Separate and combined action of lead and cobalt ions on barley seedlings

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Abstract

In the experiments, the phytotoxic and mutagenic effect of lead and cobalt ions on seedlings of barley of the spring variety Josephine was studied. The possibility of reducing the negative effects of lead with the joint presence of cobalt ions in the solution for germinating seeds has been evaluated. When evaluating the combined action of heavy metal ions, the seed soaking solution contained Pb^{2+} and Co^{2+} in concentrations corresponding to the same MPC values (0.1; 1; 10; 100). The criteria for the manifestation of phyto- and cytotoxicity were the following indicators: seed germination energy, frequency of aberrant cells, and mitotic index. It was shown that the content of lead ions in the solution for germinating barley seeds led to a significant ($p<0.05$) decrease in the germination energy, a decrease in the mitotic index and an increase in the frequency of aberrant cells in comparison with the control values already at a metal concentration equal to 0.1 MPC for drinking water. The presence of cobalt ions in the seed germination solution also led to changes in the values of these parameters, however, significant differences with the control were at an ion concentration equal to 1 MPC. A decrease in the negative effects of lead with its joint presence in a solution for germination with cobalt ions was revealed. In this case, the higher the concentration of heavy metal ions, the stronger the compensating effect of Co^{2+}. It was found that in the presence of lead ions, the quantitative yield of aberrant cells per concentration unit is 2.14 times higher compared to the combined presence of two metal ions. An assumption is made about the possibility of using cobalt-containing agrochemicals (plant growth regulators, dietary supplements, complex fertilizers) as agents that reduce the toxic effect of lead.

Keywords: barley, lead, cobalt, phytotoxicity, mutagenicity, combined action

Introduction

Various types of modern production (industrial enterprises, thermal power plants, motor transport, mining, etc.) are powerful sources of pollution of the biosphere with heavy metals [1], which negatively affect living organisms and the degree of this influence must be assessed, especially in cases when it concerns food quality issues, for example, in the production of agricultural products [2]. Heavy metal ions come from soil or nutrient solution, mainly by passive diffusion, and the competition of ions can be largely predicted based on their physicochemical characteristics and parameters of the environment from which ions enter the plant [3].

Lead is widely used in media components. According to the literature, lead binds to sulfhydryl groups of enzymes and inhibits their activity or can replace other metals that are cofactors in important enzymes, which also leads to suppression of their activity [4]. Cobalt is one of the metals with potential mutagenic and carcinogenic properties [5]. At the same time, in the ionic form of Co in living organisms it participates in many nonspecific biochemical reactions (carboxylation and decarboxylation, hydrolysis of peptide bonds, hydrolysis of phosphorus esters, transfer of phosphate groups) activated by Mn, Mg, Zn, Ni, Fe, Ca and can be replaced by ions of these metals. Through its influence on enzyme systems, cobalt participates in the exchange of amides in plants, regulates nucleic acid exchange, activates the photochemical activity of chloroplasts, and also stimulates cellular reproduction of plant leaves [3]. In addition, it participates in hormonal regulation and is able to enhance the effect of auxin, which stimulates the processes of elongation and cell division.

The combined content of two or more metals can have excellent effects on living organisms, both in terms of the strength of the effect and in its directionality. It has been shown experimentally [6-7] that synergism and antagonism are not random events of interaction between stress agents. So, with the combined action of such pairs of metals as Zn and Co, Cr and Co, Co and Cu in low concentrations on the cells of the duckweed (*Lemna minor* L.), antagonistic toxic effects were noted in comparison with their individual effects [8], which is explained by physical - chemical properties of metals (ionic radius, oxidation state) and their competition for binding sites in molecules and routes of entry into the cell.

To reduce the negative consequences of the action of heavy metals, more and more drugs are used that have multifunctional properties and affect not only growth processes, but also the resistance of plants to various stress factors of the external environment [9]. Such substances, as a rule, have physiological activity and, differing in the purpose of action and/or method of application, affect the physiological and metabolic processes in plants, contribute to the realization of the genetic potential and better assimilation of nutrients. One of these substances includes cobalt-containing fertilizers [9] or complex fertilizers containing cobalt in a chelated form.

The purpose of this work was to study the phyto- and cytotoxic effects of lead ions on seedlings of spring barley varieties Josephine and to assess the possibility of reducing negative effects due to cobalt ions.

Research methodology

The object of the study was spring barley (*Hordeum vulgare* L.), variety Josephine (brewing), included in the State Register in 2008, the applicant is the Secobra Recherches seed breeding company, France.

To assess the toxic effect of lead ions, barley seeds (50 pieces each) were placed in Petri dishes and soaked in 30 ml of lead hydroxide solution ($Pb(OH)_2$ at concentrations corresponding to 0.1 MPC for drinking water (0.0005 mg/l) , 1 MPC 0.005 g/l, 10 MPC (0.05 g/l) and 100 MPC (0.5 g/l) Seed germination was carried out in a thermostat at a temperature of 25°C for 2 days. Similarly, seeds were soaked in solutions of cobalt hydroxide ($Co(OH)_2$ at concentrations of 0.001 g/l - 0.1 MPC, 0.01 g/l - 1 MPC, 0.1 g/l - 10 MPC and 1 g/l - 100 MPC. To assess the combined action of metal ions, a seed soaking solution was prepared with the addition of lead and cobalt hydroxides in concentrations corresponding to the same MPC values. The experiment was repeated four times.

The toxic effect of the studied ions was assessed by the change in morphometric parameters - germination energy - GE - expressed in the percentage of seeds that gave roots equal to half the length of the seed and sprouts on the 2nd day of germination, the frequency of aberrant cells (FAC - the ratio of the sum of ana- and telophase cells, in of which violations were registered, to the total number of analyzed ana-telophases of the first division of meristematic cells of the root meristem of seedlings) and the mitotic index (MI - the percentage of dividing cells from the total number of analyzed). FAC and MI in the cells of the root meristem of barley seedlings were assessed after 48 h of seed germination, when the seedling length reached 5-10 mm. The samples were fixed in acetic alcohol (3:1). Cytogenetic analysis was performed in ana-telophases on temporary squashed preparations stained with acetocarmine. Compressed preparations were viewed under a microscope at 400 magnification. In each variant, from 900 to 1500 ana-telophases were analyzed. To process the research results, we used standard statistical methods of data analysis [10]. The significance of the differences was determined by the Student's test.

The antagonism coefficient (AC) was calculated using the formula [11]:

$$AC = S_{Me1+Me2} / S_{Me1} {}^{+} S_{Me2}$$

where $S_{Me1+Me2}$ – the proportion of aberrant cells under the combined action of two metals minus the value of the spontaneous background of aberrations (the proportion of aberrant cells in the control);

S_{Me1} *and* S_{Me2} – the proportion of aberrant cells under the action of one of the metals minus the value of the spontaneous background of aberrations (the proportion of aberrant cells in the control).

Results

Soaking barley seeds in a solution containing lead ions at the studied concentrations led to a significant decrease in seed germination energy (GE) and mitotic index (MI). GE (ion concentration - 0.1 and 1 MPC) was 80.1 and 60.3%, control values were 88.3%. The mitotic index at the same MPC concentrations was 6.8 and 4.2%, while in the control MI it was 8%. So, under the action of lead ions at a concentration of 0.05 g/l (10 MPC), GE decreased 3.9 times compared to the control values, MI - 3.8 times, FAC increased 9.5 times. At a lead ion concentration of 1 g/l (100 MPC), the seeds did not germinate at all.

The content of cobalt ions in the solution for germinating barley seeds caused multidirectional effects depending on the concentration of ions. Thus, pronounced phyto- and cytotoxic effects were noted at ion concentrations corresponding to 10 and 100 MPC. The germination energy decreased by 1.4 and 4.8 times compared to the control values, MI also decreased by 1.3 and 3.3 times (at $p < 0.05$). Toxic effects were proved by an increase in FAC at the given MPC concentrations - 2.7 and 3.1% compared to the control values - 0.6%. It is known that increased concentrations of cobalt lead to toxic effects, which are associated with the development of oxidative stress, suppression of assimilation processes and the translational apparatus [13]. The cytotoxic effect of metal ions can be represented as suppression of mitosis and damage to chromosomes, as evidenced by our data. At the same time, when germinating barley seeds in a solution containing cobalt ions at a concentration of 0.01 g/l (1 MPC for drinking water), their germination rate increased by 11.4% and significantly differed from the control values. There was also an increase in MI of 32.5%, while the FAC remained at the control level. It can be assumed that at a given concentration of cobalt ions, the activation of enzymes involved in matrix synthesis and in the synthesis of a biologically active substance, cobalamin, occurs, which leads to stimulating effects [8].

Germination of barley seeds in a solution containing both lead and cobalt ions led to a decrease in the negative effect caused by an increased content of lead ions. It was revealed that the presence of cobalt ions at concentrations of 1 and 10 MPC in solution together with lead ions at the same concentrations according to MPC led to a significant increase in GE (from 60.3% for the Pb2 + variant to 74.3% and from 22, 2% to 48.2%, respectively). If, when germinating barley seeds in a solution containing Pb^{2+} at a concentration corresponding to 100 MPC, a sharp inhibition of seedling development was noted (GE = 0), then the addition of cobalt ions to the solution at a concentration corresponding to the same MPC values, GE, was - 10.1%.

The same tendency to suppress the negative effect caused by lead ions during joint germination in a solution containing cobalt ions can be traced according to the MI and FAC indices. In the concentration of metal ions equal to 0.1 MPC, there is a slight increase in MI from 6.8% in the Pb^{2+} variant to 7.1% in the variant of their joint presence, but with an increase in the concentration of Co^{2+} to 1, 10 and 100 MPC, a decrease in the negative effect lead ions were more pronounced and MI increased by 1.9; 2.5 times; at a concentration of Pb^{2+} 100, the MPC MI is 1.3%. Antagonism coefficients (according to FAC) calculated under the combined action of lead and cobalt ions at concentrations equal to 0.1–10 MPC for drinking water were 0.19, respectively; 0.35 and 0.41.

Conclusion

One of the sources of the entry of heavy metals into the soil are agrotechnical measures aimed at increasing the productivity of agricultural crops (the introduction of fertilizers, pesticides, the use of wastewater for irrigation). Lead enters the soil together with mineral fertilizers and is absorbed mainly by the root system of plants. In the zones of root division and extension, there are no physiological barriers to the movement of heavy metals; therefore, the tissues of the apical site accumulate their ions, which is one of the reasons for the growth-inhibiting effect of these chemical elements [12]. Potentially, preventing the accumulation of an element in the crop, as well as reducing its toxic effect on plant growth, is possible due to the effect on the biological barrier of absorption of the element, which is associated with the properties of plants and soils, and physicochemical, which directly depends on various chemical and ion-exchange reactions.

In the case of the combined action of various pollutants, the final effect may differ significantly from the effect of the separate presence of metal in the medium. The studies presented by us, carried out on seedlings of barley of the spring variety Josephine, prove the negative effect of high concentrations of lead ions on the germination rate of seeds, cell division and the structure of chromosomes. This is consistent with the information that some heavy metals are capable of causing nuclear damage [14], disrupting RNA synthesis, and inhibiting ribonuclease activity [15]. Disorders of cell division are primarily based on the ability to bind heavy metal ions with sulfhydryl groups of fission spindle proteins and enzymes responsible for the passage of mitosis, as a result of which these proteins lose their activity. The action of cobalt ions, on the contrary, leads to both stimulation and inhibition of processes, depending on the concentration of the element in the solution for germinating seeds.

The study of the frequency of aberrant cells in the root meristem of barley seedlings showed that the joint presence of lead and cobalt ions in the solution leads to a significant decrease in the frequency of chromosomal aberrations. The positive effect of cobalt ions is most likely due to an increase in the expression of genes that control the increased synthesis in the cells of the apical meristem of proteins, like lactoferrin or transferrin, capable of inactivating the action of lead ions. With the content of lead ions, the quantitative yield of aberrant cells per concentration unit, expressed in units of MPC for drinking water, was 2.14 times higher compared to the combined presence of ions of two metals. The coefficient of antagonism, calculated according to the indicator of aberrant cells with separate and joint presence of metal in concentrations equal to 0.1-10 MPC for drinking water, ranged from 0.19 to 0.41 units. Thus, the presence of one metal (cobalt) reduced the phytotoxicity of the other (lead), which can be used for practical purposes, namely, in regulating the supply of lead to plants through the use of cobalt-containing agrochemicals at various stages of plant development. In addition, a decrease in the toxic effect of lead can completely change the direction and strength of plant organisms' responses to the action of stress agents of a different nature.

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