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Studies of the toxic effect of heavy metals contained in contaminated soil on the germination and growth of higher plants

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It is well known that the most of the heavy metals can give rise to rather well-defined toxic effects in human. They damages: the lungs in the form of pneumoconiosis; the central nervous system; the cardiovascular system; the immune system and others. Contaminated soils are the main source of heavy metals in food chains and ultimately for humans. For this reason, it is necessary to look for opportunities for the treatment of such contaminated soils, in order to enable their safe practical application. In this connection, data from the effect of contaminated with heavy metals soil on the growth and development of higher plants, through the application of pot-tests, when mixed with reference soil and compost, are presented here. Two species of higher plants were used in the study – oats (*Avena sativa*) and alfalfa (*Medicago sativa*). The obtained results show that the degree of growth and development of the experimental plants of the two plant species, are directly connected with the amount of added contaminated soil. To a greater extent, a toxic effect was found at alfalfa (*Medicago sativa*), where the test soil leads to complete suppression of germination.

Keywords: toxic effect, waste earth masses, oat *(Avena sativa)*, alfalfa (*Medicago sativa*), pot-tests.

УДК 631.45

Исследования токсического эффекта тяжёлых металлов, содержащихся в загрязнённой почве, на всхожесть и рост высших растений

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Известно, что большинство тяжёлых металлов (ТМ) и металлоидов могут вызывать у человека определённые токсические эффекты. В результате их воздействия происходят повреждение лёгких, центральной нервной, сердечно-сосудистой, иммунной систем и др. Загрязнённые почвы являются основным источником ТМ в пищевых цепях и, в конечном итоге, для человека. Поэтому необходимо искать варианты обработки таких загрязнённых почв, чтобы обеспечить их безопасное практическое применение. В этой связи в настоящей работе представлены данные о влиянии почвы, загрязнённой ТМ, на рост и развитие высших растений посредством проведения лабораторного горшечного теста при смешивании эталонной почвы с компостом. В настоящем исследовании использованы два вида высших растений – овёс посевной (*Avena sativa* L.) и люцерна посевная (*Medicago sativa* L.). Полученные результаты показывают, что степень роста и развития экспериментальных растений двух видов напрямую зависит от количества добавленной загрязнённой почвы. В большей степени токсический эффект был обнаружен у люцерны, для которой исследуемая почва приводит к полному подавлению прорастания семян.

Ключевые слова: токсическое действие, почва, овёс (*Avena sativa* L.), люцерна (*Medicago sativa* L.), лабораторный горшечный тест.

Heavy metals cause one of the most dangerous soil contaminatиon, as they are sorbed and "caught" firmly in the soil complex, forming metal salts of organic acids that remain in the surface layer. From there, the risk of damage to plants and organisms inhabiting that layer, and subsequently to humans, increases. Once in the human body, heavy metals are difficult to be removed. They are able to accumulate and may have toxic effects since they are not disposed out of human body by natural physiological mechanisms. For example: exposure to metals or their compounds damages the lungs in the form of pneumoconiosis, may have serious effects on the central nervous system, on the cardiovascular system, may also affect the immune system and others [1, 2].

According [3], over 5 million sites are considered as soil polluted and contaminated with heavy metals. For that reason, it is necessary to look for opportunities for the treatment of such contaminated soils, in order to enable their safe practical application. In soil practice, two main techniques are applied to study the effect of various soils and substances on plant growth and development: one is the technique of the field-test used by a number of authors in their studies [4, 5] and the second technique is the so-called pot-test.

The method of pot-test is one of the most common and is used to study changes in the content and forms of nutrients, the effectiveness of different types of fertilizers and improvers, etc. Also, it allows many soil differences to be studied simultaneously, which is why it is used more widely. In this type of experiment, the plants are grown in pots in specially adapted vegetation houses or greenhouses under controlled conditions in terms of moisture, heat, light, air and fertilize, they are protected from birds and are completely isolated from natural phenomena. Under such conditions, the reliability of the obtained results increases and at the same time the duration of the study is decreased. A number of authors have successfully applied the pot-tests in conducting their research on the study of various factors and substances and their impact on various plants [6–8].

In the practice, there are also standard procedures that aim to support the process of testing the effects of various substances and pollutants on the biological species – mainly plant species (ISO 17126:2005, ISO 11269-1:2012). Such is the standard ISO 11269-2:2012 whose basic principles and rules were applied in performing of the current experimental work.

The aim of the present study was to evaluate the toxic effect of heavy metal contaminated waste earth masses on the growth and development of higher plants.

Objects and methods

Sampling and analyses. For the purpose of the experiments, waste earth masses from a non-ferrous metal mine used as test soil (TS) were sampled. The samples are air-dried, sieved through a 4 mm sieve and homogenized. To interpret the test results, some basic characteristics were characterized: pH (KCl) – in accordance with Bulgarian State Standard (BSS) ISO 10390:2011; water content – according to ISO 11465:1993; cation exchange capacity – according to BSS ISO 11260:2018; total carbon content – according to ISO 10694:1995; content of nitrogen and phosphorus (BSS ISO 11261:2002, BSS ISO 11263:2002). The content of the elements Cu, Co, Ni, Zn, Al, Pb, Cr, Sn, Bi, Mn, Fe, Cd and As was determined by inductively coupled plasma optical emission spectrometry (Prodigy Teledyne, Leeman Labs) (Bulgarian State Standard EN 16170:2016).

According to the recommendations of ISO 11269-2:2012, a reference soil (RS) from an uncontaminated area near the contaminated site was used as a control. That soil also was treated as the test soil and its basic characteristics were also characterized.

Pot-tests. For the purposes of the experiment, TS was tested in a single concentration (100%), and the toxicity assessment was performed in a multi-concentration test in which series of concentrations were prepared by mixing certain amounts of test soil with reference soil and/or compost (Table 1). For comparison, a series only from the reference soil (0%) was prepared.

For the pot-tests, non-porous plastic pots with an inner diameter of 90 mm, were used. They were filled 1 cm below the upper edge, and the seeds were sown immediately after filling the pot. According to standard ISO 11269-2:2012 in each pot 10 seeds of the selected monocotyledonous species were planted – oats (*Avena sativa* L.) and dicotyledonous – alfalfa (*Medicago sativa* L.). After filling the pots, an optimal amount of distilled water was added to them, which quantities was controlled during the test. Three parallel tests were prepared for each series, for each plant species.

To avoid masking the phytotoxic effect by nutrient deficiency, after the emergence of sprouts all plants are enriched with conventional

Table 1

Contents of the initial materials, in %

Name	Contents of:		
	test (contaminated) soil (TS)	reference soil (RS)	compost
RS (0%)	0	100	0
Mixture 1	25	75	0
Mixture 2	50	50	0
Mixture 3	25	50	25
Mixture 4	50	25	25
Mixture 5	50	0	50
TS (100%)	100	0	0

commonly available mineral fertilizer – 2% ammonium nitrate solution (NH_4NO_3). After the appearance of the plants in each pot, their number has been reduced, leaving a total of five evenly spaced representative plants in the pot.

The pot-tests were conducted in the period 13.07–05.08.2020 under the following conditions: temperature 18–22 °C, humidity of air – 75–80% and soil moisture – 70–75%. During the experiment, the parameters total number of plants and stem height were monitored, and after termination of the experiment, the obtained dry mass was measured.

Results and discussion

The results for the composition of the compost from municipal waste used in the experimental work are described in detail in [9]. The results obtained from the preliminary analyses of the test (TS) and reference (RS) soil are given in Table 2.

The results for the determined heavy metal contents in the test soil are given in Table 3.

According to [10] the content of copper in the soil on industrial and production sites must not exceed 500 mg/kg. The copper content in the tested soil sample (16.84 mg/kg) is many times lower than the value for the MAC (maximum admissible concentration). In the same Regulation No. 3, the MACs for the elements Cd, Zn, Ni, Cr, Pb and As are also established, and for all of them the obtained values are below the MAC values, even for the last two they are below the detection limits of the apparatus, respectively < 0.010 mg/kg. Values for the next elements,

Table 2

Results from preliminary analysis of the soils

Parameter	TS	RS
Moisture content, %	2.34±0.01	3.93±0.01
P_{total}, %	0	0.038±0.002
$P_{soluble\ forms}$, %	0	0.009±0.001
C_{total}, %	0	8.95±0.01
Cation exchange capacity, meq per 100 g soil	1.19±0.02	20.43±0.04
pH_{KCl}	3.19±0.01	4.57±0.01
N_{total}, %	0	0.29±0.01

Table 3

Values for heavy metal content in TS

Element	Concentration, mg/kg
Cu	16.8±0.9
Co	0.378±0.019
Ni	0.069±0.007
Zn	0.717±0.032
Al	246±5
Cr	0.438±0.007
Mn	9.56±0.06
Fe	608±5

Table 4

Observation of the number of plants of oat (*Avena sativa*)

Day of observation	RS	Mix. 1	Mix. 2	Mix. 3	Mix. 4	Mix. 5	TS
	Number of plants*						
3	10	6	10	7	8	4	0
4	10	7	10	10	10	9	7
From 5 to 23	5	5	5	5	5	5	5

*Note: * The values are arithmetic-mean and are rounded to the nearest whole number.*

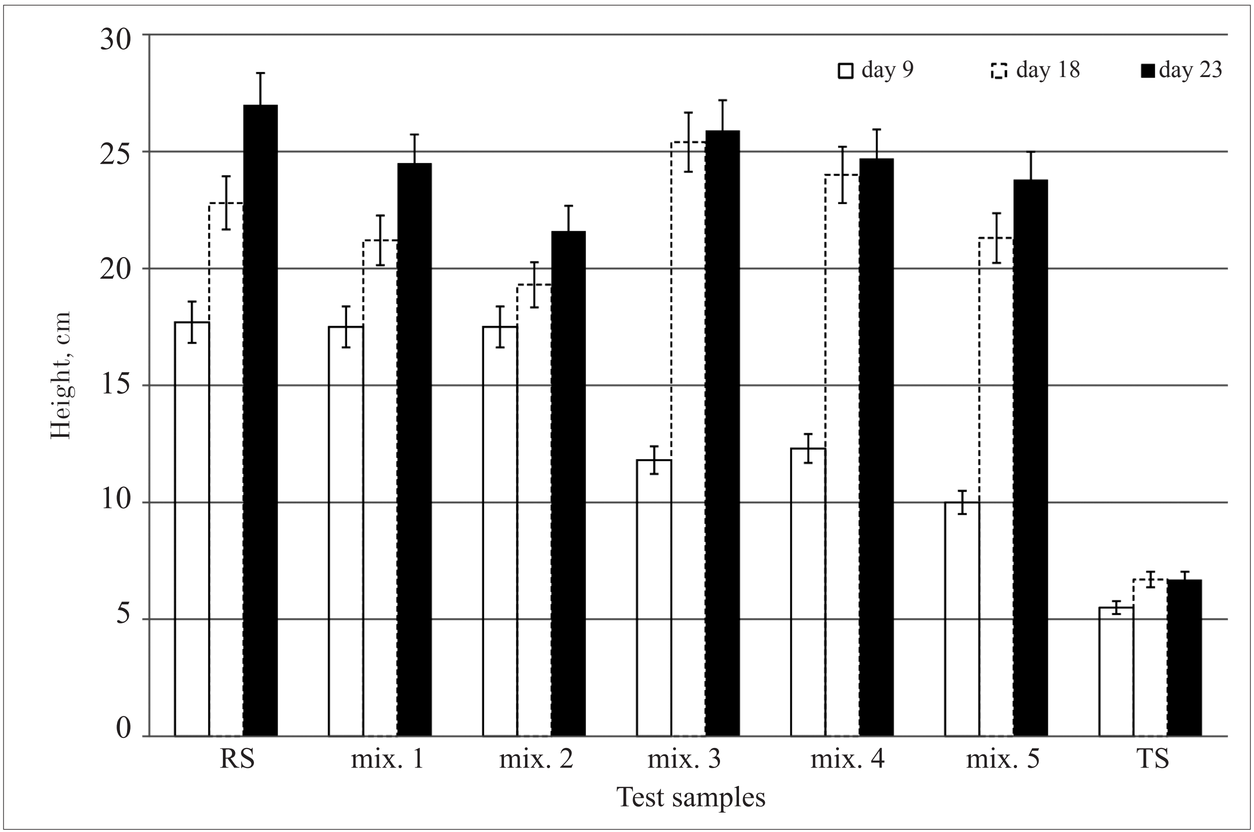

Fig. 1. Alteration of the height of the oat (*Avena sativa*) plants

also have been established below the detection limits of the apparatus: Sn < 0.010 mg/kg; Bi < 0.020 mg/kg; and Cd < 0.005 mg/kg.

The validity of the conducted pot-tests was confirmed by the fulfillment of the criteria set in ISO 11269-2:2012 regarding the comparative soil (RS): the appearance of seven healthy plants in each pot; lack of visible phytotoxic effects (chlorosis, necrosis, wilting, deformations of the leaves and stems) in plants; average survival of the emerged seedlings – at least 90% during the study.

The results from observation of the emergence and alteration of oat plants, are given in Table 4.

Table 4 shows the values from observing the emergence and alteration of oats, reported from the first to the twenty-third day after planting the seeds. After reaching the required germination on the fourth day of planting the seeds, the number of experimental plants was reduced to five per pot. Tracking the number of sprouted oat plants shows that the effect of the concentration of pollutants in the studied soils (RS, TS) and soil mixtures does not lead to a change in their number.

Figure 1 shows the growth in height (in cm) of oat plants during the experiment.

The values presented in Figure 1 show that as the concentration of contaminated soil in the mixtures increases, the height of the plants decreases – at 100% contaminated soil on day 9, the measured height is 5.5 cm, and on day 18 it reaches 6.7 cm and until the end of the experiment on 23rd day, does not change. The highest value for the height of oats during the whole experiment was found in the comparative soil, respectively on the 9th day – 17.7 cm, on the 18th day – 22.8 cm and on the 23rd day – 27 cm.

The results for the different mixtures show that the addition of real living soil (RS) and organic material leads to a clear effect on the growth of oat plants – mixture 3, shows the best values compared to other mixtures. It was established that the main substantial effect on the growth of oat plants has been obtained by addition of reference soil.

The pot-tests for both plant species were finished on day 23, after which the plants were uprooted and the yield of the respective series of experimental plants was weighted (see Tables 5 and 7) after drying in an oven at 75 °C for 16 hours.

It was found that with increasing concentration of contaminated soil (TS) in the mixtures without compost, oat yield decreases, the order is as follows: RS (0%) > mixture 1 > mixture 2. In the mixtures with compost, it is observed that with decreasing content on RS in them the yield also decreases – the order of yield is: mixture 3 > mixture 4 > mixture 5 > RS. From the values given in Table 5 it can be seen that the weight of the dry mass of oats, obtained during cultivation in the TS (100%), is lowest – 0.097 g.

The summary of the results for oats (*A. sativa*) shows that the amount of contaminated soil (TS) added affects it's growth and development, although no change in the number of experimental plants was observed until the end of the pot-tests.

In Table 6, the results from observation of the emergence and alteration of alfalfa plants, are presented.

The results show that on the third day after planting the alfalfa, the number of sprouted plants reaches the requirements of ISO 11269-2 (70 to 100%). After reaching the required germination, the number of experimental plants was reduced to five per pot, on the fifth day after planting the seeds. The observations of the alteration of the alfalfa plants (Table 6) shows that the different content of contaminated soil (TS) in the prepared mixtures affects their growth and leads to a reduction in their number. Changes in the number of plants were reported in all experimental mixtures and on the last day of the pot-test in the RS. Lack of germination was also found in the seeds planted in the TS throughout the test period.

The highest percentage of reduction in the number of plants was reported in mixture 2, where at the end of the test one specimen per pot, was found. The results show that in the mixtures with the addition of compost the reduction in the number of plants is smaller, compared to the mixtures of test and reference soil. It should be noted that when comparing the mixtures containing compost, the most significant reduction in the number of plants is observed in mixture 3, which has the lowest content of contaminated soil. A closer examination of the composition of the mixtures shows that such a significant reduction in the number of plants is also observed in mixtures 1 and 2. All three mixtures are characterized by a low content of organic materials and a predominant content of soil mineral component. That probably leads to a synergistic effect between two factors: optimal supply of nutrients in the soil solution, which enhances the absorption of the ions of the heavy metals from the root system of plants; and the absence of enough quantity of

Table 5

Obtained dry mass oat (*Avena sativa*)

Experimental series	RS	Mix. 1	Mix. 2	Mix. 3	Mix. 4	Mix. 5	TS
Weight, g	0.304	0.243	0.203	0.745	0.622	0.661	0.097

Table 6

Observation of the number of plants of alfalfa (*Medicago sativa*)

Day of observation	RS	Mix. 1	Mix. 2	Mix. 3	Mix. 4	Mix. 5	TS
	Number of plants*						
3	8	9	9	8	8	5	0
4	10	9	10	9	9	7	0
From 5 to 8	5	5	5	5	5	5	0
9 and 10	5	5	5	5	5	4	0
From 12 to 14	5	5	5	4	5	4	0
From 15 to 18	5	5	3	4	5	4	0
From 21 to 23	5	4	2	3	5	4	0

*Note: *The values are arithmetic-mean and are rounded to the nearest whole number.*

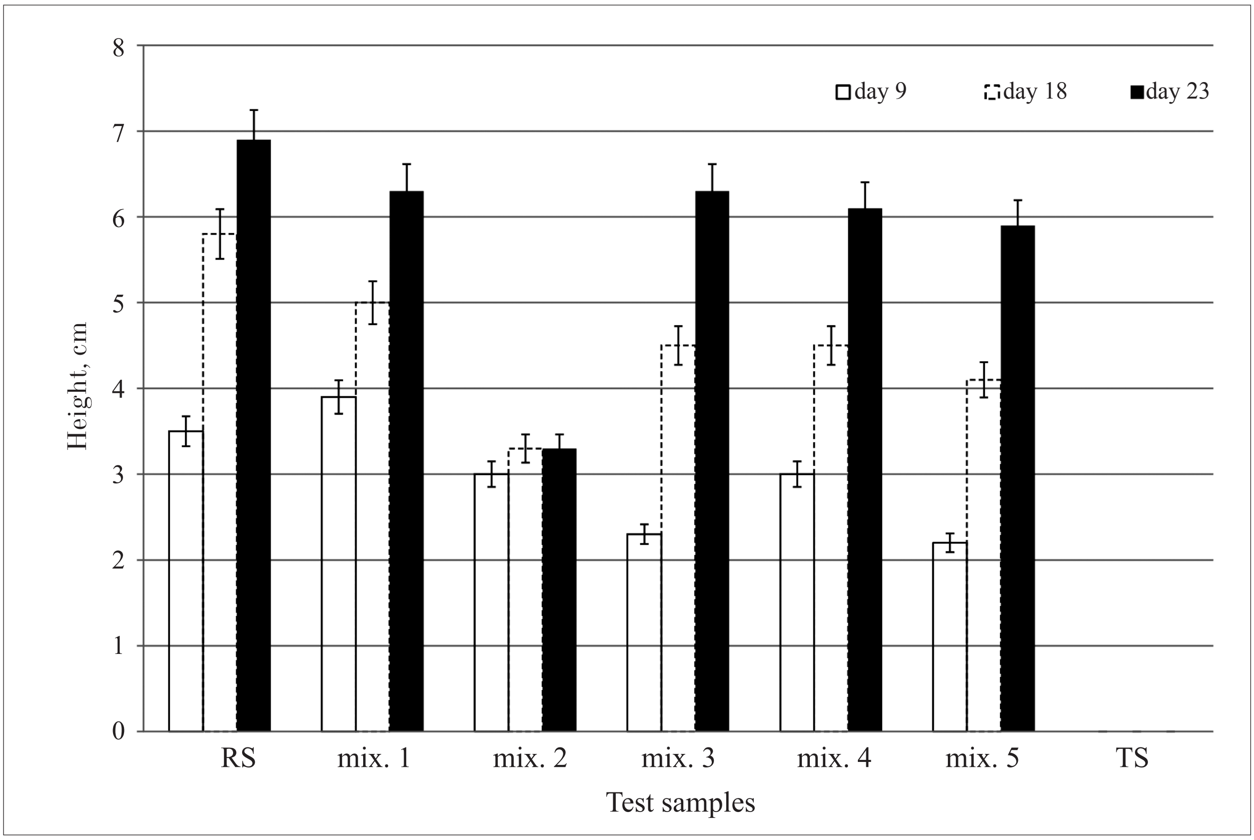

Fig. 2. Alteration of the height of the alfalfa (*Medicago sativa*) plants

Table 7

Obtained dry mass alfalfa (*Medicago sativa*)

Experimental series	RS	Mix. 1	Mix. 2	Mix. 3	Mix. 4	Mix. 5	TS
Weight, g	0.057	0.062	0.029	0.072	0.071	0.048	–

organic substances in the soil system available to block the available heavy metals in the form of metal salts of the organic acids.

On Figure 2 it is shown the growth in height of alfalfa plants. The highest value for the height of the alfalfa plants, again as with oats, was found in the reference soil (RS), respectively on the 9th day – 3.9 cm, on the 18th day – 5.8 cm and on the 23rd day – 6.9 cm. The highest initial value was found in mixture 1, which also has the highest content of RS – respectively 75%, which is most likely a carrier of mobile forms of nutrients and after their depletion a growth retardation was established.

In contrast to oats, in the alfalfa plants, the values obtained for the different mixtures show that the addition of organic material (compost) leads to a clear effect on the growth of alfalfa plants, and most likely the effect of adding a real natural soil (RS) would be significant at certain ratios (higher than those in mixture 2).

The obtained weights for the dry mass from alfalfa plants, are show in Table 7. The values of alfalfa dry mass are directly related to the number of plants left at the end of the test. This relationship shows that as the concentration of TS in the mixtures increases, the yield decreases. The order is as follows: RS > mixture 1 > mixture 2. In the mixtures with the addition of compost, the order of yield is: mixture 3 > mixture 4 > RS > mixture 5 and it is established that with the decrease of the content of reference soil in them the obtained mass also decreases. The highest reported value of the extracted alfalfa dry mass is in a mixture 3.

Based on the summarized data on the number of plants, their height and the obtained dry mass, it was found that the amount of added contaminated soil affects the emergence and growth of the alfalfa plants.

All the data presented up to now lead to the conclusion that alfalfa is more sensitive to the heavy metal contamination than oats, and the toxic effect, expressed through the observed effects of germination suppression, increased mortality of the plant specimens and slow growth in height, is more obvious.

Conclusions

The established data from the analysis for content of heavy metals in the test soil show that although available, their concentrations are below the established MAC values in the Republic of Bulgaria.

The results of the tests performed show, that the amount of added contaminated soil negatively affect the growth and development of the two types of higher plants oats (*A. sativa*) and alfalfa (*M. sativa*).

Through the observed effects of germination suppression, increased plant mortality and slow growth in height, it was found that oats (*A. sativa*) is more tolerant to heavy metal contamination than alfalfa (*M. sativa*), and the toxic effect in the second species is more obvious.

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