Citotoxicity and Genotoxicity Screening of some Aquatic Bodies in Shkodra Region Using *Allium Cepa* L. test

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Abstract *The reaction of Allium cepa (L.) genetic material to the presence of potential cytotoxic and genotoxic substances in water environment was used to screen the water toxicity presence and degree of Shkodra lake, Buna and Drini rivers, by measuring onion bulbs root length (MRL), Mitotic Index (MI), Phase Index (PI) and chromosome abnormalities (CA), grown in water samples from Shiroka, Zogaj, Shegan, Kamicë, Stërbeq, Buna bridge, Bahçellek, Zues and Dajç. All parameters were compared with filtered tap water control, using χ^2 test. Rating of samples for MRL and MI values was decreasingly: tap water, Stërbeq, Kamicë, Shiroka, Buna bridge, Zogaj, Bahçellek, Dajç, Zues, Shegan. Only the MRL and MI values of Dajç, Zues and Shegan were significant ($p<0.05$), compared to tap water. Most frequent CA types were: stickiness, bridges and fragments. Rating of samples for CA was increasingly: tap water, Kamicë, Stërbeq, Buna Bridge, Shiroka, Dajç, Bahçellek, Zogaj, Shegan, Zues. The number of abnormal dividing cells was significant ($p<0.05$) in: Dajç, Bahçellek, Zogaj, Shegan, Zues. It was noticed high level of CA rate (genotoxity) in water sample of Zogaj, which could not be screened by cytotoxic examinations (MRL and MI). The results indicated a slight water pollution in Dajç, Shegan, Bahçellek, Zogaj and Zues samples, serving as a first alert of chemical pollution environmental impact, even in low concentrations. Deeper and larger scaled monitoring network, using bioassays, has to be done in Shkodra region to protect ecosystems, biota and humans.*

Key words: *Allium test, cytotoxicity, genotoxicity, water pollution, Shkodra aquatic bodies*

1. Introduction

The human populations in developing countries have been suffering the effects of the pollution caused by urbanization and industrialization increasing. A preventive measure to detect the environmental pollutants should be made on a global scale. Many chemical substances accumulate in the environment, especially in water, because of uncontrolled releases of different industrial effluents, urban waste, as well as by drainage, intensive farming and tourism (Déportes et al., 1995; Albering et al., 1999; Vargas et al., 2001; Ohe et al., 2003). The danger of acute or chronic pollutants effects lies in the fact they may be directly toxic or carcinogenic to plants, animals and consequently to humans, causing: cancer, cardiovascular diseases, premature aging, etc. (Grover & Kaur, 1999). Even in rather low concentrations these substances can induce mutagenic effect (Smaka-Kincl et al., 1996; Kungolos et al., 2006; Žegura et al., 2009). On the other hand, only a small part of identifiable toxic and genotoxic substances (10% of more than 700), which may occur in natural water bodies (used as drinking water, house hold and irrigation sources), can be controlled by the standard physical and chemical analysis (Olson, 1993). In this context, toxicity and genotoxicity tests employing microorganisms, plant and mammalian cells have been used, alone or in combination with chemical analysis (Smaka-Kincl et al., 1996; Vargas et al., 2001; Žhegura et al., 2009).

The International Programme on Plant Bioassays (IPPB) has acknowledged, standardized and validated *Allium* test (*Allium cepa L.*, 2n=16) for monitoring and testing of environment polluters (Ma, 1999). *Allium* test is an important plant bio-test widely used for chemical water quality assessment (drinking, surface stagnant and flowing waters) and it is expressed as growth inhibition of the onion bulbs roots (cytotoxicity), nutrition uptake prevention and genotoxicity effects in root meristematic cells, caused by chemicals (Waters & Auletta, 1981, Grant, 1982; Fiskesjö, 1985b, 1997; Liu et al., 1995; Rank & Nielsen, 1997; Dovgaliuk, 2001; Rank, 2003; Palacio et al., 2005; Okorie & Makhalemele, 2009; Leme & Marin-Morales, 2009; Radić et al. 2010; Firbas, 2010). It also shows a good correlation with other bio-tests with prokaryote and eukaryote organisms as: plants, fish and mammalian, etc., (Fiskesjo 1993; Al-Sabti 1992).

Shkodra region represent a unique and complicated water system, hydrologically and ecologically interdependent, including: Shkodra Lake, Buna, Drini rivers and many streams (Dhora, 2005). The significant increase of liquid, solid and gaseous pollutants in this area during the last decades consist in a problematic concern, as demonstrated by recent

reports (UNEP, 2000; Filipoviç & Topaloviç, 2002; Bekteshi & Mijoviç, 2003; Miho et al., 2005; Neziri & Gösler, 2006; Neziri et al., 2009; Mesi et al., 2011; Mesi & Kopliku, 2011; Kopliku et al., 2011).

Based on significant increase of chemical pollution, the reaction of *Allium cepa* genetic material to the presence of potential cytotoxic and genotoxic substances in water environment was used to screen the water toxicity presence and degree of some aquatic bodies in Shkodra region.

2. Methods

2.1 Sample collection

Sampling was done during April - May 2011 and surface water was collected using Van Dorme bottles. Samples were taken from: Shkodra Lake in Zogaj, Shiroka, Shegan, Kamicë, Stërbeq (site 1, 2, 3, 4, 5); Buna River in Buna bridge (site 6), the outflow point of Buna River from the lake and the nearest sample point to Shkodra city); Bahçellek (Drini River, site 7); Zues, union point of Buna and Drini Rivers) and Dajç (site 8, 9); Dobraç (filtered tap water from Shkodra city's main water-supply station, considered as growth medium control, site 10), as Figure 1 shows.

2.2 Test organism

Equal-sized bulbs (Ø≈1.5-2 cm) of commercial onion (*Allium cepa*), not treated with plant growth regulators, were obtained from a local farm. Onions that were dry, moldy or have started shooting green leaves were discarded. Bulbs have been stored under dry conditions by +10-20°C until next harvest season.

2.3 Test procedure

The *Allium* test was carried out as described by G. Fiskesjö (1985a). The experiments were done in constant room temperature (about +20°C), with a natural light-dark regime and protected against direct sun light. The yellow-brownish outer scales have been removed, leaving the ring of root primordia intact. The peeled bulbs, twelve for each water sample, were placed in distilled water during the cleansing procedure to protect the primordia from drying. The quantity of absorbed water samples was daily replaced (0.5-1ml).

2.3.1 Day first

Each series of 12 test tubes has been filled with respective water samples. Onion bulbs have been put randomly on top of test tubes, with the root primordia downward in the liquid.

2.3.2 Day second, third

Microscopy slides for MI calculation were prepared after change of liquids *on day second*, independently of root length.

Fig. 1 Map of sampling points in Shkodra Lake, Buna and Drini rivers

From each of 5 bulbs in each series, one root tip was taken for each of 5 slides. MI slides were prepared in accordance with the standard procedure for orcein staining of squashed material: fixation and maceration/hydrolysis in a mixture of 9

parts of acetic acid 45% and 1 part of 1N HCl at +5°C for 5 min., followed by squashing in orcein 2% (in 45% acetic acid solution) was done. Slides were made permanent, mounted in Canada balsam and examined (Singh, 2000). For microscopic investigation an optic microscope Leitz-Diaplan has been used with an oil immersion objective 63/1.4 plan-APO and 500x magnification. Meristematic cells were defined as those with a nucleus bigger than a third of the cell diameter. The total number of dividing cells was examined per 400 cells in the field of view per each of 5 slides, than Mitotic Index was scored as percent ratio number of dividing cells per 1000 cells. Per each sample a Mean (± SE) of 5 slides MI values of three replicates have been scored and collected for the test report (10 water samples x 5 slides x 3 replicates). Phase Index (PI - percentage of the dividing cells in respective phases of division) values have been scored. Characterization of mitotic phases (prophase, metaphase, anaphase, and telophase) has been done.

For CA analizing 100 dividing cells per each of 5 slides and 3 replicates (of each water sample) have been examined and observed. Chromosome aberrations (CA) were characterized and classified.

2.3.3 Day forth

The length of each root bundle was measured from the outside of the test tube in each series of 10 root bundles, exceptionally shortest or longest roots were excluded (maximally two of twelve ones), using a milimetric ruler. A mean of root length (MRL ± SD) of three replicates has been scored for each sample. The data were collected for the test report (10 water samples x 12 bulbs x 3 replicates).

2.4 Determination of phytotoxicity

MRL, MI, PI and CA parameters were used to determine and compare the phytotoxic tendency of examined natural water samples. All mean values have been compared with the respective of control test ones, using χ^2 test at $p < 0.05$ level of significance. The results were plotted on graphs.

3. Results and discussion

3.1 Effects on root growth

Root growth was more or less normal in all bulb's series of tap water (Dobraç), Stërbeq, Kamicë and Shirokë. Table 1 includes MRL, MI and PI values for each sample. MRL value of Dobraç (4.17 cm) showed a linear growth, approximately 1 cm/day, being in accordance with the respective values from the literature (Fiskesjö, 1985; Kopliku et al. 2011; Mesi & Koliku, 2011; Mesi et al., 2011), whitch means good chemical quality. For that reason drinking water was used as a test control sample.

Table 1 Overview of MRL, MI and PI Means (* significant value at level 0.05)

Sample	MRL ± SD (cm)	% of contr MRL	Nr. of total divid. cells Mean ± SE	MI Mean ± SE (%)	% of contr MI	Phase Index Mean ± SE (%)			
						Prophase	Metaphase	Anaphase	Telophase
Zogaj,	3.25±0.51	78	110.6±1.34	11.06±0.17	77	22.01±0.19	32.85±0.13	21.72±0.11	23.42±0.08
Shirokë	3.67±0.53	88	123.6±1.51	12.36±0.26	86	20.93±0.07	34.16±0.16	19.93±0.13	24.98±0.17
Shegan	2.88±0.31*	69	97.7±1.10*	9.77±0.19	68	25.12±0.11*	27.59±0.05*	22.48±0.07	24.81±0.13
Kamicë	3.79±0.56	91	127.9±1.27	12.79±0.24	89	22.04±0.16	31.84±0.17	21.69±0.14	24.43±0.09
Stërbeq	3.92±0.54	94	130.8±1.62	13.08±0.19	91	19.52±0.12	33.07±0.08	20.32±0.09	27.09±0.07
B. Bridge	3.34±0.49	80	113.5±1.59	11.35±0.18	79	23.49±0.11	29.85±0.08*	22.83±0.13	23.83±0.12
Bahçellek	3.21±0.47	77	112.1±1.28	11.21±0.21	78	23.36±0.13	28.99±0.04*	24.03±0.12	23.62±0.14
Zues	3.11±0.36*	74	102±1.21*	10.20±0.23	71	23.77±0.10	29.07±0.07*	22.22 ± 0.16	24.94± 0.11
Dajç	3.08±0.37*	74	97.1±1.61*	10.35±0.25	72	24.02±0.14	28.55±0.07*	22.02±0.10	25.41±0.14
Dobraç	4.17±0.58	100	143.7±1.57	14.37±0.28	100	20.64±0.16	32.68±0.13	21.89±0.14	24.79±0.18

Rating of samples for MRL values was decreasingly: Dobraç (control) > Stërbeq > Kamicë > Shirokë > Buna bridge > Zogaj > Bahçellek > Dajç > Zues > Shegan. Only the MRL and MI values of Dajç, Zues and Shegan were significant ($p<0.05$), compared to tap water. Root length values were readily lower in Stërbeq, Kamicë and Shirokë, compared to the control test (not significant at level 0.05), showing that there is not a relevant root growth inhibition caused by chemical

water pollution**.** Concerning Buna ridge, Zogaj and Bahçellek samples, the inhibition was insufficient to register a statistically significant difference to the control (80, 78 and 77%, respectively), as a consequence, these natural samples presented virtually normal growth and were considered nontoxic. Dajç, Zues and Shegan MRL values (74, 74 and 69 %, respectively) were significally different from control test (at level 0.05), showing traces of chemical pollution. Standard deviation values were compatible with the above mentioned results: SD decrease with decreasing of MRL values (Tab.1).

3.2 Cytotoxic effects

The samples range for MI values of root meristematic cells resulted decreasingly the same as that of MRL values (Tab. 1). The differences of diving cells number to the test control showed the same significance as MRL values, fitting well with the above mentioned effects on onion root growth. The MI could be correlated with the rate of root growth (r^2 = 0.91), showing that the inhibition of root growth was induced by the inhibition of cell division. This correlation was illustrated by bar diagrams in Figure 2. Root-tip cells treated with the rivers and lake water samples also showed alterations regarding the frequency of cells in different stages of mitosis. PI characterization of Stërbeq, Kamicë and Shirokë samples did not show irregularities of dividing cell cycles. Prophase and metaphase PI values for Shegan sample were significant (at level 0.05) compared to control (25.12 and 27.59%, respectively); for Buna Bridge, Zues, Bahçellek and Dajç samples only the PI value of metaphase (29.85, 29.07, 28.99 and 28.55 %) resulted significant (at level 0.05), indicating an obstruction of metaphase, and as a consequence, irregularities in root meristematic cell cycles (Tab. 1).

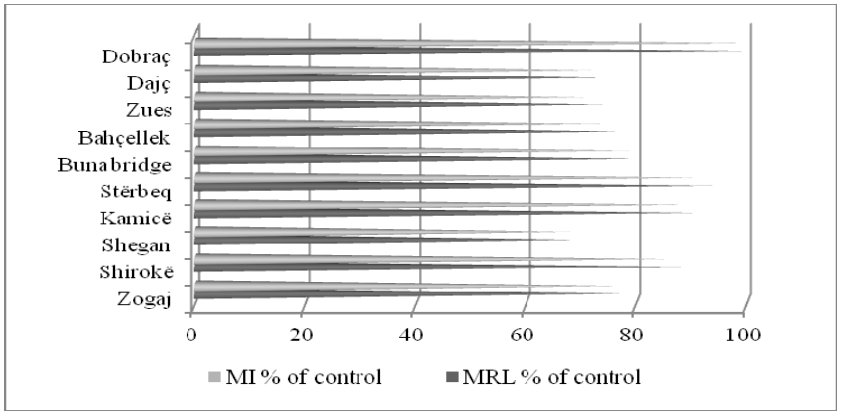

Figure 2. Bar diagram of samples MRL and MI % to control

3.3 Genotoxic effects

Characterization of chromosome aberrations (Tab. 2) showed that rating of samples for CA was increasingly: Dobraç (control) < Kamicë < Stërbeq < Buna Bridge < Shirokë < Dajç < Bahçellek < Zogaj < Shegan < Zues. The mean of normal cells per 100 observed dividing cells was significant ($p<0.05$) in: Dajç, Bahçellek, Zogaj, Shegan, Zues, compared to control (Tab. 2). It was noticed high level of CA rate (distinct genotoxity) in water sample of Zogaj (7.8%), which could not be screened by cytotoxic examinations (MRL and MI). Most frequent CA types were: bridges/fragments and stickiness, while sporadic vagrant/ring chromosomes and c-Mitosis were observed only in Shegan, Dajç, Zues and Bahçellek samples. At the sample of Zogaj it was observed the development, in the cytoplasm of certain root meristematic cells, of two extended hyaline structures generating from the nucleus material, being a typical abnormality caused by Aluminium metal solution (Fiskesjo, 1988). There are no chemical data about Al concentration and pollution in Shkodra Lake and other aquatic bodies.

Shkodra Lake, Buna and Drini rivers receive untreated wastes from municipal (mainly at sites 6, 7) and agricultural (at sites 3, 4, 5, 8, 9) operations, industrial effluents by mine activity down streaming Drini River (at sites 7, 8) and touristic activities (at sites 1, 2, 6, 7, 8). Even the significant increase of pollutants observed during the last years, above mentioned reports detected that the total quantity of heavy metals and organic pollutants in Shkodra Lake, Buna and Drini rivers were lower than EU admissible limit standards for natural waters (WHO, 2011). Anyway, the highest water and sediments concentration of heavy metals resulted in Bahçellek (Drini River) and as a consequence of natural transport in the confluence to Buna River (Zues) and in Buna outflow sector of Shkodra Lake (Buna Bridge). In the present study, the treatment of *A. cepa* roots with surface water samples from Shkodra aquatic bodies was used as an indicator of quantification and toxic tendency of chemical pollutants. The measured biological (morpho, cyto and genological) effects

in *A. cepa* appeared related to the physical and chemical characteristics of respective water bodies, being compatible with above mentioned chemical analyses, concerning Buna Bridge, Bahçellek, Zues and Dajç samples (river waters).

Table 2 Overview of dividing cells, CA rates and types (* significant value at level 0.05; c-M - c-metaphase, STC-stickness, BG & FR- bridges and fragments, VG-vagrant chromosomes,RG-ring chromosomes)

Samples	Mean of normal cells/100 dividing cells	Anormalous dividing cells (%)					Rate of CA (%)
		c- M	STC	BG/FR	VG	RG	
Zogaj,	92.2*	0.0	1.6	6.2	0.0	0.0	7.8
Shirokë	95.8	0.0	1.6	2.6	0.0	0.0	4.2
Shegan	92.1*	0.04	3.3	4.36	0.1	0.1	7.9
Kamicë	96.6	0.0	1.3	2.1	0.0	0.0	3.4
Stërbeq	96.4	0.0	1.1	2.5	0.0	0.0	3.6
Buna bridge	96.1	0.0	1.4	2.5	0.0	0.0	3.9
Bahçellek	92.3*	0.0	1.8	5.4	0.4	0.1	7.7
Zues	91.9*	0.01	3.7	3.99	0.3	0.1	8.1
Dajç	92.5*	0.02	1.7	5.58	0.1	0.1	7.5
Dobraç	96.9	0.0	1.2	1.9	0.0	0.0	3.1

Despite the possible impact of turistic activity increase and 2010 massive flooding, Stërbeq, Kamicë and Shirokë samples (lake water) showed a good water quality. The other samples caused alterations in mitotic index, chromosome and mitotic damage and also alterations in morphological parameter (MRL). Although some of them showed a certain degree of genotoxicity compared to control, the water from site 3 (Shegan), appeared to be the most damaging as evidenced by its capacity to induced chromosome and mitotic damage as well as decreasing root length and mitotic index. It might be a consequence of: herbicides and pesticides residue used in the surround area, geografic position and structure (bay lake) or a considerable unknown industrial chemical residue deposited some years ago in Bajzë railway station, near to Shegan waterside. Second some studies, even concentrations smaller than 0,1 pg/L (0,1 ppb - part per billion) of pesticide products, etc., have a partial inhibitory effect on the growth of test plant roots and are the cause of chromosomal aberrations in cells (Firbas, 2010).

4. Conclusions

The results in the present study indicate slight water pollution in Dajç, Shegan, Bahçellek, Zogaj and Zues samples, serving as a first alert of chemical pollution environmental impact, even in low concentrations.

The *Allium* plant test exhibited different sensitivities, showing some kind of correlation to lake and river water quality through their chemical parameters. The present screening provided valuable additional information about the presence of total pollutants in surface waters of Shkodra Lake, Buna and Drini rivers, by demonstrating the potential of such substances to induce macroscopic, cyto and genotoxic effects in *A. cepa* root cells.

Deeper and larger scaled monitoring network, using bioassays, has to be done in Shkodra region to protect ecosystems, biota and humans. Plant bioassays as *Allium* test should be included, along with conventional chemical analysis, in the routine monitoring programs of chemical water quality and environmental projects in Albania.

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