IMPACT OF HEAVY METALS ON THE PLANT'S POST-RADIATION RECOVERY PROCESS

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ABSTRACT

Present research discusses experimental results of simultaneous action of heavy metals' different concentrations and radiation on the intensity of post-radiation recovery in Lemna minor L. The effect caused by the heavy metals was modeling via adding cadmium and zinc ions into the plant's medium during its cultivation. It was shown that at specific concentrations zinc ions have capability to increase intenstiy of plant's post-radiation recovery under irradiation with relatively low doses. It should be noted that similar phenomenon was not detected while using cadmium ions. At all cadmium ion concentrations, used in the experiment, increase in the negative effects of gamma radiation was observed. Moreover, increasing the zinc ion concentrations resulted in nullifying of all the positive effects, as well as with the use of cadmium the negative effect caused by radiation exposure was increased.

Key words: gamma-radiation, heavy metals, post-radiation recovery, *Lemna minor L*.

INTRODUCTION

The influence of various physicochemical factors on the environment on phytocenoses is complex. In many cases, different types of damaging factors act much differently during simultaneous action than the same factors separately. This phenomenon acquires special interest when the damaging factors of different nature act on the living organism. In this regard damaging factors such as ionizing radiation and heavy metals are especially interesting.

Ionizing radiation is produced by radioactive decay, of which gamma radiation is one of types. Gamma radiation (high energy photons) has two main mechanism of action: 1) direct damage of molecules, inducing DNA molecule destruction, lipid peroxidation and/or enzyme denaturation, and 2) radiolysis of cellular water, generating reactive oxygen species (ROS) that can indirectly induce cellular damage [1]. As it is known, the total effect of the radiobiological reaction on the organism is formed by the damage caused by gamma radiation on the one hand and the potential of post-radiation recovery on the other hand [2]. Thus, great importance is attached to the study of the modifying factors of these effects.

It should be noted that a number of organic and inorganic substances undergo modification in the post-radiation recovery process and the complete metabolic process of the cell is involved in. Of particular interest in this regard are the phenomena: 1.Factors acting on the recovery process, which is naturally, involved in intracellular metabolism and 2. Factors those are completely foreign to the organism. Specific examples are cadmium and zinc.

To begin with, Zn is an essential micronutrient involved in a wide variety of physiological processes in a living organism. Here are several roles of zinc:

It is structural component of a large amount of proteins and participates in catalysis [3]; It participates in protection of an organism; Membrane-bound Zn has structural, regulatory and antioxidant functions; the latter also serves as a pool of readily available Zn [4]; Zn is an essential component to maintain proper structure of cytoskeleton; Zn plays an important role in cell signaling [5]. Zn-binding proteins constitute almost half regulatory transcription factors in the human genome [6]. Zn is an important factor for the cellgrowth as well [7].

As for the cadmium, it is a non-essential element for the living organism. It is very toxic when it comes to plants, animals or humans, but there may be some exceptions too [8]. In general, Cd toxicity may reveal in a different ways [9].

The pair of these heavy metals is also interesting in that they are very similar not only in chemical but also in biological and biochemical action [10]. It is known that Cd can substitute Zn in various proteins, which consequently provokes cell damage [11]. Moreover, in higher concentrations Cd causes oxidative stress due to existence of free radicals [12].

In conclusion, this interesting relationship between the two metals mentioned, allows us to more thoroughly investigate the impact of metals with different properties on post-radiation recovery.

MATERIALS AND METHODS

We selected the aquatic plant Lemna minor L. as our research object. The reason for our choice is the convenience of Lemna minor L as a test-system when it comes to the studying heavy metals [13], as it is characterized by revealing an instant reaction to the toxicity caused by those metals.We cultivated the plant in the Steinberg medium [14]. The multiplication of the population proceeded by growing 1 colony of the plant, thus achieving the homogeneity of the obtained cultural population. We studied the action of heavy metals by incorporating standard aqueous solutions of cadmium chloride and zinc sulfate heptahydrateinto the medium.Gamma irradiation of the study plant was performed on a machine called "gamma-capsule" where the radiation source was^{137}Cs. The experiment was recorded by counting the total plant organisms as well as the individual frond numbers of these plants. The method is based on the establishment of instant growth of the population.

The change of the latter value reflects the resistance of the environment, that is to say characterizes the sum of all the limiting factors of the environment that impede the realization (r maximum) of reproductive potential. After the exposure time passed, the total amount of the fronds is calculated on the control and each dose (including mother individuals and fronds separated from mother individuals). Based on the received results a population's instant growth coefficient (r) is calculated:

$$r = (\ln(N_t) - \ln(N_o)) / t$$

where N_o is the total amount of the fronds; N_t-the final quantitativeof the fronds; t-exposure time [13].

RESULTS AND DISCUSSION

Nowadays, when it comes to the post-radiation recovery process that occurs after plant radiation damage, scientists largely note the radiation action on individual metabolic processes. One of the examples of this is the reduction of the characteristic and necessary metals in the mentioned organisms as a result of gamma irradiation in plant systems [15]. Therefore, the heavy metals introduced into the plant after gamma irradiation have a potentially positive effect on the post-radiation recovery process. In support of this, various studies have suggested that the introduction of zinc and calcium into the plant medium has been shown to normalize the amount of reduced sulfhydryl compounds induced by gamma radiation in the plant [16].

Various factors of influence on organisms form a complex system of interaction of these organisms with real environmental situations. Synergistic and antagonistic or additive types of reactions of biological systems to the combined effect of radiation and chemical factors are natural events and most likely occur in the low dose range - typical for environmental conditions. For this reason, the actually observed level of biological effects in natural populations inhabiting radioactively contaminated areas, or even heavy metal contaminated areas, often significantly differs from the predicted separate action of factors based on the results of experimental studies. Under these conditions, both external irradiation in low doses and incorporated heavy metals have a significant effect on the level of variability of quantitative indicators of organisms in populations and the possibility of their adaptation to a specific ecological situation.

During the process of studying this issue, we used two levels of, radiation and heavy metals, effects for their doses and concentrations respectively - conditionally low and high levels: 5 Gy - 20 Gy in the case of gamma radiation, 0.07 mg/mL – 0.7 mg/mL for Cadmium and 0.5 mg/mL – 5.0 mg/mL for Zinc. These exposure levels were due to the fact that these levels resulted in adequate inhibition of the growth rate of the plant model we chose.

The main task of our experiment was to determine the impact that various heavy metals could have on the post-radiation recovery of the irradiated plant.

As can be seen from Figure 1-A, the different concentrations of cadmium introduced into the plant's medium after irradiation of the plant at a low dose (5 Gy) significantly reduced the post-radiation recovery process. In addition, the same graph shows a decrease in the recovery processes in the separate irradiation option as well. A similar pattern, however more intensively expressed, was observed in the high-dose (20 Gy) irradiation variant (Fig. 1-B).

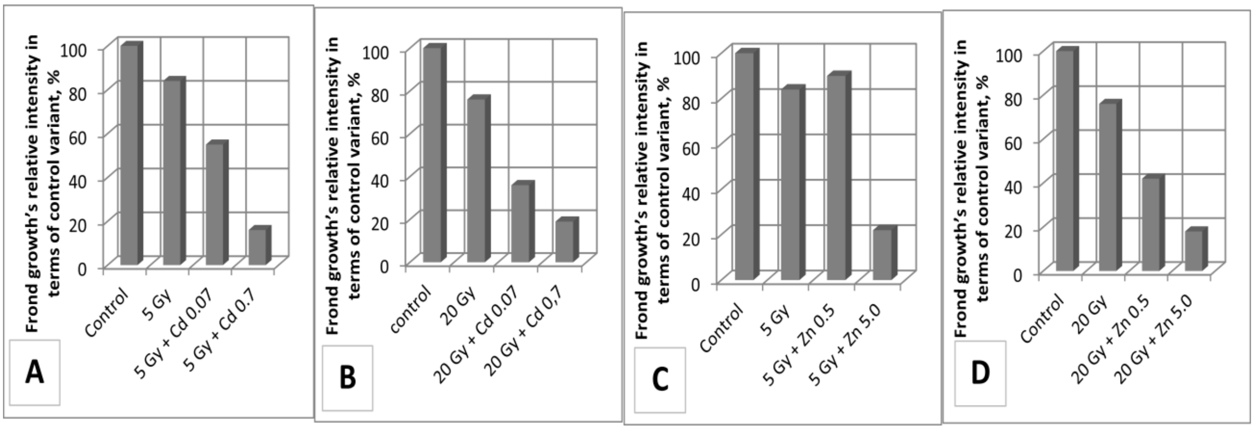

Fig.1 Impact of gamma radiation on the growth of the *lemna minor L* fronds, alongside with heavy metals.

An experiment was conducted with a similar scheme in the case of addition zinc to the experimental plant medium. It can be seen from Fig.1-C that although 5 Gy irradiation resulted in a decrease in frond growth intensity in the plant compared to the control variant, the addition of a low concentration of zinc (0.5 mg/mL) in the same variant resulted in an increase in post-radiation recovery intensity. However, in the same variant, the addition of high concentration of zinc (5.0 mg/mL) changed the potential for post-radiation regeneration to negative.In this regard, it is noteworthy that the experiment conducted by the same method, however, in the case of high doses of radiation with different concentrations of zinc, yielded identical results with respect to cadmium (Fig. 1-D).

As it is known, the response of *Lemna minor L*to inhibitory factors such as the action of heavy metals and radiation can reveal into two levels: individual fronds or population recovery levels [13]. Given this fact and the characteristics of the plant test-system we used, where the growth rate of its individual fronds indicates the level of recovery potential of the plant itself, the population effect rates caused by the same radiation exposure become important. In our case, we used quantitative indicators of plant's micro-populations of each variant as a criteria for this.As can be seen in Figure 2, which shows the results of population records of the same experimental components (as the growth characteristics of individual fronds), a variant of small concentration of zinc and a small dose of radiation is observed to show 15% growth compared to a separate low dose irradiated plants (Fig. 2-C), while in the remaining experimental elements a similar situation was observed with respect to the frond variants (Fig. 2-A, B, D).

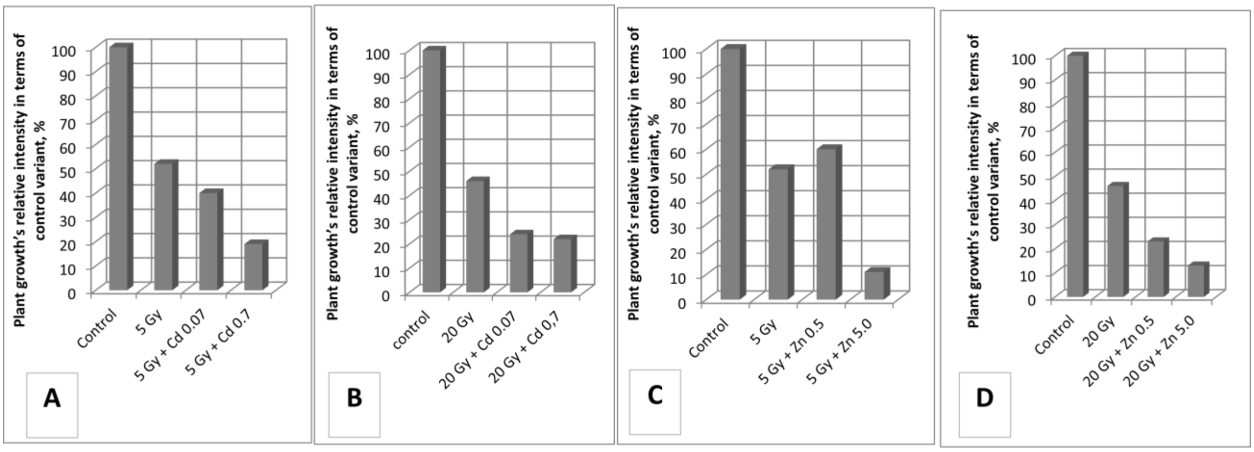

Fig.2. Impact of gamma radiation on the growth-development of separate plantsof *lemna minor L* with the presence of heavy metals.

To more fully describe the effect mentioned, we used the instant growth coefficient concept. This criterion can be used to determine what type of modifying action different concentrations of heavy metals have during the post-radiation recovery period. As can be seen from Fig. 3, both in terms of dose strength and in the presence of different concentrations of heavy metals, a heterogeneous response is observed. For example, at low-dose irradiation (5 Gy) and at low zinc ion concentrations (0.5 mg/mL), we obtained high levels of post-radiation recovery potential.

An increase in the concentration of zinc during radiation exposure of the same dose significantly reduced the level of the above phenomenon. It also sounds quite paradoxical that with an increase in the radiation dose, an adequately high concentration of zinc ions (5.0 mg/mL), if we adapt these figures to the growth dynamics of the plants used in the experiment, facilitates the recovery process at the initial stage of inhibitory action rather than deminishing it. A more contrasting ratio is obtained by the addition of cadmium ions in the plant medium. If the use of 5 Gy irradiation and low cadmium ion concentration (0.07 mg/mL) indicates a high instant growth coefficient, indicating a low inhibitory level corresponding to a low cadmium concentration, then in the case of an increase in cadmium concentration reveals a relatively high toxicity on the plant. This is evidenced by data on the combined action of high doses of radiation and both concentrations of cadmium as well.

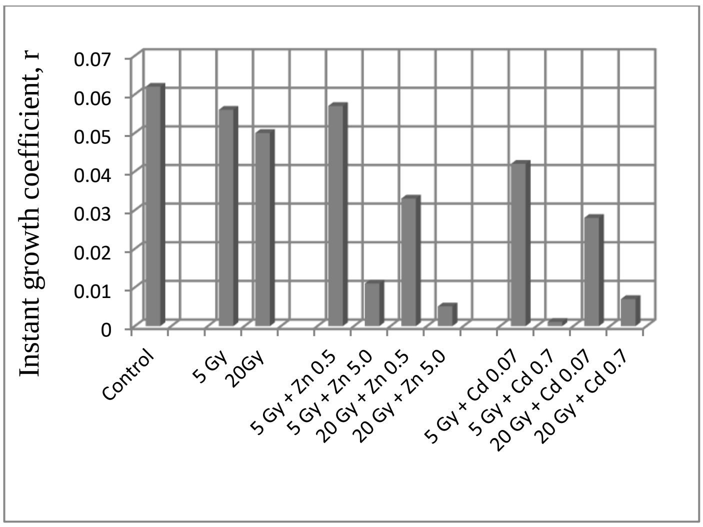

Fig.3 Joint action of heavy metals and radiation on the intensity of *Lemna minor L*'s growth

Overall, based on the results obtained, it can be concluded that the impact of heavy metals has often the opposing action. From this point of view, it can be assumed that the use of low concentrations of zinc ions (0.5 mg/mL) during low-dose irradiation (5 Gy) facilitates the recovery process of a number of radiation-induced proteins, such as alcohol dehydrogenase, carboxypeptidase, aminopeptidase, beta-amyloid, etc., in which zinc is one of the main components. The same outcome was not observed when using cadmium ions in the same proportions. The second effect that may underlie this phenomenon is the increase in the inhibitory impact of heavy metals on the dynamics of the growth intensity of the irradiated plant. Specifically, highly inhibitory concentrations of heavy metals' ions afffect more strongly on actively growing plants, than at the level of weakened growth intensity under conditions of high irradiation doses.

CONCLUSION

Based on the conducted research, we can conclude that the action of physico-chemical factors used in the experiment is often nonlinear, and is highly dependent not only on the level and capacity of the factors, but also on the interaction of processes caused by these factors themselves. These data, in our opinion, are important to monitor ecosystems with different dynamic processes caused by the pollutants, both when assessing their condition and when developing prognostic models.

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