Identifying tissue sap toxicity in certain plantain species by bioindication

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Abstract. Toxic substances influence vitally important processes in plants. Studying plant reactions to environmental pollution is one of important objectives. Among pollutants accumulated in P. major L. and P. media L. plants, a group of heavy metals (As, Cr, Cu, Mo, Ni, Pb, Sr, Zn) was separated. Each species with its genotypic differences and specific degree of flexibility reacts to environmental exposure differently. Bioindication at tissue and cell levels is based on close process limits of biotic and physiological reactions. Its advantage is high sensitivity to disruptions, which allows identifying small concentrations of pollutants. Bioindication at these specific levels enables early identification of environmental disruptions. The work examined root meristem mitosis of *Allium cepa* L. The laboratory test recorded the frequency of abnormal and normal mitoses in the cells of onion root meristems (*Allium cepa* L.) given that the onion was treated with extracts of different concentrations (0.5… 5wt.%) of *Plantago major* L. and *Plantago media* L. growing in the anthropogenic areas of Tobolsk city (Tyumen region, Russian Federation).

1. Introduction

Bioindication of the ecological state of natural complexes is one of the most promising methods for studying the different pollutions' impact on living organisms. It is a method for detecting and evaluating the abiotic and biotic factors' impact on living organisms with the help of biological systems. Bioindication may be performed on the level of macromolecules, cells, organisms, populations, communities and ecosystems. Both individual organisms and cells may serve as sensitive bioindicators. In order to understand any bioindication technique on this level, it is required to understand pollutant mechanisms [1-3]. An increased pollutant concentration in the cells of living organisms may serve as a good indicator of environmental pollution.

Information about heavy metals distribution in plant organs and tissues s is very contradictory since metal may be largely accumulated in the aerial organs [4,5] as well as in the roots [6]. Obviously, the ratio of the concentrations of elements in plants is different and associated both with the plant species specificity and with the metals' properties metals.

A popular method for studying the anthropogenic impact on plant objects is the test on root meristems of *Alium cepa* L. (Allium-test). Allium-test is simple and recommended by experts of the World Health Organization as a standard test in cytogenetic monitoring of the environment.

It is known that heavy metals affect plant cells, reducing the activity of mitosis. Mitosis is characterized by four consecutive stages: prophase, metaphase, anaphase and telophase. In this regard,

to assess mitotic activity and the frequency and spectrum of chromosomal aberrations, *Allium cepa* L. cells were studied at the interphase, prophase, metaphase, anaphase and telophase stages [7,8].

The objective of this paper is to estimate toxicity of tissue saps in *P. major* L. and *P. media* L. caused by accumulation of heavy metals and its impact on biological objects (root meristems of common onion *Allium cepa* L.).

2. Materials and methods

2.1. Selection of sites

At the stage of the field research the sites with different anthropogenic loads were determined where parameters of priority pollutants were examined. Site 1 – eastern city part adjacent to the industrial park of Tyumen Fuel and Petrochemical Refinery (Tyumen region, Russia); site 2 – roadside adjacent to the industrial park of Tobolsk City Diary Plant (Tyumen region, Russia); site 3 – reference site, edge of the mixed forest near Vinokurovo village (Tobolsk area, Tyumen region, Russia); site 4 – northern part of Tobolsk, a barren adjacent to the industrial park of Tobolsk Concrete Products Plant (Tyumen region, Russia); site 5 – roadside, southern part of Tobolsk, Tyumen region, near Nikolsky Vzvoz; site 6 – residential neighborhood 9 with modern compact multi-story planning.

2.2. Sampling

Soil samples were taken and prepared for quantitative chemical analysis in accordance with [9]. Soil samples were taken at the sites by mixing five point samples at a depth of 0-30 cm.

2.3. Sample Preparation

The samples were prepared using the speedwave MWS-2 microwave digestion system manufactured by PerkinElmer (USA).

The soil sample (m = 4.0 g) was placed in a plastic tube prior to adding of the acid solution with the ratio of HNO_3:HCl=1:3. The tube was placed in a microwave oven to decompose the sample using the program recommended by the manufacturer of the oven. The following heating conditions were used: temperature increased to 200 °C within 5 min, keeping for 5 minutes at 200 °C, cooling to 45 °C. The dissolved sample was transferred to a 15 mL test tube. The volume was brought up to 10 mL with distilled water. Then the sample was analyzed.

The sample of *Plantago major* L. and *Plantago media* L. (m = 0.3 g) was placed in a plastic tube followed by adding of the H_2SO_4:H_2O_2=1:3. The subsequent decomposition steps were carried out in the same manner as described above.

The quantitative chemical analysis of accumulated heavy metals and trace elements (As, Cr, Cu, Mo, Ni, Pb, Sr, Zn) in the soil samples and the total plant mass was conducted by the inductively coupled plasma method using the Optima 7000 DV atomic emission spectrometer manufactured by PerkinElmer (USA). Standard solutions of PerkinElmer (USA) were used for calibration.

In order to evaluate frequency and spectrum of chromosome aberrations, the cells with normally occurring anaphases and telophases and the cells with different disruptions in normal occurrence of these stages were considered. Fresh sap made of tissues, leaves and roots of *Plantago major* L. and *Plantago media* L. collected on the above mentioned sites was diluted with distilled water to required strength. The optimum moistening was achieved by adding 5 ml of the examined solution into a cup.

As a factor to be examined, the root sap extracts of *Plantago major* L. and *Plantago media* L. from plant tissues found on sites 1-6 were used in different concentrations (0.5, 1, 5.0%). After that, the seeds of *Allium cepa* L. were sowed: 20 pieces in each of the 3 cups. The experiment followed a standard aceto-orcein method. Onion roots were pulled out from the extract and placed into acetic alcohol (3 parts of alcohol and 1 part of glacial acetic acid), then washed in hydrochloric acid diluted with water (1 part of acid and 1 part of water), painted with 2% aceto-orcein (5-10 drops per 10 ml of glacial acetic acid). In the course of the work, 12 sets of experiments were run with 2,160 seeds of *Allium cepa* L. [10-13].

3. Results and discussion

Soils of examined sites showed presence of heavy metals (As, Cr, Cu, Mo, Ni, Pb, Sr, Zn). It was varying: As 0.32…0.84 (reference 0.12), Cr 1.3÷5.5 (reference 0.9), Cu 4.35…10.5 (reference 2.2), Mo 3.11…5.75 (reference 2.3), Ni 2.00÷5.5 (reference 2.3), Pb 6.18…12.61 (reference 1.2), Sr 3.25…9.08 (reference 1.36), Zn 5.22…15.23 (reference 1.3) mg/kg (figure 1).

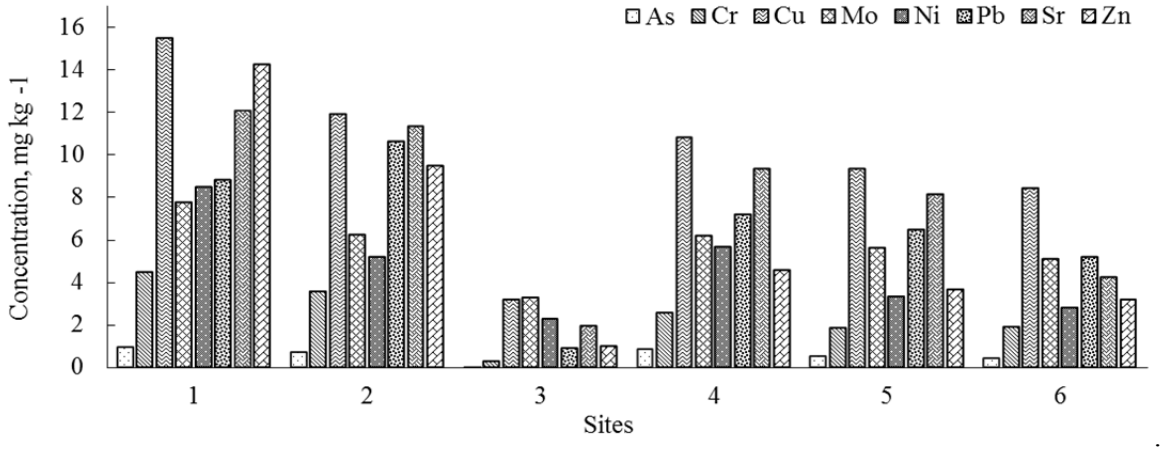

Figure 1. The content of heavy metals in soils of the observation plots 1-6.

Table 1. Frequency of Abnormal and Normal Mitoses in Cells of *Allium cepa* L. Root Meristem Treated with Extracts of *P. major* L. Growing on Various Sites in Tobolsk, n=20.

Site	Concentration, %	Total mitoses, qty	Normal mitoses, %		Abnormal mitoses, %	
			qty	%±m %	qty	%±m %
1	0.5	1080	743	68.80±1.70*	337	31.20±2.52*
2	0.5	1820	1171	64.34±1.40*	649	35.66±1.88*
3	0.5	1603	1441	89.89±0.79*	162	10.11±2.37
4	0.5	1540	1241	80.58±1.12	299	19.42±2.29*
5	0.5	1024	743	72.56±1.64*	281	27.44±2.66*
6	0.5	1214	877	72.24±1.51*	337	27.26±2.44*
1	1.0	1115	753	67.53±1.71*	362	32.47±2.46*
2	1.0	1314	926	70.47±1.50*	388	29.53±2.32*
3	1.0	1420	1322	93.10±0.70	98	16.90±3.79
4	1.0	1243	971	78.11±1.33*	272	21.89±2.51
5	1.0	963	752	78.09±1.51*	211	21.91±2.85
6	1.0	1017	793	77.97±1.47*	224	22.03±2.77
1	5.0	1013	601	59.33±2.00*	412	40.67±2.42*
2	5.0	1120	575	51.34±2.08*	545	48.66±2.14*
3	5.0	1036	991	95.66±0.65	45	14.34±5.22
4	5.0	1151	658	57.17±1.93*	493	42.83±2.23*
5	5.0	1020	713	71.15±1.70*	289	28.85±2.67*
6	5.0	1056	782	74.05±1.57*	274	25.95±2.65

Note: * - differences with the reference are true at $P<0.05$

Heavy metals are accumulated not only in the soil but also in the plants. Chemical analysis of the overall biomass of *P. major* L. and *P. media* L. determined accumulation extent for the analyzed chemical elements.

Zinc content in plants on the most contaminated sites varied from 3.22 to 14.23 mg/kg (*P. major* L.) and from 7.22 to 16.23 mg/kg (*P. media* L.). Copper accumulation varied from 8.45 to 15.5 mg/kg (*P. major* L.) and from 2.35 to 8.5 mg/kg (*P. media* L.). Content of Cr, Mo, Ni, Pb, Sr in *P. major* L. also significantly exceeded values in *P. media* L.

Based on the soil and plant analysis, the examined sites were arranged in the following order in proportion to an increase of anthropogenic loads as well as by heavy metal concentration in the plants: site 3 (reference) → 6 → 5 → 4 → 2 → 1.

Thus, the examined plants differ in their potential to accumulate heavy metals. In all cases, *Plantago major* L. accumulates heavy metals to a significantly greater extent as compared to *Plantago media* L. but the accumulative potential of the examined plants is mostly prominent in case of copper (among the examined metals) and less prominent in case of lead.

Table 2. Frequency of Abnormal and Normal Mitoses in Cells of *Allium cepa* L. Root Meristem Treated with Extracts of *P. media* L. Growing on Various Sites in Tobolsk, n=20.

Site	Concentration, %	Total mitoses, qty	Normal mitoses, %		Abnormal mitoses, %	
			qty	%±m %	qty	%±m %
1	0.5	915	546	59.67±2.22*	369	40.33±2.55*
2	0.5	918	623	67.86±1.87*	295	32.15±2.72*
3	0.5	1012	815	85.61±1.23	56	5.53±0.71
4	0.5	952	956	94.46±0.74*	137	14.39±2.99*
5	0.5	948	821	86.60±1.19	127	13.40±3.02*
6	0.5	946	785	82.98±1.34	161	17.01±2.96*
1	1.0	874	632	72.31±1.78*	242	27.69±1.52*
2	1.0	892	694	77.80±1.58*	198	22.20±2.95*
3	1.0	873	802	91.87±0.97	71	8.13±3.24
4	1.0	963	617	64.07±1.93*	346	35.93±2.58*
5	1.0	896	814	90.85±1.01	82	9.15±3.18
6	1.0	925	799	86.38±1.21*	126	13.62±3.06
1	5.0	771	514	66.67±2.08*	257	33.33±2.94*
2	5.0	954	714	74.84±1.62*	240	25.16±2.80*
3	5.0	1002	914	91.22±0.94	88	8.78±3.02
4	5.0	915	723	79.02±1.51*	192	20.98±2.94*
5	5.0	879	796	90.55±1.04	83	9.44±3.21
6	5.0	973	801	82.32±1.34*	172	17.68±2.91*

Note: * - difference with the reference are true at $p<0.05$

In the course of the experiment, the frequency of abnormal and normal mitoses in the cells of onion root meristems (*Allium cepa* L.) was recorded. In experimental samples, where *Allium cepa* L. seeds were exposed to 0.5% sap solution made of *Plantago major* L. leaves, the following results were obtained. The number of abnormal mitoses on a reference site 3 was 162. This is more than on sites with anthropogenic loads 1 (337), 2 (649) and 4 (299) (table 1).

Table 1 includes parameters that characterize the impact of *Plantago major* L. sap solutions on the number of normal mitoses in the cells of *Allium cepa* L. root meristems. The lowest (0.5%) concentration had a stimulating effect on the germination capacity whereas high concentrations depressed it. In case of 5% solution, the following results were obtained: 3 (reference) - 991; 1 - 601; 2 - 575; 4 - 658 normal mitoses.

The experiments with *Plantago media* L. extracts showed that even the lowest concentration (0.5) of the plant sap had a significant impact on plant cells (Table 2). In case of 0.5% solution, the number of abnormal mitoses in *Allium cepa* L. cells on the reference site 3 was 56 - the value is significantly lower than on the site 1 (369).

The analysis of extract impact showed that sites with a larger anthropogenic load had a decreased number of normal mitoses. Comparison of the data shows that the number of normal mitoses in *Allium cepa* L. cells is higher than abnormal. This is connected to the fact that heavy metal concentration in plant tissues changed due to contamination.

4. Conclusion

The research of soil and plant samples of anthropogenic roadside plant communities identified a group of major ecotoxicants, that is, As, Cr, Cu, Mo, Ni, Pb, Sr, Zn. When cells of *Allium cepa* L. root meristem were exposed to plantain extracts of various concentrations (0.5, 1, 5.0%), which were collected on sites with a high contamination level, an increase in the number of abnormal mitoses (19.42…48.66% for *P. major* L.; 13.40-…0.33% for *P. media* L.) was noticed as compared to reference values (5.53… 8.78% for *P. major* L.; 10.11…16.90% for *P. media* L.). Thus, regularity was revealed: the higher a contamination level is, the larger amount of heavy metals is accumulated in the plants causing an increase in the number of abnormal mitoses.

Going forward, it would be important to compare the informative value of the data obtained with different biological objects and compare peculiarities of their changes in the context of intensive anthropogenic impact on ecosystems.

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