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SOMATOSTATIC EFFECT OF HEAVY METAL CONTAMINATED WATERS IN THE REGION OF THE TOWN OF PANAGJURISHTE, BULGARIA

E. IVANOVA[a], T. STAIKOVA[a], I. VELCHEVA[a*], P. KOSTADINOVA[b]

[a] *Biological Faculty, University of Plovdiv, 24 Tzar Assen Street, 4000 Plovdiv, Bulgaria*

[b] *Agricultural University, 12 Mendeleev Street, 4000 Plovdiv, Bulgaria*

E-mail: geneiv@pu.acad.bg; tstoykova@yahoo.com; anivel@pu.acad.bg

Abstract. A study was made on the somatostatic effect of heavy metal- and cyanide contaminated waters in the region of Panagjurishte (South Bulgaria) on a plant test-system *in vivo*. A lowered cell division rate in the test sample was established. The cytogenetic method used is applicable for biomonitoring of heavy metal- and cyanide contaminated waters.

Keywords: biomonitoring, heavy metals, cyanides, somatostatic effect.

AIMS AND BACKGROUND

The continuous increase in the production and use of heavy metals on a world scale has made them one of the major environmental pollutants. The last years are characterised by a serious anxiety among scientists, related to climate changes on global and regional scales[1,2].

The falling of heavy metals into natural waters is associated with complex processes of bioaccumulation and transfer along the trophic chains of ecosystems[3,4]. The changes in organisms under the effect of these pollutants are often invisible, occurring at a cellular level .

This study examined waters from the region of the town of Panagjurishte (South Bulgaria), where copper ore mining and processing works are located. These are the copper refinery works of Assarel-Medet in Panagjurishte, the copper-processing works in Pirdop and the washery in the village of Chelopech. According to Dimov and Hristov[5], the levels of heavy metals in this region exceeded their maximum permissible concentrations for the country.

According to Ormrod[6], Boyadjiev and coworkers[7] and Kovalchuk and coworkers[8], the heavy metals such as lead, cadmium, copper, arsenic and zinc, as well as cyanides have a proven cancerogenic effect. The methods of cytogenetic

* For correspondence.

analysis enabled the analysis of cell division specificities and assessment of chromosome and genome mutations.

The objective of this study was to examine heavy metal- and cyanide contamination of local springs in the region of the town of Panagjurishte, as well as to detect the presence or absence of somatostatic effect *in vivo* using *Allium cepa* as a test-object.

EXPERIMENTAL

Heavy metals in water. We examined samples of tap water (control sample No 1) and drinking water taken from a local spring near the "Assarel-Medet" Copper Refinery Works (test sample No 2).

The chemical water analysis was conducted using the method of automatic photometry. The contents of copper, arsenic, cadmium, lead, and cyanides were determined in mg/dm^3.

Cytogenetic analysis. Rootlets of *Allium cepa* bulbs, sprouted in the control- and test-sample water, were used for cytogenetic analysis.

Temporary squash preparations were made from the root meristem of the test-object used. The sprouted rootlets (around 0.5 cm long) were fixed for 4 h in a Clarke's fixator and washed in 96 and 70% ethanol. They were hydrolysed in 3n HCl at a temperature of 25°C for 8 min and washed in distilled water. Then the roots were treated for 30 min with 45% acetic acid and stained them for 2 h in acetocarmine. After staining, the root meristems were separated and squashed it in 45% CH_3COOH. The temporary preparations were made permanent and microscopically analysed at a magnification of 40×10. A total of 12 513 meristem cells were examined. To establish the cell division rate, we calculated the mitotic index (Im) as a ratio between the number of dividing cells (N') and the total number of cells analysed (N) in promiles

$$\text{Im} = N'/N \times 1000\ ‰.$$

To compare the samples in more detail, we calculated the indices of the separate phases – prophase (I prophase), metaphase (I metaphase), anaphase (I anaphase) and telophase (I telophase). The phase indices were calculated by the formula:

$$\text{I phase} = N''/N' \times 100\ \%,$$

where N'' is the cell number in the respective phase.

RESULTS AND DISCUSSION

Heavy metals in water. The heavy metal amounts detected in the control sample exceeded the maximum permissible concentrations (MPC) for the country (Ordinance No 7, 1987). No cyanides were established.

In the test-sample, the Pb content was 0.1 mg/dm^3, exceeding five times the MPC, and that of Cu – 0.09 mg/dm^3, i.e. 1.8 times over the MPC.

Cyanide amounts of 0.025 mg were detected in the test sample (sample No 2), inadmissible for drinking water, according to the Bulgarian hygienic standards.

Cytogenetic analysis. After conducting the cytogenetic analysis, the following values of mitotic and phase indices were established for both samples (Table 1).

Table 1. Mitotic and phase indices in the analysed water samples

Sample No	Im (‰)	I prophase (%)	I metaphase (%)	I anaphase (%)	I telophase (%)
1	447	86.98	5.8	2.6	4.6
2	261	79.73	5.33	4.46	10.46

The results obtained showed a nearly twofold decrease in the mitotic index of the test sample (sample No 2) as compared to the control (sample No 1). Comparing the phase indices, we established a more significant increase in the anaphase- and telophase indices of sample No 2. The values of the prophase- and metaphase indices in both samples were relatively similar.

The chemical water analysis showed increased lead and cyanide contents in Sample No 2. In our opinion, this was the most probable reason for the decreased cell division rate. In our previous studies[9] we established also some decrease in the mitotic index of *Pisum sativum* root meristem after treating with heavy metal- and cyanide contaminated waters. These results corroborated with the data reported by Dimitrova[10] who established by morphometric analysis that the high concentrations of lead, zinc, cadmium and copper suppressed the growth of vegetative organs in plants. According to Boyadjiev et al.[7], some heavy metals (lead, cadmium, manganese, copper, nickel) exhibited reproductive toxicity in human cells. The increase in the ana- and telophase indices, detected by us in the test-sample, probably resulted from chromosome aberrations leading to the formation of ana- and telophase bridges, found by us in some of the analysed cells.

CONCLUSIONS

The tested waters from the region of the town of Panagjurishte were mainly polluted with lead, copper and cyanides.

A somatostatic effect was established on the genetic *Allium cepa* test-system *in vivo*.

The cytogenetic method used in the present study is applicable in pollution monitoring studies of heavy metal- and cyanide contaminated waters.

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