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Allium Root-Micronucleus (Allium-MCN) Test on the Genotoxicity of Soil Samples Contaminated with Heavy Metals

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Abstract: *Allium* Root-MCN bioassay was used to determine the cytotoxicity of 5 soil samples. Two types of soils were studied: contaminated one collected near of copper-smelters (the Middle Urals) and Ni-enriched soil from an area of a natural geochemical anomaly (the Polar Urals). We measured potentially mobile forms of metals in soils and water-soluble concentrations of metals in soils with standard methods. *Allium* roots were exposed in aqueous soil extracts for 30 hours with a recovery duration for 20 and 44 hours. We found an increase of frequencies of total nuclear anomalies in meristematic cells as compared to control samples. The most frequent types of anomalies in all studied samples were extrusions. Influence of high Ni concentrations leads to decrease of roots growth that was observed after recovery time also and increasing of number of micronucleus. The frequency of micronuclei in cells of root tips increased up to 6.5 fold as compared to control samples. The total number of anomalies and contribution of extrusions among them was higher for contaminated soils with prevalence of Cu. After the second recovery time point of 44 hours the number of anomalies increased dramatically, that allow recommend such period for similar experiments. In conclusion, high concentrations of Ni lead to decrease of roots growth that was observed after recovery time also and increasing of number of micronuclei.

Key words: *Allium*-test · Cytotoxicity of soils · Cu · Ni · MCN

INTRODUCTION

The study was carried out by program "Plant bioassay on the genotoxicity of contaminated water, air and soil". Plant assays Trad-MCN (Tradescantia-micronucleus), Allium/Vicia Root-MCN, Trad-SHM (Tradescantia-stamen hair mutation) were validated for monitoring of a genotoxicity of chemical contamination of the environment.

The aim of this project was to study genotoxic influence of heavy metals on meristem cells of roots of *Allium cepa*. Since soil is the growth medium for most plants, the use of *Allium* assay should be amenable for the assessment of soil genotoxicity [1]. Published data on soil genotoxicity employed more than 100 observations using *Allium* root tips assay that investigated the clastogenic effect of contaminated soils such as chromosomal aberrations or micronucleus induction. Also, there is a lack of studies that would be based on aqueous extracts and study the effect of metals in soils of natural geogenic origin [1]. Many aspects of human activity increase concentration of heavy metals in soils, including smelting of non-ferrous metals, fertilization of soils by wastewater. The *Allium* test has been used for the assessment of remediation quality of contaminated soils [2]. A dose dependent induction was reported in chromosomal aberrations, including micronuclei formation in meristem cells of roots of *Allium cepa* grown in water extracts of solid waste from metal-based industry containing high concentrations of metals [3], melted snow in polluted areas [4], other substances [5, 6].

It is currently well established that the essential micronutrients metal ions for plants are Cu, Zn, Mn, Fe, Co, Mo. However, the plants requirement for the heavy metals such as Pb, Cd, Hg is not yet proved. The significance of Ni, which is an essential element for urease

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activity, has also been established [7]. The effect of Ni for plants has been studied in Ni-enriched serpentine soils on ultrabasic rocks in the context of their toxicity. The excessive concentrations of metals determine the phytotoxicity. The degree of toxicity of heavy metals for plants decreased in the following order: Cu > Ni > Cd > Zn > Pb >Hg >Fe >Mo >Mn [8, 9].

One of the sensitive indicators of phytotoxic effect of heavy metals is inhibition of root growth. We have used the *Allium* root growth test to compare the phytotoxic and cytotoxic effects of heavy metals. The determination of the toxicity expressed as concentration obtained from 50% root growth inhibition is used [10-12]. The Cu compounds were the most toxic as for root inhibition. Frequency of aberrant cells was much higher of Cu treated roots as compared with Cd and Ni.

MATERIALS AND METHODS

Sample Collection: Five soil samples were used for this study. Two samples were collected in the Polar Urals in Yamalo-Nenets Autonomous Area, which is the region of natural geochemical anomaly with ultrabasic rocks and soils enriched by Ni. The order of samples was as following: sample 1-Vizuvshor Creek, sample 2-Makar-Ruz River. The other samples were taken from the soil surface layer (0-15cm) of the Middle Urals, in the impact areas of intensive technogenic pollution near Cu-smelter complex in the Cheljabinsk region, sample 3, (Kyshtym) and Ekaterinburg region, samples 4 and 5 (2 km from SUMZ and directly in the area of SUMZ, respectively). Samples were collected in August 2004.

The feature of toxic effects of this emission comprises coactions of metals-Cu, Cd as well as SO_2 [13]. Sulfur dioxide acidifies the soils and increases the mobility and consequently biological assessability of metal ions and is very toxic to biota. It was shown that mobility of heavy metals in polluted soils near smelter complex increases at the lowest pH value [14].

Soil Chemical Properties: Samples of surface layer of soils from ultramafic rocks and contaminated soil samples were air-dried and passed through sieve with diameter 1 mm. De-ionized water was used to determine water soluble forms of chemical elements in soils (soil-water ratio 1:10). Aqueous suspensions from soil samples were extracted 24 hours after the incubation at 1 hour in a rotary shaker. After filtration through paper filter, solutions were analyzed by atomic absorption spectrophotometry. The concentrations of metals (mg/l) in water solutions were expressed as M. Potentially mobile forms of metals in soils were extracted by 1N HNO_3 for 1 hour in a rotary shaker. Exchangeable forms of Ca and Mg in soils were extracted by ammonium acetate buffer with pH 7.0 [15]. pH of water extracts was measured after 24 hours (soil-water ratio 1:2.5). Loss-on ignition of soil samples was determined at 450°C after 12 hours in a muffle. The results are averages of two replications. Results of chemical analysis were confirmed by the analysis of Certified Reference Material 281 (Trace elements in Rye Grass). For pH measurements we used a calibration with standard reference solutions. The relative standard deviations for pH measurements were <1.5%.

Indicator Plant: The bulbs of common onion *Allium cepa* L. (variety Radar) were used for the assay. The bulbs were grown on filtered tap water for 2 days. Root length of bulbs selected for the experiment was approximately 1.5-2 cm. The three bulbs were treated in each group. They were exposed on water extracts from soils and grown for 30 hours at artificial daylight (day-10h, night-14h) at 21°C. They then were moved to filtered water for a recovery time of 20 and 44 hours. Control plants were grown on filtered tap water.

The following fixation of root tips was used: the first fixation after 30 hours exposition on soil extracts, the second fixation after 20 hours of a recovery time on filtered tap water. It is estimated that it is the time required for duration of one cell cycle at 21°C [16]. The third fixation was performed after 44 hours of a recovery time as previously defined by Ma [17]. Root tips were fixed for 24 hours in aceto-ethanol (glacial acetic acid: ethanol, ratio 3:1). Roots were then stained by Feulgen's reaction with Shiffs reagent. Some experimental groups were stained with DAPI (Serva) [18].

We have analyzed region of meristem zone of approximately 1 mm in length that is known to possess maximum number of mitotic cells. These cells are distant from root tip by 1 mm as reported by Jense and Kavaljan [19] (Fig.1).

The temporary slides were prepared from root tips for microscopic examination. The frequency of micronuclei and other anomalies was expressed in terms of the number of cells per 1000 scored cells resulting from 10 roots for each sample. The mitotic index was determined as number of mitotic cells among the total amount of scored cells and is shown as a percentage. The observations were carried out with a Carl Zeiss light microscope and a LUMAM luminescent microscope.

The results were statistically analyzed with χ^2-test and F-test of analysis of variance (ANOVA) using "Statistica", StatSoft Inc.

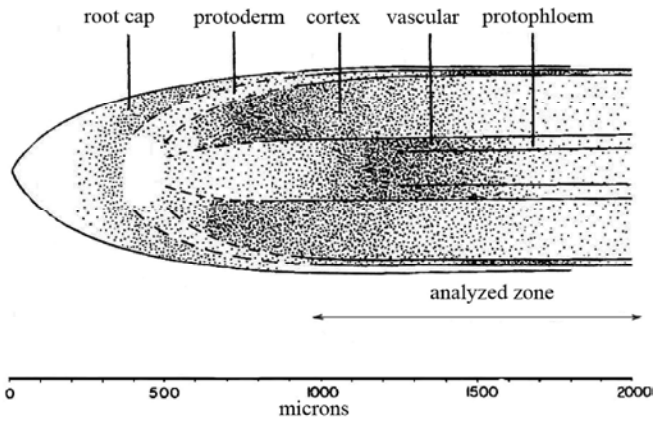

Fig. 1: *Allium cepa* L. root tip anatomy by Jense and Kavaljan [19]. The frequency of dividing cells is indicated by the light intensity.

RESULTS AND DISCUSSION

Chemical and Physicochemical Properties of Soils: The results of chemical analysis of soils tested for heavy metals have revealed elevated concentrations of Ni in the samples 1 and 2 and high concentration of Cu in the samples 3, 4 and 5 in the two types of extracts, acid soluble forms and de-ionized water soluble forms (Table 1). The highest concentration of water extractable Cu-5.7 x 10^{-5} M had sample 5, with lowest pH 4.05, but the highest concentration of potentially mobile of Cu-1170 Mg•g^{-1}-sample 3, possibly due to influence of some different type of anthropogenic pollution. Sample of soil 3 contains 409 Mg•g^{-1} of acid-soluble Pb, whereas soil 4-only 15.5, soil 5-32 Mg•g^{-1} of Pb. Concentration of available Mg and Ca, pH were also higher in this sample of soil 3. Complex nature of contamination indicates presence of water-soluble Cd in soil samples 3 and 5. Sample 2 from the Polar Urals has highest concentration of Ni.

The concentration of water-soluble Ni and Cu in samples of soils ranged widely. It was previously shown that the content of Ni including forms connected with silicates is unavailable for plants [20]. The concentration of water-soluble Ni in soils represents a small part of acid soluble Ni.

It is currently established that mobility of metal depends on the soil acidity and the soil contamination. Soil sample 5 had concentration of water-soluble Cu which was 10 times higher and the lowest pH 4.05, some concentration of Cd and low content of Ni.

There is a proportional dependency of acid soluble potentially mobile and water-soluble forms of Ni. In our experiments, the concentration of water-soluble Ni of soils 1 and 2, 0.34 and 0.93 Mg?ml^{-1} (5.8 x 10^{-6} and 1.6 x 10^{-5} M) was higher than the concentration of Ni in soil solutions of other ultrabasic soil-0.13-0.67 Mg?ml^{-1} (2.2 x 10^{-6}-1.1 x 10^{-5} M) [21]. The root inhibition tests with *Allium cepa* were showed that the Cu^{2+} ions at the concentration 2.7 x 10^{-6} M was the most toxic of the other

Table 1: Concentration of water-soluble (M) and acid-soluble metals in 1N HNO_3 (Mg•g^{-1}) metals in soils

	Chemical element						
	Cu		Ni		Cd		
Soil sample	M	Mg•g^{-1}	M	Mg•g^{-1}	M	Mg•g^{-1}	pH H_20
Control	< 2.4 x 10^{-7}	-*	< 6.8 x 10^{-7}	-	< 4.4 x 10^{-8}	-	6.42
1	6.9 x 10^{-6}	5.5	5.8 x 10^{-6}	254	<<	n.d.	6.38
2	0.94 x 10^{-6}	6.2	1.6 x 10^{-5}	540	<<	n.d.	6.10
3	2.0 x 10^{-6}	1170	<<	18.7	8.9 x 10^{-8}	4.71	7.12
4	5.3 x 10^{-6}	43.7	<<	2.9	<<	0.42	7.65
5	5.7 x 10^{-5}	560	7.0 x 10^{-7}	1.1	2.7 x 10^{-7}	0.90	4.05

*-filtered water was used as a control

<<-below the detection limit Ni-0.4, Cu-0.015-Mg•ml^{-1}, n.d.-not determined.

Table 2: Basic characteristics of tested soils (mean±SD)

	Ca		Mg			
Soil sample	M	meq/100g	M	meq/100g	Ca/Mg	Loss-on-ignition, %
control	<2.4 x10^{-6}	-*	<4.0 10^{-6}	-	-	-
1	2.1 x 10^{-5}	3.1±0.31	6.0 x 10^{-5}	25.7±0.10	0.12	17.4±0.16
2	4.7 x 10^{-5}	5.0±0.20	9.5 x 10^{-5}	47.0±0.08	0.11	26.1±0.44
3	1.8 x10^{-4}	13.2±0.05	1.2 x 10^{-4}	1.8±0.06	7.2	3.68±0.14
4	2.3 x10^{-5}	8.4±0.34	3.8 x 10^{-5}	1.5±0.11	5.7	1.85±0.09
5	2.5 x10^{-4}	11.7±0.01	1.2 x 10^{-4}	1.0±0.02	11.7	9.53±0.13

*-filtered water was used as the control

Influence of soil extracts enriched by metals on root growth and mitotic index

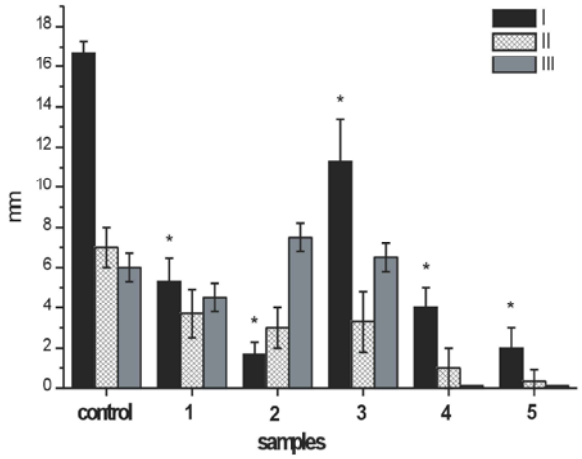

Fig. 2: Root growth (mm) of *Allium cepa* after exposition on water extracts of soils containing Ni and Cu and the different time intervals of recovery growth on tap water
I-after 30 hours of exposition, II-20 hours of recovery è III-44 hours of recovery.
*-statistically significant at p<0.05.

ions (EC_{50}) and the high degree of toxicity at the concentration Ni^{2+} ions at 1.7 x 10^{-5} M [22]. The concentration of Cu in water extract of sample 5 is higher than given concentration, 5.7 x 10^{-5} M.

Thus, the concentration of potentially mobile forms of metals in all tested soil samples is relatively high. The elevated concentrations of water-soluble metals correlate with concentrations of acid soluble forms in all studied soils with the exception of Cd in samples 1 and 2. The background concentrations of Cu, Cd and Ni are much lower than these of the contaminated soils and soils of natural geochemical anomalies [8, 13, 24].

We found that the concentration of exchangeable and water-soluble forms of Ca and Mg in studied soils varied widely (Table 2). In soils from ultrabasic rocks of the Polar Urals the ratio of available Ca/Mg was lower than 1, but this Ca/Mg ratio in Cu-contaminated soils was higher than 1. High concentrations of Ni are important as a primary cause of toxicity of soils on ultrabasic rocks although the significance is also attached to the low Ca/Mg ratio [23]. These features are also typical for soil on ultrabasic rocks in Polar Urals [24].

The tested soils characterized by a different value of loss-on-ignition. This parameter was lower in Cu-contaminated soils (1.85-9.53%) (Table 2). It can be explained of the weak accumulation of organic compounds in the surface layer soils exposed to emission near smelters due to toxicity of Cu for plants and the degradation of soils of these sites.

Our results showed that there is a significant inhibition of the root growth of *A. cepa* L. in all tested soils as compared to control after 30 hours. The intensive root growth was observed on extracts from soil samples 1 and 3 at 30 hours of exposition. The lowest root growth showed samples 2 and 5, indicating the highest concentrations of Ni and Cu. It should be noted that degree of inhibition of root growth correlates with a total effect of unfavorable factors. The low Ca/Mg ratio in soil extracts from ultrabasic rocks one of the main chemical causes of decreased plant growth on these soils besides high content of Ni.

The root growth inhibition correlates with the exposition time on water extracts containing metals and concentration of metals. The root growth of seedlings of *Zea mays* L. had stopped at the concentration of Ni^{2+} 2 x 10^{-5} M within 10 hours [25]. Concentration of water-soluble Ni of soil sample 2 is higher, than that sample 1. The highest concentrations of water-soluble Ni^{2+} 1.6 x 10^{-5} M in soil sample 2 inhibit growth effectively after 30 hours exposition compared with the control growth response than those of the soil sample 1 (Fig. 2).

The strong inhibition of root growth is affected by the excess of Ni in the solutions: the concentration of Ni 1 x 10^{-4} M acted on the reduction of elongation, whereas effect on the division in meristem affected to a lesser degree [26]. The concentrations of Cu^{2+} in the solutions in a range of 1 x 10^{-7}-10^{-5} M led to the root growth inhibition. Also, the limit of tolerance for majority of higher plants is the concentration of Cu^{2+} at 1 x 10^{-6} M [27].

The influence of Cu exerts the most pronounced toxic effect in cells as compared to the action of Ni. It was found that the frequency of chromosomal aberrations even at very low concentrations of $CuSO_4$, 1x 10^{-6} M is considerably increased [12]. The inhibition of growth of roots of *Allium cepa* is expressed to a higher degree at the treatment with $CuSO_4$ [28]. The merisrematic cells were destroyed even at the concentration of 5 x10^{-6} M [11]. The 50% reduction of growth was observed even at the low concentration of Cd-3.1 x 10^{-5} M [22].

The growth of roots showed the reduction in all tested samples after growing on water for 20 hours (Fig.2). The lowest growth response, as defined by increasing root length, was observed in samples 4 and 5 exposed on soil extracts enriched by Cu after growing on water. The decrease of mitotic index cells versus control to 0.66% was detected in roots treated with high concentration Cu of sample 5. The concentrations of water-soluble Cu of sample 5 are comparable with the range of Cu concentrations, which were deleterious influence [11].

The ability of roots to resume the growth after growing for 20 hours on water was found even in roots exposed on soil extract from sample 2 with high concentration of Ni and also the recovery of growth was on sample of soil 1. The strong root growth reduction and loss of ability to resume after 44h on water was showed the sample 4 with the high concentration of Cu and the highest concentration of Cu and Cd of sample 5. The root growth on the water extract of soil sample 2 was higher than that of soil sample 1 possibly due to stronger negative influence of Ni and Cu of soil 1.

As expected, the highest concentrations of water-soluble metals of soils were the most toxic for the root growth. However, we found the resumption of roots growth on samples from water extract of soil with high concentration of Ni 1 and 2 and also on one sample with lowest concentration of Cu. The loss of resumption of the growth was observed in sample with highest concentrations of Cu.

Nuclear Anomalies of Cells: In order to compare toxic effects of soils on meristem cells, we scored the frequency of cells with nuclei anomalies induced by water extracts of soils contaminated by heavy metals at the different recovery time periods, 20 and 44 hours. We analyzed the following parameters: micronucleus (MCN), anaphase and telophase bridges, separated fragments of chromosomes, extrusions, pycnotic degradation of nucleus. Such phenomenon as extrusion of chromatin was described for cells in mitosis and meiosis phases [29]. Phenomenon of pycnotic degradation of nucleus that sign of meristem cells necrosis was found in the interphase cells after the treatment with lethal concentrations of metal compounds as well $CuSO_4$ at the concentration 1 x 10^{-5} M as $CdCl_2$ at the concentration 1 x 10^{-4} M [11]. Nuclei with geterochromatic structure were caused by Cu ions (7.5 x 10^{-6} M) in *A. cepa* root meristem cells [22]. Micronuclei were found in onion root cells after the treatment for 24 hours with solution 5 x 10^{-5} M $CdCl_2$. This effect could be due to the consequence of the laggard chromosomes or decondensation of chromosome fragments [12].

Results of our analyses showed that the total amount of cells with anomalies of all tested soils was significantly increased after growing roots on soil extracts after 20 and 44 hours of a recovery period as compared to control samples (Table 3,4). After 20 hours of a recovery time in samples 1 and 3 were the highest total number of anomalies. These samples were also remarkable of the highest growth in comparison to other samples. The most frequently observed anomalies among of total number were extrusions in samples 1 and 3, while treatment with the highest concentration of Cu resulted in pycnotic degradation in sample 5 (Table 3). The interphase cells in sample 5 had an abnormal appearance with nuclei of a dispersed structure. That is a result of treatment with high concentration of Cu and could be due to an influence of Cd, which was increased in this sample 5. Also, number of cells with anomalies in anaphase and telophase in samples 3 and 4 was significantly higher as compared to a control. The frequency of cells with micronucleus after 20 hours of a recovery time in sample 1 was increased up to 4 times relative to control with significance level at $p<0.17$. There was the 2-fold increase in MCN frequency in sample 5 relative to control.

We found that the total number of cells with anomalies as well as certain types of anomalies after 20 hours of a recovery time was not depended on mitotic index, with exception of samples 3 and 5. High frequency of a number of anomalies in anaphase of sample 3

Table 3: Mitotic index (%) and average number of nucleus anomalies (per 1000 cells) in meristem cells of onion roots, growing 30 hours on water extracts of soils and 20 hours of a recovery time, in parenthesis standard deviation, (SD)

Soil sample	MI	MCN/ 1000 cells	anomalies in anaphase and telophase	extrusions	pycnotic degrada-tions	Total number of anomalies
Control	5.01 (2.1)	0.2 (0.4)	1.2 (1.1)	0.6 (0.9)	0	2.0 (1.4)
1	3.80 (2.5)	0.8 (1.1)[a]	1.8 (1.9)[b]	7.4 (5.5)**	0	10.0 (5.1)**
2	4.64 (1.6)	0	2.0 (2.2)[a]	0.6 (0.8)	0	2.6 (1.9)*
3	5.70 (2.1)	0.2 (0.4)	5.3 (4.2)**	4.6 (5.0)*	0	10.1 (7.7)**
4	4.06 (0.35)	0.1 (0.4)	3.5 (1.6)**	0.8 (0.9)	0	4.4 (1.6)**
5	0.66 (0.98)**	0.4 (0.7)	0.1 (0.4)	0.4 (0.7)	20.5 (24)**	21.4 (25)**

Significant difference by χ^2-test at **-p<0.01; *-p<0.05, [a]-p<0.17, [b]-p<0.26.

Table 4: Mitotic index (%) and average number of nucleus anomalies (per 1000 cells) in meristem cells of onion roots, growing 30 hours on water extracts of soils and 44 hours of a recovery time, in parenthesis standard deviation, (SD)

Soil sample	MI	MCN/ 1000 cells	anomalies in anaphase and telophase	extrusions	pycnotic degradations	Total number of anomalies
Control	5.12 (1.4)	0.2 (0.2)	1.2 (1.1)	0.6 (0.9)	0	2.0 (1.4)
1	3.73 (1.4)	1.3 (1.8)**	0.3 (0.8)	1.9 (2.9)	0	3.5 (3.0)**
2	4.89 (1.7)	0.8 (1.0)[a]	2.3 (1.7)[a]	9.5 (12)**	0	12.6 (12)**
3	6.64 (3.4)	1.0 (1.3)*	1.1 (1.3)[b]	15.1 (7.3)**	0	17.2 (7.4)**
4	2.87 (1.8)	0.8 (1.4)[a]	1.2 (1.0)[b]	12.5 (13)**	0	14.5 (14)**
5	2.95 (0.91)	0.5 (0.5)[a]	0.3 (0.5)	8.7 (7.2)*	0	9.5 (7.2)**

Significant difference by χ^2-test at **-p<0.01, *-p<0.05, [a]-p<0.17, [b]-p<0.26.

correlated with the high mitotic index. The lowest frequency of number of cells with anomalies in anaphase in sample 5 was noticed at the low mitotic index of 0.66% (Table 4). The frequency of the total number of anomalies in cells in this sample correlated with the highest number of cells with pycnotic degradation of nucleus, which is known to be induced by a high concentration of Cu compounds.

Already after 20 hours of a recovery time we observed the highest frequency of cells with micronucleus-0.8 cells per 1000 in sample 1 with Ni. As it is mentioned in literature, MCN in meristem cells can be found of onion roots right away after 24 hours of exposure in solution of $CdCl_2$ with concentration 5 x 10^{-5}M [12]. The lowest total number of anomalies in our case, sample 1, was observed after 44 hours of a recovery time. It can be explained by a movement of cells with anomalies in basal zone of root of the limits of analyzed zone. After 20 and 44 hours of a recovery time, the frequency of cells with extrusions was gradually decreased.

Figures 3 shows damages in mitotic and interphase cells detected in root slides.

After 44 hours of a recovery time, number of micronucleus in all samples exceeded the control 2.5-6.5 times. However, the significant difference was found in sample 1 only, whereas others samples differed from control at p<0.17. The results after 20 and 44 hours of a recovery time showed the increase of frequency of MCN cells in all samples after 44 h.

We also detected a tendency to a decrease of MCN cells in samples with increasing concentrations of Ni (from 1 to 2) and in samples with elevated concentration of Cu (from 3 to 5), where a strong inhibition of root growth took place (Table 3, 4; Fig. 2). It should be noted that number of cells with micronucleus in both samples with enhanced concentration of Ni depends on time and increase from the first (20h) to the second period (44h) of a recovery time. The frequency of MCN cells differed significantly in sample 1 enriched with Ni (5.8 x 10^{-6} M) and was much higher than in a control after 44 hours of a recovery time.

Extrusions were the most prominent type of anomalies observed after 44 hours of a recovery time. In all samples with exception of sample 1, the number of cells with extrusions significantly differed from control samples (Table 4). It should be noted that the number of cells with this type of anomalies for sample 3 increased from 20 to 44 hours of a recovery time.

The differences in frequencies of recorded anomalies for different samples after 20 and 44 hours of a recovery time can be explained by various rates of root growth. We noted that samples which had a significant inhibition of root growth after exposition, samples 2, 4, 5 recovered slowly, whereas samples 1 and 3, which had continued roots growth after exposition, recovered more strongly. The last group of samples is marked by a high number of extrusions, already after 20 hours of a recovery time (Table 3).

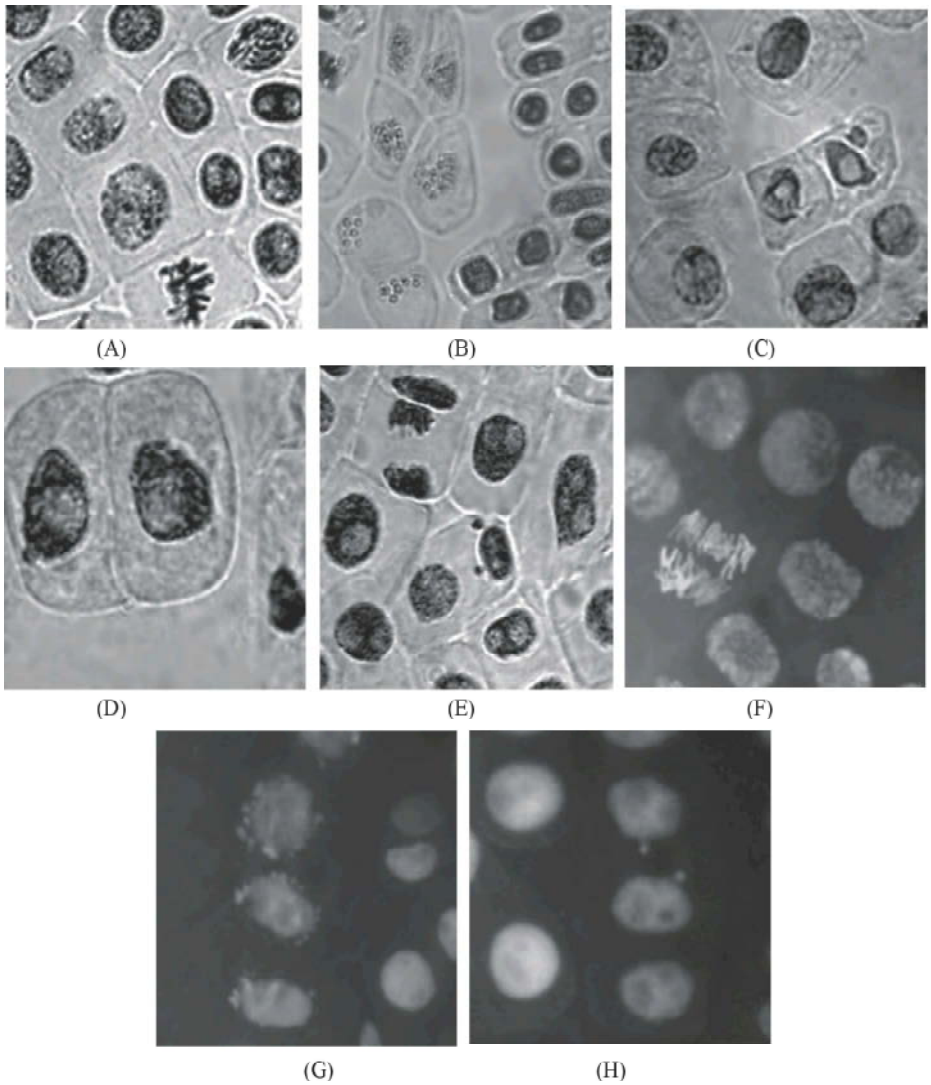

Fig. 3: Influence of growing of *Allium cepa* L. roots on meristem cells by exposure 30 h to soil extracts and 20 hours of a recovery time
a-control; Carl Zeiss light microscope, stained by Feulgen, b-sample 5, pycnotic degradation of nucleus (Ñu 5.7.0 x 10^{-5} M); c-sample 5, micronucleus; d-sample 5, extrusions; e-sample 2, micronucleus (Ni 1.6 x 10^{-5} M);
f-control; LUMAM lumenescent microscope, stained by DAPI; g-multiple micronucleus, sample 1, 30 h exposition without recovery time (Ni 5.8 x 10^{-6} M); h-single micronucleus, sample 3, 30 h exposition without recovery time (Cu 2.0 x 10^{-6} M);

Toxic effect of Cu and Ni ions is caused by the mechanism of their entry and distribution in plant tissues. Niñkel enters in shoots of plants relatively quickly [30]. Ni-hyperaccumulating plants, e.g. genus *Alyssum*, growing on ultrabasic soils of the Polar Urals have an ability to accumulate extremely high concentration of Ni in their leaves [31, 32]. High toxicity Cu correlates with inhibition of active transport of ions in roots and an increase in penetrability of plasmatic membranes [24].

In our experiments with meristem cells of roots of *Allium cepa* L., growing in water extracts of soils with Cu and Ni, ions of heavy metals had a toxic effect leading to formation of nuclei anomalies. High concentrations of Ni ions inhibited roots expansion and let to a drastic decrease of root growth rates, which resumed only after 44 hours of growth in water. High concentrations of Cu ions not only led to the reduction of root growth, but also affected its recovering and inhibit growth of the root completely.

Influence of Cu and Ni ions on root cells of *Aliium cepa* had different aspects. After 20 and 44 hours of a recovery time all samples had the total number of nucleus anomalies significantly higher as compared to control samples. High Cu concentration affected the division of cells and caused the highest number of irregularities related with a process of nucleus destruction. Nickel influence on cells already after 20 hours of a recovery time resulted in a high number of anomalies with prevalence of extrusions. In samples with relatively low Cu concentration, which growth was not inhibited sharply, anomalies in anaphases were the prevalent type after 20 hours.

The number of MCN in meristem zone of roots at different recovery time was variable. Number of cells with micronucleus after 20 hours of a recovery time in sample 1, enriched with Ni, was 4-fold higher as compared to control and in sample 5 with Cu, 2-fold higher. After 44 hours of a recovery time, the main types of anomalies were extrusions and micronuclei. Number of extrusions significantly exceeded control in all samples with exception of sample 1. Number of MCN cells was higher in all samples by 2.5-6.5 folds relative to a control.

In conclusion, high concentrations of Ni lead to decrease of roots growth that was observed after recovery time also and increasing of number of micronucleus. Complex nature of industrial pollution of soils from the Cheljabinsk and Ekaterinburg region with high concentration of Cu was accompanied by the highest number of anomalies in meristem cells of roots of *Allium cepa* with a predominance of extrusions. Concentrations of water-soluble Ni and Cu in soils were more different than other metals. Higher number of anomalies was observed after 44 hours of recovery time, therefore such time can be recommended for fixation of experimental material at concentration of Cu compounds approximately-10^{-6} M, Ni-10^{-5}-10^{-6} M. The number and dynamic of anomalies in meristem zone of root tips of *Allium cepa* correlates with inhibition of root growth, concentration of metals in soils and their toxicity. Concentration of water-soluble Cu in soils near smelter complex "SUMZ", 5.7 x 10^{-5} M, can be qualified as lethal for roots. Water extracts of heavily polluted soils with Cu need to dilute for testing of cytological parameters. The present data show that growth inhibition test should be carried out before test of cytotoxicity.

Allium-test is sensitive method as for testing of soils of natural geochemical anomalies as for monitoring of soils of impact areas of technogenic contamination. Studied soils may have cytotoxical effect on plant species of these sites.

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