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Mixture toxicity of different metal ions in cytogenetic biotests

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Abstract. The separate and combined impacts of aluminium and selenium ions on different barley cultivars and onion (*Allium cepa* L.) seedling as well as ions copper, zinc and nickel on onion have been studied. The experiments have been performed in several series with different solutions of ions: aluminium have been used in concentration of 0.5 mg/l (Russian MPC for cultural-domestic water), ions of Fe, Mn, Se, Ag and their combinations with ions of Al have been used in concentrations corresponding to the values of Rus MPC for cultural-domestic water. At the same condition were chosen the concentrations of copper, zinc and nickel. The phytotoxic and genotoxic effects (as mitotic index – MI and aberrant cell frequency – ACF) were estimated. It was showed that aluminium ions promote higher phytotoxic and genotoxic effects, but in combined with another ions the negative effects on barley and *Allium cepa* reduced. This confirms that the universal mechanism the interaction between two metals (aluminium and other) and their detoxification effect in plant. The essential metals Cu and Zn and the toxic metal nickel provide a strong cytogenetic effect which results to ACF increasing and MI suppression. We assume that the mechanisms of detoxication should be initiated by metals themselves.

1. Introduction

Metal ions in various combinations are always present in natural water bodies used for fishing, cultural and domestic purposes. As a rule, regulatory actions of each ion concentration do not consider their combined impact on people, aquatic animals and plants and it probably appears as synergism, antagonism and additive effects [1]. It demands the study of these reactions in biotests and regulation of water pollution.

Aim – detection and qualitative cytogenetic analysis of a combined impact of metal ions.

2. Materials and methods

Cytogenetic methods of biotesting are used to assess the direct and combined effects of metal ions in water. The mitotic index (MI) and the aberrant cell frequency (ACF) are determined in the root meristem of barley and onion (Allium-test) seedlings during their sprouting in metal solutions [2]. Aluminum which at the concentration of 0.5 mg/l (MPC for cultural-domestic water) and pH = 4.5 has a strong cytogenetic effect is taken as the primary acting agent [3]. Coupled with Al, ions of Fe, Mn, Se and Ag and their combinations have been used in concentrations corresponding to the Russian MPC values for cultural and domestic water.



Other experiments with Stuttgart Risen onion seedlings made it possible to assess the cytogenetic changes after sprouting in zinc, copper and nickel solutions in concentrations (according to the metal ion) which correspond to MPC values for cultural-domestic water. The coupling factor (antagonism or synergism) is found from $K=S/S_1+S_2$, where S is the measured ACF value (here minus from a reference value); S_1 and S_2 are the corresponding ACF values under the impact of each metal particularly (minus reference values). The statistical data processing from three independent experiments is realized using the Stat Graf program.

3. Results

Table 1 presents the experimental results for seedlings of two barley cultivars and Stuttgart onion; the data are assessed from the mitotic index (MI) of root meristem cells and in *Allium*-test.

Table 1. Mitotic index in the root meristem of barley and onion seedlings.

Species	MI (%)			
	Blank	Al ³⁺	Se ²⁺	Al+Se
Bios, barley	6.5±0.3	2.5±0.2	4.3±0.2	5.5±0.3
	Blank	Al ³⁺	Fe ²⁺	Al+Fe
Bios, barley	8.4±0.6	3.2±0.3	8.9±0.7	5.4±1.0
Getman, barley	10.0±0.3	6.1±0.2	9.3±0.8	7.2±0.3
	Blank	Al ³⁺	Mn ²⁺	Al+Mn
<i>Allium cepa</i>	8.2±0.6	5.1±0.5	6.2±0.7	7.4±0.7
	Blank	Al ³⁺	Ag ²⁺	Al+Ag
<i>Allium cepa</i>	10.1±0.9	5.9±0.5	6.6±0.9	7.0±0.6

It was earlier established with wheat seedlings that Al³⁺ ions found in the water environment at pH = 4.5 have a profound cytogenetic effect [3]. This regularity is also confirmed by barley and onion seedlings: in the concentration used the ions of Al suppress the mitotic index of Bios barley which is sensitive to its effects by 62% and the mitotic index for cells of the resistant Getman variety goes down by 39 % only. For the root meristem cells of onion the degree of suppression in various experiments ranges from 38 to 42%. Ions of other metals (Se, Mn, Ag) also suppress the mitotic activity of meristem cells, however, in case of a combined (with aluminum) effect the mitotic activity rises as compared to the action of Al alone. The same effect is observed in a combined action of aluminum and ferrous iron, the latter having no cytotoxic effect. One of the expected reasons for suppressing the plant cell division is the disrupted chromosome integrity and mitoses caused by the indirect impact of various oxide radicals [4]. The aberration rate has been studied to test this assumption of disruption in the genetic apparatus of dividing cells of the root meristem seedlings. These results are presented in table 2.

It is shown that selenium characterized by pronounced antioxidant properties has demonstrated its protective effect for Bios barley in combination with aluminium. In this case the antagonism factor is 0.66. If aluminium and ferrous iron ions are present in the solution simultaneously, there is a pronounced effect of antagonism in case of Bios ($K = 0.47$) which is more sensitive to Al ions as compared to the resistant variety Getman ($K = 0.77$). Even more pronounced antagonism ($K = 0.20$) is observed in a combined impact of bivalent manganese and silver ions on onion seedlings.

The following series of experiments allowed the separate and combined effects of essential (Cu, Zn) and certainly toxic (Ni) metals (table 3) to be studied. The problem consisted in observing the highest effect of antagonism from the cytogenetic indices under investigation.

Table 2. Cytogenetic effect of metal ions and their combinations on barley and onion seedlings.

Species	ACF (%)				K
	Blank	Al ³⁺	Se ²⁺	Al+Se	
Bios, barley	1.6±0.4	6.3±0.6	3.1±0.4	4.1±0.6	0.66
	Blank	Al ³⁺	Fe ²⁺	Al+Fe	
Bios, barley	1.7±0.3	6.8±0.4	1.5±0.4	4.1±0.7	0.47
Getman, barley	0.7±0.4	3.4±0.3	0.7±0.5	2.8±0.4	0.77
	Blank	Al ³⁺	Mn ²⁺	Al+Mn	
<i>Allium cepa</i>	1.4±0.8	4.1±1.4	1.3±0.6	1.9±0.9	0.20
	Blank	Al ³⁺	Ag ²⁺	Al+Ag	
<i>Allium cepa</i>	1.2±0.8	4.0±1.9	3.5±1.2	2.2±1.1	0.20

ACF – aberrant cell frequency, K – antagonism factor.

Table 3. Genotoxic effect of Cu, Zn and Ni ions on *Allium cepa* root meristem [2].

Version	Number of ana-telophase cells	Aberrant cell frequency (%)	Mitotic index (%)	Type of chromosome aberration
Cu	314±4	6.56±0.82	7.24±0.46	m, f
Zn	333±3	5.16±0.90	8.77±0.41	m, f, 3p
Ni	438±4	7.76±1.37	9.96±0.40	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
Reference	781±8	0.58±0.23	15.12±0.73	M

Cu – 1 mg/l, Zn – 1 mg/l, Ni – 0.02 mg/l.

As seen from table 3, both the essential metals (copper and zinc) and the plant toxic nickel used in given concentrations (MPC for cultural-domestic water bodies according to the Russian health legislation) cause a strong cytogenetic effect which results in the certain ACF increase and MI suppression. A significant decrease of cytogenetic as well as cytotoxic effects can be achieved by combining these metals in germination solutions. However, the damaging effect of all three metals could not be fully eliminated even in their combined action. There were no tripolar mitoses (as most destructive to cell activity) under the action of essential metals as well as their combinations with admittedly toxic nickel.

4. Discussion

In 1909 the German agrochemist E. Mitcherlich had published the paper formulating ‘the law of additive effects of environmental factors’. The law says: the well-being of species, population, organisms depends not only on a particular, though a limiting factor, but simultaneously on the totality of ecological factors. The discovery of any law of nature, including this one, can be considered as a certain key to one of the intentions of the Creator-the Author of the Universe. In this instance it can be assumed that in planning the Universe creation in its current form the Creator knew in advance that it

would be based on 100 chemical elements. Supposing that the initial optimum amount of each this element is exceeded by 1% (in their biological effect) it would result in almost complete disappearance of life, plant life in particular. To prevent the expected adverse developments, the existence of living things is based on special defense mechanisms. Being under a pleiotropic genetic control and, hence, considering a vast variety of their manifestations, these mechanisms should cancel and reduce the effect of increased values (higher than the optimal ones) of aluminum concentrations. The mechanisms of detoxication should be initiated by metals themselves. If one of the metals suppresses the plant growth and development, the neighbor metal would cancel or reduce the adverse effects. The preliminary results on a combined action of various metals or fluorine on barley and wheat were presented in 2009 in Syktyvkar at the 100 anniversary of E. Mitcherlich law [5]. Antagonistic manifestations of induction effects of different chromosome aberrations followed by an increased mitotic cell activity were demonstrated at the cytogenetic level. Recently similar regularities have also been found by Belgian scientists in the experiments with a simultaneous impact of four metals (copper, nickel, cadmium and zinc) on wheat seedlings [6].

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