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THE STUDY OF BARLEY RESISTANCE TO CADMIUM AND ZINC

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Abstract: The rich resource and raw material potential of Kazakhstan was the basis for the development of a powerful industry. However, it is industrial centers that are the areas of the highest pollution of the environment with heavy metals. A significant increase in the content of heavy metals in the environment is accompanied by their accumulation in plants, which has a negative impact on their growth, development and productivity. In this regard, the study of the reaction of plants to the action of heavy metals causes not only great scientific, but also practical interest. Different varieties of barley have been studied under conditions of natural soil contamination with heavy metals. The aim of the study is to identify resistant to cadmium and zinc barley varieties. The objects of the study are four varieties of barley from the collection of the East Kazakhstan Research Institute of Agriculture: Siberian Avant-Garde, Modern, Omsk, and Donetsk-8. The yield and survival of barley plants during the spring-summer vegetation were studied. Determination of physiological parameters was carried out according to the method of field experience. Productivity was determined by the direct method. Heavy metals (cadmium and zinc) were determined by atomic absorption with atomization in a flame and graphite furnace. The largest crop among the studied varieties has Modern variety of barley. The best survival is also demonstrated by Modern variety of barley. Donetsk-8 barley variety accumulates the least cadmium in the seeds. Siberian Avant-Garde barley variety accumulates the minimum amount of zinc in the seeds and at the same time is characterized by good survival during the spring-summer vegetation under conditions of polymetallic soil contamination.

Keywords: barley, cadmium, zinc, yield

Introduction

Among the many pollutants of the environment, one of the main places is occupied by heavy metals, which are highly toxic and capable of entering the human body and animals through food chains, thereby posing a serious threat to their health.

In recent decades, due to the rapid development of industry around the world, the pollution of the environment with heavy metals has increased on a scale that is not characteristic of nature. Because of this, the increase in their content in the environment is becoming a serious environmental problem of our time *(Dobrovolsky, 1992, Bashkin and Kasimov, 2004).* The most dangerous sources of anthropogenic pollution for populations of any organisms are industrial enterprises. The East Kazakhstan region is the industrial center of Kazakhstan, where a large amount of heavy metals enters the soil, while the soils of agricultural communities can be contaminated. *(Alybaeva, 2007).*

Among heavy metals, there are elements necessary for plant life (Cu, Zn, Co, Cr, Mn, etc.), as well as elements (Cd, Hg, Pb, etc.), the functional role of which is currently unknown *(Alekseev, 1987, Kabata-Pendias and Pendias, 1989).* Trace elements (Co, Cr, Cu, Fe, Mn, Ni and Zn) are involved in almost all processes taking place in the plant cell: energy metabolism, primary and secondary metabolism, hormonal regulation, signal

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transmission, etc. It should also be noted that 25–50% of all proteins work only in the presence of metal ions *(Blindauer and Schmid, 2010)*, of which the largest number (more than 1200) is functionally related to zinc *(Krämer, et al, 2007, Hänsch and Mendel, 2009, Husted, et al, 2011).* Usually, the concentration of trace elements in plants is small (0.001 % of the dry weight of the cell and below), but with an increase in their level in the environment, they become toxic to living organisms *(Williams, Salt, 2009).* In contrast, heavy metals, which are not trace elements, including the most important environmental pollutants – Cd, Hg and Pb, have a negative impact on plants even in relatively low concentrations *(Hassan and Aarts, 2011).*

Despite the fact that many heavy metals are not necessary for plants, they can be actively absorbed by them, accumulate and enter the human body through the food chains *(Nesterova, 1989, Ilyin, 1991, Grant, et al, 1998).* The danger of metals is exacerbated by the fact that they have a cumulative effect and retain toxic properties for a long time *(Mineev, et al, 1981, Yagodin, et al, 1989).*

Heavy metals are characterized by very uneven distribution in natural environments. With a relatively low natural content of heavy metals in the environment, in the areas of ore deposits the concentrations of some of them (Cu, Pb, Zn, Mo, Ni, etc.) can be hundreds of times higher than the background values *(Kositsin and Alekseeva-Popova, 1983).* In addition, it should be borne in mind that the intensive development of modern industry and agriculture is inevitably accompanied by an artificial increase in their content in the environment *(Prasad, 1995, Sanità di Toppi and Gabbrielli, 1999).* Heavy metals belong mainly to dispersed chemical elements, therefore not only the ground surface, in particular, the soil cover, but also the hydrosphere and atmosphere are polluted by them *(Dobrovolsky, 1983, 2004).* Because of this, the increase in their concentration in the environment due to natural or anthropogenic inputs can be global. The natural sources of heavy metals include rocks (from the products of weathering which formed the soil cover), volcanoes, space dust, soil erosion, evaporation from the surface of the seas and oceans, their release by vegetation *(Bogdanovsky, 1994).* Anthropogenic sources of income are mainly associated with the work of enterprises of coal mining, metallurgical, chemical industry and energy complex. Important sources of environmental pollution with heavy metals are various vehicles, as well as agrotechnical measures, in particular, the application of fertilizers and pesticides containing these elements in their composition *(Merrington and Alloway, 1994, Nicholson, et al, 1994, Nikiforova, 2003).*

The problem of obtaining safe food products remains relevant for a very long time. Even with an impeccable biochemical composition, deserving the highest praise of physiologists for nutritional and biological value, plant products can be considered dangerous to human health if its ashes contain unacceptable amounts of lead, cadmium and other metals *(Sokolova, et al, 2006, Pugaev, 2013).*

A long period of self-cleaning of soils and the high cost of their artificial cleaning make humanity look for new ways to solve the problem of soil pollution with heavy metals. The most promising direction in this area is the study of the genetic potential of plants and the identification of plant objects characterized by minimal accumulation of heavy metals. Individual varieties of different types of food crops show significant differences in resistance to the action of soil pollutants *(Barsukova, 1997, Yang, et al, 2000).* These properties can be used in environmentally friendly production. By selecting the most metal-resistant cultures that accumulate the minimum amount of pollutants, it is possible to obtain environmentally safe products *(Lukin, et al, 1999).* Metal-resistant forms can also be used in breeding to create varieties of plants that are tolerant to pollutants *(Molchan, 1996, Clarke, et al, 2002, Ozkutlu, et al, 2007).*

Methods

The experiment studied 4 varieties of barley from the collection of the East Kazakhstan research Institute of agriculture (EKRIA): Siberian Avant-garde, Modern, Omsk and Donetsk-8. The experiments were carried out in the field conditions of natural soil contamination with cadmium and zinc, which are one of the priority soil pollutants of the East Kazakhstan region. The plants were grown at the research and testing site of the EKRIA, in the suburban area of Ust-Kamenogorsk, East Kazakhstan region, North-East direction, and 3 km from the border of the city. The area of the experimental plot is 15 m^2 in triple repetition. The width of the aisles is 15 cm, the space between the rows is 50 cm. Soil is ordinary black, heavy loamy, slightly humus, the content of NO_3 - 5.3 mg/g of soil, P_2O_5 - 3.4 mg/g, K_2O – 29.8 mg/g. Early spring harrowing, cultivation, pre-sowing cultivation. Care of plants (rolling, weeding) was made manually.

Analysis of physiological parameters. Determination of physiological parameters was carried out by the method of field experience *(Dospekhov, 2011).* Plant survival was determined. Plants were counted in the phase of full shoots and before harvesting. The number of preserved plants (%) was calculated by the formula:

B= (Cx100) / A, where

B - number of plants preserved for harvesting, %; A - number of plants in the phase of full germination, units/m^2, C - number of plants to harvest, pcs/1 m^2.

Yields were determined by direct method. The grain obtained from each plot, poured into the bag. Bags of grain were weighed with an accuracy of 0.01 kg. Grain moisture was determined by weight method. From each plot, grain samples were taken in aluminum cups with a tight lid, weighed and dried at a temperature of 100-105° C to a constant weight of about 4-6 hours, then calculations were carried out according to the formula:

X = B : H, where

X - grain moisture, %; B - mass of evaporated water, g; H - crude sample, g. The yield was recalculated to the standard 14% moisture by the formula:

X = Y x (100-b) / 100-c, where:

X - is the yield given to the standard humidity; Y - is the yield obtained; b - is the yield humidity (%); C - is the standard humidity for this object.

Determination of heavy metals. Heavy metals (cadmium and zinc) were determined by atomic absorption with atomization in a flame and graphite furnace on an “A-Analyst 300” device from “Perkin Elmer” company. Sample preparation was carried out using a “Hot Block” heating unit with the addition of concentrated nitric and hydrochloric acids at a temperature of 90 ± 5 ° C, according to standard operating procedures. Samples were placed in tasting disposable glasses; 5 ml of 50% nitric acid and 0.5 ml of concentrated hydrochloric acid were added. The samples were well mixed to the state of liquid clay, covered with watch glass and placed in the heating block; the samples were heated to a temperature of 90±5 ° C and evaporated for 10-15 minutes, not reaching the boiling point. After that, the samples were cooled; 5 ml of concentrated HNO_3 were added again and reheated for 30 minutes. The contents of the containers were evaporated, not reaching the boiling point, at a temperature of 90 ± 5 ° C, up to about 5 ml within 2 hours, avoiding foaming. After that, the samples were cooled and the volume was adjusted to 50 ml with deionized water. To calibrate the device, a calibration form consisting of deionized water and 1% HNO_3 solution and standard samples of “High Purity” company was used. After calibration of the device, the indicators of the analyzed samples were taken. The accuracy of the analysis was checked by the Merck verification standard. The heavy metal content in the sample was calculated using the following formula:

C, mg / kg = C device × V sample × FD / m, where

C device – indicator of device (mg / l); V sample - final sample volume (ml); FD - dilution factor; m - sample weight (g).

The "Data analysis" package of Microsoft Excel was used, which was used to determine such indicators as average value, representativeness error and variance. Then, in order to establish the least significant difference (LSD), the parameters of difference error and Student's t- criterion for each data group were found. LSD = t0.5 × Sd – the formula for calculating the least significant difference.

Research results

The content of cadmium and zinc in the soil of the root zone. The results showed that the content of heavy metals in the root zone of the soil of different varieties of barley is not the same.

The content of cadmium in the root zone of the soil of different varieties of barley is different. The excess of the maximum permissible concentration (MPC) of this metal is observed in the soil of the root layer of barley varieties: Siberian Avant-Garde, Modern and Donetsk-8. And only in the soil of the habitat zone of the roots of the Omsk variety, there is no excess of MPC (Figure 1).

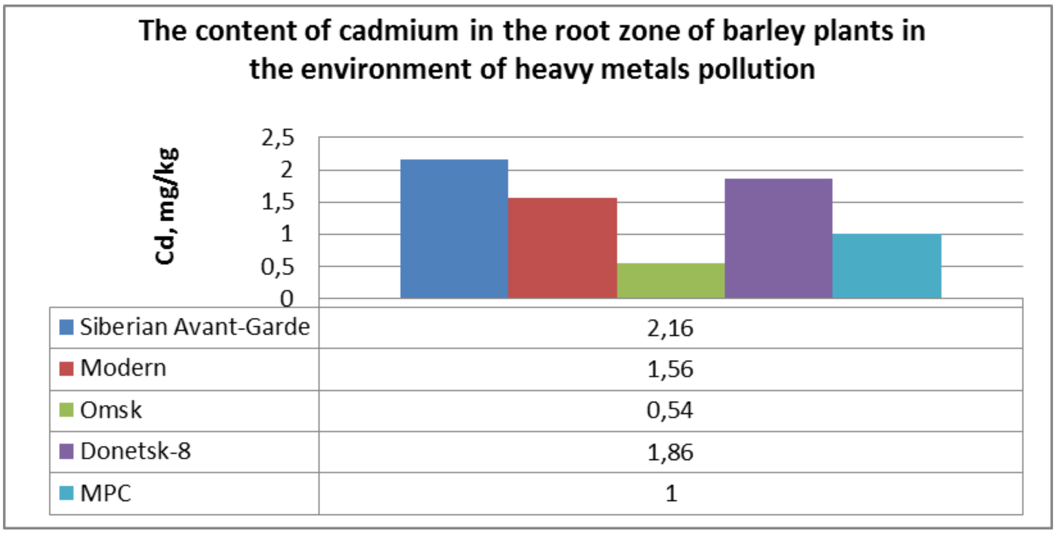

Figure 1: The content of cadmium in the root zone of the soil of the studied varieties of barley from the EKRIA collection in relation to the MPC.

Analysis of the metal content in relation to the MPC does not always give an objective picture. Using Regional Clark can provide more objective information. The Regional Clark content of elements shows the content of this element in the soil of the region. Since the varieties of barley zoned in the region are studied, it can be assumed that they are adapted to the Regional Clark content of these elements in the soil and the true excess, which can affect the barley plants, may be excess relative to the Regional Clark. In relation to the MPC, which is about 1.4 times higher than the Regional Clark content of this metal (0.7 mg/kg in the soil of the East Kazakhstan region), the cadmium content in the soil of the habitat zone of roots of various barley varieties exceeds Regional Clark.

The study of zinc accumulation in the soil of the habitat area of the roots of different genotypes of barley from the collection of EKRIA in conditions of natural pollution of the environment with heavy metals showed that the content is uneven. In almost all variants of the experiment, the MPC of this element is exceeded in the soil, except for the soil of the root zone of the Omsk variety. The MPC is exceeded from 1.73 to 1.89 times (Figure 2).

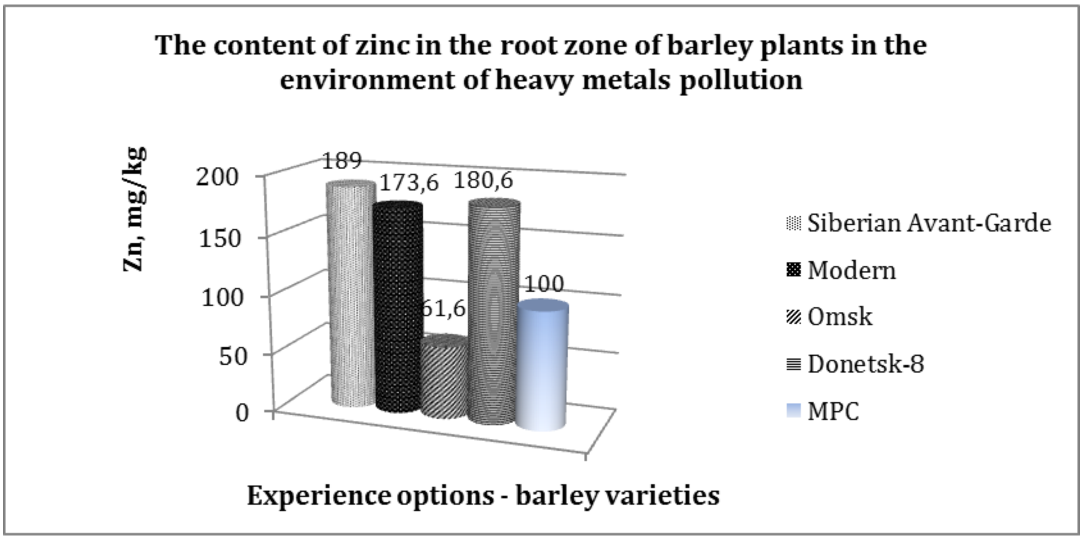

Figure 2: The content of zinc in the root zone of the soil of the studied varieties of barley from the EKRIA collection in relation to the MPC.

In relation to the Regional Clark content of this metal (42.4 mg/kg in the soil of the East Kazakhstan Region), the zinc content in the soil of the root zone of all the considered barley varieties is exceeded.

Thus, studies of the content of cadmium and zinc in the soil of the root zone of various barley genotypes from the EKRIA collection showed that the excess of the MPC of cadmium and zinc is not observed only in the soil of the habitat of the roots of the Omsk variety compared to other varieties.

The content of cadmium and zinc in barley seeds. Studies of the accumulation of cadmium in the seeds of barley plants from the EKRIA collection under polymetallic soil contamination have shown that this metal accumulates in relatively large quantities. Its content exceeds 11.4 times the MPC for grain in the case of barley variety Siberian Avant-Garde. The average excess of MPC is observed in the varieties Modern and Omsk. A slight excess of MPC was revealed in the case of the Donetsk-8 variety (Figure 3).

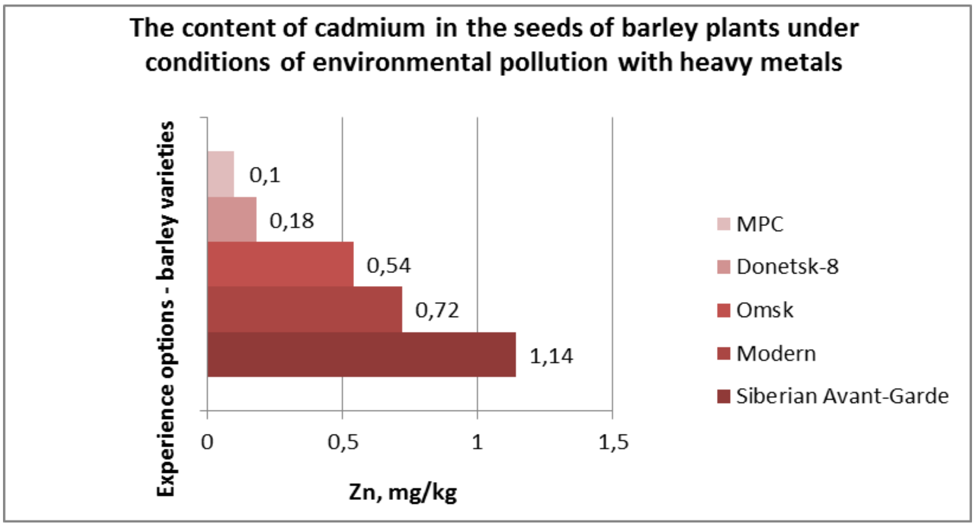

Figure 3: The cadmium content in seeds of different genotypes of barley from the collection of EKRIA.

At the same time, it should be noted that cadmium content in the soil of the root layer is also significant and exceeds the MPC in almost all barley varieties, except for the Omsk variety (Figure 1). The greatest amount of cadmium is accumulated by the seeds of Siberian Avant-Garde plant.

The content of zinc in the seeds of different genotypes of barley is the most important, since the grain of barley is used in the food industry.

Studies of the accumulation of zinc in the seeds of barley plants from the EKRIA collection under polymetallic soil contamination conditions have shown that this metal accumulates in a small amount. Its content exceeds the MPC more than 2 times in the seeds of all barley genotypes. The greatest excess of MPC (almost 2.6 times) is observed in the Donetsk-8 variety. The Omsk genotype revealed an average excess of MPC (2.38 times). The lowest excess of MPC is observed in the Siberian Avant-Garde and Modern varieties (Figure 4).

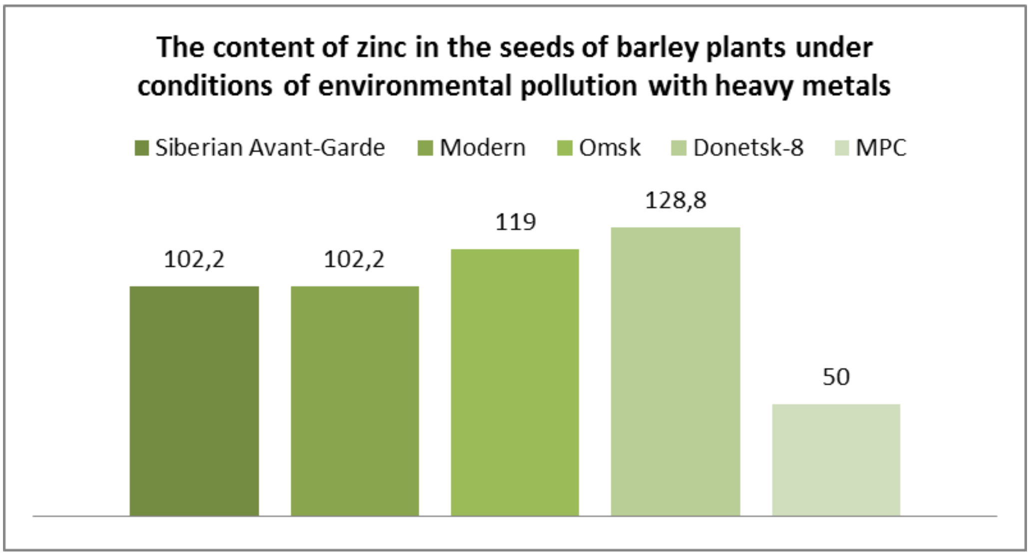

Figure 4: The zinc content in seeds of different genotypes of barley from the collection of EKRIA.

In accordance with the foregoing, the seeds of all barley plants are contaminated with zinc ions. However, the barley varieties Siberian Avant-garde and Modern accumulate zinc in the smallest amount compared to other varieties, so they can be recommended for use in breeding and genetic studies as the most resistant to the accumulation of zinc in the seeds.

Thus, studies on the accumulation of cadmium and zinc in barley seeds from the EKRIA collection have shown that, on the basis of cadmium accumulation, the most stable barley variety is Donetsk-8. In terms of zinc accumulation, the most stable varieties were Siberian Avant-Garde and Modern.

The Barley variety Donetsk-8 can be recommended for further use in breeding as the most resistant to cadmium accumulation in grain. Varieties of barley Siberian Avant-Garde and Modern can be recommended for further use in breeding as the most resistant to the accumulation of zinc in grain.

The physiological parameters of barley plants. Physiological parameters of barley plants were also investigated in order to characterize their other economically significant traits - yield and survival in the conditions of spring-summer vegetation.

The survival of barley plants. The number of plants before harvesting in comparison with their number before tillering shows survival during the spring-summer vegetation. A study of the number of plants before tillering showed that the largest number of plants is observed in the variety Modern (Figure 5). The average number of plants, compared with other varieties, is observed in Omsk and Donetsk-8 varieties. The smallest number of plants in the variety - Siberian Avant-Garde.

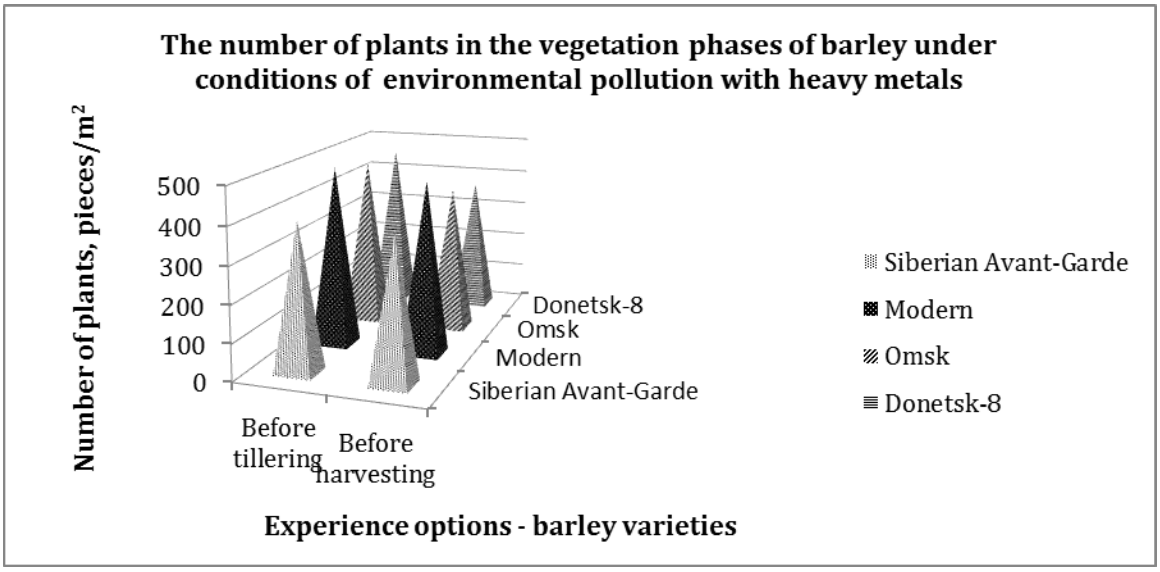

Figure 5: The number of plants of different genotypes of barley in the phases of vegetation during their cultivation in conditions of polymetallic soil pollution.

Determination of the number of plants before harvesting showed that the greatest number of plants is also observed in the variety Modern (Figure 5). The average number of plants, compared with other varieties, is observed in Omsk and Siberian Avant-Garde varieties. The smallest number of plants in the variety - Donetsk-8 (Figure 5).

The calculation of the percentage of survival showed that the Siberian Avant-Garde variety has the greatest survival. The barley variety Modern shows an average survival rate. The lowest survival rate is shown by varieties – Omsk and Donetsk-8 (Figure 6).

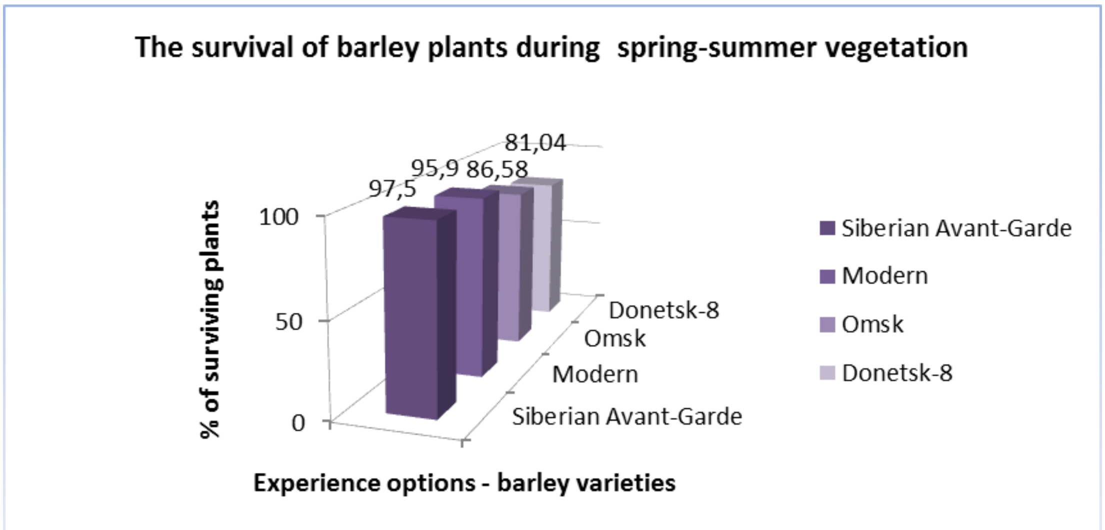

Figure 6: Percentage of different genotypes of barley preserved before harvesting when grown in conditions of polymetallic soil contamination.

The yield of barley plants. The study of the harvest showed that among the varieties of barley from the EKRIA collection, the yield from the plot was the largest in the Modern variety (Figure 7). The lowest yield in terms of soil contamination with heavy metals is demonstrated by the Donetsk-8 variety. The average yield compared with other varieties of barley was found in varieties: Omsk and Siberian Avant-Garde.

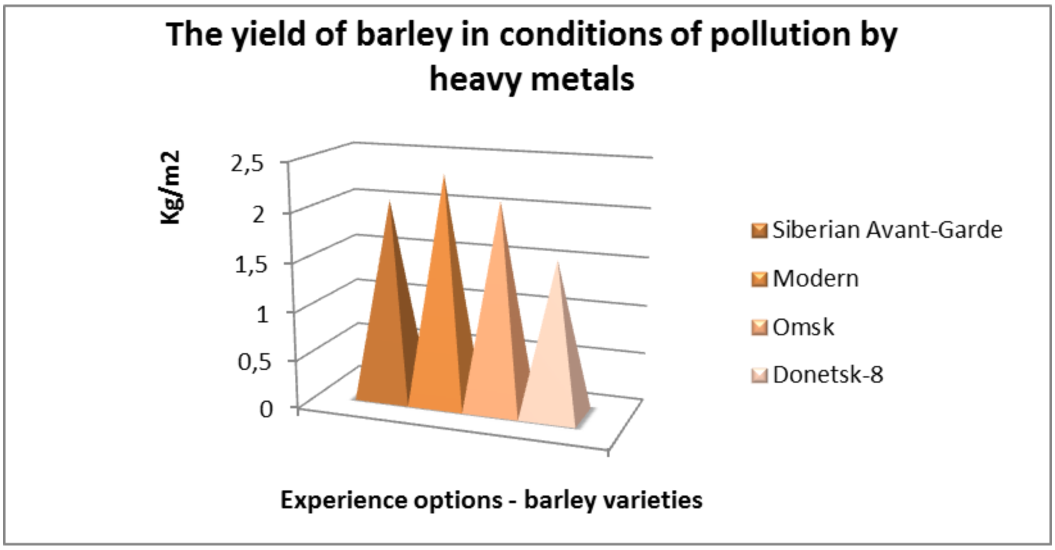

Figure 7: The yield of different barley genotypes from the EKRIA collection when grown under natural environmental conditions.

Discussion

First of all, the content of cadmium and zinc in the root zone of the soil of different barley genotypes was studied in order to have an idea whether the barley plants experience stress from the excess of heavy metals in the soil. Research results have shown that barley plants are under stress from elevated levels of cadmium and zinc in the soil. Also, according to literature data, it is known that soils are contaminated with other priority heavy metals for this region *(Alybaeva, 2007).* Thus, barley plants of different varieties, grown in conditions of natural pollution by heavy metals, are under stress from the complex effects of many metals.

It is important to have the varietal specificity of the response of plants to changes in environmental factors, in particular, soil and plant pollution with ecotoxicants. In this regard, the attention of researchers is increasingly focused on the study of the behavior of plant organisms in relation to the accumulation of chemical elements of different nature in commercial products.

The study of the content of cadmium and zinc in the seeds of different genotypes of barley is the most important, as barley grain is used in the food industry. The results showed that the content of cadmium and zinc in barley seeds in all variants exceeded the MPC of these metals in the grain (Figure 3 and Figure 4). The varietal specificity for this trait was revealed.

On the basis of cadmium accumulation in grain, the most resistant barley variety is Donetsk-8 (Figure 3). This fact can be considered an advantage, since cadmium content in the soil of the root habitat is high, despite this, this variety does not accumulate a significant amount of cadmium in the seeds. In terms of the accumulation of zinc in the grain, the Siberian Avant-Garde and Modern varieties are the most resistant (Figure 4). This fact can also be considered an advantage, since, it should be noted that in the root zone of the soil the zinc content is significant and exceeds the MPC in almost all varieties. Despite this, a significant amount of zinc does not accumulate in the seeds of barley varieties Siberian Avant-Garde and Modern.

Resistance to climatic factors of the environment is an important feature in identifying and creating high-yielding barley varieties, since the combination of these characteristics will determine the ability of the variety to fully realize its productive potential in various growing conditions. It is interesting to study the parameters of growth and development of these varieties to identify tolerance to weather conditions and agronomic stability of varieties under polymetallic stress. This can contribute to the identification of varieties that maintain good yield, survival during the spring-summer growing season and at the same time accumulate heavy metals in the grain a little. In this regard, the biological characteristics of the varieties of barley were studied.

The calculation of the percentage of survival showed that the Siberian Avant-Garde variety has the greatest survival. The average survival rate is demonstrated by the Modern barley variety. The lowest survival rate is shown by varieties – Omsk and Donetsk-8 (Figure 6). The study of barley productivity showed that the greatest yield from the plot was at the Modern barley variety (Figure 7). This is due to the large number of spikelets and grains in the ear, a high mass of grains of the main spike and lateral stems, as well as high productive bushiness and good survival during the spring-summer growing season.

Conclusion

Based on the results, the following conclusions were made:

1. The Donetsk-8 variety of barley accumulates the least cadmium in the seeds.
2. The Siberian Avant-Garde and Modern varieties of barley accumulate the least zinc in the seeds and at the same time are characterized by high survival during the spring-summer growing season.
3. The Modern variety of barley is characterized by high yield and survival during the spring-summer vegetation period in conditions of polymetallic soil contamination, which may be associated with a large number of spikelets and grains in the ear, high grain weight of the main spike and lateral stems, as well as high productive bushiness.

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