CYTOTOXICITY AND GENOTOXICITY OF SOIL IN SHUMEN CITY PARK

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Abstract: Sedentary lifestyle has become a great concern to human health. City parks play a great role in the solution of this problem, but exposure to urban pollutants leads to the necessity to monitor environmental quality.

Purpose of Study The aim of the study was to evaluate the possible cytotoxicity and genotoxicity of soil samples collected from Shumen city park using *Allium cepa* L.-test.

Methods The following microscopic parameters were used: mitotic index, index of each phase of mitotic division, mitotic abnormalities and interphase cells with micronuclei or two nuclei.

Findings and Results The decline of the mitotic activity and changes in the proportion of mitotic phases indicate soil cytotoxicity. Various mitotic abnormalities and binucleated cells revealed genotoxicity.

Conclusions and Recommendations Established cytotoxicity and genotoxicity of soil revealed a potential health risk to park users. Further analyses should be provided, since positive results from *Allium*-test serve as an alarm.

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Keywords: Shumen city park, *Allium*-test, soil cytotoxicity and genotoxicity

Introduction

More than a half of mankind now lives in towns. A sedentary lifestyle has become a great concern to human health (Owen et al., 2010). Physical inactivity is a major factor for various diseases. City parks play a great role in the solution of this problem. Numerous studies have documented the positive influence of urban green spaces on physical and mental health (Kabisch and Haase, 2013). On the other hand, being located in the towns, parks are exposed to various pollutants due primarily to traffic, industrial and domestic emissions (Galušková et al., 2011). Park demand determines the necessity to monitor environmental quality. Air pollution is deeply studied, but soil pollution has remained in the background (Science Communication Unit, 2013). It should be noted that citizens have direct contact with soils using recreational areas and adverse effects are generally associated with inhalation of dust (Moosavi and Zarasvandi, 2009). Exposure to contaminated soils may pose a health risk, especially for children because of the additional soil-hand-mouth pathway (Ljung et al., 2006).

Recently, a lot of studies have been focused on urban soil pollution (Karim et al., 2014; Luo et al., 2012; Yang and Zhang, 2015). The standard analytical approach is not appropriate for evaluation of health risk of complex mixtures like soil – bioassays are useful in such studies (Soodan et al., 2012). Special attention has been paid to soil mutagens (Monarca et al., 2002). A lot of studies have established genotoxic pollutants in soils (Watanabe and Hirayama, 2001; White and Claxton, 2004). Higher plant bioassays are used as genetic models for testing toxicity of various environmental contaminants (Chahal et al., 2014; Corneanu et al., 2009). The *Allium cepa*-test has been widely used to study of cytotoxicity and genotoxicity of different pollutants (Fiskesjö, 1993; Tedesco and Laughinghouse IV, 2012; Leme and Marin-Morales, 2009).

The present study is focused on an urban recreational area – Shumen city park (Bulgaria). A "site by site" approach is recommended in such studies, because of specific features of the particular site (Science Communication Unit, 2013). In the case of present study, the park is located near the center of the town of Shumen – a medium-sized city, an important transport hub with well-developed light industry. The park provides a variety of functions including spaces for relaxing, meeting friends, play areas for children, etc.

The aim of this study was to evaluate the possible cytotoxicity and genotoxicity of soil samples collected from Shumen city park using *Allium cepa*-test.

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Materials and Methods

Studied area and soil sampling

The study area in the present investigation is City Park in Shumen (CP), located in northeastern part of Bulgaria. Soil samples from CP (SCP) were collected during August 2015 under dry weather conditions. The latitude and longitude at sampling site were recorded using a handheld global positioning system (GPS) – N43°16'15.88" E26°56'21.47".

Sampling was provided by digging soil to depth of 0–10 cm (soil horizon A). Ten topsoil sub-samples were taken and bulked to give a composite sample. Sub-samples were taken in an 'S-shape' pattern at a distance of 5 m. The samples were air-dried at room temperature in laboratory to constant weight, pulverized and then sieved to < 1 mm particle size.

Allium cepa-test

The onion bulbs were purchased from a biofarm certified to the BCS Öko Garantie; GLOBALG.A.P and IFS Food. The outer scales of the bulbs and the old dry roots were removed without destroying the root primordia. The bulbs were kept for root germination in deionized water for 24 h. Bulbs with new roots with length of 1.5 cm were placed on soil:water suspension (25:1) and were allowed to root for 24 h at 25 ± 1 ºC. The root tips were washed thoroughly with distilled water, fixed in Clarke's fixative (95% ethanol: acetic acid glacial, 3:1) for 90 minutes and hydrolyzed in 1N HCl for 8 min and in 45% acetic acid for 60 min at room temperature. Then they were stained for 90 min in 1% aceto-orcein and the terminal root tips (1-2 mm) were excised and squashed in 45% CH_3COOH. Each soil sample and control group consisted of 9 meristems from 3 bulbs. At least 1000 cells of each root meristem were analyzed. The mitotic index was determined as a ratio between the number of cells in mitosis and the total number of analyzed cells, expressed as a percentage. The index of each phase of mitotic division was calculated as a ratio between the cell number in the respective period and the number of dividing cells. The frequency of aberrant cells was calculated as a percentage of the total number of analyzed cells. The following abnormalities were scored: mitotic cells with chromosome bridges, fragments, vagrant chromosomes, multipolar anaphase/telophase, diagonal spindle, C-mitosis and interphase cells with micronuclei or two nuclei.

Statistical analysis

Results were expressed as the mean ± standard deviation (SD), and significance was analyzed using Student's t-test where significance was accepted at $P \leq 0.05$.

Results and discussion

Effects of a soil sample from CP on mitotic index and phase indices in root meristematic cells of *Allium cepa* L. are summarized in Table 1. The mitotic index decreased in comparison with the negative control. The lower mitotic division could be associated with various factors (as listed by Lamsal et al., 2010): inhibition of protein synthesis, influence on enzyme function, reduction of oxidative phosphorylation. Several checkpoints in mitotic cycle ensure proper distribution of genetic material. If there is DNA damage or inhibition of DNA synthesis, the cells are blocked in G_2 phase (Selmi et al., 2014). Inhibition of mitotic activity is accepted as an indication of a cytotoxic effect (Olorunfemi et al., 2011). The treatment with soil suspension changed the mitotic phase distribution. The notable effect was a decrease of the telophase index. Changes in the proportion of mitotic phases also indicate the occurrence of a cytotoxic effect (İlbaş et al., 2011).

Table 1: Mitotic index and phase indices in root meristematic cells of *Allium cepa* L. exposed to soil water suspension for 24 h.

Sample	Mitotic index (*% ± SD*)	Prophase index (*% ± SD*)	Metaphase index (*% ± SD*)	Anaphase index (*% ± SD*)	Telophase index (*% ± SD*)
Control	5.51 ± 0.76	34.31 ± 14.48	20.86 ± 6.42	11.89 ± 8.15	32.94 ± 9.65
SCP	4.14 ± 1.06*	42.15 ± 10.24	25.65 ± 6.85	14.40 ± 4.83	17.80 ± 9.36*

SCP – water suspension of soil from City Park of Shumen; Control – deionized water. Data are expressed as means ± SD (standard deviation), *P≤0.05.

Source: Authors

Exposure to soil sample increased ~2.5-fold the percent of chromosome aberrations in comparison to negative control (Table 2). Different kinds of mitotic abnormalities were observed (Figure 1).

Diagonal metaphases and anaphases were the most frequent abnormalities. The occurrence of mitotic cells with diagonal position of spindle upon treatment with different compounds were reported in other studies (Bhatta and Sakya, 2008; Lamsal et al., 2010; Tripathy et al., 2013). Presence of vagrant chromosomes was notable in the treated cells. Vagrant chromosomes served as a sign of spindle disturbances (Yildiz and Arikan, 2008). C-mitoses and bridges in ana-telophase also were scored. Disturbed metaphases may be caused by inhibition of spindle formation (Selmi et al., 2014). Bridges may be consequence of DNA breaks (Maluszynska and Juchimiuk, 2005). Fragments and multipolar anaphases were not detected. As can be seen, soil samples caused spindle disturbances (vagrant chromosomes and disturbed metaphases and anaphases) rather than clastogenicity (bridges and fragments).

Treatment did not affect percent of micronuclei (extranuclear bodies of chromatin material) as compared to negative control (Table 2). On the other hand, soil sample from CP increased the number of binucleated cells ~1.5 fold. Binucleated cells result from failure of mitotic cells to complete cytokinesis and are accepted as a sign of cytotoxicity (Leme и Marin-Morales, 2009).

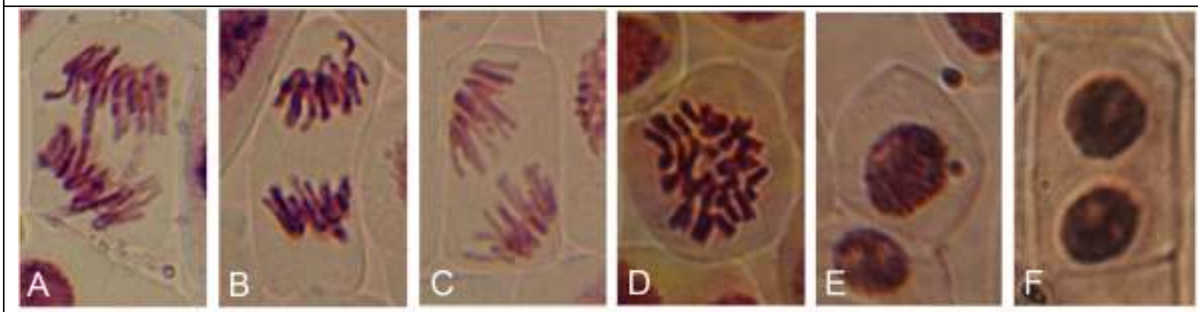
Table 2: Abnormalities in root meristematic cells of *Allium cepa* L. exposed to soil water suspension for 24 h.

Sample	Dividing cells							Interphase cells	
	B (%)	V (%)	F (%)	MP (%)	CM (%)	DS (%)	Total (*% ± SD*)	Micronuclei (*% ± SD*)	Two nuclei (*% ± SD*)
Control	0.58	2.73	0.00	0.00	0.00	2.73	6.04 ± 4.71	0.01 ± 0.04	0.25 ± 0.15
SCP	1.05	4.45	0.00	0.00	3.14	6.28	14.92 ± 6.67*	0.01 ± 0.04	0.38 ± 0.39

SCP – water suspension of soil from City Park of Shumen; Control – deionized water. B – Bridges; V – vagrant chromosomes; F – Fragments; MP – multipolar anaphase/telophase; CM – C-mitosis; DS – diagonal spindle. Data are expressed as means ± SD (standard deviation), *P≤0.05.

Source: Authors

Figure 1: Aberrations induced by soil sample in *Allium cepa* root tips: A – bridge; B –vagrant chromosome in anaphase; C – diagonal anaphase; D – C-mitosis; E – micronucleus in interphase cell; F – binucleated cell.



Source: Authors

Studies on soil pollution could contribute to a municipal policy on environmental management (Žigová et al., 2008). Since soil pollutants can have harmful effect on human health, there is a necessity to find out proper bioindicators for ecotoxicological analyses (Fontanetti et al., 2011). Present study confirmed other data that *Alium cepa*-test can be recommended for detection of cytotoxicity and genotoxicity of complex mixtures (Fiskesjö, 1993; Leme and Marin-Morales, 2009;

Tedesco and Laughinghouse IV, 2012). In our study, we compared effects of soil samples from CP on root meristematic cells with those of deionized water (as a negative control). The decline of the mitotic activity, changes in the proportion of mitotic phases, various mitotic abnormalities and binucleated interphase cells revealed cytotoxic and genotoxic effects. These observations indicate the existence of a health risk for people.

Conclusion

Established cytotoxicity and genotoxicity of soil sample from City Park revealed a potential risk to park users. Our study confirmed that bioassays could provide basic information about hazards of complex mixtures like soil. Further analyses on soils in City Park should be provided, since positive results from *Allium*-test serve as an alarm.

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