

Original Article

The use of selenium for protective and stimulating effects on plants when soil is contaminated with cadmium

O uso de selênio para efeitos de proteção e estímulo nas plantas com o solo contaminado por cádimo

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Abstract

At the present time one of the tasks of modern agricultural industry consists in obtaining the ecologically safe and clean products. Contamination of soils with heavy metals due to an anthropogenic impact drives up their content in the composition of plant products. This shapes not only a reduction in crop yields, but also a deterioration in products quality. Within the terms of vegetation research in soil culture, there has been studied the protective and stimulating effect of sodium selenite upon the adaptive capacity of spring wheat plants of the variety Zlata under conditions of oxidative stress due to the soil contamination with cadmium. There has been studied the effect of different methods of sodium selenite application on the yield of spring wheat and the plants photosynthetic activity, depending on the level of soil contamination with cadmium. The object of research was a spring wheat variety Zlata. Plants have been cultivated in a greenhouse trial under soil culture conditions in Mitscherlich-vessels with a capacity of 6 kg of soil. Sod-podzolic soil has been used for research. Sodium selenite was introduced in three ways: pre-sowing seed treatment, foliar treatment of vegetative plants at the beginning of stage VI of organogenesis - the end of the tillering phase - the beginning of the stem-extension phase and the application of a salt solution into the soil when packing the vessels. The control samples represented variants without sodium selenite. To assess the plants photosynthetic productivity, there has been determined the chlorophyll content in plant leaves. The research results made it possible to determine the protective effect of sodium selenite on the adaptive capacity of plants under conditions of oxidative stress due to the soil contamination with cadmium. The increase in the adaptive capacity of plants manifested itself through the decrease in yield diminishing due to the improvement of conditions for fertile florets and ear initiations on the vegetative apex, as well as the development of flowers into grains, which contributed to increase in the grain content of the spike. The stimulating effect of selenium on the intensity of photosynthetic processes has been revealed, which showed not only the increase of chlorophylls content, but also the ratio changes of chlorophylls *a* and *b*.

Keywords: protective and stimulating effect, sodium selenite, spring wheat, oxidative stress, cadmium.

Resumo

Atualmente, uma das tarefas da indústria agrícola moderna consiste em obter produtos ecologicamente seguros e limpos. A contaminação dos solos com metais pesados devido ao impacto antrópico aumenta o seu conteúdo na composição dos produtos vegetais. Isto molda não apenas uma redução no rendimento das colheitas, mas a deterioração da qualidade dos produtos. No âmbito da investigação da vegetação em cultura do solo, estudou-se o efeito protetor e estimulante do selenito de sódio sobre a capacidade adaptativa de plantas de trigo de primavera da variedade Zlata em condições de stress oxidativo devido à contaminação do solo com cádmio. Tem sido estudado o efeito de diferentes métodos de aplicação de selenito de sódio na produtividade do trigo de primavera e na atividade fotossintética das plantas, dependendo do nível de contaminação do solo com cádmio. O objeto de pesquisa foi uma variedade de trigo de primavera Zlata. As plantas foram cultivadas em um teste de estufa sob condições de cultura do solo em vasos Mitscherlich com capacidade para 6 kg de solo. Solo sod-podzólico tem sido usado para pesquisa. O selenito de sódio foi introduzido de três maneiras: tratamento de sementes pré-semeadura, tratamento foliar de plantas vegetativas no início da fase VI da organogênese - final da fase de perfilhamento - início da fase de extensão do caule e aplicação de solução salina no solo ao embalar os vasos. As amostras de controle representaram variantes sem selenito de sódio. Para avaliar a produtividade fotossintética das plantas, determinou-se o teor de clorofila nas folhas das plantas. Os resultados da pesquisa permitiram determinar o efeito protetor do selenito de sódio sobre a capacidade adaptativa de plantas em condições de estresse oxidativo devido à contaminação do solo com cádmio. O aumento da capacidade adaptativa das plantas se manifestou pela diminuição do rendimento diminuindo devido à melhoria das condições para floretes férteis e iniciações de espigas no ápice vegetativo, bem como o desenvolvimento de flores em grãos, o que contribuiu para o aumento do grão conteúdo da espiga. O efeito estimulante do selênio na intensidade dos processos fotossintéticos foi revelado, o que mostrou não apenas o aumento do teor de clorofilas, mas também as mudanças na proporção de clorofilas *a* e *b*.

Palavras-chave: efeito protetor e estimulante, selenito de sódio, trigo de primavera, estresse oxidativo, cádmio.

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1. Introduction

At present one of the main tasks of modern agricultural industry in the world consists in obtaining the ecologically safe and clean products. Contamination of soils with heavy metals due to an anthropogenic impact drives up their content in the composition of plant products, and hence in the diet of humans and animals. Therefore, the aspects of studying protective and stimulating mechanisms for reducing the toxic effect of heavy metals on plants and reducing the accumulation of toxic elements in crop production is of paramount importance (Shumilin, 2016).

It is known that areas near large plants of heavy industry, large agricultural enterprises, highways, and motorways with high traffic are characterized by a huge man-made burden. Sources of environmental pollution with heavy metals also include the discharge of effluents into the wild nature, the widespread release of garbage, the growth of areas for stationary landfills without technologies for processing and recycling waste, the irrational use of chemical ameliorants, large amounts of fertilizers (Shumilin, 2016).

Based on the level of heavy metals (HM) accumulation, all plants may be divided into 3 groups subject to their ability to accumulate: accumulators, indicators, excluders (Verbruggen et al., 2009).

The chemical and granulometric composition of the soil, the level of high humus content of the soil, and its pH have the greatest effect on the accumulation of toxicants (Titov et al., 2007). The amount of cadmium accumulation directly depends on the indicators of soil acidity. It was shown that cadmium was more accessible for plants in soils with a more acidic reaction of the environment with a total equal gross content of the toxicant in the soil than in soils with a neutral and close to neutral reaction of the environment. In the latter case, cadmium accumulates in the soil in a large amount, but is mostly inaccessible to plants (Shumilin, 2016).

The toxicity of cadmium in soil is due to its valence (II), which defines the chemical affinity of this element with other divalent cations, such as zinc. It easily penetrates plants through the soil, accumulates both in roots and above-ground parts of plants in large quantities. Cadmium is not a necessary element and does not participate in the nutrition processes and metabolism of the cells of higher and lower plants. When accumulated in plants, cadmium inactivates several enzymes, including glycolysis enzymes (Kolesnichenko and Kolesnichenko, 2011; Shumilin, 2016). The presence of cadmium ions leads to a decrease in the level of oxygen uptake by plant roots, the transport of electrons and protons in mitochondria (Farouk et al., 2011).

Furthermore, cadmium causes lipid peroxidation in the plant organism and, under certain conditions, acts as a cofactor for the formation of reactive oxygen species, triggering cascade peroxide processes in the plant cell. These processes have as the consequences in the plant organism the damage of DNA structure, pigments, and other cellular components. Thus, it can be argued that the main mechanism of the HMs negative effect is their ability to induce oxidative stress in plants (Pourrut et al., 2013). Under these conditions, the physiological and

biochemical processes in the plant organism slow down. There has been identified a disruption of the processes of photosynthesis, including a decrease in the content of photosynthetic pigments, the rate of their accumulation in leaves, inhibition of the photosynthesis intensity, photorespiration. In this case, the negative effect is reasoned by the possibility of substitution of cadmium for magnesium cations in the composition of green plant pigments, which leads to disruption of their metabolism (Baryla et al., 2001; Myśliwa-Kurdziel and Strałka, 2005).

A decrease in the intensity of photosynthesis processes in conditions of increased content of cadmium in the soil is usually reasoned by disturbances during the Calvin cycle reactions with inactivation of the main enzyme within the dark fermentation, which is ribulosebiphosphate carboxylase. At the same time, we also identify a change in the synthesis of glyceraldehyde-3-phosphate dehydrogenase due to the damage of the protein quaternary structure (Monnet et al., 2001; Shumilin, 2016).

Furthermore it is to be mentioned the negative impact of cadmium associated with the accumulation of this element in economically valuable parts of plants, which are usually not suitable for human and farm animals' nutrition. The negative effect of cadmium on humans and animals is also due to the fact that it is practically not eliminated out of plant and animal organisms, therefore its danger to human health increases (Ulyanenko et al., 2012).

One of the mechanisms of a plant organism resistance is a cascade of reactions with the formation of products for synthesis of specific enzymes in cells. The main enzymes to resist the oxidative stress represent antioxidant enzymes, the main of which are catalases, peroxidases, and some other oxidoreductases. Substances, elements, and enzymes of the antioxidant system are involved in the reactions to resist oxidation: polyphenols, ascorbic acid, vitamin E, tocopherols, selenium, etc. (Alscher et al., 2002; Shumilin, 2016; Belopukhov et al., 2017).

Thus, at present, it is necessary to study the protective-simulating effect of various agrochemical agents on increasing the resistance of plants to the negative effects of oxidative stress caused by soil contamination with HMs. The goal of our research was to study the protective and stimulating role of sodium selenite for reducing the toxic effect of cadmium on the yield and photosynthetic activity of spring wheat plants.

2. Research Methods

Spring wheat was grown in a model experiment in the growing house of the Russian State Agrarian University-Moscow Timiryazev Agricultural Academy. The object of research was spring wheat (*Triticum aestivum* L.) variety Zlata. The plants were grown in Mitscherlich vessels with a capacity of 6 kg of soil. The plants in the vessels were watered every day until the optimum state. For the research, a soddy-podzolic medium loamy soil with the following agrochemical characteristics was used: humus content according to Tyurin - 2.9% (GOST 26213-91), pH_{KCl} - 5.5 (GOST 26483-85); Soil hydrolytic acidity (Hg) (according to Kappen) - 1.3 mg-eq / 100 g of soil (GOST

26212-91); S (according to Kappen-Gilkovits) - 12.0 mg-eq / 100 g of soil (GOST 27821-88); V - 92.5%, soil nitrogen easily hydrolysable form (Nlg) - 55–60 mg / kg of soil (GOST 26107-84). The content of general forms of selenium was 0.32 mg/kg of soil. The content of common forms of cadmium was 0.43 mg/kg of soil. The saturation of the soil with mobile forms of phosphorus and potassium (according to Kirsanov) was 180 (V class) and 150 (IV class) mg / kg of soil, respectively (GOST 26207-91). Agrochemical analysis of the soil was carried out under generally accepted methods.

The level of mineral nutrition in all vegetation experiments were created by adding ammonium nitrate (NH_4NO_3), monosubstituted potassium phosphate (KH_2PO_4), and potassium chloride (KCl). In all variants, NPK was introduced at the rate of 100 mg / kg of soil for each element.

Sodium selenite was used by treatment of seeds before sowing in the form of soaking seeds with a 0.01% solution, by foliar treatment of vegetative plants at the beginning of stage VI of organogenesis – end of the tillering phase – beginning of the stem-extension phase and, adding a salt solution at a dose of 0.07 mg / kg soil when packing vessels. Variants without sodium selenite served as control.

Different levels of soil contamination with cadmium were created by introducing a solution of cadmium nitrate ($\text{Cd}(\text{NO}_3)_2 \times 4\text{H}_2\text{O}$) into the soil at doses of 10 and 50 mg / kg of soil. This is 5 and 25 times more than the maximum permissible content of cadmium in the soil. Cadmium was not added to the control variant.

Sowing of seeds was carried out with dry seeds at the rate of 30 seeds per 1 vessel. Then, in the tillering phase, the shoots were thinned out to 15 plants in a vessel. Each variant was laid in four repetitions.

To determine the photosynthetic activity of plants during the growing season, the content of chlorophylls a and b was determined in the 5th and 6th leaves of spring wheat in the booting phase.

After harvesting, the plants went through the stage of field-harvest ripening for one month. After reaching the post-harvest ripeness of the grain, the mass of grain (g/vessel) and straw (g/vessel), the structure of the crop (the length of the ears, the number of grains and spikelets in the ear, the weight of 1000 grains) were determined. To prove the reliability of the experiments, we used mathematical processing by the method of two-dimensional analysis of variance. This is a classic example of studying the dependence of crop weight on the level of soil contamination with cadmium and seed treatment with selenium. Observed feature in this case, the change in the mass of the spring wheat crop. The task is to constructing a statistical model of the dependence of the wintering of the crop mass on the two indicated above factors. Reliability was assessed using the significant difference indicator (SD_{05}).

3. Research Results

Obtaining high yields of spring wheat in most regions is associated with the conditions of its cultivation, among which anthropogenic factors have the greatest influence.

With the accumulation of cadmium in plants, the activity of growth enzymes is inactivated, which results in a decrease in growth rates and impaired development of generative organs. This translates to decrease in the plant productivity. The results of our research regarding the study of the sodium selenite protective effect on the productivity of plants when soil contaminated with cadmium are presented in Table 1.

In various studies, the effectiveness of the use of selenium preparations as a microfertilizer has been revealed. Studies have shown that selenium had a positive effect on the yield of spring wheat. It can be observed from the Table 1 that the use of sodium selenite in the control variant without cadmium contributed to an increase in the productivity of wheat plants of the variety Zlata. An increase in grain weight by 25% was obtained after the seed treatment before sowing, by 17% after the foliar treatment of vegetative plants.

Evaluating the effect of a high level of cadmium content in the soil, a decrease in the weight of grain was identified at both doses of its introduction into the soil in comparison to the control variant without cadmium. The effect of cadmium on wheat yield depended to a large extent on the level of soil contamination. If at a dose of 10 mg / kg of cadmium it occurred a decrease in grain weight by 37%, then the weight of straw even increased by a factor of 1.7 times. In this variant, the share of grain in the yield structure decreased significantly, as evidenced by the coefficient of economic efficiency of photosynthesis (K economic) (14.3% versus 31.4% in the control without cadmium). With the content of cadmium in the soil at the level of 50 mg / kg for this soil, the grain weight decreased by 21%, and the straw weight by 12%. The grain ratio also decreased, but by a smaller amount to 29.0% versus 31.4% in the control without cadmium.

It was found that a decrease in wheat yield under conditions of soil contamination with cadmium had occurred, on the one hand, because of a disturbance in the processes of florets initiation and their development in grains, which determined a decrease in the ear grain content, on the other hand, because of a disturbance in the outflow of assimilates from vegetative organs into generative ones. This defined a decrease in 1000 kernel weight.

The application of sodium selenite in case of soil contamination with cadmium fostered the stimulation of plant defense mechanisms. The increase in weight of plants was identified in almost all variants of studies and yet given 10 mg of cadmium per 1 kg of soil, the effect of selenium was manifested both in increase of grain weight by 11-54%, and in increase of straw weight by 7-16%, which led to a decrease in the grain ratio in the structure of the crop, as evidenced by the coefficient of photosynthesis economic effectiveness.

The variants where a high level of cadmium content (50 mg / kg of soil) was created, show that the use of selenium realized to a greater extent in increase in grain weight by 30-47%, while the straw weight did not change relative to the control without selenium, which contributed to the increase in grain ratio in the wheat yield structure. The coefficient of the photosynthesis economic

Table 1. Influence of different ways of using selenium on the yield and yield structure of spring wheat variety Zlata within the conditions of soil contamination with cadmium.

Trial variant		Weight, g / vessel		Number per spike, pcs		Spike length, cm	1000 kernel weight, g	K economic*, %
mode of application	dose of cadmium, mg / kg soil	grains	straw	spikelets	grains			
without treatment	-	5.09	11.1	8.6	8.1	6.0	27.7	31.4
seed treatment before sowing		6.41	17.3	10.7	12.4	6.9	23.9	27.0
foliar treatment of plants		5.99	16.8	10.0	11.0	6.6	21.9	26.3
soil application		4.35	14.7	6.0	8.7	7.0	29.0	22.8
without treatment	10	3.19	19.1	3.2	12.6	6.9	17.1	14.3
seed treatment before sowing		4.92	22.2	5.3	11.4	7.6	20.3	11.8
foliar treatment of plants		3.54	20.5	12.3	10.0	7.0	28.8	13.1
soil application		4.56	17.2	6.8	9.4	6.8	25.7	21.0
without treatment	50	4.01	9.8	7.1	7.7	6.1	22.5	29.0
seed treatment before sowing		5.22	6.4	8.8	9.9	6.8	26.4	44.9
foliar treatment of plants		5.88	9.4	11.7	8.6	6.4	22.7	38.5
soil application		4.0	12.8	5.6	7.8	5.6	32.1	23.8
SD** ₀₅		0.6	1.4	0.9	0.9	0.6	2.1	

*K economic - coefficient of economic efficiency of photosynthesis, %. **SD₀₅ - significant difference at significance 05.

effectiveness increased to 44% under the seed treatment with selenium before sowing (versus 29.0% in the control without selenium).

The most effective ways of using sodium selenite within the conditions of 10 mg of cadmium per kg of soil were the use of seed treatment before sowing, within the conditions of 50 mg of cadmium - foliar treatment of vegetative plants.

Thus, the results of our research made it possible to identify the main mechanisms of negative effect of cadmium on the formation of wheat yield. It was revealed that being exposed to cadmium, the processes of formation and development of plants reproductive sphere are inhibited due to reducing the number of florets resulting from decrease in the intensity of cell division, as well as the processes of florets development into grain (Shumilin, 2016). When changing the spike morphostructure, i.e. simultaneous spike elongation and increasing the number of spikelets, the spike reduces its grain content, which leads to a decrease in the effective spike activity. On the other hand, a decrease of 1000 kernel weight by 19 - 38% leads to disturbance in the outflow of assimilates from vegetative organs into generative ones.

It was found that within the conditions of 10 mg / kg of cadmium in the soil, the processes of generative organs formation are more disturbed, at a dose of cadmium of 50 mg / kg of soil it damages the intensity of the green matter accumulation. This leads to decrease of the grain ratio in the yield structure, which involves deterioration of conditions for harvesting and threshing the wheat yield within this growth environment.

It was shown that the use of selenium in most variants enhanced the activity of protective-stimulating

mechanisms, allowing to increase the resistance of wheat plants to the negative effects of oxidative stress. It was revealed that within the conditions of soil contamination with cadmium the most effective way was the seeds treatment before sowing and foliar treatment of vegetative plants with sodium selenite.

It is known that the productivity of grain crops is largely determined by both the total area of the photosynthetic surface and the content of green pigments in plant leaves. Chlorophylls *a* and *b* are the main pigments in chloroplasts of plant leaves, which determine the photosynthetic activity of the plant leaf surface. As the components of the photosynthetic apparatus, they are evaluated primarily like photosynthesis pigments, which are present in the plant photosystems determining the storage of sunlight energy in ATP molecules. Cadmium has an inhibitory effect on the pigments biosynthesis and, as a consequence, reduces the accumulation of ATP. In a plant cell, chlorophylls *a* and *b* are included in photosystem 1 and photosystem 2 with different amounts. The functional activity of the photosynthetic apparatus can largely depend on the distribution and ratio of the complexes. Cadmium inhibits the reaction center of photosystem 2 and the electron outflow around photosystem 1, resulting in disruption of the photosynthesis light reactions. Thus, the phytotoxicity of cadmium is manifested in the inhibitory effect of cadmium on the light phase (Heldt, 2011; Shumilin, 2016).

The assessment of the protective and stimulating role of sodium selenite on the photosynthetic activity of the leaf surface of wheat plants under conditions of soil contamination with cadmium presented effects in the tillering phase (Table 2).

Table 2. Chlorophyll content in the tillering phase in the leaves of spring wheat varieties Zlata with the use of various selenium compounds in conditions of soil contamination with cadmium, mg / kg wet weight.

Trial variant mode of application	dose of cadmium in soil, mg / kg soil	Chlorophyll content		The sum of chlorophylls <i>a + b</i>	Chlorophyll <i>a/b</i> ratio
		<i>a</i>	<i>b</i>		
without processing	-	0.19	0.07	0.52	2.66
seed treatment before sowing		0.18	0.06	0.50	2.80
soil application		0.19	0.07	0.54	2.73
without treatment	10	0.19	0.07	0.53	2.62
seed treatment before sowing		0.16	0.06	0.45	2.81
soil application		0.18	0.07	0.51	2.76
without treatment	50	0.17	0.06	0.46	2.77
seed treatment before sowing		0.18	0.07	0.50	2.78
soil application		0.18	0.07	0.50	2.60
SD * ₀₅		0.02	0.01	0.04	

*SD₀₅ - significant difference at significance 05.

The tillering phase, when using selenium, the degree of chlorophyll accumulation in the leaves of spring wheat plants of the Zlata variety increased, providing a protective potential for the transformation of energy necessary for the vital processes. This led to both an increase in productivity and an improvement of the key quality indicators of spring wheat variety Zlata. Furthermore, the ratio of photosynthetic apparatus photosystems is also of importance. A change in the ratio of chlorophyll *a* to *b* was revealed in favor of an increase in the proportion of chlorophyll *a* and, as a consequence, the activity of photosystem 1, which may be evidence of enlarging the antenna structures in order to increase the accumulated energy.

An increase in the level of soil contamination with cadmium led to a decrease in the content of chlorophyll in plant leaves. Thus, in the variant where 50 mg / kg of cadmium soil was added to the soil, the decrease in the concentration of chlorophyll *a* reached 12% compared to the variant where the cadmium content was at the background level for the given soil, chlorophyll *b* by 17%, the sum of chlorophylls *a* and *b* by 13%. The ratio of chlorophylls also changed for increasing the proportion of chlorophyll *a* in the chlorophyll complex structure, which may be considered as a consequence of adaptation processes triggered by oxidative stress associated with an increased content of cadmium in the soil. In the conditions of soil application with 10 mg / kg of cadmium, the increase in ratio of chlorophyll *b* in comparison to chlorophyll *a* regarding the control variant is observed. Thus, the use of selenium in the variants with the soil application of 10 mg / kg of cadmium increased this ratio from 2.62 in the control variant to 2.80 when applying seed treatment before sowing, which means the increase in the proportion of photosystem 1 in the structure of the pigment complex, and as a result thereof the Plants could not only receive more energy by the light-harvesting complex, but also use more excitons to reduce NADP + to NADP * N thanks to

the activity of photosystem 1, which could subsequently bring on increasing the area of flag and subflag leaves (Shumilin, 2016).

The protective and stimulating effect of selenium is also observed at a higher content of cadmium in the soil. The use of sodium selenite made it possible to increase the accumulation of green pigments (the sum of chlorophylls *a* and *b*) in plant leaves by 9% in the tillering phase when applying seed treatment before sowing, and when applying selenium to the soil by 8% compared to the control variant. The use of selenium in conditions of soil contamination with cadmium also reflected the change in the ratio of chlorophylls *a* and *b* in the structure of the photosynthetic apparatus. This determines the protective effect of the microelement, which allows increasing the resistance of plants to oxidative stress due to the soil contamination with cadmium.

Thus, the protective effect of selenium manifested itself not only through the direct accumulation of chlorophylls in plant leaves, which subsequently made it possible to accumulate more energy for its development in products, but also changed the ratio of chlorophylls *a* and *b*, which may indicate a change in the composition of photosystems of the leaves pigment complex for specific plant response to oxidative stress.

4. Conclusion

1. The protective effect of sodium selenite on the adaptive capacity of plants within the conditions of oxidative stress due to the soil contamination with cadmium has been determined. The increase in the adaptive capacity of plants manifested itself through the decrease in yield diminishing due to improvement of the conditions for fertile florets and ear initiations on the vegetative apex, as well as the development of flowers into grains, which contributed to increase in the grain content of the spike.

2. The stimulating effect of selenium on the intensity of photosynthetic processes has been revealed, which showed not only the increase of chlorophylls content, but also the ratio changes of chlorophylls *a* and *b*. At the same time, within the optimal growing conditions, the proportion of chlorophyll *a* in the structure of the chlorophyll complex increased, though within the conditions of oxidative stress, the proportion of chlorophyll *b* increased, what is considered as one of the mechanisms of plant adaptation to stress.

3. It was found that the use of selenium triggered the acceleration of plant repair processes both by increasing the accumulation of chlorophylls in the leaves and by reducing damage to the cell walls during lipid peroxidation caused by the formation of reactive oxygen species.

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