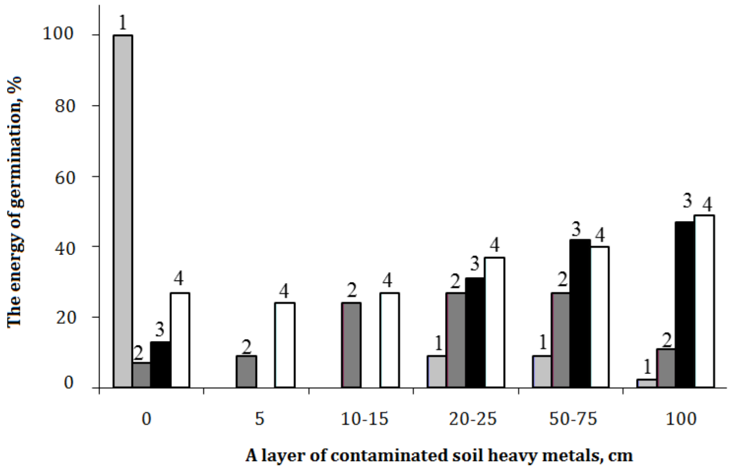

Figure 2. Lettuce seed dependence on the contaminated heavy metals soil layer: 1 – haplic arenosol; 2 – mollic gleyic fluvisol; 3 – gleyic chernozem; 4 – typical heavy black earth

Among the indicators of seed germination under GS soil contaminated conditions in a model proved to be the indicators of germination and their duration (Figure 3). The stimulating average effect of metals on the lettuce growth up to 7 days was stronger but its toxic action was weaker. Increasing the period of lettuce growth from 7 to 30 days, the growth character of the plants varies: the significant metal depressing effect is greater. The germination index in almost all cases of tested soils is fairly high: 9–73 % in the haplic arenosol sandy soil; 7–28 % in the mollic gleyic fluvisol loam; 13–42 % in the gleyic chernozem; 20–45 % in heavy loamy typical chernozem (Figure 3a). However, the duration index of germination is not high enough in all tested soil layers: 0,3–16 % in haplic arenosol; 1–4,4 % in the mollic gleyic fluvisol; 1,2–5.2 % in the gleyic chernozem; 0,9–6,0 % in the typical heavy black earth (Figure 3b).

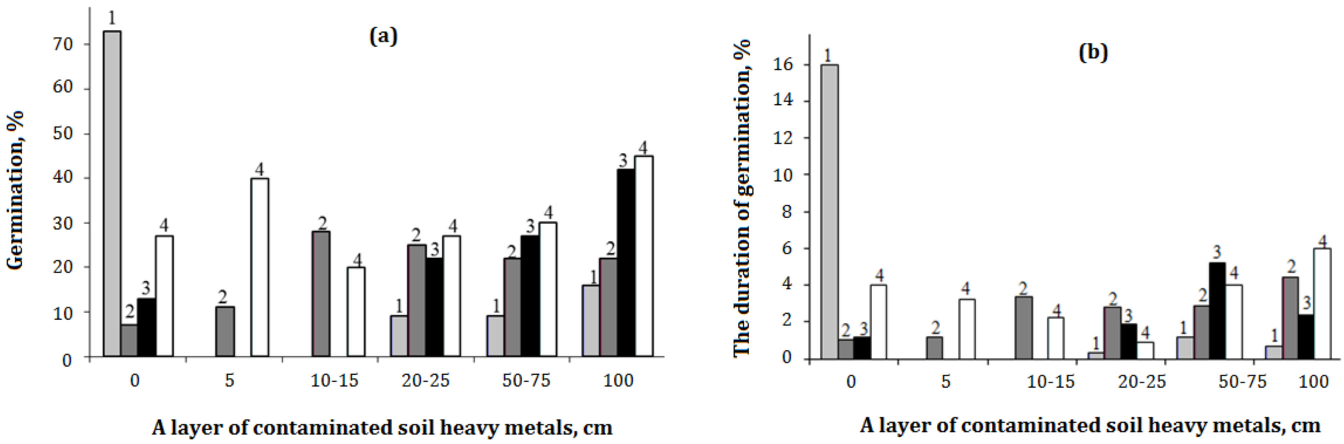

Figure 3. The dependence of lettuce seed germination (a) and there duration (b) on contaminated heavy metal soil: 1 – haplic arenosol; 2 – mollic gleyic fluvisol; 3 – gleyic chernozem; 4 – typical heavy black earth

The most informative among the growth and development of the tested culture in the model soil contamination is the germination activity indicator (Figure 4). The simultaneous of seed germination is relatively high in black chernozem soils: 1.3–4.7 % in gleyic chernozem; 3.4–7.0 % in heavy loamy chernozem. The same indicator is significantly lower in sandy soils and amounts to: 0.3–1.2 % in the haplic arenosol (except the control sample); 0.2–1.9 % in the mollic gleyic fluvisol.

Such indications of germination, growth and seed development of tested plants under heavy metal contamination can be explained by the fact that Zn and Cu belong to the metal group of average absorption by plants (Gruzdev, 2010). That is why at the initial terms the seeds possess sufficient nutrient potential to suppress the heavy metal effect. However, the inhibitory effect of metal-toxicants increased at the later development stages.

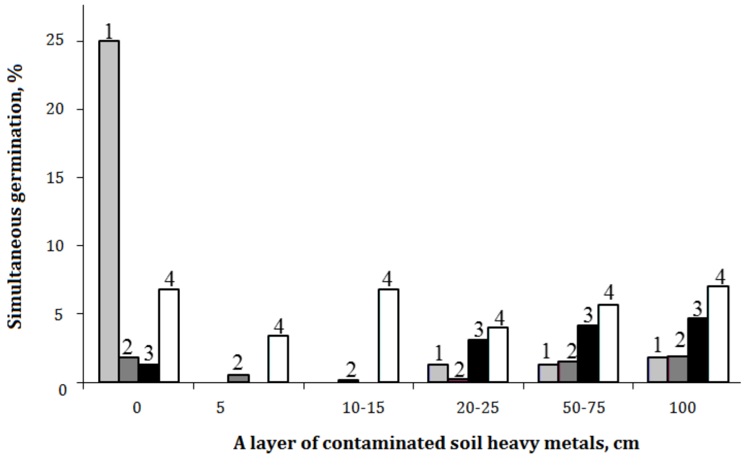

Figure 4. Seed germination activity dependence on the contaminated heavy metals soil of lettuce from the layer: 1 – gleyic fluvisol; 2 – mollic gleyic fluvisol; 3 – a typical heavy black earth, 4 – haplic arenosol

It should be also noted that for black earth typical loam (Figure 1) in the upper layers (5, 10–15 cm) the highest Cu and Zn content was obtained. The excess of their LC is equal to: 5.54–36.57 for Cu; 7.53–229.30 for Zn. However, the germination indicators, their activity energy and germination duration (Figure 2-4) are higher than those in other types of soils with lower LC. This may be due to the fact that clay soil characteristics are related to the soils possessing high adsorption properties and can bind heavy metals, protecting vegetable products from contamination (Baiseitova and Sartayeva, 2014).

According to modern concepts there are no toxic and non-toxic chemical elements in nature but there are only toxic and non-toxic concentrations. Depending on concentration and metal type, metal ion valence, solubility and duration of exposure, copper and zinc in small amounts contribute to the plant growth and development being essential microelements for them. But high Cu and Zn concentration can suppress them, breaking their vital functions (Black et al., 1999; Gruzdev, 2010).

As the results show, the data obtained confirm the above findings and point to the correlation between the content of heavy metals in soil and the growth activity of test plants. The comparison of maximum LC concentration values of copper and zinc in all test soils with germination, growth and development of lettuce seeds indicators showed the reducing of their concentration in the test soil layers slows down metal toxic effect. However, such correlation is noticeable not in all cases. Such dependence can be traced only in a single soil and it is not observed in other types of soil. It may be explained by natural interrelation between physical and physicochemical properties of the soil and tested culture parameters.

It should also be noted that copper and zinc effect has different focus on the activity for tested plants. Copper and zinc are known to be the most toxic pollutants in excess concentration. According to (STATE STANDARDS 2.2.7.029–99, 1999) copper is assigned to the low class of danger, and zinc to the high one. It is shown in the experiment that when LC (Cu) exceeds LC (Zn) 2–7 times (Table 1), the indices of germination and growth of test cultures are relatively higher (Figure 2-4) than in soils LC (Cu) < LC (Zn).

However, it should be noted that this dependence is not observed in all layers of soil. High indices of test culture germination are observed in the layers 10–15, 20–25 cm in mollic gleyic fluvisol where LC (Cu) exceeds LC (Zn) 0.05–0.06 times, and also in calcic voronic chernozem layers 5, 10–15 cm where the excess is 0.16–0.7 times. Thus, it should be assumed that the ambiguity of the correlation between copper and zinc content in the soil above their LC and active growth of test culture is connected with the complex action of copper and zinc. When combined action of the two metals occurs under unfavorable conditions and in harsh plant doses it may have both strengthening and weakening toxic effects. The synergistic action of zinc and copper may be determined by the location of these elements in the adjacent periodic system. As the authors suggest (Gladkov, 2010), copper possesses particularly high phytotoxicity and in the presence of zinc toxic effect increases sufficiently. That is why such contradictory ambiguous evidence about copper and zinc impact the test cultures and require further and more detailed studies.

Conclusion

The effect of heavy metals contained in the technogenic contaminated soils on plants under controlled conditions is determined, namely:

- soils contaminated by copper and zinc have integrated phytotoxic effect. The combined effect of copper and zinc is manifested as the inhibition or stimulation of lettuce growth processes and is determined, first of all, by the level and nature of contamination, soil properties and biological specificity of the test culture;
- the initial terms of the seed test culture have the potential of nutrients to suppress the effect of heavy metals. However, during later development the dampening effect of metal toxicants becomes stronger;
- there is both inhibition and stimulation of lettuce growth of the vegetative part and root system. Haplic arenosol is characterized by significant inhibition of the test culture, and for calcic voronic chernozem almost all layers showed significant stimulation;
- the seed sensitivity of the test object for copper and zinc is significantly manifested in the vigor testimony. Accoding to this parameter the investigated soils can be arranged in series: a haplic arenosol < mollic gleyic fluvisol < gleyic chernozem < calcic voronic chernozem**;**
- the indicator of seed germination in almost all cases of tested soils is high (7-73 %). However, the duration of the germination rate has the highest rate (0.3-16 %) depending on the type of soil;
- the germination period of seeds is relatively high in the black soils (1.3-7.0 %), but it is much lower in sandy soils (0.2-1.9%).

Evidence-based data provided in the work, are of interest in terms of the methods of bioassay environmental pollution due to the openness and relevance of this issue at the present stage of ecology development. These results confirm the need for agroecological monitoring to prevent possible negative consequences of human activities on the environment.

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