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Anila Mesi
Associate Professor
University of Shkodra Luigj Gurakuqi
Albania

Ditika Kopliku
Associate Professor
University of Shkodra Luigj Gurakuqi
Albania

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Athens Institute for Education and Research
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Toxic Potency Evaluation of Metal-doped River Water (Cr, Cu and Pb), on *Allium cepa* L. - An Albanian Case

Anila Mesi
Associate Professor
University of Shkodra Luigj Gurakuqi
Albania

Ditika Kopliku
Associate Professor
University of Shkodra Luigj Gurakuqi
Albania

Abstract

Different natural and anthropogenic sources, have progressively increased metal concentration, posing a serious environmental and ecological concern with regard to bio-accumulation tendency and toxicity.

In the present research the *Allium cepa* L. test was used to assess the toxicological tendency of some surface water samples taken in rivers, Drini and Buna (North Albania) and to evaluate the toxic potency of three metals (copper, chromium and lead) experimentally added in analyzed natural waters. The roots of onion bulbs were grown in river samples mixed with three doses, representing the corresponding ¼ EC_{50}, ½ EC_{50} and EC_{50} concentrations of $K_2Cr_2O_7$, $CuSO_4$, $Pb(NO_3)_2$ salts (EC_{50}-s were evaluated in a preliminary root growth inhibition test for 96 h). Toxicity endpoints on roots exposed to unloaded and metal-loaded samples, such as: length and morphological aberrations, mitotic index, frequencies of micronuclei, chromosomal aberration and types were evaluated and compared.

A. cepa assay exhibited different sensitivities according to water quality of river samples, metals and corresponding concentrations. A certain difference of cytogenetic endpoints between two groups of natural samples was detected. The results showed strong toxic potency of these metals, revealing that metal excess in natural waters as the Drini and Buna Rivers could cause remarkable phyto- and genotoxic effects on onion roots meristem. The most frequent chromosomal abnormalities resulted: c-mitosis, stickiness, bridges/fragments and micronuclei, demonstrating high genotoxic and clastogenic induced effects.

This approach proved to be valuable and appropriate in early warning detection and in bio-monitoring the heavy metal pollution in natural water bodies.

Key words: river water quality, metal pollution, *Allium cepa* test, toxicity, EC_{50}

Introduction

The advent of the industrial era for newer materials has enormously increased the contamination of water, air and soil by heavy metals (Patra *et al*., 2004). Heavy metals are considered the most prevalent and consistent environmental pollutants. Their concentrations can even be enhanced through food chains of terrestrial and aquatic ecosystems, due to bio-accumulation tendency, posing a serious concern. Consumption of food containing high levels of heavy metals may lead to chronic toxicity (Kungolos *et al*., 2006). According to toxic potency, metals can be categorized as: essential and relatively nontoxic (*e.g*., zinc and iron); essential, but extremely toxic at high concentrations (*e.g.*, copper, chromium, cobalt and nickel); non essential but strongly cyto- and genotoxic (*e.g.*, lead, aluminum, arsenic and mercury). Typical symptoms induced by metal excess in plants include: inhibition of photosynthesis, biomass reduction, upsets of mineral nutrition and water balance, hormonal status and membrane structure and permeability (Patra *et al*., 2004; Sharma & Shanker, 2005; Shanker *et al*., 2005; Ahmad *et al*., 2011; Santos & Rodriguez, 2011).

The preservation of fresh water quality is important for the drinking water supply, various water uses and food production. Fresh waters as in rivers and lakes have been reported to be easily contaminated by human activity-generated heavy metals especially in developing countries, showing often no apparent indications on biota (Alloway & Ayres, 1993; Monte Egito *et al*., 2007; Ivanova *et al*., 2008; Žegura *et al*., 2009; Radić *et al*., 2010; Eyckmans *et al*., 2012; Kopliku & Mesi, 2012; Barberio, 2013; Mesi *et al*., 2013). With respect to Albania, Skoulikidis (2009) emphasized that the pollution pressures in the western Balkan basins are less extensive than in the central and eastern ones, with the exception of the Drini River, where mineral mining and processing are intense. The treatment of mining and industrial wastewaters is overall missing, while the whole country faces significant problems in solid waste managing. Moreover different untreated and/or inadequately treated municipal wastes even in large towns as Shkodra (North-West Albania) are directly discharged into surrounding fresh waters, such as in Shkodra Lake and the Buna River (UNEP, 2000).

Chromium, copper and lead are naturally occurring metallic elements of the earth’s crust (Alloway & Ayres, 1993). Globally, Cr is mainly mined in the former Soviet Union, Albania and Africa (Chandrra & Kulshreshtha, 2004; ABIA, 2010), while Pb is usually found in ore with zinc, silver and (most abundantly) copper, and is extracted together with these metals. Locally Cr and Cu mines are mostly situated in north and north east Albania, close to the Drini River basin. As some of the most widely used metals in the world, Cr, Cu and Pb pollution is mainly related to anthropogenic activities including mining, smelting, agrochemicals, industrial and automobile emissions. By the early 1990s, the worldwide annual release of Cu and Pb had reached 954,000 and 796,000 tons, respectively (Alloway & Ayres, 1993), while 112,000 tons of Cr was discharged annually into the world’s aquatic ecosystems (Ahmad *et al*., 2011).

Considering the effects of these metals on plants, animals, and humans, it is important to monitor their presence in the environment through bio-assays. The relationship between increasing environmental concentration of heavy metals and the inducement of genotoxicity and carcinogenicity has been investigated by many eco-toxicological studies (Kungolos *et al*., 2006; Žegura *et al*., 2009). In this context, higher plant-based assays have shown to be quick, simple, reliable, less expensive and similar in chromosome morphology and sensitivity to mammals, making them ideal for risk assessment of potential environmental mutagens and even good predictors of carcinogenicity (Grant, 1999; An, 2006; Barberio, 2013). *A. cepa* L. (2n=16) is a widely used bio-test, due to the high reaction of genetic material to hazards present in natural and drinking waters, different industrial effluents and experimental solutions (Monte Egito *et al*., 2007; Leme & Marin-Morales, 2009; Radić *et al*., 2010). The *Allium* test has been successfully applied to assess the induced toxicity of heavy metals through multiple endpoints, such as: growth inhibition, nutrition uptake and genotoxicity (Fiskejsö, 1988; Wierzbicka & Antosiewicz, 1993; Liu *et al*., 1995; Palacio *et al*., 2005; Espinoza-Quiñones *et al*., 2007; Kopliku & Mesi, 2013; Mesi & Kopliku, 2013).

In the present study the *Allium cepa* L. bio-assay was used to evaluate the eco-toxicological tendency of some riverside water samples collected from the Drini and Buna Rivers and to assess the phytotoxic and genotoxic potency of chromium, copper and lead, experimentally added in analyzed natural waters.

Materials and Methods

Sampling Collection

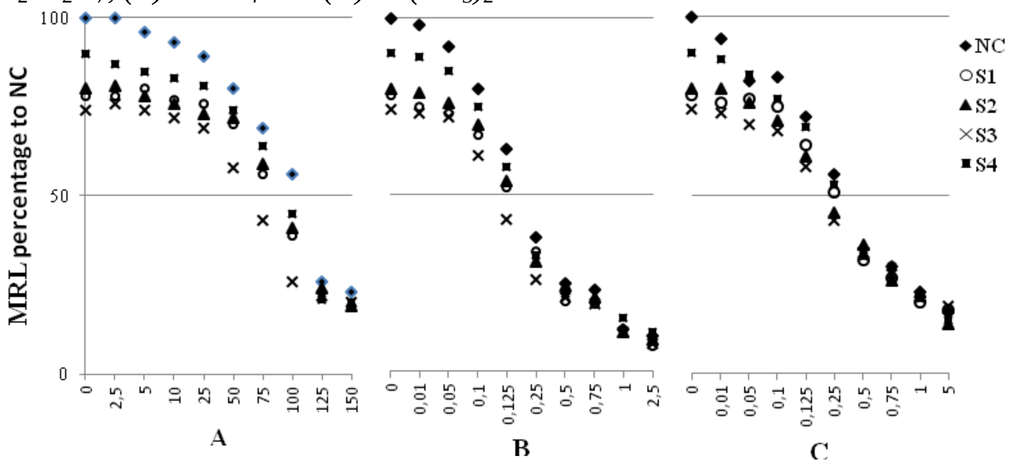
The sampling of surface river waters was done during April 2013. The monitoring stations were as follows: Fierza (S_1) - HPP Fierza Lake down streaming Drini River, collecting mostly effluents of chromium, copper and nickel/iron reserves and mine industry; Bahçellek (S_2) - Drini River, down streaming closed to Shkodra town and containing municipal sewage water; Zues (S_3) – confluent point of the Buna and Drini Rivers, containing runoff from soil contaminated by agricultural practices and municipal and touristic waste water; the Buna River mouth (S_4) – the mouth of Buna River to the Adriatic Sea, accumulating leaching from soil contaminated by agricultural use (Fig. 1). Drinking water was used as negative control (NC).

A. cepa Test Procedure for the Evaluation of Metal Toxicity Concentrations

The *Allium cepa* L. (2n = 16) test was performed using the method described by Fiskesjö (1988). Heavy metal's EC_{50} endpoint (effective concentration of different chemicals and mixtures, permitting 50% growth of the sample under study in relation to control) of all water samples were assessed by using $K_2Cr_2O_7$, $CuSO_4$ and $Pb(NO_3)_2$ salts and a preliminary *A. cepa* root growth inhibition test for 96 h. Nine definitive concentrations of each salt in tap water: 0.25-15 mg/L for Cr salt and 0.01-2.5 mg/L for Cu and Pb salts, were chosen. EC_{50} values were statistically evaluated by plotting on graphs the mean root length values as a percentage to NC against concentrations. The polynomial equations (order 3), which had the biggest R^2 value, were chosen for this evaluation. Three replications were done. EC_{50} values of loaded water samples resulted as illustrated in Figure 2 and summarized in Table 1. After that drinking and river waters were mixed again with the respective salt solutions to be diluted in their corresponding ¼ EC_{50}, ½ EC_{50} and EC_{50}.

Figure 1. *Map of Sampling Points Down Streaming Drini and Buna Rivers and Mine Sites in North Albania. S_1-S_4 - River Water Sampling Points*

Figure 2. *Statistical Evaluation of EC_{50} Endpoint per Each Salt-doped Water Sample using a Preliminary Growth Inhibition Test of A. cepa Roots: (A) $K_2Cr_2O_7$, (B) $CuSO_4$ and (C) $Pb(NO_3)_2$*



MRL-mean root length; NC-negative control; S_1-S_4 – river water samples

Table 1. *Summary of EC_{50} Values of Salt-doped Water Samples*

EC_{50} mg/L	Samples				
	NC	S_1	S_2	S_3	S_4
$K_2Cr_2O_7$	10.11	8.34	8.56	6.98	8.92
$CuSO_4$	0.190	0.128	0.132	0.118	0.161
$Pb(NO_3)_2$	0.34	0.27	0.23	0.20	0.31

NC-negative control; S_1-S_4 – river water samples

Morpho- and Cytogenetic Analysis of A. cepa Roots and Statistical Analysis

The assessment of phyto- and genotoxic effects on *A. cepa* roots of the three salt doses ($K_2Cr_2O_7$, $CuSO_4$, $Pb(NO_3)_2$), representing the corresponding ¼ EC_{50}, ½ EC_{50} and EC_{50} of each water sample was done. The toxic tendency of the river samples was evaluated as well. The microscopic investigation was done after 48 h. Root tips (10 mm) taken from 5 bulbs randomly chosen in each series, were placed on slides and the terminal root tips (1-2 mm) were cut off and used for further preparation of microscopy slides. Five slides per each treatment were prepared in accordance with the standard procedure for Feulgen staining of squashed material. Cytogenetic endpoints in root meristem, such as: mitotic index (MI), frequencies of interphase cells with micronuclei (MNC) and aberrant mitotic cells (FAC) with chromosome aberrations (CA), were observed and evaluated. By scanning slides, data related to MI and FAC were collected on a number of dividing cells out of 1000 cells per slide, while for the formation of micronuclei and their frequency (MNC) at least 1500 interphase cells were counted (Ma *et al.*, 1995). All parameter values have been scored as a percent ratio. Root morphological aberrations were observed after 96 h.

Analysis of Variance (One-way ANOVA) and post-hoc Student Newman-Keuls (SNK) tests were used to test for significant differences of all evaluated parameters. All the results were expressed as the mean of three replicates per sample ± standard deviation (SD). Parameter differences between exposure treatments and corresponding NC were considered statistically significant at level 5%.

Results

The present study was performed to evaluate the eco-toxicological potency of some metal (Cr, Cu and Pb)-enriched surface waters collected from two rivers (in North Albania) by using *Allium cepa* bioassay. Figure 2 and Table 2 represent all data related to the morphological and cyto-genetic analyzes of *A. cepa* roots, exposed to unloaded and salt loaded water samples ($K_2Cr_2O_7$, $CuSO_4$, $Pb(NO_3)_2$). The results showed a certain sample and dose-dependence of all screened endpoints compared to unloaded negative control (using ANOVA and SNK tests). Moreover all river samples induced similar adverse effects with corresponding loaded NC at the highest concentrations of chosen salts.

Table 2. *Cyto- and Genotoxic Effects Induced by Unloaded and Metal-loaded Water Samples (at ¼ EC_{50}, ½ EC_{50} and EC_{50} of $K_2Cr_2O_7$, $CuSO_4$ and $Pb(NO_3)_2$ Salts) on A. cepa Root Meristem.*

Samples			MI (%)	MNC (%)	FAC (%)
Unloaded		NC	14.52	0.07	2.7
		NS	10.93-12.92	0.09-0.14	2.9-8.0
$K_2Cr_2O_7$ (mg/L)	**1/4 EC_{50}**	NC	13.07	0.09	4.6
		NS	10.45-11.61	0.11-0.15	6.1-9.1
	1/2 EC_{50}	NC	10.89	0.14	9.2
		NS	9.73-10.81	0.15-0.20	9.9-12.7
	EC_{50}	NC	6.22	0.21	13.1
		NS	6.39-7.41	0.22-0.25	13.5-14.6
$CuSO_4$ (mg/L)	**1/4 EC_{50}**	NC	13.50	0.10	4.9
		NS	10.69-11.91	0.12-0.16	6.5-9.4
	1/2 EC_{50}	NC	11.47	0.15	10.0
		NS	10.02-10.85	0.16-0.22	10.6-13.7
	EC_{50}	NC	8.28	0.23	14.1
		NS	7.11-8.42	0.25-0.28	14.4-15.8
$Pb(NO_3)_2$ (mg/L)	**1/4 EC_{50}**	NC	13.79	0.11	3.5
		NS	11.28-12.31	0.14-0.20	4.4-8.3
	1/2 EC_{50}	NC	11.76	0.19	6.8
		NS	10.31-11.62	0.22-0.27	6.7-9.4
	EC_{50}	NC	8.13	0.29	9,9
		NS	7.38-8.57	0.27-0.32	10.5-11.4

NC-negative control; NS-natural samples; MI-mitotic index; FAC-frequency of aberrant cells with chromosome aberrations; MNC- frequency of micronucleated interphase cells

Root growth inhibition is randomly used to evaluate the phytotoxic effects of pollutants. Related to root growth as the combination of cell division and elongation, reduction of length over 55% and the decrease of roots number are strong indexes of rhizotoxicity caused by most heavy metals (Fiskesjö, 1988). Metal-doped tap water samples strongly affected *A. cepa* root growth at 10.11, 0.190 and 0.34 mg/L concentrations (respective EC_{50}-s of tested Cr, Cu and Pb salts), being in agreement with former findings (Fiskesjö, 1988; Liu *et al.*, 1995; Espinoza-Quiñones *et al.*, 2007; Kopliku & Mesi, 2012; Kopliku & Mesi, 2013; Mesi & Kopliku, 2013). Moreover, these effective concentration values (used as phytotoxicity thresholds) should be decreased by a factor of: 1.21, 1.18, 1.44 and 1.13 ($K_2Cr_2O_7$); 1.48, 1.43, 1.61 and 1.18 ($CuSO_4$); 1.26, 1.47, 1.70 and 1.10 ($Pb(NO_3)_2$) to obtain the same inhibition effect corresponding to S_1-S_4 natural samples, respectively. As suggested by Palacio *et al*. (2005) this factor could be used as an indicator for quality assessment in polluted river water.

The morphology of *A. cepa* roots grown in unloaded water samples was more or less normal, except for slight bending and sporadic stunted roots detected in S_2 and S_3 samples. Observations of metal-induced toxic effects revealed structural damage, which varied across tested water samples, salts and concentrations. Wholesale root bending was detected at ¼ EC_{50} Cr- and Cu-loaded S_3 samples, while disorientated root bending started in all ½ EC_{50} metals-loaded samples, except in S_4 and tap water (at EC_{50}). Root stunting and stiffing, color changing (particularly by Cu and Pb) and reduction of root number per bundle, as general symptoms of heavy metal toxicity (Fiskesjö, 1988; Liu *et al.*, 1995), were mostly recorded in onion bulbs exposed to all EC_{50} loaded water samples.

The reduction of mitotic activity in root meristem is a general phenomenon associated with most of the heavy metals. A decrease below 50% of MI (compared to control) inducts sublethal effect, while below 22% it causes lethal effect on test organisms. These effects are probably due to either disturbances in the cell cycle or chromatin dysfunction induced by pollutant–DNA interactions. Concerning unloaded river samples, the cytological analyses of exposed root meristem (Tab. 2) showed that only S_1 and S_3 could significantly reduce the mitotic index, compared to NC (respectively 23 and 25% of NC, $P<0.05$ using ANOVA test). On the other hand data revealed a rapid and significant decrease of MI through all metal-loaded samples and salts compared to unloaded NC ($P<0.05$ and 0.001). The cytotoxic effects of salts resulted particularly strong at ½ EC_{50} and EC_{50}, decreasing the MI parameter respectively: 67 (S_3) – 74% (S_4) and 44 (S_3) – 51% (S_2) for $K_2Cr_2O_7$; 69 (S_3) – 75% (S_4) and 49 (S_3) – 58% (S_4) for $CuSO_4$; 71 (S_3) – 80% (S_4) and 51 (S_2) – 59% (S_4) for $Pb(NO_3)_2$. The highest depressed cellular proliferation was induced by EC_{50} Cr- and Cu-doped S_3 river samples, with demonstrating even sublethal activity against treated onion roots. The results fitted well the above mentioned phytotoxic effect on onion roots, proving that reduction of length was a consequence of mito-depressive activity of metals.

Genotoxicity assessment is necessarily requested in eco-toxicological studies, since the genotoxic effects, mainly during chronic exposure, are developed in lower concentrations than toxic ones. In the present investigation the metal-induced genotoxic effects were assessed by evaluating the frequencies of abnormal cells with chromosomal aberrations (FAC) and micronuclei (MNC) in root meristematic cells of *A. cepa*. The rate of aberrant dividing cells resulted significantly higher than corresponding NC ($P<0.05$) in S_1, S_2 and S_3 unloaded samples. Moreover, all tested doses of salt-loaded samples increased the frequency of chromosome aberrations, demonstrating a positive dose-effect relationship, as well (Table 2). FAC values significantly exceeded the corresponding unloaded NC (of 2.7%) by: 2.3 (S_4) - 5.4 (S_2), 2.4 (S_4) - 5.9 (S_3) and 1.6 (S_4) - 4.2 (S_2) folds across concentration and river sample treatments, respectively for $K_2Cr_2O_7$, $CuSO_4$ and $Pb(NO_3)_2$ salts ($P<0.05$ and $P<0.001$).

Additionally, all metal salts could induce a wide spectrum of chromosomal aberrations (CA), varying in a certain dose- and metal-dependent manner. The most frequent CA types observed in this study were: c-mitosis, stickiness, bridges involving one or more chromosomes, fragments, laggard and vagrant chromosomes, demonstrating both aneugenic and clastogenic activity of chromium, copper and lead on onion roots. There was a drastic increase of physiological (stickiness and c-mitosis) and clastogenic (bridges and fragments) aberrations at the highest concentrations of each metal-doped sample. Polyploidy, bi-nucleated cells, irregularly shaped nuclei, disintegrated nuclei and disrupted nuclear membranes had a lower frequency and were mostly observed at ½ EC_{50} and EC_{50} metal-loaded samples. C-mitosis was due to spindle failure, while stickiness, bridges and fragments were due to chromatin dysfunction. Stickiness is considered a common sign of highly toxic effects on chromosomes, an irreversible type, probably leading to cell death, reported in *Allium* root cells after treatment with various heavy metals (Fiskesjö, 1988). Meanwhile chromosome bridges are probably formed by chromosome and/or chromatid breakage and fusion (Leme & Marin-Morales, 2009). Concerning c-mitosis, Liu *et al.* (1995) suggested that metal excess decreases the tissue distribution of Ca^{2+} ions by displacing them from exchange sites, preventing as a consequence, calmodulin (CaM), to activate the key enzymes of mitotic spindle which in turn leads to the disturbance or inhibition of mitosis.

Micronuclei formation (MNC) is considered an important cytogenetic endpoint of observed toxicity by indicating the level of accumulated genetic damage during the cells cycle. MNC often results from the acentric fragments or laggard chromosomes that fail to be incorporated into the daughter nuclei during the telophase stage and can cause cellular death due to the deletion of primary genes. MN formation is considered a true indicator of mutagenic effect (Ma *et al.*, 1995). As noted in Table 2, there was a relatively high MNC frequency in root cells treated with S_2, and S_3 unloaded river samples (significant compared to respective NC of 0.07%, $P<0.05$). In addition, there was a significant increase ($P<0.05$ and 0.001) of MNC frequency through whole salt-loaded samples exposure, resulting in: 1.6 (S_4) - 3.6 (S_2) by $K_2Cr_2O_7$, 1.7 (S_4) - 4 (S_2) by $CuSO_4$ and 2 (S_4) - 4.6 (S_2) by $Pb(NO_3)_2$) folds higher than unloaded NC.

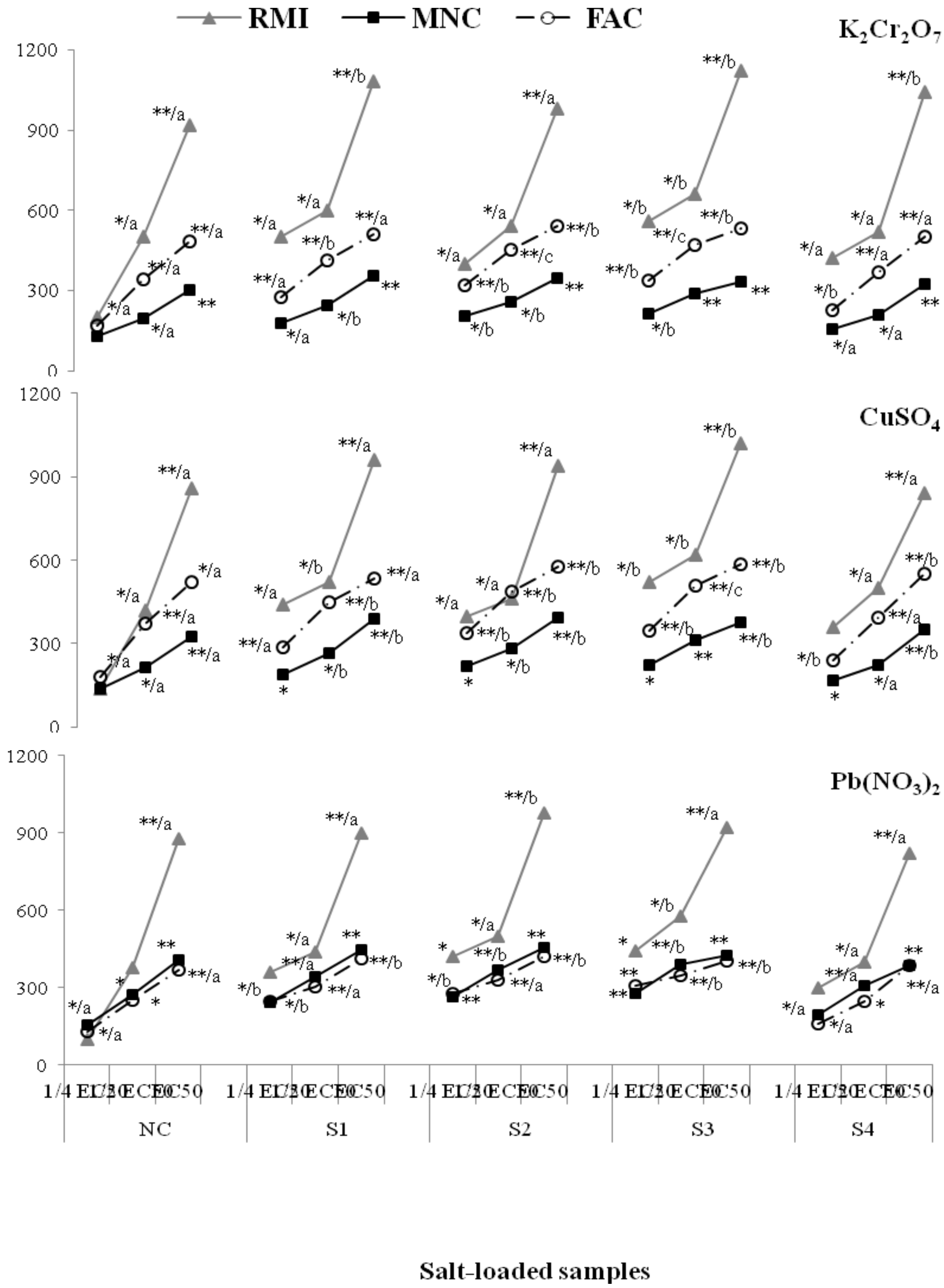
Discussion

The results of the present study indicated really strong and cumulative phyto- and genotoxic activity of all investigated metals. The observed inhibition of root growth might be due to metal ions interference with meristematic cell cycles, including inducement of abnormal mitosis, chromosomal aberrations and micronuclei.

Figure 3 illustrates the relationship between cyto- and genotoxic effects induced by tested metal salts and river water samples on *A. cepa* root tip meristem according to selected loaded concentrations (¼ EC_{50}, ½ EC_{50} and EC_{50}). Based on the data, metal-induced genotoxic effects (evaluated as frequencies of chromosome aberrations and micronuclei) appeared most positively depending on concentrations. Unlike phytotoxic effects, induced genotoxicity was significantly different to NC ($p<0.05$) at the lowest tested dose of the three salts (¼ EC_{50}). The toxicity range of analyzed metals in this investigation descended in the order: Cr > Cu > Pb with the respect of cytotoxicity and Cu > Cr > Pb for genotoxicity. Excluding Pb, the range of genotoxic effects (expressed as FAC) exerted by Cr and Cu salts on onion root cells were greater than for mutagenic ones (as MNC) irrespective of treatment concentration. Our findings on *A. cepa* roots following the treatment with these metals generally confirmed the statements from related studies with different plant bioassays (Liu *et al*., 1995; Patra *et al*., 2004; Palacio *et al*., 2005; An, 2006; Mahmood *et al*., 2007). Fargašová (2004) and Gomes (2011) emphasized that even though Cu and Cr accumulation in roots is lower than for Pb, their unfavorable toxic effects on root growth are much stronger. According to the differences of the action mechanism on genetic material between these metals, Wise *et al*. (2008) hypothesized that chromium reacts directly with DNA, causing DNA damage by forming various adducts. Meanwhile other metals such as copper and lead mostly act through the inhibition of DNA repair machinery (Santos & Rodriguez, 2011).

The biological (morpho-, cyto- and genetic) effects on *A. cepa* roots observed in the current investigation appeared related to the physical and chemical characteristics of respective analyzed natural water bodies. (Bushati *et al*., 2012). The most polluted samples resulted in Zues (S_3, union point of Drini and Buna Rivers) and Bahçellek (S_2, Drini River) which are the nearest sampling points to Shkodra city, while Fierza (S_1, upstream of Drini River and the nearest point to mines) induced mostly lower toxic activity. Despite the possible impact of agricultural practices and successive flooding during 2010-2012 in Nën-Shkodra lowland, the Buna River mouth sample (S_4) showed in general a good water quality. As summrised in Table 2, a certain positive correlation between MI and FAC was observed: unloaded samples that did not induce any obvious citotoxic effects (drinking water and river mouth), did not produce significant genotoxic effects, as well. Likewise Zues and Fierza induced simultaneous cyto- and genotoxicity. The distinct cyto/genotoxic effects caused by the Zues sample may be due to the interfering of pollutants from water bodies of Buna (flowing out from Shkodra Lake) and Drini Rivers. In the Bahçellek sample the recorded cytotoxicity was not positively correlated to the corresponding genotoxicity. The relatively high mitotic activity of root cells grown in this sample might be due to temporary stimulating effects of nitrate, nitrite, ammonium and phosphate on the proliferation of onion root-tip cells, because of the abundant discharge of municipal and tourism wastes. Based on this data, a certain amelioration of river water quality down was noted in the downstream Nën-Shkodra lowland, probably due to the self-regeneration of the Buna River. This phenomenon has been screened by physical-chemical and microbiological analyses, as well (Bushati *et al*., 2012). Additionally, *A. cepa* test exhibited different sensitivities following treatment even with metal-doped river waters. On the basis of phytotoxicity parameters, the samples could be arranged in the rank order as follows: $S_3 > S_1 > S_2 > S_4$ for Cr^{6+}/Cu^{2+} and $S_3 > S_2 > S_1 > S_4$ for Pb^{2+} ions. Concerning the induced genetic damage, the increasing order of samples had the same results for all analyzed metals: $S_4 < S_1 < S_2 < S_3$ (Fig. 2, 3 and Tab. 1, 2).

Figure 3. *Relationship between Cyto- and Genotoxic Effects (Expressed in Percentage to Corresponding NC-s) Induced by Selected Concentrations (¼ EC_{50}, ½ EC_{50} and EC_{50}) of Cr (VI), Cu and Pb salts on A. cepa Root Tip Meristem.*



Means labeled with asterisks and letters are significantly different respectively from NC according to One-Way ANOVA test (* $P<0.05$; ** $P<0.001$) and between samples at the same treatment concentration in SNK test (a, b $p<0.05$). NC-negative control; S_1-S_5 – lake surface and waste water samples; RMI-reduction of mitotic index; FAC-frequency of aberrant cells with chromosome aberrations; MNC-frequency of micronucleated interphase cells.

Physicogeographic and hydrochemical conditions differ substantially with respect to the basins of the rivers under study. The Buna River flows out from Shkodra Lake, traversing all Nën-Shkodra lowland with slow effluence due to the small declivity. The Drini River comes down from North-East Albania, a craggy mountainous and erosion suffering region, where chromium, copper and nickel mines are mostly situated. Both rivers flow together into the Adriatic Sea. The rich hydrological network coupled with climate conditions and water flow result in this area being potentially affected by flooding (Fig. 1). Over the last decades, fluctuated precipitation and mismanagement due to administrative and structural constraints, poor environmental planning, inspection and awareness, have imposed significant pressures on the rivers under study. The treatment of municipal wastewaters, mining and industrial effluents is insufficient and has caused a dramatically uncontrolled discharge of hazardous pollutants into water bodies. In general, lowland river sections have been hydro-morphologically modified and are at the greatest pollution risk, significantly affecting the function of food chains in general and the economic sectors, such as agriculture and fisheries. Meanwhile, upstream areas mostly retain their natural conditions. The water bodies are randomly used as irrigation sources and in many cases as drinking and house hold waters, so the chemical water quality assessment and periodic bio-monitoring is of critical importance for population health, as well.

Conclusions

The present eco-toxicological study provided preliminary comparative information about the toxicity induced by the short-term exposure of *Allium cepa* roots to the Drini and Buna River samples experimentally enriched with chromium, copper and lead. The *A. cepa* test demonstrated different sensitivities, showing some kind of correlation to river water quality. The evaluated genotoxicological endpoints in onion root cells were generally more sensitive than the phytotoxic counterparts. The more pronounced phyto- and genotoxic effects of the Bahçellek and Zues samples reflected the complex nature of the potential pollution present in these fresh waters. The recorded differences in the potential toxicity of Cr, Cu and Pb should be taken into account in the biomonitoring and ecological risk assessment of this study area. The results should serve as an indicator of mutagenic and clastogenic impact that biota and human health may incur by natural and anthropogenic heavy metals discharge in water bodies, especially in developing countries such as Albania.

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