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Acute Genotoxic Effects of Effluent Water of Thermo-Power Plant "Kosova" In *Tradescantia Pallida*

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Abstract: The aim of this study was the evaluation of acute genotoxic effect of effluent water of thermo-power plant by means of *Tradescantia* root tips micronucleus test (MN), mitotic index and cell aberrations. *Tradescantia*, was experimentally treated (for 24 h), with effluent water of thermo-power plant in different dilution ratios (negative control – distilled water; primary untreated effluent water and 1:1; 1:2; 1:3; 1:4; 1:5; 1:6 and 1:7 respectively). Number of aberrant cells, and frequency of micronuclei (MN), in meristematic root tip cells of treated plants (*Tradescantia*), were significantly increased ($P<0.001$; $P<0.001$ respectively), while the mitotic index in all treated plants was progressively decreased in comparison to the negative control. The results of present study indicate that Tradescantia root-tip micronucleus assay with direct exposure of intact plants is an appropriate method which enables to detect genotoxic effects of effluent waters.

Keywords: Tradescantia; MN-test; cell aberration; mitotic index

INTRODUCTION

During last years, mutagenecity assays were also used for the investigations of complex environmental mixtures including ground-surface and drinking waters and industrial effluents, and specific procedures have been developed for this purpose (Zahen et al., 1992; Helma et al., 1994). A number of studies have shown that Tradescantia – micronucleus assays (Ttad-MCN tests) can detected the clastogenic effects of a variety of environmental relevant industrial chemical commonly found in waste sites, such as pesticides and heavy metals (Ma, 1982; Sandhu et al., 1989). Furthermore, it has been demonstrated, that this test procedure is a useful tool for the identification of genotoxic contaminants of water samples (Ruiz et al., 1992).

The Tradescantia micronucleus assays (Ma, 1982), are currently a widely used plant bioassay for environmental biomonitoring (Helma et al., 1994). The meristematic mitotic cells of plant roots are appropriate and efficient cytogenetic materials for the detection of clastogenicity of environmental pollutants, especially for in situ monitoring of water contaminants. Among several cytological endpoints, in these fast dividing cells, such as chromosome/chromatid aberrations, sister chromatid exchanges and micronuclei, the most effective and simplest indicator of cytological damage is micronucleus formation (Ma et al., 1995).The major advantage of these tests procedures in comparison to ctytogenetic tests with mammals and bacterial mutagenicity assays for water testing is that plants can be exposed directly to the natural state of the water sample without any concentration procedure of filtration process in laboratory.

Tradescantia and Vicia MN tests are suitable for the detection of a variety of direct acting compounds including certain pesticides and chemicals commonly found at waste dumps sites as well as heavy metal compounds which cannot be detected in most vitro systems (Eckel, 1995; DeMarco et al., 1988), but are not suitable for the detection of many environmental compounds which require mammalian metabolic activation.

In addition to the huge modification of the landscape due to the open-cast mining and large dumps for overburden and solid wastes (ash and sludge), the Kastriot industries emit important quantities of air pollutants (dust, sulphur dioxide, and nitrogen oxides), and cause considerable water pollution of Sitnica river with ammonia and phenols. The aim of this study was to evaluate the acute genotoxicity effect of effluent water of thermo- power plant "Kosova" in Kastriot using the Tradescantia root – tip micronucleus test.

MATERIAL AND METHODS

Coal and lignite-mining, coal burning, industry, a nearby thermal power plant "Kosova", traffic, and farming left ecotoxicological burdens in

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Kosovo industrial – rural region of Kastriot municipality, and surrounding regions. The newest data shows that lignite resources in Kosovo reach 15 billion t. (Rizaj et al., 2008).

Lignite is the most important energy resource in Kosovo, providing about 87 % of electric energy production in two thermo-power plants (6 units with 1.470 MW installed capacity).

The discharges of liquid organic waste (measured as chemical oxygen demand, COD), from the industrial thermo-power complex in Kastriot , 5-10 tones per day, are , however , largely exceeded by the discharges of untreated urban sewage from the city of Prishtina: up to 700 tone per day. In addition to the huge modification of the landscape due to the open-cast mining and large dumps for overburden and solid wastes (ash and sludge), the Kastriot industries emit important quantities of air pollutants (dust, sulphur dioxide, and nitrogen oxides), and cause considerable water pollution of Sitnica river with ammonia and phenols.

The effluent water samples were taken from the main pipe that removes water from thermo-power plant to the Sitnica River. Forty five (45) Tradescantia , intact plants were removed from the cultivation pots, the roots rinsed with deionized water, (5 per each treatment) embedded for 24 h, either in negative control (distilled water), primary effluent water (un-diluted effluent water), and in effluent water diluted samples (1:1; 1:2; 1:3; 1:4; 1:5; 1:6; and 1:7 respectively) and fixed thereafter.The procedure for slide preparation for Tradescantia root-tip micronucleus test was made according the method of Ma (1982). To isolate the interphase nuclei the daughter cells (F1) of the treated cell generation, the root cap (calyptra) and the first mm of the meristematic region of the root tip was removed (with a razor blander). The next 2 mm of the root tip are cut of and fixed in an aceto-ethanol solution (1:3), for 24 h and stored in 70% ethanol Subsequently, the roots are washed with distilled water for 10 minutes and then hydrolised in 5 N HCl for 15 min., than they are placed on slide to which a few drops of an aceto-orcein solution (1 % orcein in 45 % acetic acid) are added. After 1 min, a cover slip is placed on top of the slide and the tissue squashed by exerting gentle pressure with the hand. The cells are spread evenly over the surface of a slide to ensure proper evaluation. Scoring was carried out fewer than 400- fold magnification. Since the scoring criteria for MN have not defined specifically for plant bioassays, but criteria developed for MN experiments with mammalian cells were adapted (Tolbert et al., 1992). The minimum number of cells evaluated per treatment was 2000 cells from five slides.

To monitor inhibition of cell division, the number of mitotic cells was determined in parallel. In the same slides are scored the cell aberrations in all mitosis phases, micronuclei and number of cells in Prophase, Metaphase, Anaphase and Telophase.

Results of this study are expressed as main values (X) and ± standard deviation (DS). MN frequencies observed after exposure to different effluent diluted water samples were compared by Student's t-test after arcsine transformation (Shokal and Rolf, 1981). Statistical data processing was carried out by computer, using software for statistical data processing, Sigma Stat 2004.

RESULTS AND DISCUSSION

The results of this study (Table 1.), show that effluent water of all dilutions ratios induced decrease mitotic index. The lowest percentage, of mitotic index is recorded in Tradescantia treated with effluent un-treated water (MI 3.7 %) and in the effluent diluted waters samples from the ratio 1:1; 1:2 and 1:3 (7.1, 6.7 and 7.1 % respectively) in comparison to positive control. In the Tradescantia treated with effluent diluted water from the ratio 1:4; 1:5; 1:6 and 1:7 the mitotic index is progressively increased (10.1, 11.8, 14.5, and 17.4 % respectively) but is still lower than in positive control (20%). The cell aberrations show progressive increased from the samples treated with un-treated effluent water toward the dilution ratio. The highest values of cell aberrations were established in the Tradescantia treated with effluent diluted water ratios from 1:2 (38±3.5) which is 9.5 times higher in comparison to control (4.0±1.2). However, the number of cell aberrations is higher in all treated samples in comparison to the control. The frequency of micronuclei was significantly increased in all treated plants in comparison to control.

Table 1. Genotoxic effects of effluent water of thermo-power plant "Kosova" in *Tradescantia pallid*

D	NI	N'	N" P	N" M	N" A	N" T	MI %	P %	M %	A %	T %	CA X±SD	MN X±SD
Co	1663	337	233	38	27	39	20	69.1	11.2	8	12	4 ±1.2	0.8 ±0.8
Un	1929	71	48	3	4	16	3.7	67.6	4.2	5.6	23	11*** ±1.3	4.6*** ±1.1
1:1	1869	132	99	23	6	4	7.1	75	17.4	4.5	3	21*** ±5.4	5.0*** ±1.6
1:2	1875	126	96	7	3	20	6.7	76.1	5.5	2.3	16	38*** ±3.5	3.6*** ±1.1
1:3	1868	132	115	9	5	3	7.1	87.1	6.8	3.7	2.3	30*** ±3.9	3.8*** ±1.3
1:4	1817	184	125	14	27	18	10.1	67.9	7.6	15	9.7	19*** ±2.2	3.2*** ±0.8
1:5	1789	211	122	12	34	43	11.8	57.8	8.2	16	20	12** ±3.6	1.8*** ±0.4
1:6	1747	254	191	24	17	22	14.5	75.1	9.4	6.6	8.6	9* ±2.0	1.4** ±0.5
1:7	1703	297	184	63	23	27	17.4	61.9	21.2	7.7	9	11** ±2.3	1.2* ±1.1

Note: Plant number tested (5); D-water dilution ratio; Co- negative control; Un-untreated effluent water; NI –number of interphase cells; N'- number of dividing cells; N''-number of cells in respective phase; P-prophase; M-metaphase; A-anaphase; T-telophase; MI – mitotic index; C.A. –Cell aberrations; MN- mean ± SD of micronuclei/2000 cells. * $P<0.05$; ** $P<0.001$; *** $P<0.001$

Our results of decreased mitotic index in all treated Tradescantia according to Odeigah et al (1997), can be explain with increasing concentration and cones quently, increasing toxicity, there was an inhibitory effect on cell division. This might occur in pre-prophase, where cells are prevented from entering prophase or there may be prophase arrest where cells enter into mitosis but are arrested during prophase resulting in a high frequency of prophase cells. It is suggested that prophase –arrest is most likely explanation, as it could also explain the decline of cell aberrations, without any parallel decline in the mitotic index values. According to Odeigh et al. (1997), the impact of genotoxic wastewater of the environment and the significance to human health are difficult to predict, because wastewater are complex mixtures of chemical substances. Complete interpretation of their effect often requires, in addition chemical analysis of the constituents that may indicate the components of wastewater that can persist and accumulate in biota and potentially pose a hazard to human health.

Our results of increased frequency of micronuclei, higher value of cell aberrations and decreased mitotic index in root tip cells of Tradescantia treated with effluent water are in accordance with results of several authors who after treatments of plants; Alium cepa, Vicia faba and Tradescantia , with different toxicants , surface waters, ground waters (, industrial effluent waters , river waters, waste waters , and heavy metal contamination of soils (De Rainho, et al., 2010; Matsumoto et al., 2006; Samka-Kinci et al., 1996; Samuel et al., 2010; Egito et al., 2007; Ivanova et al., 2005, Knasmuller et al., 1998; Haider et al., 2002; Steinkellner et al., 1999; Majer et al., 2002; Knasmuller, 1998), established higher frequency of micronuclei, higher value of cell aberrations and decreased mitotic index .

Our results related to higher frequency of micronuclei in the Tradescantia root tips treated 24 h with effluent thermo-power plant diluted water are in accordance with results of our studies (Elezaj et al., 2003), when we determined higher incidence of micronuclei (MN) in peripheral erythrocytes of three fish species (Barbus barbus and Perca fluviatilis) , collected in two different locations of downstream of Sintnica river (Lummadh/5 km and Maxhun/7km far from "Kosova Power Plant), and in erythrocytes of fish (Onconhyrchus mukiss Walbaum) exposed for 24 h with different diluted effluent water of thermo-plant "Kosova" (1:1; 1:2; 1:3; 1:4; 1:5; 1:6; 1:7 respectively).

This study has shown that the genotoxic potential of effluent water of thermo-power plant can easily be detected using the Tradescantia root tip micronucleus test, and cell aberrations assay. It would be beneficial to apply Tradescantia micronucleus assay as a tool for monitoring the genotoxic effects of industrial and wastewaters thereby providing information on the need for environmental managers to further subject treated industrial effluent to Toxicity Identification Evaluation (TIE) and Toxicity Reduction Evaluation (TRE) before they are finally discharged. This will enable proper chemical analysis of industrial effluent in order to identify the constituent that is really genotoxic and its prompt removal from the effluent before discharge. Finally these observations have raised concern that direct stream of effluent waters of thermo-power plant "Kosova" in Kastriot without chemical, biological treatment and without dilution with "clear" water may pose risk for biota of downstream Sitnica River and human inhabitants (of this area) who use the ground water for agro and other purposes.

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