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Potential Toxicity Investigation of Copper -Doped River Water of Nën-Shkodra Lowland (Albania) on a Plant Bio-test

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Abstract: Various natural and anthropogenic copper sources have progressively increased its environmental concentration, getting a hazardous threat to plant, animal and human health due to bio-accumulation tendency and toxicity. In the present investigation the *Allium cepa* L. test was used to assess the toxicological tendency of some riverside water samples taken in Nën-Shkodra lowland (Albania) and to evaluate the toxic potency of copper, experimentally added in analyzed natural waters. Roots of onion bulbs were exposed for 48 h to three doses, representing respective ¼EC_{50}, ½ EC_{50} and EC_{50} of $CuSO_4$-loaded samples. Macro and microscopic endpoints of onion roots grown in unloaded and copper-loaded samples, such as: morphological aberrations, mitotic and phase indexes, interphase nuclear volume and DNA content, chromosomal aberration frequency and types were evaluated and compared. There was a distinct difference of toxicity rate between two groups of natural samples. The results showed obvious metal concentration-dependence of all parameters, revealing that excess copper can cause strong phyto-, cyto- and genotoxic effects on onion roots. Chromosome stickiness, bridges and fragments, c-mitosis and disintegrated nuclei were mostly detected. This approach resulted to be successfully applicable for biological monitoring of water pollution, especially in developing countries as Albania.

Keywords: water pollution, *Allium cepa* test, copper, phytotoxicity, genotoxicity, nuclear DNA content

Introduction

The water quality assessment is closely related to the application of monitoring and remediation projects which aim to reduce the risk that aquatic ecosystems incur by hazardous substances. Over the last few decades, contamination of water bodies by heavy metals has increased drastically (Kovalchuk *et al.*, 2001; Albanis *et al*., 2009). Heavy metals like copper, lead, cadmium, manganese, zinc, nickel, etc., are a reliable index of environmental pollution, although in low concentrations some of them are vitally important elements.

Copper is a naturally occurring metallic element of earth's crust. It is an essential micronutrient for all living organisms required by a number of enzymes involved in specific oxidase type reactions, but it is extremely toxic at elevated concentrations, causing deleterious effects both morphologically and physiologically. Various anthropogenic sources, including industrial (smelting and refining) and urban wastes, mine drainage, agricultural practices (copper-based pesticides), antifouling paints and atmospheric emissions, have contributed to a progressive increase of copper concentrations in several environments (Andrade *et al.*, 2004). Since 1990s, the worldwide annual discharge of copper had reached 954,000 tons (Kovalchuk *et al*., 2001), being a potential threat to biota and human life due to its bio-accumulation tendency and bio-availability, which may lead as a consequence to mutagenicity and carcinogenicity.

Evaluation of contamination degree in aquatic environments should not be based only on physical and chemical characterization but it should be combined with biological assays (Fiskesjö, 1988; Kuzmanov & Ivanova, 2006). Bio-assays can assess the potential toxic effects from all the water components (including those due to unknown substances and their synergic, antagonistic or additive effects) and allow an integrated evaluation of the impact in populations and communities. Higher plants, providing an appropriate genetic system, have been widely used as biomonitors and bioindicators of environmental contaminants (Grant, 1994). *Allium cepa* L. is considered to be suitable for *in situ*

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monitoring, to detect the mutagenic potency of heavy metals and other chemicals, helping even in cancer research due to the good correlation with mammalian bio-tests (Fiskesjö, 1988).
The *Allium* test has been recently introduced in Albania to screen the waters quality of Shkodra region (Mesi *et al*., 2011; Kopliku & Mesi, 2012; Kopliku *et al*., 2012; Mesi *et al*., 2013). Shkodra region (North-West Albania) is distinguished for a unique and complicated water system, hydrologically and ecologically interdependent, which includes: Shkodra Lake (a transboundary lake with Montenegro), Buna and Drini Rivers and many streams. Nën-Shkodra lowland is part of Shkodra region, located between Shkodra city and Adriatic Sea. The hydrological network of this important agricultural area is particularly rich because of the climate conditions and water flow, getting it potentially affected by flooding. Buna River flows out from Shkodra Lake, traversing all the lowland with slow effluence due to the small declivity. Meanwhile Drini River comes down from North-East Albania, a craggy mountainous and erosion suffering region, where are situated most part of copper, chromium, iron and nickel mines (Fig. 1). Reports of recent chemical analyzes have shown significant increase of heavy metals and other pollutants concentration in Drini and Buna water bodies and sediments (UNEP, 2000; Neziri & Gössler, 2006).

In this study the *Allium cepa* L. bio-test was used to evaluate the toxic tendency of some riverside water samples collected in Nën-Shkodra lowland (North-West Albania) and to assess the phytotoxic and genotoxic potency of copper, experimentally added in analyzed natural waters.

Material and Methods

Sampling collection

Sampling was done during March 2013. Surface water samples were collected from: (1) Bahçellek (Drini River, the nearest point to Shkodra city); (2) Zues (union point of Drini and Buna rivers), (3) Oblikë, (4) Dajç and (5) Pentar (located down streaming Buna River along Nën-Shkodra lowland agricultural area); (6) Delta (Buna river mouth to Adriatic Sea). (Figure 1) Tap water was used as negative control (NC).

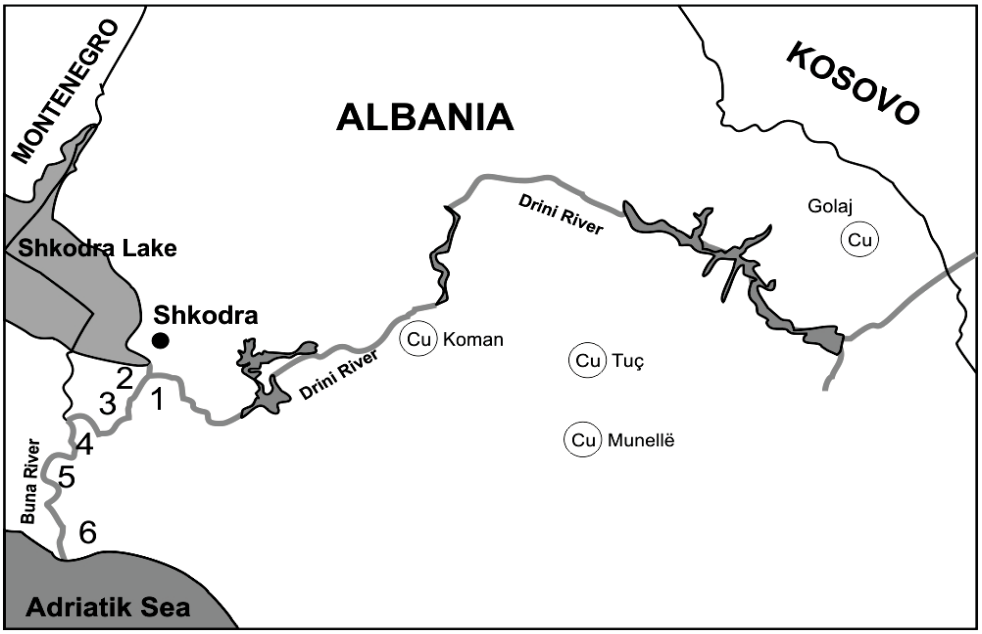

Figure 1. Map of water sampling points in Nën-Shkodra lowland and copper mine sites in North Albania (*Notes: 1-6 – sampling sites: 1-Bahçellek, 2-Zues, 3-Oblikë, 4-Dajç, 5-Pentar, 6-Delta; Cu – copper mine sites*)

Biological material and test procedure

The *Allium cepa* L. test was performed second the method of Fiskesjö (1988). Equal-sized bulbs (3-4 g) of common onion were grown and observed in the laboratory. All experiments were set up in a completely randomized design with twelve test tubes per sample. On top of each test tube one onion bulb has been put with the root primordia downward in the liquid.

Preliminary range of toxic copper concentrations finding by using Allium test to determine EC_{50} endpoint

EC_{50} endpoint (effective concentration of different chemicals and mixtures, permitting 50% growth of the sample under study in relation to control) of Cu-loaded tap water and natural samples were assessed by using $CuSO_4$ salt and a preliminary growth inhibition test of *A. cepa* roots for 96 h. Nine definitive concentrations of Cu salt (0.01-2.5 mg/L), were chosen. A set of 1000 mg/L $CuSO_4$ standard solutions was prepared in the laboratory. To obtain the test solutions loaded with metallic ion, drinking and river water samples were mixed with the standard solutions, diluted in chosen treatment concentrations. EC_{50} values were statistically evaluated by plotting on graphs mean root length values as percentage to negative control against concentrations. The polynomial equations (order 3), which had the biggest R^2 value, were chosen for this evaluation. Three replications were done and as illustrated in Figure 2, the EC_{50} values of loaded samples resulted: 0.12 mg/L (Bahçellek), 0.114 mg/L (Zues), 0.14 mg/L (Oblikë), 0.124 mg/L (Dajç), 0.15 mg/L (Pentar), 0.154 mg/L (Delta) and 0.17 mg/L (tap water). After that drinking and river waters were mixed again with the standard solutions, diluted in corresponding ¼ EC_{50}, ½ EC_{50} and EC_{50}.

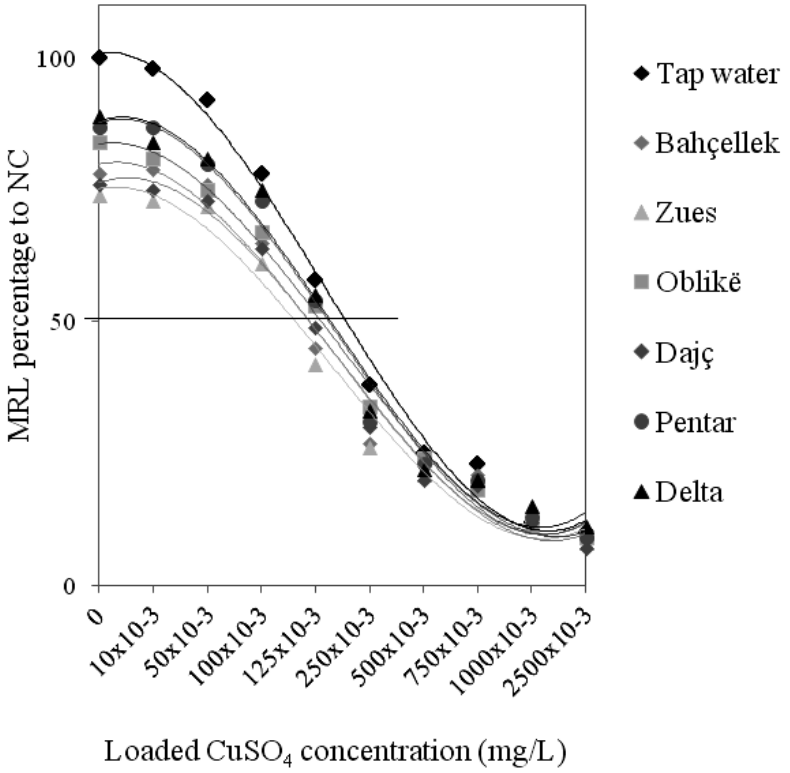

Figure 2. Assessment of EC_{50} endpoint per each Cu salt-loaded water sample (tap water and six natural water samples) by using a growth inhibition test of *A. cepa* roots (*Notes: MRL-mean root length; NC-negative control*)

Morphological and cytogenetic analysis of A. cepa roots

The phytotoxic and genotoxic potency of Cu and the toxic tendency of natural samples were assessed by measuring and comparing some macro/microscopic endpoints in *A. cepa* roots, such as root morphological aberrations and EC_{50}, mitotic and phase indexes (MI and PI), interphase nuclear volume (INV), nuclear DNA content (DNAC), frequencies of aberrant cells with chromosome aberrations (FAC) and chromosome aberration types (CA).

Microscopic investigations were done under an optic microscope Leitz-Diaplan using a 500x oil-immersion lens. Microscopy slides were prepared 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. Slides were prepared in accordance with the standard procedure of squashing the Feulgen-stained material. The total number of dividing cells (NDC) was determined in

1000 examined cells in the field of view per each slide, than MI and PI (of prophase, metaphase and ana/telophase) were scored as percent ratio of NDC per 1000 cells. For INV investigation it was obtained the mean of two diameters of each selected stained nucleus, observed at right angles to each-other and measured under oil-immersion objectives. INV was evaluated using the formula $4/3r^3$, where *r* is the radius of the nuclei. DNAC was estimated by cytophotometric measurement of 2C telophase nuclei, using a microspectrophotometer at a wavelength of 550 nm. The Feulgen stained root-tips were washed in three changes of SO_2 water for 10 min. each and dried briefly on absorbent paper. Darkly stained ones were squashed in a drop of 45% glacial acetic acid. 100 2C telophase nuclei were measured in each slide (Van't Hof, 1965). Means of MI, PI, INV, and DNAC values of 5 slides per sample have been calculated. 1500 dividing cells (300 cells per each of 5 slides per sample) have been observed for the characterization and classification of chromosome aberrations (CA). FAC and frequency of CA types were expressed as percent ratio per 1000 cells. MI, FAC and frequency of CA types have been estimated in onion roots exposed to unloaded and ¼ EC_{50}, ½ EC_{50} and EC_{50} Cu salt-loaded samples, while PI, INV and DNAC only at unloaded and EC_{50}-loaded ones. Morphological aberrations of onion roots treated with each unloaded and loaded water sample were observed after 96 h.

Statistical analysis

Analysis of Variance (ANOVA) and post-hoc Student Newman-Keuls (SNK) tests were used to test for significant differences in mitotic and phase indexes, interphase nuclear volume and nuclear DNA content of *A. cepa* roots exposed to different samples and $CuSO_4$ concentrations, while Mann–Whitney U test for significant differences in frequencies of aberrant cells. All the results were expressed as the mean of three replicates per sample ± standard deviation (SD). Differences from corresponding control were considered statistically significant at 5%.

Results and Discussion

The morphology of *A. cepa* roots grown in unloaded water samples was normal, except a slight bending detected in Bahçellek and Zues samples. Observations of roots treated with different concentrations of $CuSO_4$-loaded samples revealed structural damage, which varied depending on salt concentration. Stunted root growth, as a general symptom of copper toxicity (Fiskesjö, 1988; Liu et al., 1995), was detected in all loaded samples. Disorientated root bending started only in natural samples at ¼ EC_{50}, while changing of color to slight blue-green and brownish of tips was mostly observed after EC_{50} treatments application.

Root growth and elongation are essential for plants exploring for water and mineral nutrients. Meanwhile root growth reduction over 55% strongly indicates the presence of phytotoxic substances (Fiskesjö, 1988). Growth inhibition is a general phenomenon associated with most of heavy metals. In the present investigation the $CuSO_4$ EC_{50} values were used to determine and compare the tendency of copper amount and bio-availability in examined natural samples. Depending on this phytotoxic parameter, copper EC_{50} of tap water sample should be decreased by a factor of: 1.49, 1.42, 1.37, 1.21, 1.13 and 1.10, to obtain the same root growth inhibition effect as corresponding EC_{50} of: Zues, Bahçellek, Dajç, Oblikë, Pentar and Delta, respectively.

Tables 1 and 2 represented all data about the cytogenetic analyzes of *A. cepa* root meristem exposed to unloaded and ¼ EC_{50}, ½ EC_{50} and EC_{50} $CuSO_4$-loaded samples. The mitotic index reflects the frequency of cell division and it is regarded as an important parameter when determining the rate of root growth. It is considered to reliably identify the presence of cytotoxic pollutants in the environment. Reduction of 50% (to control) is a limit MI value: a decrease below 50% inducts sublethal effect (Panda & Sahu, 1985), while below 22% causes lethal effect on test organism (Antonise-Wiez, 1990). These effects are probably due to either disturbances in the cell cycle or chromatin dysfunction induced by pollutant–DNA interactions. As shown in Table 1, there was obvious dose-dependent mitotic activity of root cells: the detected inhibition came progressively increasing with addition of copper salt in all water samples ($p<0.05$, using post-hoc SNK test). The reduction of MI at ¼ EC_{50} and ½ EC_{50} resulted 10-29% and 20-35% compared to unloaded NC, respectively. Moreover the MI values of root meristem exposed to EC_{50}-doped samples of Zues, Bahçellek, Dajç and Oblikë samples resulted 44-49%, demonstrating sublethal effect of copper salt. It is recommended not scoring chromosome aberrations if MI value is too low. This paper data confirmed that EC_{50} is an appropriate toxicity

endpoint to select the test concentrations for genotoxicity assays, because there were present enough mitotic cells to permit the microscopic investigation.

Table 1. Mitotic index, frequency of abnormal cells and chromosomal aberration types of *A. cepa* root meristem exposed to unloaded and ¼ EC_{50}, ½ EC_{50} and EC_{50} $CuSO_4$ loaded samples after 48 h treatment application

Samples	$CuSO_4$ cc (mg/L)	MI ± SD (%)	MI % to control	FAC ± SD (%)	CA types (%)				
					cM	Br	Fr	Stk	Dnc
NC	0	13.80±1.06^{a}	100	2.3±0.37	-	1.8	-	0.5	-
	1/4 EC_{50}	12.42±0.82^{a}	90	4.1±0.46*	1.8	0.4	0.2	1.7	-
	1/2 EC_{50}	11.04±0.13^{c}	80	8.9±0.28**	1.9	0.6	0.3	6.1	
	EC_{50}	7.45 ±0.27^{g}	54	12.7±0.22**	1.5	1.2	1.6	7.9	0.5
Bahçellek	0	12.14±0.81^{ab}	88	7.9±0.33*	0.4	3.3	2.5	1.5	-
	1/4 EC_{50}	10.49±0.38^{d}	76	8.3±0.27*	1.6	0.7	0.1	5.9	-
	1/2 EC_{50}	10.07±0.35^{d}	73	11.8±0.56**	2.2	0.9	1.8	6.7	0.2
	EC_{50}	6.62±0.41^{ij}	48	14.2±0.18**	1.7	1.3	1.9	8.5	0.8
Zues	0	10.21±0.25^{d}	74	7.2±0.25*	0.5	2.9	2.4	1.5	-
	1/4 EC_{50}	9.79±0.29^{ef}	71	7.8±0.31*	1.9	0.2	0.5	5.2	-
	1/2 EC_{50}	8.97±0.39^{f}	65	11.4±0.26**	2.0	1.4	1.1	6.9	-
	EC_{50}	6.07±0.23^{j}	44	13.9±0.18**	1.4	2.9	0.3	8.7	0.6
Oblikë	0	11.59±0.31^{b}	84	3.3±0.37*	0.1	1.3	1.2	0.7	-
	1/4 EC_{50}	11.45±0.29^{bc}	83	5.4±0.66*	1.6	0.4	0.8	2.6	-
	1/2 EC_{50}	10.44±0.25^{d}	76	9.1±0.24**	1.9	0.3	1.0	5.9	-
	EC_{50}	6.76±0.17^{i}	49	13.2±0.20**	1.5	2.1	0.9	8.2	0.5
Dajç	0	10.63±0.35^{cd}	77	7.1±0.41*	0.7	3.3	1.9	1.2	-
	1/4 EC_{50}	10.04±0.27^{de}	73	7.5±0.16*	1.3	0.5	-	5.7	-
	1/2 EC_{50}	9.11±0.32^{f}	66	10.9±0.53**	1.7	1.4	1.1	6.6	0.1
	EC_{50}	6.35±0.22^{j}	46	13.7±0.39**	1.4	1.3	0.6	9.5	0.9
Pentar	0	12.70±0.33^{a}	92	2.9±0.24	0.1	1.5	0.9	0.3	-
	1/4 EC_{50}	11.18±0.38^{bc}	81	5.7±0.48*	1.5	1.1	0.9	2.2	-
	1/2 EC_{50}	10.35 ±0.26^{d}	75	9.5±0.12**	1.8	0.8	0.5	6.4	-
	EC_{50}	7.04±0.18^{gi}	51	12.4±0.25**	1.6	1.7	1.2	7.6	0.3
Delta	0	12.28±0.48^{ab}	89	3.1±0.62	-	1.2	1.3	0.6	-
	1/4 EC_{50}	11.08±0.67^{bc}	80	6.2±0.29*	1.3	1.5	0.5	2.9	-
	1/2 EC_{50}	9.94±0.29^{e}	72	10.1±0.14**	2.1	1.7	0.2	6.1	-
	EC_{50}	7.18 ±0.21^{g}	52	12.9±0.17**	1.4	2.1	1.2	8.2	0.7

*Notes: Means labelled with different superscript letters along the column are significantly different ($p<0.05$) in the SNK test, while means with asterisks are significantly different from control: *$p<0.05$, **$p<0.001$ according to Mann–Whitney test; MI-mitotic index; FAC-frequency of aberrant cells; CA-chromosome aberrations; cM-c-mitosis; Br-bridges; Fr-fragments; Stk-stickiness; Dnc -disintegrated nuclei; SD-standard deviation*

Root growth inhibition and impaired penetration of roots into the soil due to metal toxicity could be due to inhibition of root cell division/root elongation or to the extension of cell cycle. As shown in Table 2, PI characterization of *A. cepa* cells exposed to all unloaded samples did not demonstrate irregularities of dividing cell cycles. Meanwhile loaded samples at EC_{50} concentration caused significant changes in all mitotic phases compared to corresponding unloaded ones ($P<0.01$, using ANOVA test). It was noted remarcable decrease of metaphase (47-56%) and certain increase of prophase (117-135%) rates, compared to NC. These results demonstrated an obvious obstruction of metaphase stage, and probably an inhibition of mitosis or extension of cell cycles. This may be attributed to the blocking of cell division by Cu at the end of the prophase. In this case, this metal can be considered as pre-metaphase inhibitor. That might be the reason of MI reduction observed in treated *A. cepa* roots. The evaluation of interphase nuclear volume and DNA content showed a significant reduction of nuclei size (34-41%) and DNA weight (28-37%) in onion root cells exposed to all EC_{50} loaded samples compared to control ($P<0.01$ using ANOVA test)**.** However the analyses revealed lower decrease of these endpoints compared to MI. Recorded INV and DNAC reduction of onion cells exposed to EC_{50}-loaded samples may be attributed to the mito-depressive action of cooper, by blocking the G_1 stage and suppressing DNA synthesis.

Table 2. Phase index, interphase nuclear volume and DNA content of *A. cepa* root meristematic cells exposed to unloaded and EC_{50} $CuSO_4$-loaded samples after 48 h treatment application

Samples	$CuSO_4$ cc (mg/L)	PI (%)			INV±SD	DNAC±SD
		Pro	Meta	Ana/Telo	(μm^3)	(pg)
NC	0	39.12	41.25	19.63	578.21 ± 0.51	92.61± 1.38
	EC_{50}	49.33	21.95	28.72	416.31 ± 0.75	74.09± 0.52
Bahçellek	0	41.02	38.87	20.11	522.14 ± 0.44	95.16± 0.67
	EC_{50}	55.38	18.68	27.21	355.06 ± 0.36	68.52± 0.75
Zues	0	38.15	40.01	21.84	508.42 ± 0.57	89.94± 0.79
	EC_{50}	50.04	19.81	30.15	350.81 ± 0.65	65.66± 0.18
Oblikë	0	37.24	39.95	22.81	492.37 ± 0.48	86.48± 0.27
	EC_{50}	49.53	17.69	26.94	344.67 ± 0.19	64.96± 0.31
Dajç	0	39.93	40.47	19.60	538.16 ± 0.36	98.12± 1.14
	EC_{50}	50.92	18.05	31.03	376.71 ± 0.89	73.59± 0.72
Pentar	0	38.78	42.53	18.69	593.40 ± 0.38	90.63± 1.06
	EC_{50}	45.34	20.26	34.40	421.31 ± 0.65	69.78± 0.44
Delta	0	38.85	39.16	21.99	547.05 ± 0.48	82.79± 0.96
	EC_{50}	48.47	19.35	32.18	382.94 ± 0.91	65.40± 0.39

Notes: PI-phase index; Pro-prophase; Meta-metaphase; Ana/Telo-anaphase/telophase; INV-interphase nuclear volume; DNAC- DNA content; SD-standard deviation

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 study all tested doses of $CuSO_4$ loaded samples increased the frequency of chromosome aberrations in onion root cells, demonstrating even positive dose-effect relationship (Table 1). FAC values resulted significantly higher than corresponding NC ($p<0.05$, using Mann–Whitney test) in unloaded Bahçellek, Zues, Dajç and Oblikë samples and all ¼ EC_{50} loaded samples. Meanwhile ½ EC_{50} and EC_{50} increased the frequency of chromosome aberrations at about 4-5 and 5.5-6 folds of NC, being significant at $p<0.001$. The most frequently observed CA types were: stickiness, bridges involving one or more chromosomes, fragments, c-mitosis and disintegrated nuclei, demonstrating mutagenic and clastogenic activity of copper. It was detected a notable increase of sticky chromosomes with the addition of Cu concentration (Table 1). Disintegrated nuclei and disrupted nuclear membranes had the lowest frequency and were mostly detected at EC_{50} loaded samples. Stickiness, bridges and fragments were due to chromatin dysfunction, while c-mitosis to spindle failure. 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. Concerning c-mitosis, Liu et al. (1995) suggested that copper excess decrease 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 disturbance or inhibition of mitosis.

The present study provided for the first time data about the cyto/genotoxicity of copper experimentally added in some river water samples of Nën-Shkodra lowland by using a plant bio-test. Additionally the biological (cytological and genetic) effects observed in *A. cepa* roots appeared related to the physical and chemical characteristics of respective natural water bodies (Neziri & Gössler, 2006; Bushati et al., 2012). The results demonstrated a certain difference of corresponding mitotic index values and frequency of aberrant cells between two groups of unloaded natural waters. The most polluted resulted Bahçellek, Zues, Dajç and Oblikë samples. Despite the possible impact of agricultural practices and successive flooding during 2010-2013 in Nën-Shkodra lowland, Pentar and Delta samples showed in general a good water quality. A certain positive correlation between the inhibition of mitotic activity and chromosomal aberrations was observed: unloaded samples that did not induce any obvious citotoxic effects (Pentar and Delta), did not produce also significant genotoxic effects; the significant genotoxic potency was revealed only in unloaded samples, which caused lower mitotic activity (Bahçellek, Zues, Dajç and Oblikë). Considering the second group of samples, in the case of Bahçellek (the nearest point to Shkodra city) cytotoxicity was not strictly correlated to genotoxicity. The relatively higher mitotic activity of root cells grown in this sample compared to the others might be due to temporary stimulating effects of nitrate,

nitrite, ammonium and phosphate on the proliferation of *A. cepa* root-tip cells, because of the discharge of abundant municipal and tourism wastes. The high cyto/genotoxic effects caused by Zues sample may be due to the interfering of pollutants from water bodies of Buna (flowing out from Shkodra Lake) and Drini Rivers. It was noted a certain amelioration of river water quality down streaming Nën-Shkodra lowland, screened by physical-chemical and microbiological analyzes as well (Bushati et al., 2012), probably due to self-regeneration of Buna River. Buna and Drini Rivers collect wastes from municipal, agricultural and touristic activities, and different industrial effluents. Additionally large quantities of metal compounds have been discharged for years from mines located down streaming Drini River, because of erosion and flooding. These water bodies, commonly used as irrigation sources and in many cases as drinking and house hold waters, suffer uncontrolled discharge, lack of pretreatment and deficiencies in disposal of waste effluents.

Conclusions

The present study provided valuable information about the morpho/cyto and genotoxic effects of copper on *A. cepa* roots, grown in river water of Nën-Shkodra lowland, experimentally enriched with $CuSO_4$ salt. The results should be considered a warning of risk the environment, biota and human health may incur by natural and anthropogenic copper discharge in water bodies. *A. cepa* test demonstrated different sensitivities, showing some kind of correlation to rivers water quality. From a methodological point of view, the measured genotoxicological parameter in onion roots was more responsive than its cytological counterpart. This investigation clearly indicated that sensitive, short-term and low cost plant bioassays are useful and complementary approach to determine the environmental impact of contaminants, especially in developing countries as Albania.

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