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Phytoremediation Potential of Flowering Plants in Relation to Copper

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ABSTRACT

Heavy metals such as cadmium, chromium, nickel, mercury, lead, copper, zinc and others are among the priority environmental pollutants. Determination of their content in its main subsystems is an obligatory component in environmental monitoring and certification of agricultural products. On the other hand, all metals are natural components of soil-forming rocks, and some metals are classified as biogenic microelements, and their absence provokes functional disorders in living organisms. This article describes the results of studying the phytoremediation potential of flowering plants in relation to copper ions under laboratory conditions. The following flowering plants were selected as test crops: tansy phacelia, white mustard, small-flowered marigolds and a mixture of cereal grasses consisting of red fescue, perennial ryegrass and bluegrass in a ratio of 40%, 50%, 10%, respectively. Within the experiment, copper ions in concentrations of 2 and 10 maximum permissible concentration (MPC) were introduced into the soil sampled from the territory of agricultural lands (leached medium-thick heavy loamy chernozem with a high level of humus, mobile phosphorus and exchangeable potassium and a low level of nitrate nitrogen, copper in gross and mobile forms). It was found that all plants selected as test crops are capable of accumulating copper ions from the soil to varying degrees, which makes it possible to use them in phytoremediation of agricultural lands planned for organic farming. The ability to accumulate copper ions increases in the following order: white mustard < small-flowered marigolds < tansy phacelia < mixture of cereal grasses. The maximum effect of soil phytoremediation was revealed in the variant with a mixture of cereal grasses. When they are grown, the content of copper ions in the soil with the introduction of 2 MPC decreases by 38.8%, with the introduction of 10 MPC the concentration decreases by 47.8%.

KEYWORDS: ACCUMULATION COEFFICIENT, BIOLOGICAL ABSORPTION COEFFICIENT, COPPER, FLOWERING PLANTS, HEAVY METAL IONS.

INTRODUCTION

Most plants have ability to accumulate copper ions, while this accumulation effect is lower for some more toxic elements, since copper is a biogenic element and is directly involved in vital processes in plant cells; therefore, its concentration in phytomass is controlled by the plant itself (Olshanskaya et al. 2013). Copper is a typical nutrient. It plays an important role in the life of plants,as it participates in the processes of respiration, photosynthesis, moisture balance and other vital functions. Disorders of physiological processes caused by a lack of copper such as photosynthesis and respiration affect the energy metabolism of a plant and negatively affect the processes of their growth and development (Printz et al. 2016; Mosa et al. 2018). Lack of copper in human and animal organisms can lead to disorders of the central and peripheral nervous system, dysfunction of the immune system, decreased bone density, joint diseases and other negative phenomena. However, the excess intake of copper in living organisms is no less dangerous than its deficiency and causes their poisoning (Bityutskiy 1999; Shtangeeva et al. 2020).

According to the interstate standard in force in the Russian Federation, chemical substances, according to the degree of danger, can be classified as highly hazardous, moderately hazardous or low hazardous for at least three indicators (toxicity, persistence in soil or plants, MPC, migration, impact on the nutritional value of agricultural products). Copper according to this classification is classified as moderately hazardous (GOST 17.4.1.02-83 2008; Wang et al. 2019; Amirova et al. 2019). At the end of the 20th century, the share of techno-genic copper in the environment was 75% and the main sources of its emission are high-temperature processes in nonferrous and ferrous metallurgy, combustion of fossil fuels, ore dressing wastes, copper-containing pesticides used in the practice of intensive

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agriculture (Yakovchenko et al. 2017; Asati et al. 2016; Shtangeeva et al. 2020).

Intensive agriculture, which has been practiced for a long time on the territory of Russia, has led to the fact that in most constituent entities of the Russian Federation there is a deterioration in the condition of agricultural land, a decrease in soil fertility continues, and their resistance to destruction and the ability to self-regenerate decreases. To preserve and restore the "health" of soils in order to use them in an organic land use system, a scientifically based approach to maintaining and increasing their fertility is required. Therefore, studies of the heavy metals' behavior in the system soil-plant allow scientists not only to assess environmental risks and make environmental forecasts regarding the accumulation of heavy metals in environmental objects, but also to apply new approaches and ways to eliminate them which is especially important in the production of agricultural products. In recent years, there has been a tendency for agricultural producers to switch to organic farming, which provides for high requirements for soil quality (Guidelines for determination of heavy metals 1989; SanPiN 2.1.7.1287-03 2004; Hygienic standard 2.1.7.2041-06 2006; Federal Center for Hygiene and Epidemiology of RosPotrebNadzor 2009; Vodyanitskiy 2011; GOST 12038-84 2016; Musilova et al. 2016; Wang et al. 2019; Amirova et al. 2019).

One of the most promising methods used for the restoration of agricultural land within the framework of organic agriculture can be phytoremediation - a method that is environmentally friendly and, importantly, economically beneficial. Phytoremediation is based on the ability of plants to extract harmful substances and elements from various components of the environment, accumulate them in their cells, tissues and organs without visible signs of oppression or convert them into safe compounds - metabolites (Volkov et al. 2013; Baycu et al. 2015; Yakovchenko et al. 2017; Kirichkova 2017; Poktsepay et al. 2017; Suman et al. 2018; Wang et al. 2019; Amirova et al. 2019; Shtangeeva et al. 2020). The purpose of this study is to determine the phytoremediation potential of some flowering plants in relation to copper ions.

MATERIAL AND METHODS

The study was carried out in the laboratory of the Department of Landscape Architecture of the Kuzbass State Agricultural Academy in the period from 15.04.2020 to 15.05.2020. Ornamental, green manure plants and weeds were test crops to study their hyperaccumulative potential for copper ions: small-flowered marigolds (*Tagetes patula* L.), tansy phacelia (*Phacelia tanacetifolia* Benth.), white mustard (*Sinapis alba* L.), and a mixture of cereal grasses. For the mixture of cereal grasses, the following plants was selected: red fescue (*Festuca rubra* L.), perennial ryegrass (*Lolium perenne* L.) and bluegrass (*Poa pratensis* L.) in a ratio of 40%, 50% and 10%, respectively. The choice in favor of these crops was due to their high ecological plasticity, the availability of seed for agricultural producers, and the simplicity of the cultivation technology.

Before sowing, the bioenergetic potential and laboratory germination were determined in the seeds of the studied crops (GOST 12038-84 2016). For laboratory studies, soil was sampled from the territory of agricultural land, planned in the future to be used for organic farming. Soil samples were taken from the arable horizon. Soil sampling was carried out by the Envelope method (GOST 17.4.4.02-2017 2018). In the soils taken for the study, the main agrochemical parameters and the content of copper ions were determined. Soil analysis was carried out in accordance with the following regulatory documents: mass fraction of organic matter, mass fraction of total and nitrate nitrogen, pH of salt extract, mass fraction of mobile phosphorus and exchangeable potassium, and content of gross and mobile forms of copper. To simulate soil contamination with copper ions, we used aqueous solutions of copper sulfate. The concentration of solutions was prepared based on the MPC for mobile forms of copper in soils (MPC Cu = 3.0 mg/kg of soil), in this experiment we used solutions with conditional concentrations of 2 and 10 MPC (GOST 26483-85 1986; GOST 26951-86 1986; GOST 26213-91 1993; PND F 16.1:2.3:3.11-98 1998; GOST R 54650-2011 2011; GOST R 58596-2019 2019).

The preparation of soils and plants for the control of copper ions in them was carried out in accordance with the method proposed by Lindemann et al. (2008). In accordance with the methodology, one kilogram of soil was placed in previously prepared plastic containers measuring 20×30×10 cm, which was thoroughly moistened before adding 50 ml of a solution with an appropriate concentration of copper ions. Soils from agricultural lands were used as a control group. After that, the studied cultures were sown in three replicates in the prepared soil in accordance with the experimental scheme (Table 1). The control test of the copper ion content in the soil and phytomass of the studied crops was carried out 30 days after sowing the plants using the method of atomic emission spectral analysis using an emission spectrometer with inductively coupled plasma OPTIMA model 2100 DV (PND F 16.1:2.3:3.11-98 1998). Accumulation coefficients characterizing soil remediation were determined as the ratio of the copper content at the end of the experiment to its content in the soil at the beginning of the experiment. Biological absorption coefficients characterizing plants' phytoremediation potential were determined as the ratio of the copper content in plant ash to the total copper content in the soil (Selyukova 2019).

RESULTS AND DISCUSSION

The crop seed quality was determined by the value of such indicators as germination and germination energy. These parameters further determine the most important sowing characteristics, such as the quality of seedlings, their amicability and evenness, which is directly related to the survival of plants in agro-phytocenosis. The observations and measurements of experimental plant samples showed a high bio-energetic potential of the seed, the germination energy and laboratory germination varied within 66-76% and 79-92%, respectively. The germination rate of white mustard seeds in laboratory conditions was significantly higher than that of other crops. In addition, this culture showed high germination energy, as evidenced by the highest values of this indicator (Table 2) (Amirova et al. 2019).

Table 1. Scheme of the laboratory experiment

№ option		Test crops		
Control	tansy phacelia	white mustard	mixture of cereal grasses	small-flowered marigolds
2 MPC Cu	tansy phacelia	white mustard	mixture of cereal grasses	small-flowered marigolds
10 MPC Cu	tansy phacelia	white mustard	mixture of cereal grasses	small-flowered marigolds

Table 2. Bioenergetic potential of crop seeds

Crop	Emergence rate, %	Laboratory germination, %	Terms for indicator determination, days	
			Emergence rate	Laboratory germination
White mustard	76±2,1	92±2,6	3	6
Tansy phacelia	72±1,2	90±3,5	4	10
Small-flowered marigolds	66±3,2	83±1,9	3	7
Perennial ryegrass	70±3,1	85±2,8	5	10
Bluegrass	71±2,7	89±2,5	7	21
Red fescue	68±2,5	79±3,4	7	14

Table 3. Agrochemical indicators of the upper horizon (0-20 cm) of the soil sampeled for laboratory research

Indicators	Content	Level of concentration
pH of the salt extract	5,7	close to neutral
Mass fraction of nitrate nitrogen, mg/kg of soil	4,2	low
Mass fraction of mobile phosphorus, mg of P_2O_5 per 1kg of soil	127,5	increased
Mass fraction of total nitrogen, %	0,2	increased
Mass fraction of exchangeable potassium, mg of K_2O per 1kg of soil	180,1	increased
Mass fraction of organic matter, %	9,1	high

Table 4. Content of copper ions in soil intended for laboratory research in comparison with the Clarke number and APC/MPC

Standard indicators of HM content in soils				HM content in the soil of the experimental plot	
Clarke number in Earth's crust, mg/kg		Total APC, mg/kg	Mobile MPC, mg/kg	Total, mg/kg	Mobile forms, mg/kg
According to Vinogradov (1957)	According to Kabata-Pendias (1989)				
20,0	26,0	132	3,0	25,9±4,4	0,23±0,06
Note. Accuracy indicators (±Δl at P=0,95)					

The soil that was used in the laboratory experiment was leached medium-thick heavy loamy chernozem with a high level of humus in the upper horizon (0-20 cm) (9.1%), an increased level of mobile phosphorus (127.5 mg P_2O_5/kg of soil) and exchange potassium (180.1 mg K_2O/kg of soil) and a low level of nitrate nitrogen (4.2 mg/kg of soil). The soil solution reaction was according to hygienic standard 2.1.7.2511-09 2009 and was close to neutral pH 5.7. (Table 3) (Amirova et al. 2019).

When comparing the data on the content of copper total forms in the studied soil with the literature data, it was found that their value was higher than the Clarke number according to Vinogradov, but it is lower than this value given by Kabat-Pendias (Dai et al. 2017; Lia et al. 2018). According to the standards in force in the Russian Federation, the approximate permissible content (APC) of copper total forms in soils, with a soil solution reaction of >5.5 (close to neutral), was 132 mg/kg. In the course of the study, it was found that in the studied soil samples the reaction of the soil solution is close to neutral (pHsol 5.7), and the content of copper total forms is 25.9 mg/kg of air-dry soil, which was five times lower than the APC value for this type of soil. The content of mobile forms of copper was less than 1.0% of the total one and 13 times lower than the MPC (Table 4) (Shtangeeva et al. 2020).

After introduction of copper ions into the soils taken for the study with concentrations in solutions of 2 and 10 MPC, its content at the beginning of the experiment was 34.0±6.0 mg/kg and 65.9±9.2 mg/kg of air-dry soil, respectively (Table 5). Comparative analysis of soil and plant material samples by the content of copper ions in a laboratory experiment showed that all plants taken for the research (weeds, ornamental and green manure plants) are capable, to one degree or another, of accumulating copper ions from soils (Shtangeeva et al. 2020).

In the options of the experiment when growing white mustard, the lowest value of the accumulation indicator of the studied pollutant in plants on the control was noted (2.5±0.4 mg/kg of air-dry phytomass). The highest value of this indicator in dry phytomass was found in the option with tansy phacelia (7.2±1.4 mg/kg of air-dry phytomass).

Table 5. Comparative analysis of soil and plant material samples by the content of copper ions based on the laboratory experiment

Experimental options		Copper ion content		
		Soil, mg/kg		Plants, mg/kg of dry phytomass end of the experiment
		beginning of the experiment	end of the experiment	
Tansy phacelia	Control	25,9±4,4	19,4 ±3.9	7,2±1,4
	2 MPC	34,0±6,0	21,5±3,6	10,2±1,8
	10 MPC	65,9±9,2	43,1±7,2	21,2±4,2
White mustard	Control	25,9±4,4	23,7±4,1	2,5±0,4
	2 MPC	34,0±6,0	28,2±5,6	5,9±1,2
	10 MPC	65,9±9,2	56,6±9,5	6,7±1,3
Mixture of cereal grasses	Control	25,9±4,4	21,0±4,2	3,7±0,7
	2 MPC	34,0±6,0	20,8±4,7	17,0±4,1
	10 MPC	65,9±9,2	34,4±7,5	38,3±7,7
Small-flowered marigolds	Control	25,9±4,4	21,2±4,1	5,0±1,0
	2 MPC	34,0±6,0	21,4±5,7	10,1±2,0
	10 MPC	65,9±9,2	50,2±9,3	21,5±4,3

Note. Accuracy indicators (±Δl at P=0,95)

This indicator was 1.9 times higher than in the option with a mixture of cereal grasses and 1.44 times in the option with small-flowered marigolds. It should be noted that with an increase in the concentration of copper ions in the soil, the accumulating ability of plants to this pollutant increases in all experimental groups. However, the study noted significant differences in the experiment options. Thus, in the option with an increase in the copper ion concentration in the soil (2 MPC), the highest accumulative capacities were observed in plants of the cereal family. The indicator of their accumulative ability in relation to copper ions was more than by 1.6 times higher than in the options with tansy phacelia and small-flowered marigolds and more than by 2.8 times in the option with white mustard.

In addition, an increase in the concentration of copper ions in the soil (10 MPC) increased the accumulation indicators of this pollutant in the phytomass of cereals more than by 4 times compared with similar indicators in the options with small-flowered marigolds and more than by 2.5 times in the option with phacelia tansy and white mustard. Thus, at low levels of copper ion content in soils (control), the ability to accumulate copper by plants increased in the following order: white mustard - a mixture of crop grasses – small-flowered marigolds - tansy phacelia; and at concentrations of 2 and 10 MPC, the tendency to minimal accumulation of copper persists for white mustard, but varies for cereals (Shtangeeva et al. 2020).

Their phytomass showed the highest content of the investigated heavy metal - 17.0±4.1 and 38.3±7.7 mg/kg, respectively. Analysis of the results of the copper ion concentration changes in the soil according to the options of the experiment showed that in the control group there is a significant decrease in its content only in the option with tansy phacelia (25.0%). When a copper ion concentration was 2 MPC, its significant decrease in the soil was noted in options with the cultivation of cereal grasses, the studied indicator decreased by 38.8%. In the experiment options with tansy phacelia and small-flowered marigolds, there was observed a decrease in the concentration of the pollutant by 36%. At acopper ion concentration of 10 MPC, a significant decrease in its content in the soil was revealed as well (Shtangeeva et al. 2020).

Figure 1: Phytoremediation potential of flowering plants in relation to copper ions in laboratory conditions (K_{ba} is the coefficient of biological absorption)

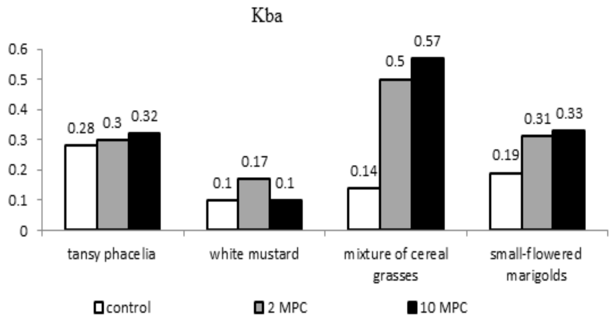
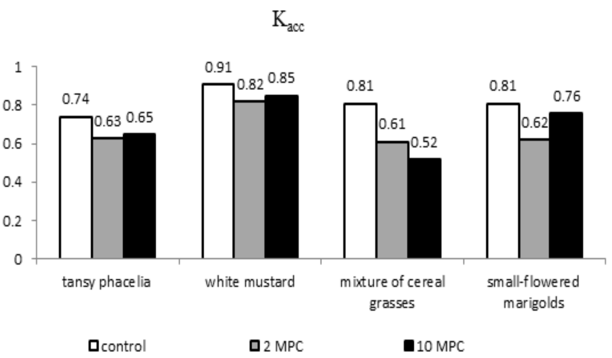


Figure 2: Efficiency of soil phytoremediation in relation to copper ions in laboratory conditions (K_{acc} - accumulation coefficient)



When growing a mixture of cereal grasses, this indicator decreased by 47.8%, when growing phacelia tansy - by 34.6%, and when growing small-flowered marigolds - by 23.8%. In the course of data analysis, a correlation was established between the decrease in the content of the studied copper ions in the soil and their accumulation in plant material, which is quantitatively confirmed by the coefficient values of biological absorption (Kba) and of accumulation (Kacc) (fig. 1-2). Comparative analysis of the values of the biological absorption coefficients of different crops showed that, under laboratory conditions, the ability to accumulate copper ions by plants increases, when the pollutant concentration increases in the soil (Vityaz et al. 2021).

At the same time, it was found that with an increase in the concentration of copper ions in the soil up to 2 MPC, the accumulative capacity of plants in the option with small-flowered marigolds increases 1.5 times (Kba = 0.3), and in the option with a mixture of cereal grasses - 3.5 times (Kba = 0.5) compared with the corresponding control groups. A further increase in the copper ion content in the soil (up to 10 MPC) leads to a significant increase in the accumulative capacity of plants in the option with a mixture of cereals, where the biological absorption index reaches 0.57, which is 4 times higher than the value of this indicator in comparison with the corresponding control group (Vityaz et al. 2021).

The study revealed that when the copper ion concentration in the soil increases by 2 MPC, the accumulative ability of plants increases in the following order: white mustard < tansy phacelia < small-flowered marigolds < mixture of cereal grasses; at a concentration of copper ions of 10 MPC - the order changes: white mustard < small-flowered marigolds < phacelia tansy < mixture of cereal grasses. According to the analysis of the accumulation coefficient values of the studied crops it follows that with a low (background) content of copper ions in the soil, a significant decrease in its concentration is revealed only in the option with tansy phacelia, which is confirmed by the highest value of the accumulation coefficient (Kacc = 0.74).

With the content of copper ions in the soil at concentrations of 2 MPC, its significant decrease is found in the option with a cereal mixture and small-flowered marigolds, which is confirmed by the corresponding values of Kacc (0.61 and 0.62). When the content of copper ions in the soil at a concentration of 10 MPC, its significant decrease is found in the option with a cereal mixture (Kacc = 0.52). Thus, the maximum effect of soil phytoremediation was revealed in the option with a cereal mixture, where the content of copper ions in the soil decreases by 38.8% with the introduction of 2 MPC, and with the introduction of 10 MPC - by 47.8%. The results obtained in the course of the study are consistent with the literature data on the high phytoremediation potential of representatives of the cereal family in relation to heavy metals (Vityaz et al. 2021).

CONCLUSION

The findings of the present study found that weeds, ornamental and green manure plants, due to their accumulative capabilities in relation to heavy metals, can be used as phytoremediators to improve the agrochemical parameters of agricultural soils intended for organic farming. When the (background) content of copper ions was low, a significant decrease in its concentration in the soil (25.0%) was revealed only in the variant with tansy phacelia, which is also confirmed by the value of the accumulation coefficient (Kacc = 0.74). According to the ability to accumulate copper ions, the tested plants form the following row: white mustard < small-flowered marigolds < tansy phacelia < mixture of cereal grasses.

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