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SCREENING OF SPRING WHEAT VARIETIES FOR RESISTANCE AGAINST COPPER AND LEAD UPTAKE IN A CONTAMINATED SOIL

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Abstract

The best way to obtain clean crop products on soils polluted with heavy metals is to identify and use varieties that concentrate small amount of heavy metals in the consumed parts of plants. In this regard, varieties of spring wheat under the conditions of the East Kazakhstan region, the metallurgical center of Kazakhstan, were studied to identify metal-resistant forms that could be recommended to production and selection process. 10 zoned varieties of spring wheat were studied. The experiments were performed in the field conditions of natural pollution of the soil with copper and lead. The following was studied: content of heavy metals in the soil of the root zone and in plant seeds, the survival rate during the spring-summer growing season, the yeld and its structure. Studies have shown that copper accumulates in the seeds and its content exceeds the MPC from 1.3 to 1.8 times in almost all genotypes, except for the Kutulukskaya and Ulbinka-25 varieties. Lead also accumulates in the seeds of wheat and its content exceeds the MPC from 3 to 8 times in almost all variants, except for the varieties Erythrospermum-616 and Kutulukskaya. The Glubochanka variety has the highest survivability - 98.6%. The harvest is greatest for the Ulbinka-25, Glubochanka and Erythrospermum-616 and Altai varieties of spring wheat 300-317 g/m^2. The Ulbinka-25 variety can be recommended for cultivation on soils polluted with copper, and Erythrospermum-616 variety - lead, as this variety accumulates the least of all the corresponding metal in the seeds and at the same time is characterized by high yield and good survival during the spring-summer growing season. Kutuluka variety can be recommended for cultivation on soils polluted with copper and lead.

Key words: Wheat genotypes, Screening, Metal resistance, Survival, Productivity, Promising forms.

Introduction

Currently, ecologists around the world are paying increasing attention to the pollution of agronomic soils by heavy metals and their subsequent negative impact on agro-ecosystems (Oves *et al.,* 2016). The sources of anthropogenic pollution, the most dangerous for populations of any organisms, are industrial enterprises. East Kazakhstan region is the industrial center of Kazakhstan, where a large number of heavy metals enter the soil (Alybayeva, 2007), and soils of agrocenoses can be polluted.

As a result of increasing technogenic streams, the excess accumulation of pollutants occurs in economically useful parts of the plant growing products (Zhao *et al.,* 2012; Khaled *et al.,* 2016; Khan *et al.,* 2016; Xie *et al.,* 2016). A significant amount of contaminants, including heavy metals, enters to the plant from the soil, as well as falling out of the atmosphere are absorbed by plants (Khan *et al.,* 2008; Zhang *et al.,* 2010; Tian *et al.,* 2012).

Soils are the main absorber of heavy metals released into the environment. Most metals in soil are not biodegradable (Kirpichtchikova *et al.,* 2006). Heavy metals are present in the soils for a long time after their arrival (Adriano *et al.,* 2001). According to modern research, the half-removal period (i.e., removing half of the initial concentration) of heavy metals from soils varies greatly and is as follows: for zinc from 70 to 510 years, for cadmium from 13 to 1100 years, for copper from 310 to 1500 years, and for lead from 740 up to 5900 years (Kabata-Pendias & Pendias, 2001).

Soil contamination with heavy metals can be dangerous for human and ecosystems. The accumulation of heavy metals in the soil disrupts the physicochemical equilibrium of the natural system (Kabata-Pendias & Pendias, 2001). From the soil, heavy metals are assimilated by plants, which then fall into food, that decreases the nutritional value of food due to their toxicity, lowers the effectiveness of crop production, causes problems of food security (McLaughlin *et al.,* 2000a,b; Ling *et al.,* 2007).

The toxic effect of metals is manifested in their damage to the structure of proteins involved in metabolic processes (Flora *et al.,* 2008). They distort the course of metabolic processes, disrupt the synthesis and work of substances important for metabolism (Rehman *et al.,* 2011; Andosch *et al.,* 2012; Ilyin, 2012).

There is information about the effect of environmental pollution by cadmium and copper ions on the activity of PS II. Cadmium ions affect both the reaction center of PS II and the light-collecting complex, which can lead to an inefficient transfer of energy from the light-collecting complex to the reaction center (Katnoria *et al.,* 2008). Studies have shown that under conditions of different levels of polymetallic stress (Pb^{2+}, Cd^{2+}, Hg^{2+}), lipid peroxidation plant *Bruguiera gymnorrhiza* accelerated the process (Shu *et al.,* 2012). The introduction of Cu into the growing medium and its absorption by plant tissues led to a decrease in the content of photosynthetic pigments, stimulation of lipid peroxidation and an increase in the permeability of the membrane (Gupta & Sinha, 2009). Studies have shown that impact of Cd, Pb and Hg leads to polyploidy, karyokinosis, chromosome fragmentation, chromosome fusion, the formation of micronuclei in beans, garlic (*Allium sativum*) and onions (*Allium cepa*) (Ellinger-Ziegelbauer *et al.,* 2009). These pollutants cause a decrease in the supply of necessary elements (Siedlecka, 1995). They violate the structure and function of membranes, which leads to disruption of transport processes. (Sazanova *et al.,* 2011; Mroczek-Zdyrska & Wójcik, 2012; Rozentsvet *et al.,* 2012).

For many decades, food safety has been an issue that has attracted the attention of scientists and farmers. If plant products contain heavy metals in quantities exceeding the limits, then such products are not considered acceptable, even if the nutritional value of the biochemical composition is excellent (Sokolova *et al.*, 2006; Pugaev, 2013). For example, for lead and copper, the MPCs in grain of leguminous crops are 0.5 and 10 mg/kg, respectively (Suldina, 2016)

Self-cleaning of soils from pollution takes time and artificial purification is time-consuming and expensive. This necessitates the search for other environmentally friendly technologies that allow obtaining pure crop production. The best way to obtain clean crop products on soils polluted with heavy metals is to identify and use varieties that concentrate a small amount of heavy metals in the consumed parts of plants. Studies have found that different species and varieties of plants exhibit different resistance to soil ecotoxicants (Barsukova, 1997, Yang *et al.,* 2000, Inelova *et al.,* 2018). Some varieties of different types of food crops show significant differences in resistance to soil contaminants (Barsukova, 1997; Yang *et al.,* 2000).

The ability of such varieties of plants not to accumulate heavy metals in economically valuable parts of plants can be used in environmentally friendly production. The identification of metal-resistant varieties that accumulate little toxicants makes it possible to obtain an environmentally friendly technology for their cultivation and the production of products suitable for consumption (Lukin *et al.,* 1999). Such varieties can also be used in breeding as donors for resistance to certain metals (Molchan, 1996; Clarke *et al.,* 2002; Ozkutlu *et al.,* 2007).

The aim of our study was to identify metal-resistant forms that could be recommended in production and selection process.

Materials and Methods

In the experiment, 10 of spring wheat from the collection of the East - Kazakhstan Agricultural Research Institute (EKARI) were studied: Lyazzat, Nargiz, Samal, Kutulukskaya, Omskaya-18, Zaulbinka, Altai, Ulbinka-25, Glubochanka, Erythrospermum-616. Varieties are regionalized in the East-Kazakhstan region. The experiments were carried out in field conditions of the natural soil contamination by copper and lead, which are some of the priority pollutants of the East Kazakhstan region soil (Alybayeva, 2007*)*. Plants were grown on scientific test section of the East - Kazakhstan Agricultural Research Institute, in a suburban area of the Ust-Kamenogorsk city, East Kazakhstan region, north-eastern direction, 3 km from the city limits. The area of the experimental plots is 5 m^2, in three replications. Seeding mechanized, pushed plot, seeding rate is 5-6 million germinating seeds per 1 ha. The width between rows of 15 cm, between the pushed plot space of 50 cm. The soil is ordinary black soil, heavy loamy, low humus content, the content of NO_3 - 5.3 mg/g soil, P_2O_5 - 3, 4 mg/g, K_2O - 29.8 mg/g. The predecessor - after autumn plowing black fallow (23-25 cm). Early spring harrowing, cultivation, cultivation before sowing. Plant care (row packing, weeding) was done manually.

Analysis of physiological parameters: Determination of physiological parameters was carried out by the method of field experiment (Dospechov, 2011). The survival of plants was determined. Counted plants in the phase of full shoots and before harvesting. The number of surviving plants (%) is calculated by the formula:

$$D = \frac{(Cx100)}{B}$$

where D - the number of the surviving plants to harvest, %; B - the number of plants in the phase of full shoots, pcs. per m^2; C - the number of plants to harvest, pcs. per 1 m^2.

Yields were determined by a direct method. The grain received from each plot is poured into the bag. Bags with grain are weighed to the accuracy of 0.01 kg. The moisture content of the grain was determined by the weight method. From each plot, the grain samples are taken into aluminium cups with a tight lid, weighed and dried at a temperature of 100° to 105°C to a constant weight of about 4 to 6 h, then calculated by the formula:

$$X = B : H$$

where: X - moisture content of the grain, %; B - mass of evaporated water, g; H - crude sample, g.

The standard 14% humidity is recalculated according to the formula:

$$X = Y \text{ x } (100\text{-}b)/100\text{-}c,$$

where: X - yield, reduced to standard moisture; Y - the obtained crop; B-moisture content of the crop (%); S is the standard humidity for the given object.

Determination of heavy metals: The experiment used an atomic absorption method using an AAnalyst 300 instrument ("Perkin Elmer") with flame atomization in a graphite furnace. Sample preparation was carried out according to standard operating procedures using the "Hot Block" digestor, at a temperature of (90 ± 5)°C. The samples were placed in glasses, concentrated nitric acid (5 ml) and concentrated hydrochloric acid (0.5 ml) were poured, stirred, covered with glass and evaporated for 10-15 minutes at a temperature of 90 ± 5°C. After that, the samples were cooled and re-heated with the addition of 5 ml of concentrated nitric acid for half an hour. Samples in a glass were heated for two hours. 50 ml of deionized water was added to the cooled samples.

The instrument was calibrated using standard samples from High Purity, deionized water and a 1% solution of HNO_3.

The amount of the test element in the sample was determined using the following formula:

$$C \text{ mg / kg} = \frac{C \text{ x } V \text{ x } FD}{m}$$

where: m is the sample weight (g), C is the instrument reading (mg/l); V– sample volume (ml); FD - breeding factor.

Statistical analysis: Statistical analysis was performed using "Data Analysis» package of Microsoft Excel program, by means of which were defined such indicators as average value, an error representativeness and dispersion. To establish the Least Significant Difference (LSD), we found the error parameters of the difference and Student's t-test for each data group. LSD = t 0.5 was used for calculating the Least Significant Difference" (Dospechov, 2011).

Results

Copper and lead content in soil of the root zone of wheat plants: Studies have shown that in the field of natural pollution, the studied heavy metals in the root soil of spring wheat varieties accumulate in various ways.

The copper content in the root zone of the soil of different varieties of spring wheat is not the same. Exceeding the maximum allowable concentration (MAC) of this metal is observed in the soil of the root layer of the varieties Glubochanka, Zaulbinka, Kutulukskaya, Nargiz, Ulbinka-25, Altai and Erythrospermum-616 and only in the soil of the habitats of the roots of the varieties Lyazzat, Samal, Omskaya-18 of this excess is not observed (Fig. 1).

Analysis of the metal content in relation to the MAC does not always give an objective picture. Using Regional Clark can provide more objective information. The Regional Clark content of the elements shows the content of this element in the soil of the given region. Since we are exploring varieties of wheat regionalized in this region, we can assume that they are adapted to the Regional Clark content of these elements in the soil and the true excess, which can affect wheat plants, may be excess relative to Regional Clark. Regional Clarke copper content is more than 2 times higher than the MAC (maximum allowable concentration) value. Thus, for lead and copper, the MPC is 35 and 23 mg/kg, and Regional Clark is 22 and 59 mg/kg, respectively. The content of this element in the soil of habitat zone of roots of various spring wheat varieties is above the values of Regional Clark. We found that the copper content in the soil is from 180.6 to 260.0 mg/kg.

The study of lead accumulation in soil habitats of roots of different genotypes of spring wheat collection of the East Kazakhstan Institute of Agricultural Research in natural pollution conditions showed that the amount of this element is different, but for all studied varieties its content in the soil is higher than the MAC of the metal in the soil. Excess of maximum concentration limit makes from 1,4 to 4,5 times (Fig. 2).

In relation to the Regional Clark content of this metal, there is a higher lead content in the soil of the habitat zone of the roots of various varieties of spring wheat than the value of Regional Clark, although it is higher than the MAC value.

The accumulation of the investigated metals in the seeds of wheat: Studies on accumulation of copper in the seeds of plants of spring wheat varieties of the EKARI collection under conditions of soil contamination with heavy metals showed that this metal accumulates in an amount exceeding its maximum permissible concentration in almost all genotype seeds, except for the Kutulukskaya and Ulbinka-25 varieties (Fig. 3).

It should be noted that at the same time in the soil of roots habitat layer of these varieties of copper content exceeds a MAC and Regional Clark.

The highest copper content in the seeds is observed in Omskaya-18, Samal and Altai varieties. At the same time, in the roots habitat soil layer of Samal and Omskaya-18 varieties the excess of MAC and Regional Clark is not observed. Average amount of copper compared to other varieties, but also higher than the MPC observed in seed of Lyazzat, Nargis, Zaulbinka, Glubochanka, Erythrospermum-616 varieties.

The study of lead accumulation in the seeds of different varieties plants of EKARI collection in a polymetallic polluted soil have shown that the metal is accumulated in significant amounts and its content exceeds MPC for grain in almost all variants except Erythrospermum-616 and Kutulukskaya varieties (Fig. 4). At the same time, the lead content in the soil of root zone exactly in the case of these varieties is the greatest.

The greatest amount of lead accumulated in the seeds of varieties Samal, Nargiz and Lyazzat. The average amount of lead, compared to other varieties, accumulate the seed of such varieties as the Omskaya-18, Zaulbinka, Glubochanka, Ulbinka-25 and Altai.

Physiological parameters of wheat plants in conditions of soil contamination with heavy metals: Physiological parameters of wheat plants were also investigated in order to characterize their other economically important features like productivity, resistance and survival in the conditions of the spring-summer growing season.

Survival of wheat plants in conditions of soil contamination with heavy metals: The number of plants before the harvest, in comparison with their number before tillering, shows the survival rate during the spring and summer growing season.

Studies of the quantity of plants before tillering showed that the largest number of plants were observed in variety Glubochanka.

The average number of plants, in comparison with other varieties, is observed in varieties - Zaulbinka, Omskaya-18, Altai, Ulbinka, Kutulukskaya, Samal. The smallest number of plants is at the varieties - Erythrospermum-616, Lyazat, Nargis. Determination of the plants number before harvest. The average number of plants, in comparison with other varieties, observed the varieties Zaulbinka, Omskaya-18, Altai, Ulbinka-25 and Kutulukskaya. The smallest number of plants is at the varieties - Erythrospermum-616, Samal, Lyazat, Nargis.

The survival rate counting showed that the highest preservation has variety of spring wheat - Glubochanka. Median survival demonstrated varieties of spring wheat: Zaulbinka, Omskaya-18, Ulbinka-25, Kutulukskaya, Lyazat, Nargiz and Altai. The lowest survival rates show varieties - Erythrospermum-616 and Samal (Fig. 5).

Harvest of wheat plants in conditions of soil contamination with heavy metals: Among the varieties of spring wheat of EKARI collection, the harvest from the plot is greatest in the varieties of spring wheat Ulbinka-25, Glubochanka and Erythrospermum-616 and Altai. The lowest yields in conditions of soil contamination with heavy metals are demonstrated by varieties: Lyazzat, Zaulbinka, Samal, Nargiz. The average yield in comparison with other varieties of spring wheat is revealed in varieties: Kutulukskaya and Omskaya 18 (Fig. 6).

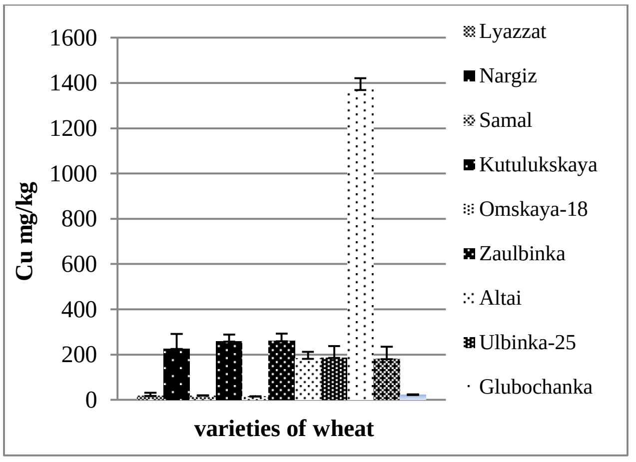

Fig. 1. The copper content in the root zone soil of studied varieties of EKARI collection in relation to MPC.
Vertical bars represent ± SD of three replicates (n=3); LSD =86,82 at p<0,05

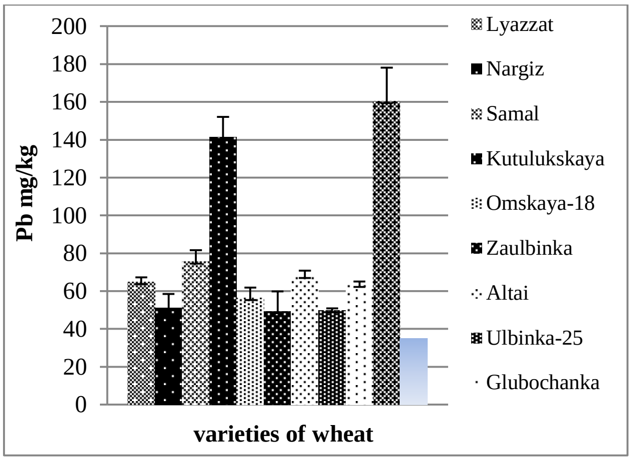

Fig. 2. The lead content in the root zone soil of studied varieties of EKARI collection in relation to MPC.
Vertical bars represent ± SD of three replicates (n=3); LSD = 82,74 at p<0,05

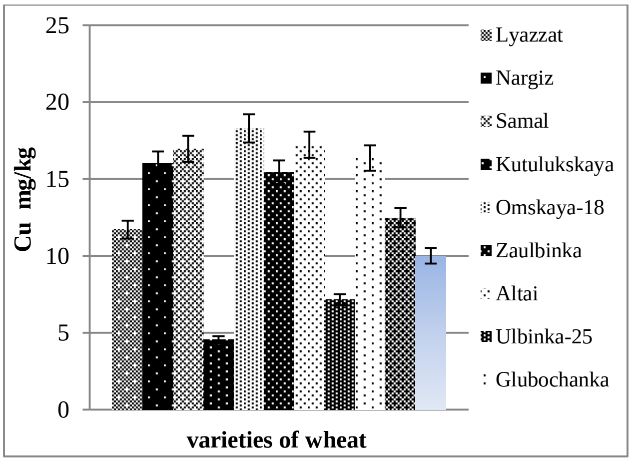

Fig. 3. The copper content in the seeds of different varieties of spring wheat of EKARI collection under polymetallic soil contamination
Vertical bars represent ± SD of three replicates (n=3); LSD = 3,03 at p<0,05

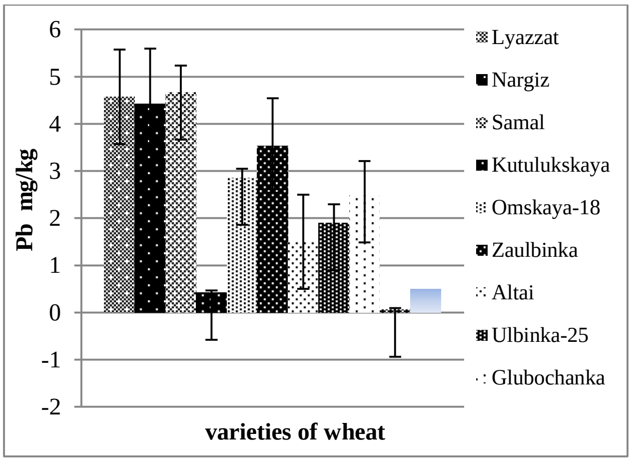

Fig. 4. The lead content in the seeds of different varieties of EKARI collection under polymetallic soil contamination
Vertical bars represent ± SD of three replicates (n=3); LSD =2,33 at p<0,05

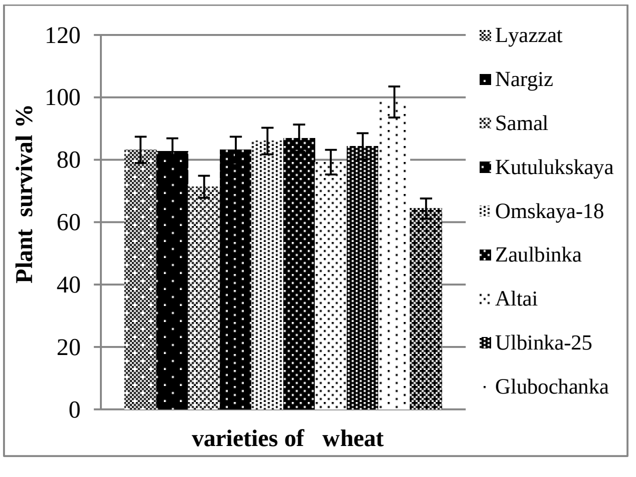

Fig. 5. Percentage of surviving plants before harvesting of different spring wheat genotypes of EKARI under polymetallic soil contamination
Vertical bars represent ± SD of three replicates (n=3); LSD =30,96 at p<0,05

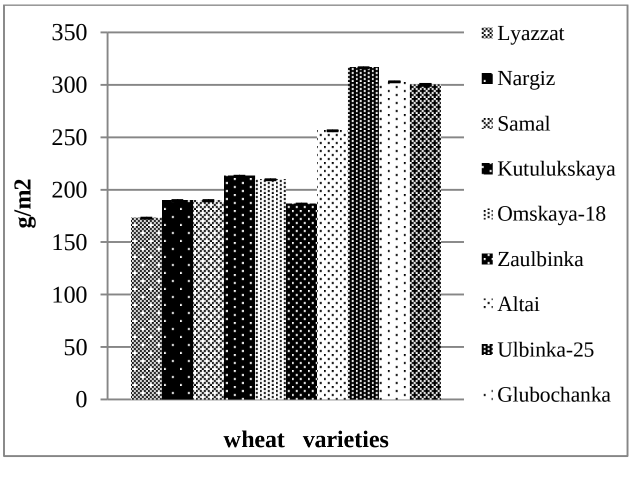

Fig. 6. Harvest of different spring wheat genotypes of the EKARI collection, when grown in conditions of polymetallic soil contamination
Vertical bars represent ±SD of three replicates (n=3); LSD =0,13 at p<0,05

Discussion

The problem of formation and enhancement of the biological and adaptive potentials of agricultural plants has become the center of attention of researchers (Goncharova & Khodorenko, 2003; Pivovarov & Dobrutskay, 2003; Shabala *et al.,* 2016). Now, when contamination of cultivated soils has become relatively common and is likely to continue, the identification and creation of varieties that do not have the ability to store heavy metals for contaminated areas becomes practically the only real solution to emerging environmental problems (Gamzikova & Shumny, 1994; Yang *et al.*, 2000; Wu & Zhang, 2002; Zhuchenko, 2003; Ishikawaa *et al.,* 2012; Zhan *et al.,* 2013).

Screening of crops and varieties resistant to soil contaminants is becoming especially important in industrially developed and techno genic polluted areas, large settlements where regional pollution of soils and the environment occurs (Tian, 2015; Narenova *et al*., 2016; Xu *et al*., 2017).

The East Kazakhstan region is characterized by a developed metallurgical industry, therefore the environment and, in particular, the soils are contaminated with heavy metals. In this region, wheat is grown for the needs of the region, which can be contaminated with heavy metals. Identifying and cultivating wheat varieties that accumulate minimal amounts of priority heavy metals in the commercial part of the crop can help solve the problem of possible contamination of agricultural products. To do this, it is necessary to study the genetic stock of samples cultivated, wild-growing and mutant plants and to reveal instances capable of concentrating very little pollutants in the economic part of the plants (Molchan, 1996; Yang *et al*., 2000, Ishikawaa *et al*., 2012; Al Khateeb & Al-Qwasemeh, 2014).

First of all, the content of copper and lead in the root layer of soil of various spring wheat genotypes was studied in order to have an idea of whether wheat plants are stressed by excess of heavy metals in the soil. The results of the research showed that wheat plants experience stress from the increased content of lead, copper and cadmium in the soil. Also, according to the literature, it is known that the soils are contaminated with other heavy metals in priority for this region (Alybaeva, 2007). Thus, wheat plants of different varietiesthat are grown under conditions of natural contamination of the environment with heavy metals, are stressed by the complex effects of many metals.

The presence of varietal specificity of the reaction of plants is important to changes in environmental factors, in particular pollution of soil and plants with ecotoxicants. In this regard, the attention of researchers is increasingly directed at studying the behavior of plant organisms in relation to the accumulation of chemical elements of different nature in food commodity. In practical terms, it is of interest to study the levels of accumulation of heavy metals in the vegetative and reproductive organs of plants that enter the food ration.

There is no strict dependence between the content of heavy metals in plants and their gross content in the soil, because plants have a selective accumulation of elements (Shkolnik & Popova, 1983; Golia *et al*., 2003; Krupskaya *et al*., 2014). It is known that the ability to absorb, accumulate, and use chemical elements in plants is genetically determined (Gamzikova & Barsukova, 1994; Gamzikova, 1996; Zhang & Song, 2008). Research by O. Gamzikova with employees revealed a significant variability in the stability of Triticum to heavy metals at species and varietal levels. Based on the material obtained from the screening of the gene pool of wheat and the use of genetic models, the authors develop ideas on the possibility of controlling the signs of edaphic stability using the breeding method (Gamzikova & Barsukova, 1994, Gamzikova, 1996).

The study of copper accumulation in plant organs of spring wheat varieties of the EKARI collection in conditions of polymetallic soil contamination showed that copper accumulates in all plant organs. Investigation of lead accumulation in plant organs of spring wheat varieties has shown that lead accumulates mainly in roots, leaves, and smaller amounts in stems and grain.

Investigation of copper and lead content in seeds of different genotypes of spring wheat is the most important because wheat grain used in the food industry. The results showed that the content of copper and lead in wheat seeds in almost all cases exceeded MAC of these metals in the grain (Figs. 3 and 4). Varietal specificity for this feature is identified.

On the basis of the accumulation of copper, the varieties of spring wheat Kutulukskaya and Ulbinka-25 are the most resistant (Fig. 3). This fact can be considered an advantage, since in the soil root zone habitat copper content increased, despite that these varieties do not accumulate significant amounts of copper in the seeds. According to the accumulation of lead, the most resistant varieties are Erythrospermum-616 and Kutulukskaya (Fig. 4). This fact can also be considered an advantage, as it should be noted that in the root layer of the soil the lead content is significant and exceeds the MAC in all varieties. Despite this, the seeds of spring wheat varieties Erythrospermum-616 and Kutulukskaya not accumulate significant amounts of lead. The variety of spring wheat Kutulukskaya exhibits resistance to both types of metal accumulation. Excess amounts of heavy metals in the seeds of plants have been identified as in other studies. For example, cadmium in quantities significantly above the MAC set for cereals has been found in wheat grains (Harris & Taylor, 2001), rice (Shah & Dubey, 1998) and barley (Chen *et al.,* 2007) during the growth of those species in metal containing soils.

Spring wheat varieties Kutulukskaya and Ulbinka-25 can be recommended for further use in breeding for resistance to accumulation of copper in the grain. Varieties of spring wheat Erythrospermum-616 and Kutulukskaya can be recommended for further use in breeding for resistance to accumulation of lead in the grain. Kutulukskaya can be recommended for further use in breeding for resistance to the accumulation of both types of metals.

The success of the research depends on the quality of the material being studied, the basis of which is the search for forms with the maximum combination of economically valuable traits (Chursina, 2012). Important features in identifying and creating high-yield wheat varieties is resistance to climatic factors of the environment, since a combination of these characteristics will determine the ability of the variety to fully realize its productive potential in different growing conditions. It is of interest to study the parameters of growth and development of these varieties for the detection of tolerance to weather conditions and agronomic resistance of varieties under conditions of polymetallic stress. This can help to identify varieties that retain good productivity and survival during the spring and summer growing season and at the same time, few accumulate heavy metals in grain. In this connection, the biological features of the invistigated wheat varieties were studied.

Counting the percentage of survival showed that the greatest survival has variety Glubochanka. Medium survival exhibit the spring wheat varieties: Zaulbinka, Omskaya-18, Ulbinka-25, Kutulukskaya, Lyazat, Nargiz and Altai (Fig. 5). A study of wheat productivity showed that the highest yield from a plot in spring wheat varieties: Ulbinka-25, Glubochanka, Erythrospermum-616 and Altai (Fig. 6). This is apparently due to a large number of grains per ear, a high mass of grains per ear and high productive tillering and good survival during the spring-summer growing season.

Of particular interest to us are the physiological parameters of the metal-resistant wheat varieties that we detected, which contain heavy metals in seeds in quantities that do not exceed the MPC for wheat grains: Kutulukaya and Ulbinka-25-copper and Erythrospermum-616 and Kutulukaya - lead. The results of the conducted researches showed that the Ulbinka-25 spring wheat variety has a high yield and good survival in the period of spring-summer vegetation. The genotype of spring wheat Kutulukskaya has good yield and survival. Variety of spring wheat Erythrospermum-616 has a high yield and a low survival rate in the period of spring-summer vegetation.

Conclusion

Ulbinka-25 can be recommended for cultivation on soils contaminated with copper, as this sort of spring wheat accumulates the least copper in the seeds and at the same time is characterized by high yield and good survival in the period of spring-summer vegetation in conditions of polymetallic soil contamination. The variety Erythrospermum-616 can be recommended for cultivation on soils contaminated with lead, as this varaity of spring wheat accumulates the least amount of lead in seeds and at the same time is characterized by high yield. Kutulukskaya variety can be recommended for cultivation on soils contaminated with copper and lead, as this sort of spring wheat accumulates the least copper and lead in seeds and at the same time is characterized by good yield and survival in the spring-summer vegetation in conditions of polymetallic soil contamination.

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