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Water pollution

STUDY ON THE MUTAGENIC EFFECT OF WATERS POLLUTED WITH HEAVY METALS AND CYANIDES ON *Allium cepa* PLANT SYSTEM *in vivo*

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Abstract. A study was made on the mutagenic effect of heavy metal- and cyanide contaminated waters in the region of Panagjurishte (South Bulgaria) on a plant test-system *in vivo*. A high frequency of chromosome mutations in the test-sample was established. The cytogenetic method used is applicable for biomonitoring of heavy metal- and cyanide polluted waters.

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

AIMS AND BACKGROUND

Heavy metals are pollutants of high bioaccumulation rate. When fallen into the organism, they are slowly released, causing a number of negative, often invisible, damages[1,2] .

The region of the town of Panagjurishte, the object of this study, is subjected to anthropogenic pressure, associated with the presence of heavy metals in the environment. The major pollution sources are connected with the copper ore mining and processing in Assarel-Medet, Panagjurishte, the Copper-Processing Works in Pirdop, and the washery in the village of Chelopech. According to Dimov and Hristov[3], a number of heavy metals were found as permanent components in the ground and surface waters of this region.

The problem of mutations is one of the fundamental problems of modern genetics. Taking into consideration the increased environmental pollution with chemical mutagens, we could say that mutations pose a real threat to the presence and future of mankind[4]. Proved mutagenic and cancerogenic effects are established in the heavy metals lead, cadmium, copper, arsenic, and zinc[5-7]. Therefore, the studies on the mutagenic effect of heavy metals on different test-objects are of present interest. The cytogenic methods of chromosome- and genome mutation analysis are widely used also for ecological monitoring studies.

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The objective of the present work was to study the mutagenic effect of heavy metal- and cyanide contaminated waters in the region of Panagjurishte on an *Allium cepa* plant test-system *in vivo.*

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 root meristem. 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 for determining the mutation frequency, calculated as percentage of the total number of analysed cells for each variant tested. A total of 12 513 meristem cells were examined.

In accordance with the purpose of the study, we used anaphase- and micronucleus tests for mutagenicity.

RESULTS AND DISCUSSION

Heavy metals in water. The heavy metal amounts detected in the control sample were below 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.

Frequency of chromosome mutations. Using micronucleus analysis we established the presence of micronuclei in interphase cells with frequencies of 0.15% – for sample No 1, and 0.48% – for sample No 2, and in prophase cells with frequencies of 0.12% – for sample No 1, and 0.43% – for sample No 2 (Figs 1, 2). Prophases with fragments of 0.02% frequency were also observed in the sample No 2.

Using anaphase analysis, we established anaphase- and telophase bridges, as well as chromosome fragments, which might be in result of different types of chromosome aberrations. In Sample No 1, anaphase fragments of 0.09% frequency were found. In sample No 2 we established: metaphases with fragments – 0.1%; anaphases with one fragment – 0.44%; anaphases with two or more fragments – 0.13%; anaphase bridges – 0.23% (Fig. 2); telophases with one fragment – 0.13%; telophases with two or more fragments – 0.03%, and telophase bridges – 0.04%.

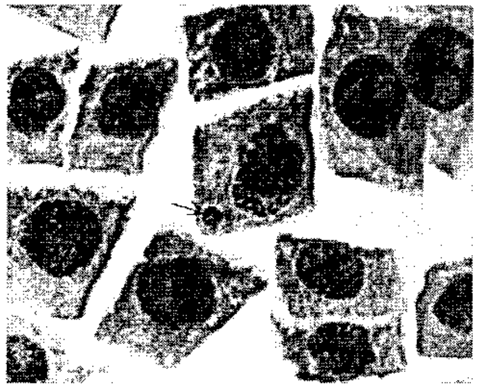

Fig. 1. Prophase with micronucleus

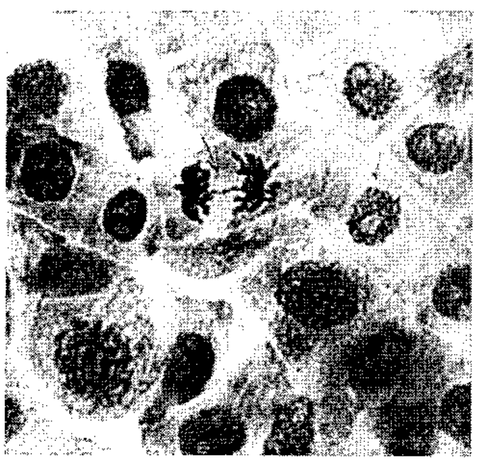

Fig. 2. Anaphase bridge and prophase with micronucleus

The results obtained showed the highest micronucleus frequency (a total of 0.91%) in sample No 2. The micronuclei resulted from two main mechanisms: the lagging of whole chromosomes and the immobility of large acentric fragments. While the formation of acentric fragments resulted from different chromosome aberrations, the lagging of chromosomes was induced by disturbances in the mitotic spindle and the centromere as well as by chromosome non-disjunction after translocation stickings.

The frequency of all mutations established by both methods of analysis was 0.36% for sample No 1 and 2.07% for sample No 2. In our opinion, the chromosome anomalies observed in sample No 1, resulted from a spontaneous autogenic process. The total frequency of the mutations established in sample No 2 was more than five times higher than that in sample No 1. Based on the chemical analysis, we suggest that this was due to the increased contents of lead and cyanides in the analysed water.

The results obtained by the present study corroborate the data reported by Kovalchuk and coworkers[7] who established that the pollution with heavy metals salts (Cd^{2+}, Pb^{2+}, Ni^{2+}, Zn^{2+}, Cu^{2+} and As-203) induced an increase in the frequency of somatic intrachromosomal and point mutations. They also confirmed our previous studies[8] concerning the mutagenic effect of waters, contaminated with heavy metals and cyanides, on *Pisum sativum*.

The high frequency of chromosome mutations, established by us in this region, corresponds also to the data obtained by Boyadjiev and coworkers[6], who reported that the lead had proven embryotoxic and gonadotoxic effects.

CONCUSIONS

Taking into consideration the fact that the water examined by us is used for drinking, we should note that, in our opinion, the established water pollution is a potential threat to human health. This suggestion supports the opinion of Ananoshtev and coworkers[9] who think that the increased cancer rate in the studied region is directly related to the increased contents of heavy metals detected in samples of tap water and open water sources in the region.

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