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CYTOTOXICITY AND GENOTOXICITY OF HEAVY METAL AND CYANIDE-CONTAMINATED WATERS IN SOME REGIONS FOR PRODUCTION AND PROCESSING OF ORE IN BULGARIA

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Abstract

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This study generalizes the data of investigations on cytotoxic and genotoxic effect of heavy metal- and cyanide-contaminated waters in 2001, 2003 and 2005. The water samples were collected from different water sources in the region of “Assarel-Medet” Copper Refinery Works. The contents of copper, arsenic, cadmium, lead, and cyanides ($mg.dm^{-3}$) were determined using the method of automatic photometry. The *Allium cepa* and *Pisum sativum* plant systems were used for testing of the cytotoxicity and genotoxicity of heavy metals and cyanides. A lower mitotic index and a higher frequency of chromosome aberrations were established in all test samples than in the control ones. Chromosome fragments, anaphase and telophase bridges, micronuclei, lagging chromosomes and C-mitotic effect in cells were observed. It was concluded that the pollution found in the regions for production and processing of ore has cytotoxic and genotoxic effect on cells and it could be a potential threat to water ecosystems and human health.

Key words: cytotoxicity, genotoxicity, biomonitoring, heavy metals, cyanides

Introduction

The dangerous pollutants of the environment having a highly toxic effect on living organisms are lead, cadmium, copper, zinc and arsenic (Ormrod, 1988). The same are with proved mutagenic and carcinogenic effect (Boyadziev et al., 1990; Kovalchuk et al., 2001). The open-cast mining of ores and minerals is at the root of the mining industry in Bulgaria. Heavy metals (Pb, Zn, Cd, Cu, Às) and cyanides are one of the main components found in waste waters of many industrial and mining productions. Nowadays about 80% of the ferrous-and non-ferrous metal ores in the country are provided by the mines of “Elatzite” EAD, “Kremikovtzi AD and Assarel-Medet” EAD (Dimov and Hristov, 1998). These regions are subjected to anthropogenic pressure, associated with the presence of heavy metals in the environment. According to Dimov and Hristov (1998), a number of heavy metals were detected as permanent components in the ground

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and surface waters of these regions, at levels surpassing the maximum permissible concentrations.

The investigations made by various authors pointed out that industrial pollution induced reduction in the number of populations and restricted the distribution of species from the fauna of these regions (Mecheva et al., 1987).

There is a number of studies concerning the problem of the relationship between the increased heavy-metal amount in nature and industrial environment, their mutagenic and carcinogenic effects and the increased cases of malignant tumour formations in man. Mitrov and Chernozemski (1985), Vodenicharska et al. (1992), Tzonevski et al.(1998), Bruning and Chronz (1999), Chernozemski and Shishkov (2001), Ivanova et al. (2005) and others discussed the problem of the relationship between the heavy metal amounts in natural and industrial environment, the increased frequency of chromosome mutations and the carcinogenic processes in the organism.

The ecological monitoring uses different methods for establishing the degree of pollution and the effect of heavy metals and cyanides on organisms. A number of authors (Sabti, 1989; Smaka-Kinkl et al., 1996; Chang et al., 1997; Rank and Nielsen, 1998; Cotelle et al., 1999; Moraes and Jordao, 2001) suppose that different plant test systems are useful for the studying of the cytotoxicity and genotoxicity of heavy metals. It has been established that *Allium cepa* and *Pisum sativum* test systems are useful tools for detection of potentially genotoxic substances in water screening programs (Fiskesjo, 1985, 1993a, 1993b, 1994, 1997, Ivanova et al., 2002a, 2002b).

This study generalizes the data of the investigations on the cytotoxic and genotoxic effect of heavy metal-and cyanide-contaminated waters in 2001, 2003 and 2005.

Materials and Methods

The water samples were collected from different water sources in the region of "Assarel-Medet" Copper Refinery Works: Panagyurishte town (water, taken from the municipal water supply system and from Luda Yana River); a close region near Panagyurishte town (spring waters, used by the local population for drinking and irrigation); a region near the copper mine (spring waters and water from Assarelska River); the roads Panagyurishte-Sofia and Panagyurishte–Plovdiv (waters from roadside springs and from Luda Yana River) and they were tested by chemical and cytogenetic analysis. Tap water samples were used as control samples.

Chemical water analysis

The contents of copper, arsenic, cadmium, lead, and cyanides (mg.dm^{-3}) were determined using the method of automatic photometry (apparatus SQ 118-Merk). The results for the contents of the heavy metals and cyanides tested were compared to the hygienic norms adopted for the country (maximum permissible concentrations–MPC) according to the Ordinance No 7/1986.

Cytogenetic analysis

The *Allium cepa* plant system (Fiskesjo, 1985, 1988, 1995; Staykova et al., 2005) and the *Pisum sativum* plant system (Ivanova et al., 2002a, 2002b) were used for testing of the cytotoxic and genotoxic effects of heavy metals and cyanides. More than 40 000 *Allium* and *Pisum* rood meristem cells have been studied during the cytogenetic investigation.

Results and Discussion

The data generalized for the contents of Cu, Às, Cd, Pb and cyanides as the maximum permissible concentrations (MPC) for these metals (according to Ordinance No 7/1986) are given in the Table 1.

We ascertained the high content of copper in a water sample from Assarelska River in 2005. The presence of Cd and CN was increased in most of the water samples studied during the three periods of this investigation. We established highest content of lead in the water samples collected from the roadside springs near the roads from Panagyurishte to Sofia and to Plovdiv, including Assarelska River, which crosses the Panagyurishte-Sofia road.

Table 1

Content of heavy metals and cyanides in the water samples studied, mg.dm^{-3}

Period	Cu	As	Cd	Pb	CN
2001	< 0.05 – 0.06	< 0.05	< 0.025	0.1 – 4.5	0.011– 0.028
2003	< 0.05 – 0.09	< 0.05	< 0.025	0.1	0.0 – 0.025
2005	< 0.05– 0.17	≤ 0.05	≤ 0.025	0.3 – 3.8	0.011– 0.037
Bulgarian standards	0.1	0.05	0.01	0.05	0.0

Table 2

Mitotic index (Im) and total frequency of chromosome aberrations, %

Period	Mitotic index – Im		Total frequency of chromosome aberrations	
	Control samples	Test samples	Control samples	Test samples
2001	350	150 - 300	0.056	0.54 - 2.01
2003	447	261	0.09	0.157
2005	536 - 871	442 - 719	0 - 0.027	2.03 - 3.85

Table 3

Frequency of different types of chromosome aberrations analyzed by the Allium test, %

Period	Cells with micronuclei		Prophase with chromosome fragments		Anaphase bridges and chromosome fragments		Telophase bridges and chromosome fragments	
	Control samples	Test samples	Control samples	Test samples	Control samples	Test samples	Control samples	Test samples
2003	0.27	0.92	0	0.02	0-0.09	0.80	0	0.20
2005	0-0.018	0.27-2.82	0	0.02-0.17	0	0.11-0.98	0	0.097-0.20

Tables 2 and 3 present summarized results from the cytogenetic analysis.

The mitotic index (Im) is a parameter for the intensity of cell division. We established a lower mitotic index of all test samples than that of the control ones.

Taking into account the results from the chemical analysis, we suggested that the combined presence of heavy metals and cyanides in the waters tested, provoked an effect of reduction in the cell division rate (Table 2). These results confirm the opinion of Fiskesjo (1994) about the decrease of mitotic index in *Allium cepa*-roots growing in chemical contaminated waters. The lower value of Im was in correlation with the increase in the pollution obtained (Table 1 and 2). Studying the effect of heavy metals on the morphological and anatomical traits in some plants, Dimitrova (1993) reported that the high concentrations of lead, zinc, cadmium and copper suppressed the growth of vegetative organs. Dimitrova and Ivanova (2003) also reported that the increased heavy metal amounts in soil decreased not only the growth of vegetative organs, but also the rate of their cell division, inducing chromosome aberrations in *Linum usitatissimum.* Different types of chromosome aberrations were established by means of cytogenetic analysis: chromosome fragments (in prophase, anaphase and telophase); anaphase bridges; telophase bridges and micronuclei (Table 3). Lagging chromosomes and C-mitotic effect in cells from some test samples were ascertained, too.

The total frequency of chromosome aberrations in the samples tested in comparison to the control ones were 1.8 (2003) to 143 times (2005) higher – (Table 2), which proves a genetoxic effect of heavy metals and cyanides. It was interesting to notice that the lower frequency of chromosome mutations in 2003 in comparison to the other two years (2001 and 2005) was in correlation with the lower value of a lead and cyanide contamination in the same period. These results are in agreement with Kovalchuk et al. (2001) who reported that pollution with heavy metal salts induced an increase in the frequency of intrachromosomal mutations. (Figures 1, 2, 3, 4, 5 and 6)

The micronucleus test showed the presence of micronuclei in 0.27% (2003) and 0.018% (2005) of the cells from the control samples; in 0.92% (2003) and in 0.27% to 2.82% (2005) of the cells, respectively (Table 3.). The comparative analysis of these

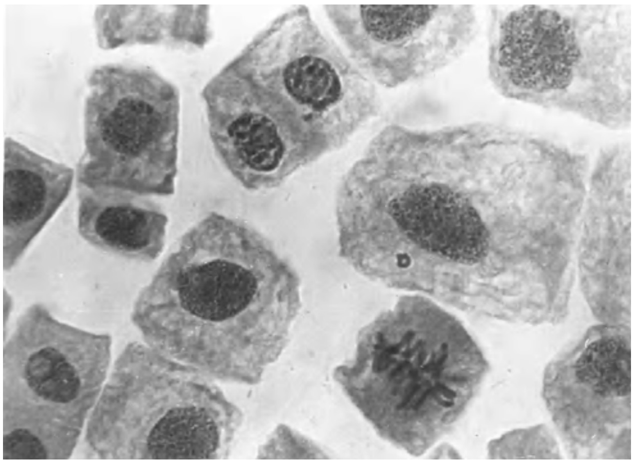

Fig. 1. Cytogenetic effect of heavy-metal- and cyanide-polluted waters in *Allium cepa* root meristems, x 400. Prophase with micronucleus; prophase with ring chromosome fragment; late telophase with chromosome fragment

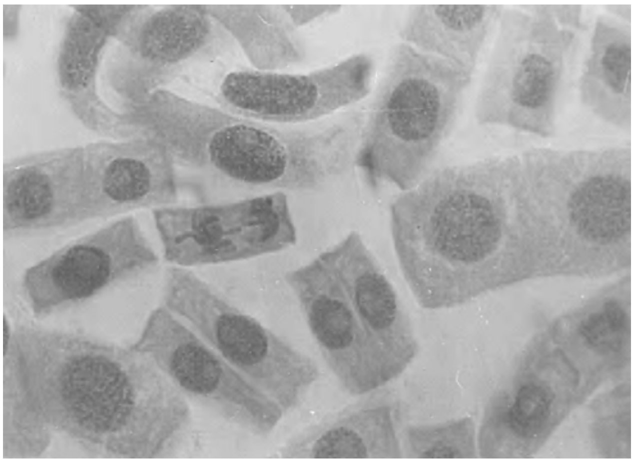

Fig. 2. Cytogenetic effect of heavy-metal and cyanide-polluted waters in *Allium cepa* root meristems, x 400. Anaphase with broken chromosome bridge and lagging chromosome

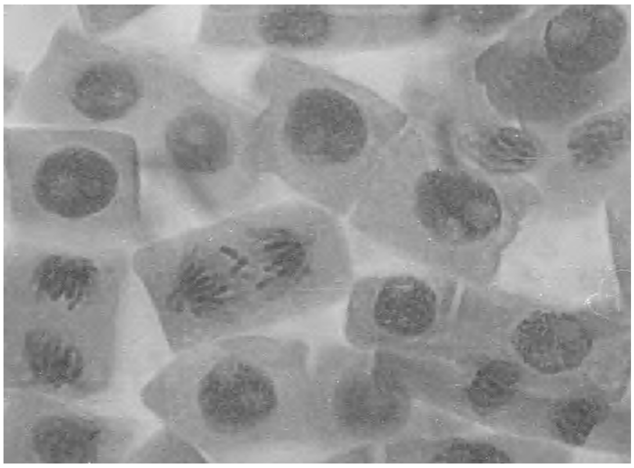

Fig. 3. Cytogenetic effect of heavy-metal- and cyanide-polluted waters in *Allium cepa* root meristems, x 400. Anaphase with chromosome fragments

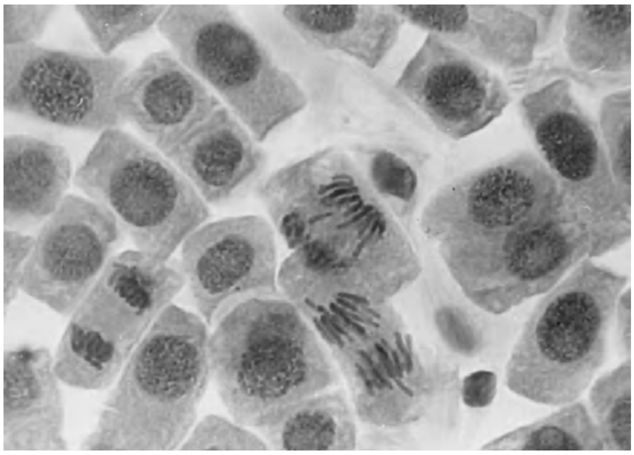

Fig. 4. Cytogenetic effect of heavy-metal- and cyanide-polluted waters in *Allium cepa* root meristems, x 400. Anaphases with chromosome fragment and lagging chromosomes

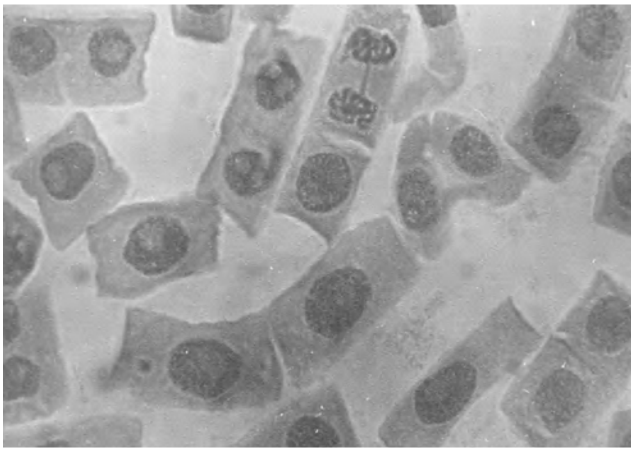

Fig. 5. Cytogenetic effect of heavy-metal- and cyanide-polluted waters in *Allium cepa* root meristems, x 400. Prophase with micronucleus; telophase bridge

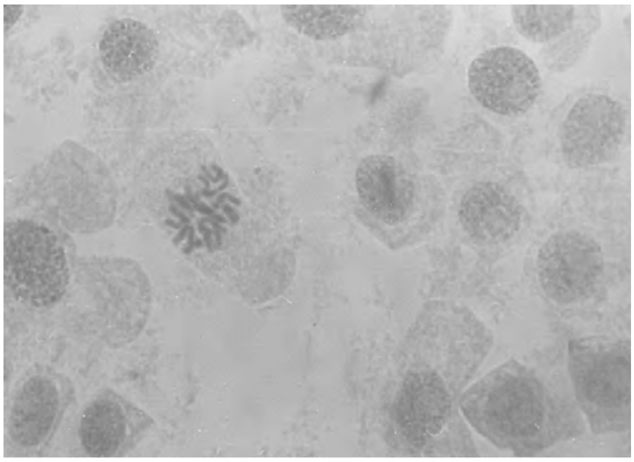

Fig. 6. Cytogenetic effect of heavy-metal and cyanide-polluted waters in *Allium cepa* root meristems, x 400. C – mitotic effect

data demonstrated over a triple increase in 2003 and from 15 to 157 time-increase in 2005 in the micronuclei frequency in the tested water. In our opinion, the high micronuclei frequency observed in the test samples was induced either by the immobility of large acentric fragments or by the lagging of whole chromosomes. The lagging of chromosomes was caused by disturbances in the mitotic spindle or the centromere. In this connection, it was said by Fiskesjo (1994) that the chromosome lagging was induced by a weak C-mitotic effect and they indicate a risk of aneuploidy. According to Schmid (1975); Fenech (1990); Ma et al., (1992) the occurrence of micronuclei (MNi) has been used as a single parameter for the recording of genotoxic damage. The screening of MNi is said to be a more rapid method than the screening of chromosomal aberration (Fiskesjö, 1997).

Among the other chromosome mutations, the cells with anaphase bridges and fragments were with the highest frequency (to 0.98%). We suggest that the anaphase and telophase bridges established, as well as the chromosome fragments resulted from different types of chromosome aberrations, associated with a loss of genetic material. Our suggestion corroborated the data reported by Hoga et al. (1991), who considered the anaphase bridges as obtained from structural changes of deficiency- and translocation type, some of them surviving to late telophase, which is indicative of their stability. Using anaphase-telophase assay, Rank and Nielsen (1998) established that the genotoxicity correlates to the industrial load and the concentrations of the heavy metals (Pb, Ni, Cr, Zn, Cu, Cd) correlate with the toxicity. Chang et al. (1997) using *Allium cepa* test prove the genotoxic effect of a lead-contaminated soil, too. According to Boyadjiev et al. (1990), it was the lead that had embryotoxic and gonadotoxic effects and, the cadmium–teratogenic effect.

Conclusions

Based on the data presented for the cytotoxic and genotoxic effect of the heavy metals and cyanides in tested waters, we could conclude that the pollution found in the regions for production and processing of ore is a potential threat to water ecosystems and to human health.

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