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CYTOGENETIC EFFECTS IN *Allium cepa* L. RESULTED FROM SEPARATE AND COMBINED EXPOSURE TO Cu, Zn AND Ni

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Abstract

Heavy metal contaminations of agricultural lands necessitate the study of phyto- and genotoxic effects in plants of different types. The impact of elevated concentrations of biologically essential metals, e.g. Cu and Zn, and the metals with a pronounced toxic effect even at low concentrations is of special importance. Generally, the model objects (e.g. *Allium cepa* L.) are used to simulate the impact of one of the metals. In this case the concentration chosen is greater (100 times or more) than the levels of actual contamination and maximum permissible concentrations in different environments. Data on the combined action of metals at concentrations actually existing in the environment are practically not reported, which prevents the development of standards to limit their impact on ecosystems and agrobiocenoses. Our objective was to compare the cytogenetic changes in the root meristem of *Allium cepa* exposed to different concentrations of Cu, Zn and Ni separately and combinedly. Experiments were carried out in 4-fold replication. The roots of onion plants (10 per replication) were germinated in distilled water (control) or $\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$, $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ and $\text{Ni}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ solutions. Salt concentrations corresponded to the maximum permissible concentrations in water for fish-farming (Cu — 0.001 mg/l, Ni and Zn — 0.01 mg/l) and for household purposes (Cu and Zn — 1.0 mg/l, Ni — 0.02 mg/l). Chromosome aberrations were viewed in 180–790 cells of onion root tips in fresh crush preparations after acetic-orcein staining. We estimated the mitotic index calculated as the fraction of mitotic cells to the total number of cells in the root meristem, the frequency of aberrant cells, and the types of chromosomal aberrations. The influence of each element and their various combinations on cytogenetic parameters was compared that allowed us to determine the coefficient of antagonism. It has been shown that Cu, Zn and Ni ions, as depending on their properties, inhibited cell division in onion root meristem to varying degrees. At relatively low concentrations of metals, equal to the MPC in water for fish-farming, the frequency aberrant cells was about 3 to 7 times higher as compared to the control. An increased metal concentrations (1000-fold, 100-fold, and 2-fold for Cu, Zn and Ni, respectively) did not lead to a proportional increase in the frequency of aberrant cells which was only two times as much as that in control. Changes in the mitotic index were also disproportionate to the metal concentration in the solution. When combined, the metal ions had lower genotoxic effect as compared to their individual activity. The differences between separate and combined effects are indicative of ion competition. The calculated coefficients of antagonism in the experiment ranged from 0.20 to 0.40.

Keywords: *Allium cepa* L., heavy metals, genotoxic effect, coefficients of antagonism

Intensification of economic activity and degradation processes may lead to increase in pollutant content, including heavy metals (HM) in natural environments. For example, heavy metal soil contamination values in several regions of the Russian federation are rather high. The greatest soil contamination (as a percentage of examined area) is observed with regard to Cu (3.8 %) and Ni (2.8 %), while this parameter for Zn is lower (0.2 %) [1].

HM toxicity for living organisms is determined by their concentration and physical and chemical properties, including migration capacity and bioavailability, which finally determine the value of chemical elements accumulation in

organs and tissues [2]. Accumulation of HM in soil affects growth and development of plants [3, 4], as well as soil microbiota [5], which leads directly or indirectly to farm crops yield loss. To date, the main mechanisms of HM fixation in solid phase of soil have been determined [6]. It ensures development of measures aimed at decrease of HM content in the soil solution and reduction of toxicity risk for agricultural plants. Phytotoxic effect has been studied sufficiently for only a few metals [7-9]. The lack of information on the patterns of biological systems response to effect of a wide range of HM complicates evaluation of their actual hazard for plants and animals.

As a rule, experimental studies are conducted using model objects, with impact of one metal in various concentrations, including high ones, which is aimed at quantitation of the effect. However, in natural conditions elevated concentrations of metals with negative impact on cenosis components are generally lower than those selected for an experiment. Polyelemental impact is considered even to a lesser extent, as in this case forecasting of risks is much more difficult, because of possible mutual interactions of metals and synergism or antagonism processes [10]. The studies of barley and wheat sprouts [11] have demonstrated decrease in phytotoxic effect of Al^{3+} ions in the presence of Fe^{3+} ions, which may be explained by induced synthesis of proteins similar to transferrin or lactoferrin in animals, organic acids or phytochelators, inactivating Al^{3+} ions. Sensitivity of barley varieties to the effect of Al ions of genetic pleiotropic nature has been detected [12].

The impact of elevated concentrations of biologically essential metals (Cu, Zn) or the metals with a pronounced toxic effect even at low concentrations (Ni) is of special importance for agricultural practice. It has been established that these elements have pronounced mutagenic properties [13-15]. Their compounds may cause both clastogenic and aneugenic effects (including disturbances of mitosis and cytokinesis) [16]. Inhibition of mitosis induced by Ni and Zn is explained by redistribution of cell (tissue, organism) energy resources for metallothionein synthesis, while Cu ions increase mitotic activity [17]. Significant correlation between DNA damage and Cu content was detected [18].

Special attention is paid to search of phytoremediation agents [19, 20], examination of heavy metal consumption and distribution patterns in various parts of plants [21, 22], including depending on the varietal characteristics [23], examination of selective transport of elements from rhizosphere to leaves in ontogenesis [24], mechanisms of heavy metal detoxication in various plant species [25, 26].

Examination of genotoxicity of various chemical substances for cytogenetic and morphometric parameters of *Allium cepa* L. test object is of importance [27-29]. The data on toxic effects of Cu, Zn and Ni compounds, both independently (at CuCO_3 concentration of 5 mg/l, ZnCl_2 concentration of 10 mg/l, NiCO_3 concentration of 0.7 mg/l) [17] and in various combinations, were obtained using this test system. It has been noted that in case of joint effect of metal ions on root meristem of *Allium cepa* the mitogenicity in tissue changes and protein synthesis in cells increases.

As a rule, rather high concentrations of metals are selected for work. For example, according to R.F. Garipova [17], these significantly exceeded the maximum permissible values in water for fish-farming (100-1000-fold) and household (more than 2-fold) purposes. Examination of effects of small metal concentrations and determination of their genotoxicity value is more relevant, as it is important from both scientific and practical point of view, especially in case of polyelemental contamination. Apart from that, the lack of data on antagonism coefficients for various combinations of elements prevents comprehensive determination

of interdependent effect; at that, synergism and antagonism shall be taken into account in determination of risk of heavy metal impact on living organisms.

We first evaluated the impact of Cu, Zn and Ni in concentrations, corresponding to their maximum permissible content in water of various economic purposes, on cytogenetic parameters of *Allium cepa* L. This approach ensures comparison of HM effects at concentrations, differing 1000-fold, 100-fold, and 2-fold, and identification of mutual inhibition of toxic effect of various metals in case of their combined action.

Our work focused on examination of the impact of Cu, Zn and Ni on development of cytogenetic effects in root meristem of *Allium cepa* L. at various concentrations of metals, in conditions of mono- and polyelemental contamination, as well as determination of antagonism coefficients.

Techniques. Plants of *Allium cepa*, variety Stuttgarter Riesen, were the study object. The experiments were conducted in quadruplicate in laboratory conditions. A total of 10 pcs of planting onion of similar size (1.5-1.7 cm) were selected for each replication of the experiment variant. The roots of onion plants were grown in distilled water (control) or $\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$; $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ and $\text{Ni}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ solutions. During examination of joint effect the solution contained $\text{Zn}^{2+} + \text{Ni}^{2+}$, $\text{Zn}^{2+} + \text{Cu}^{2+}$, $\text{Cu}^{2+} + \text{Ni}^{2+}$ and $\text{Zn}^{2+} + \text{Ni}^{2+} + \text{Cu}^{2+}$ ions. Salt concentrations corresponded to the maximum permissible concentrations in water for fish-farming purpose (MPC_{ff}) (0.001 mg/l for Cu ions, 0.01 mg/l for Ni and Zn ions) and in water for household purpose (MPC_{h}) (1 mg/l for Cu and Zn, 0.02 mg/l for Ni).

Chromosome aberration analysis was conducted in root meristem of onion plants. To do so, the roots of onions about 1 cm long were cut off in 72 hours, fixed in acetic alcohol (3:1) and washed with 80 % alcohol. Fresh crush preparations with acetic-orcein staining were examined. Mitotic index (MI), i.e. the proportion of cells at mitosis stage in the total number of root meristem cells, as well as aberrant cells frequency (ACF) and types of chromosome aberrations were considered. A total of 180-790 ana-telophase cells were reviewed in each variant.

The effect of heavy metals was evaluated by a deviation of variant average values of the respective parameters from their control values. Antagonism coefficient (AC) was calculated as per the formula [31]:

$$\text{AK} = S_{\text{Me1+Me2}} / (S_{\text{Me1}} + S_{\text{Me2}}),$$

where $S_{\text{Me1+Me2}}$ is the proportion of aberrant cells in case of joint effect of two metals minus the spontaneous aberration background value (the proportion of aberrant cells in control); S_{Me1} and S_{Me2} are the proportion of aberrant cells in case of effect of one metal minus the spontaneous aberration background value (the proportion of aberrant cells in control). In case of effect of three metals antagonism coefficient was calculated as per the formula:

$$\text{AK} = S_{\text{Me1+Me2+Me3}} / (S_{\text{Me1}} + S_{\text{Me2}} + S_{\text{Me3}}).$$

Statistical processing of the data was performed using Microsoft Excel. Significance of differences between variant was determined based on paired two-sample *t*-test for mean values. Mean values ($\bar{X}$) and standard error of mean ($\pm x$) are presented in the tables.

Results. It has been shown that Cu, Zn and Ni ions present in the germination solution inhibited cell division in onion root meristem to varying degrees (Table 1).

Genotoxic effect of metals essential for plants (Cu and Zn) was nearly similar, while genotoxic effect of Ni was significantly higher: ACF (%) in onion root meristem at Cu concentration of 0.001 mg/l has increased 5.3-fold as compared to the control, at Zn concentration of 0.01 mg/l 4.7-fold (see Table 1). In

case of increase of Cu and Zn concentration up to 1 mg/l (MPC_h) ACF increased 11.3-fold and 8.9-fold, respectively, as compared to the control (see Table 1). A 7.3-fold and 13.4-fold ACF increase was observed for Ni at MPC_{ff} and MPC_h, respectively.

1. Cytogenetic parameters in case of effect of Cu, Zn and Ni in various combinations on root meristem of *Allium cepa* L. ($\bar{X} \pm x$)

Variant	The number of anaphase cells reviewed	Aberrant cells frequency, %	Mitotic index, %	Chromosome aberration type
Water for fish-farming purpose				
Cu	725±7	3.10±0.39 ^{a, b}	14.53±0.58 ^{b, d, e}	m, f
Zn	500±5	2.72±0.46 ^{a, b, e}	10.91±0.41 ^{a, e}	m, f
Ni	424±4	4.23±0.60 ^{a, c, d, e}	10.43±0.38 ^{a, d, e}	m, f
Cu + Zn	585±6	1.78±0.21	9.91±0.48	m, f
Zn + Ni	550±5	2.58±0.51	12.41±0.66	m, f, 3p
Ni + Cu	279±3	2.92±0.49	7.82±0.65	m, f
Cu + Zn + Ni	571±6	2.82±0.34	9.09±0.47	m, f
Control	781±8	0.58±0.23	15.12±0.73	m
Water for household purpose				
Cu	314±4	6.56±0.82 ^{a, b, d, e}	7.24±0.46 ^{a, d}	m, f
Zn	333±3	5.16±0.90 ^{a, b, d, e}	8.77±0.41 ^{a, e}	m, f, 3p
Ni	438±4	7.76±1.37 ^{a, d, e}	9.96±0.40 ^{a, c, d, e}	m, f, g
Cu + Zn	391±4	3.03±0.20	8.97±0.45	m, f
Zn + Ni	324±3	3.04±0.39	7.35±0.34	m, f
Ni + Cu	177±2	3.83±0.80	6.32±0.42	m, f
Cu + Zn + Ni	347±4	3.85±0.54	7.13±0.40	m, f
Control	781±8	0.58±0.23	15.12±0.73	m

Note. Concentrations equal to maximum permissible concentrations in water for fish-farming (MPC_{ff}) and household (MPC_h) purpose were used. MPC_{ff}: Cu — 0.001 mg/l, Zn — 0.01 mg/l, Ni — 0.01 mg/l; MPC_h: Cu — 1 mg/l, Zn — 1 mg/l, Ni — 0.02 mg/l; control — distilled water; f — fragment, m — bridge, g — lagging, 3p — tripolarity.

a, b, c, d, e The differences are significant at $p < 0.05$ with regard to control, Cu + Zn, Zn + Ni, Ni + Cu and Cu + Zn + Ni, respectively.

MI decrease was observed with increase of metal salt concentrations in the solution from MPC_{ff} up to MPC_h. MI decreased 2.0-fold in case of Cu and 1.3-fold in case of Zn; the differences were insignificant for Ni (10.43±0.38 and 9.96±0.40 for MPC_{ff} and MPC_h, respectively). Thus, MI changes were not proportional to increase in metal content in the solution. Similar phenomena were observed for other metals, for example, Cd [32, 33].

HM salt effect on plants is characterized with complexity and a wide range of targets, which explains various disturbances observed at all organization levels of a biological object [17, 34]. Zn and Cu are bioactive (essential) chemical elements of major importance, involved in various metabolic processes. The role of Ni is known to a lesser extent. As a rule, Ni is considered as a toxic element for most plants [8]; its participation in some enzymatic reactions in legumes has been confirmed. The majority of plant species is characterized by high resistance to excess of metal salts in soils due to metal consumption limitation mechanisms and their detoxication in cells. According to laboratory studies, HM in 10⁻⁶-10⁻³ M concentrations cause cytogenetic disturbances in apical meristem cells of *Allium cepa* L. [16]. The rating of HM salts with regard to their ability to induce chromosome aberrations is as follows:

At that, the strongest antimitotic effect was observed for Ni (the percentage of chromosome lagging, multipolar anaphases and K-mitoses increased nearly 70-fold as compared to the control). Ni ions also were the strongest inducers of cytokinesis disturbance [16].

In our study chromosome lagging was only detected for Ni in concentrations equal to MPC_h (see Table 1). Two types of aberrations, i.e. acentric fragments and bridges, were observed by us during anaphase and telophase.

Simultaneous presence of two metals in growth solution decreased the

toxic effect of each of them. In variants with the solution containing Cu ions chromosome aberration frequency in onion root cells in the presence of Zn decreased significantly, i.e. 1.7-fold and 2.2-fold for MPC_{ff} and MPC_h, respectively, as compared to ACF for Cu. In combination of Cu and Ni ions ACF decreased less significantly, i.e. 1.06-fold and 1.70-fold. In case of simultaneous effect of Cu, Zn and Ni in concentration equal to MPC_{ff} ACF did not reach the values of individual effect of metals (for Cu and Ni) or was comparable with them (for Zn) (see Table 1). In case of MPC_h and joint effect of metals ACF was always below the values of their individual effect (see Table 1). ACF_{Ni} and ACF_{Cu+Zn+Ni} differed 2-fold.

2. Antagonism coefficient in case of effect of Cu, Zn and Ni in various combinations on root meristem of *Allium cepa* L.

Variant	MPC _{ff}	MPC _h
Cu + Zn	0.31	0.26
Zn + Ni	0.37	0.24
Ni + Cu	0.40	0.27
Cu + Zn + Ni	0.28	0.20

Note. Concentrations equal to maximum permissible concentrations in water for fish-farming (MPC_{ff}) and household (MPC_h) purpose were used. MPC_{ff}: Cu — 0.001 mg/l, Zn — 0.01 mg/l, Ni — 0.01 mg/l; MPC_h: Cu — 1 mg/l, Zn — 1 mg/l, Ni — 0.02 mg/l. Antagonism coefficient was calculated by aberrant cells frequency.

three metals (Cu, Zn and Ni) MI was lower than that of Zn or Ni. Such differences in response to separate and combined presence of metals in the solution suggest competition of metal ions [34]. At that, a new type of chromosome disturbances (tripolarity) was observed in Zn + Ni (MPC_{ff}) pair. In case of metal concentration increase up to MCP_h tripolarity was only observed in Zn.

Antagonism coefficient values calculated by ACF (Table 2) varied from 0.20 (for Cu + Zn + Ni) to 0.27 (for Ni + Cu) at MCP_h. At MPC_{ff} this parameter was from 0.28 (for Cu + Zn + Ni) to 0.40 (for Ni + Cu). It should be noted that maximum and minimum antagonism coefficient values, irrespective of concentrations, were observed in the same components of metal mixture.

Thus, genotoxic effect of heavy metal ions on root meristem cells of *Allium cepa* has been established at the concentrations of elements, corresponding to the respective MPC values in water for fish-farming and household purposes. Even at relatively low concentrations of metals (0.001 mg/l for Cu, 0.01 mg/l for Ni and Zn) the aberrant cells frequency was about 3 to 7 times higher as compared to the control. Mutual inhibition of effects, which was the most prominent in Cu + Zn variant, was observed in case of joint effect of metal ions. Increase in metal concentration up to MPC_h led to 2-fold increase in genotoxicity values, but was disproportionate to the increase in concentration of elements. At that, different spectra of chromosome aberration for essential (Cu, Zn) and toxic (Ni) elements were observed (chromosome lagging was detected for Ni at MPC_h). Antagonism coefficient values (by aberrant cells frequency) at MPC_h varied depending on the combination of metals. The revealed patterns of individual and joint effect of metals on root meristem shall be considered during monitoring of both water ecosystems and soil biocenosis subject to joint effect of heavy metals, as well as during evaluation of environmental risk for various ecosystems under multiple-factor effect of chemical elements.

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Mitotic index for MPC_{ff} in case of joint effect of metals was below MI in case of individual effect, except for MI of Zn + Ni, where increase of the parameter was observed as compared with both MI of Zn and MI of Ni. On the contrary, the changes of MI for MPC_h in case of joint effect of two metals varied, and in case of joint effect of

three metals (Cu, Zn and Ni) MI was lower than that of Zn or Ni.

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