Cytogenetic damage and heavy metal bioaccumulation in molluscs inhabiting different sites of the Neris River

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Cell aneuploidy, polyploidy and meiotic injuries were studied in somatic and gonadal cells of bivalves *Anodonta cygnea* and *Unio tumidus* and gastropods *Viviparus viviparus* inhabiting different sites of the Neris River. The amount of cytogenetic injuries in indigenous molluscs from contaminated sites was 1.8–4 times higher than in organisms from the upstream zones. The statistical tests showed insignificant differences in the cytogenetic damage of cells of molluscs from Raudondvaris and Verkiai localities. Significant differences in numerical changes of chromosomes were found in molluscs inhabiting the upstream sites of the Neris at Gariūnai and Rykantai ($P < 0.0001$). Hypodiploidy of cells was the main type of aneugenic effects in the tissues of the molluscs studied. The amount of hypodiploid cells in the tissues of *A.cygnea* collected from the river below Vilnius (at Gariūnai) reached 29%, *U. tumidus* 30%, *V. viviparus* 35%. The highest frequency of polyploid cells (42%) was determined in gonadal cells of viviparids from Gariūnai locality. The concentrations of heavy metals (Cd, V, Cr, Ni, Cu, Mn, Zn, Fe, Sr and Hg) were determined in soft tissues of molluscs that were used for the cytogenetic studies. The highest concentrations of Mn, Cu, Cd, V, Cr, Zn and Ni were found in tissues of molluscs collected in the Neris River below the Vilnius City, while in samples from the Neris by the Rykantai, the concentrations of Hg and Sr were higher than in molluscs inhabiting the upstream sites.

Key words: cytogenetic damage, aneuploidy, chromosomes, molluscs, heavy metals, bioaccumulation

INTRODUCTION

The main part of pollutant intake into the Neris River comes from the Vilnius city. The elevated levels of heavy metals (Hg, Pb, Zn, Cu, Cd, Cr, Ni, V, As) were determined in soils of territories of industrial enterprises, near highways and in the Neris River sediments [9, 15]. Comparatively high concentrations of Pb, Zn and As were found in tissues of molluscs inhabiting the Neris sites below Vilnius [5].

A series of genotoxic chemicals exerts effects on the cells of various vertebrate and invertebrate organisms [7, 10, 12, 13]. It is known that nickel, lead, cobalt, chromium and arsenic compounds are cancerogenic and genotoxic to organisms. Metal ions exhibit a high affinity to catalyze the formation of free radical species, to interfere with the DNA repair and replication processes and to induce cytogenetic damage [7].

Molecular and cellular biomarkers are early-warning indicators of environmental pollution. A limited number of analyses of aquatic organisms exposed to polluted media have implicated aneugenic effects as a relatively sensitive and broad specificity biomarker of genotoxic substances.

Identification and assessment of man-made and other toxicants is crucial for a comprehensive study of pollutants within the freshwater or marine environment. The relationship between genotoxic impacts and the exposure of organisms to contaminants in aquatic media was examined in fish, molluscs, crustaceans and other animals. Molluscs, due to their filtration activity, sedentary, detritus-feeding style of life, weak metabolizing systems can accumulate high amounts of harmful substances and achieve tissue concentrations 100 to 1000 times higher than water concentrations [7].

The objective of this study was to examine the environmental genotoxicity in selected sites of the Neris River differing by the ecotoxic exposure. Resident bivalve and gastropod molluscs were collected from two heavily polluted sites below Vilnius and

from four significantly less contaminated areas in the Neris upstream zone. In order to demonstrate the genotoxic impact of Vilnius industrial and municipal activities, molluscs from Verkiai locality (at the entry of the urban area) and from a heavily polluted site below Vilnius at Gariūnai were studied. At the cellular level, the chromosome set has been identified as a target for the effects of genotoxic compounds.

Heavy metal bioaccumulation was studied in the soft tissues of the same mollusc specimens that were used in cytogenetic studies.

MATERIAL AND METHODS

Cytogenetic disturbances were assessed in somatic and gonadal cells of molluscs inhabiting different zones of the Neris River. A locality at Raudondvaris served as a reference site. Sampling places at Nemenčinė and Verkiai are characterized as comparatively unpolluted, while the Neris below Vilnius at Gariūnai and Rykantai is heavily polluted by municipal and industrial contaminants [14]. A direct influence of rain water effluents from the big Vilnius City area is observed in the study site at Trinapolis (Fig. 1).

Sixty-three specimens of *A. cygnea*, 30 specimens of *U. tumidus* and 89 specimens of *V. viviparus* were examined from different sites of the Neris River during June–July 1998 and 1999. The chromosomes of 4841 mitotic metaphase spreads and meiotic nuclei from somatic and gonadal tissues of molluscs were counted.

Fig. 1. Map of the sampling localities in the Neris River

Blocking somatic (including embryonic) and gonadal cell divisions at metaphase was achieved by injection of a 0.1–0.2% aqueous solution of colchicine into the adult molluscs 4–10 h before they were dissected. Juvenile molluscs were placed directly into 0.01–0.02% solution of colchicine. Pieces of the sample tissues were dissected from molluscs and prepared according to modified methods previously used in karyological studies of trematodes [3] and molluscs [4]. Hypotonization of mollusc tissues was performed in distilled water at room temperature for 40–90 min. The material was fixed with 3:1 ethanol acetic acid solution, which was changed three times: after 30 min, after 1 h and after 24 h. Tissues were dissociated in 45% acetic acid and the cells were smeared on slides, slightly heated up to human body temperature on a flame. The slides were stained with 4% Giemsa for 30–50 min, using a phosphate buffer solution, pH 6.8. The mitotic metaphase and meiotic stages were examined with a Jena Med cytology microscope. Numerical changes of chromosomes in mitotic and meiotic nuclei, such as cell hypo-, hyperploidy and polyploidy were determined.

The chromosome number variability p was counted as a percentage according to the formula:

$$p = (\Sigma a \ / \ \Sigma x)\ 100\%,$$

where a is the abnormal / normal cell number, x stands for all examined cells.

For the evaluation of standard deviation (SD) of data, the following formula was used (Rokickij, 1974):

$$SD = (p\ (100–p) \ / \ \Sigma x)^{1/2}.$$

The statistical analysis was done employing INSTAT statistical package using Chi-square, Fisher's and Bonferroni tests.

Determination of microelement concentrations in mollusc soft tissues was performed by atomic absorption method. 0.5 g of dry sample powder was placed in ceramic crucibles and moistened with a few drops of bidistilled water. Concentrated acids were then added: 5 ml of fluorine acid (HF), 5 ml of nitrogen acid (HNO_3), 3 ml of chlorine per acid ($HClO_4$), 1 ml of sulfuric acid (H_2SO_4). The crucibles were placed into sand tanks, hermetically closed and

left till full disintegration (for 20–30 min). For a more rapid disintegration the samples were periodically stirred. When disintegration was over, the condensate from the lids of crucibles was rinsed with bidistilled water into the crucibles. The crucibles were again placed into the sand tanks and evaporated till the moist salts. After adding concentrated hydrochloric acid (HCl) the samples were kept in a hot bath till full dissolution. The transparent solution was poured into a 100 ml measuring flask and diluted with bidistilled water. The blank test (acid background) was done for each set of samples.

For control tests, the standard samples with a known content of microelements were used. They were prepared in the same way as other samples.

The obtained transparent water solution was combusted in a graphite furnace and the concentrations of microelements were determined using a Saturn 3P atomic-absorption spectrophotometer.

RESULTS

Modal chromosome numbers 2n = 38 of the *A. cygnea* species appeared in 52.8–81.2% of the cells studied. The highest level of aneugenic effects was detected in molluscs inhabiting the Neris below Vilnius by Gariūnai and Rykantai. Cytogenetic damage in gonadal cells is more frequently than in somatic ones (Figs. 2–4). About 20% less of cytogenetical disturbances were determined in the bivalves sampled from the Neris River at Raudondvaris (Fig. 5). A comparatively high amount of cytogenetic lesions was found in tissues of clams inhabiting the Neris River at Trinapolis. All statistical tests (Bonferroni, Fisher's Exact and Chi-square) showed that the level of cytogenetic damage in cells of *A. cygnea* from Raudondvaris and Verkiai localities was not significant. The differences were not significant also between molluscs from Trinapolis and Gariūnai sites. Significant differences in cytogenetic damage were observed in all other comparisons ($P < 0.0001$).

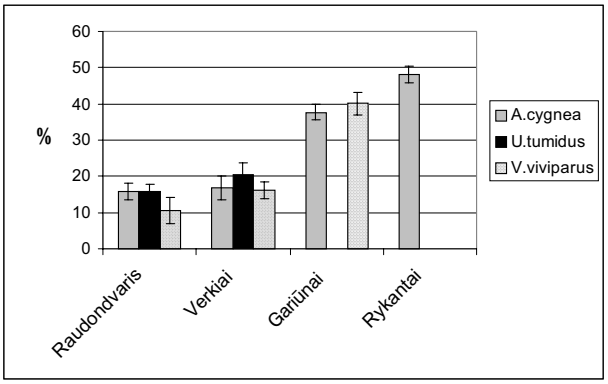

Fig. 2. Aneugenic effects in somatic cells of molluscs from different sites of the Neris River

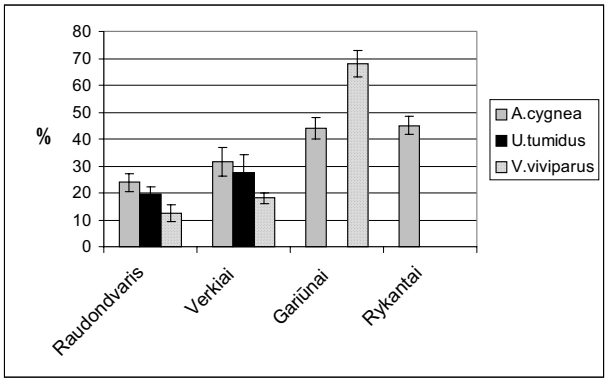

Fig. 3. Aneugenic effects in gonadal cells of molluscs from different sites of the Neris River

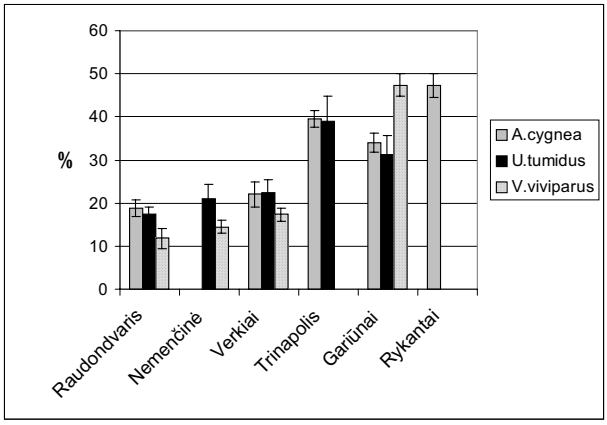

Fig. 4. Cytogenetic disturbances in somatic and gonadal cells of molluscs

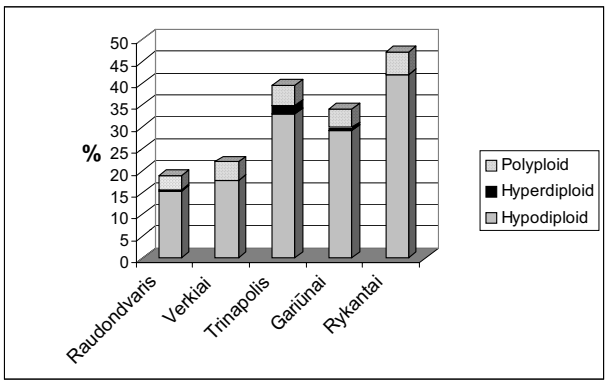

Fig. 5. Aneugenic effects in the cells of *Anadonta cygnea*

The diploid chromosome complement of *U. tumidus* consists of 38 chromosomes. The most damaged karyotypes (in over than 60% of the cells studied) were observed in tissues of molluscs collected from the Neris site at Gariūnai (Fig. 6). These bivalves from the Neris at Raudondvaris possessed more than four times less cytogenetic injuries than those from the river below Vilnius. The amount of aneup-

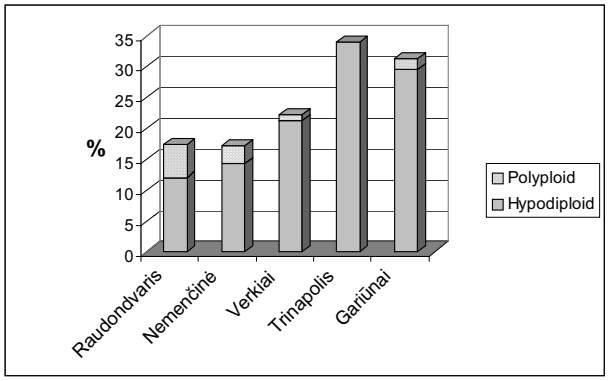

Fig. 6. Aneugenic effects in the cells of *Unio tumidus*

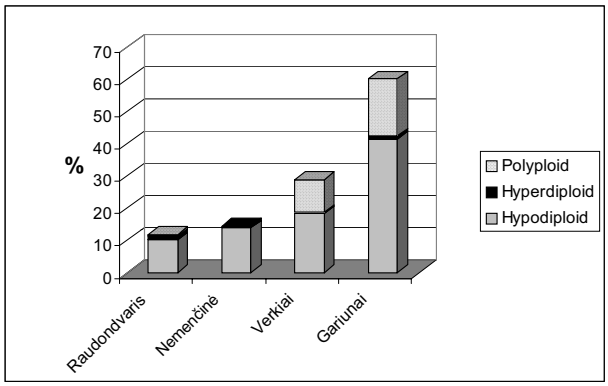

Fig. 7. Aneugenic effects in the cells of gastropod snails *Viviparus viviparus*

loid and polyploid cells in tissues of *U. tumidus* collected from the Neris at Nemenčinė was two times lower than in specimens sampled from Gariūnai locality. Differences between the populations of molluscs inhabiting comparatively uncontaminated sites of the Neris River upstream from Vilnius (at Verkiai, Nemenčinė and Raudondvaris) were insignificant. Significant differences ($P < 0.0001$) were found between cytogenetic damage in the cells of *U. tumidus* from the Neris below Vilnius at Gariūnai and all the other sites studied.

The numerical changes in chromosome sets of *V. viviparus* species were studied in the somatic metaphase and meiotic nuclei of gonads. The diploid chromosome number of *V. viviparus* equals to 18. Such a number was found in 52.6–88.2% of the cells studied. The highest level of aneugenic effects was determined in snails sampled from the Gariūnai locality (Fig. 7). The cytogenetic damage in gonadal cells of viviparids from contaminated sites was 4 times higher than in organisms from the upstream zones. Cytogenetic damage in viviparids from this site was significantly different from other two localities ($P < < 0.0001$).

Hypodiploidy of cells was the main type of aneugenic effects in the tissues of the molluscs studied. The amount of hypodiploid cells in tissues of *A.cygnea* collected from the river below Vilnius reached 29%, *U. tumidus* 30%, *V. viviparus* 35% (Fig. 6). The highest frequency of polyploid cells (42%) was determined in gonadal cells of viviparids from Gariūnai locality. It was also noticed that polyploid cells were more prevalent in viviparid males than in embryos from all studied locations, while hypodiploid cells were more frequent in embryos and gill tissues. Chromosomal aberrations were most frequent in molluscs inhabiting heavily polluted areas.

Analysis of heavy metal concentrations was performed in tissues of molluscs that were used in the cytogenetic investigations. The highest concentrations of Mn, Cu, Cd, V, Cr, Zn and Ni were found in tissues of molluscs collected from the Neris River below Vilnius (at Gariūnai), while in samples from the Neris by Rykantai the concentrations of Hg and Sr were higher than in molluscs inhabiting the upstream sites (Table).

Table. **Concentrations of heavy metals in soft tissues of molluscs (μg/g dry weight; Fe, %)**

	Cd	Cr	Cu	Fe	Ni	V	Zn	Mn
Raudondvaris	0.3	<1	4	0.06	<1	<1	72	1923
Verkiai	0.4	1	3	0.15	<1	1	72	1969
Gariûnai	0.88	3	4.4	0.10	0.9	4	300	5660
Rykantai	0.7	1	4	0.05	<1	1	107	4139

DISCUSSION

Organisms inhabiting contaminated sites can accumulate a variety of chemicals, radionuclides, including those with mutagenic and cancerogenic modes of action. *In vivo* studies of the eco-genotoxicity of aquatic media performed on organisms such as molluscs, fish, crustaceans and others represent the direct testing of real environmental sites, and such factors as bioavailability and metabolic transformation could be integrated directly in the response of organisms to the action of xenobiotics. Thus, *in vivo* studies of genotoxic impacts of environmental contamination are suitable to evaluate cumulative effects of complex mixtures of pollutants usually occurring in natural systems [8, 11].

Genotoxicity studies of aneuploidy may potentially produce different results, depending upon the chromosome selected for analysis, if chromosome-specific sensitivities to chemical exposure exist. Any chromosome specificity characteristics that predispose to aneuploidy might interact with environmental exposures in additional different ways related to the mechanism of aneuploidy induction [12].

In this study, we have shown that environmental genotoxic effects exist in the tissues of molluscs inhabiting different sites of the Neris River. It is noteworthy that the aneugenic influence in this river below Vilnius was 1.8–4 times higher than in other studied sites upstream from Vilnius. It means that genotoxic pollutants originate mainly from Vilnius. Cell hypodiploidy (cells consisting of one or more chromosome less than in diploid sets) was a predominant type of cytogenetic damage in the tissues of molluscs. A comparatively high frequency of hypodiploid (24.1%) and polyploid (18.4%) cells was found in gonads of *A. cygnea* inhabiting the Trinapolis zone. The rain water sewerage is the main pollution source at Trinapolis locality. The inflow of pollutants originates from the big Vilnius region. In earlier studies it has been established that hypodiploid and polyploid cells usually are more frequent in molluscs inhabiting areas highly polluted by different contaminants [2]. A comparatively high amount of polyploid cells was observed in gonads of the filter-feeding bivalves *A. cygnea* from the Neris at Raudondvaris. This site was selected as the reference area. In this area there is no industrial activity and about 10 km above there is the estuary of the Žeimena, which is characterized as one of the cleanest rivers in Lithuania. However, there is an increasing evidence that an intake of genotoxic compounds comes from the Žeimena River (perhaps from the former military areas) or from the territory of Belarus.

The contamination of aquatic ecosystems by heavy metals has gained increasing attention in resent decades. Heavy metals represent a significant ecological and public health concern due to their toxicity and ability to accumulate in living beings [1, 10].

The concentrations of heavy metals (Cd, V, Cr, Ni, Cu, Mn, Zn, Fe, Sr and Hg) were determined in soft tissues of molluscs, which were used for the cytogenetic studies. The highest concentrations of Mn, Cu, Cd, V, Cr, Zn and Ni were found in tissues of molluscs collected from the Neris River below Vilnius, whilst in samples from the Neris by the Rykantai the concentrations of Hg and Sr were higher than in molluscs inhabiting the upstream sites.

Chronic exposure to and accumulation of heavy metals by aquatic biota can result in tissue burdens that have adverse effects on the exposed organisms [6]. The aneugenic and clastogenic effects of cadmium chloride, cadmium sulphate, nickel chloride, nickel sulphate, chromium chloride have been shown [13]. All these compounds increased the frequency of micronuclei in a statistically significant way. However, an increase in kinetochore-positive micronuclei frequencies was higher than in kinetochore-negative ones [13].

The present work demonstrates the cumulative genotoxic ability of heavy metals and other environmental contaminants. Thus, studies of aneugenic effects in the tissues of aquatic organisms could be a useful tool in the ecological risk assessment *in situ*, especially in contaminated sites of rivers located below cities or in the areas of agricultural activities.

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CITOGENETINĖS PAŽAIDOS IR SUNKIŲJŲ METALŲ BIOAKUMULIACIJA MOLIUSKUOSE, GYVENANČIUOSE SKIRTINGOSE NERIES UPĖS ZONOSE

S a n t r a u k a

Pagrindinis šio darbo tikslas yra nustatyti citogenetines pažaidas ir sunkiųjų metalų bioakumuliaciją gėlųjų vandenų moliuskuose, kurie gyvena santykinai neužterštuose ir labai užterštuose biotopuose. Moliuskai tyrimams buvo renkami Neries upėje ties Raudondvariu (kontrolinė zona), virš Nemenčinės (30 km nuo Vilniaus), prie Trinapolio (lietaus kanalizacijos išleidėjas) ir žemiau Vilniaus ties Gariūnais bei Rykantais. Chromosomų rinkinių pakitimai buvo tiriami *Anadonta cygnea, Unio tumidus* bei *Viviparus viviparus* žiaunų bei gonadų ląstelėse. Nustatyta, jog žemiau Vilniaus miesto gyvenančių moliuskų audiniuose aneuploidinių, poliploidinių ląstelių bei mejozės pažaidų yra 1,8–4 kartus daugiau nei moliuskų, gyvenančių kitose tirtose Neries zonose. Chromosomų eliminacija (hipodiploidija) buvo vyraujantis aneugeninių pažaidų tipas. Tirtų moliuskų audiniuose buvo nustatoma sunkiųjų metalų bioakumuliacija. Didžiausios Mn, Cu, Cd, V, Cr, Zn ir Ni koncentracijos buvo nustatytos audiniuose moliuskų, gyvenančių žemiau Vilniaus miesto ties Gariūnais. Tuo tarpu Hg ir Sr didžiausi kiekiai buvo rasti Neryje prie Rykantų gyvenusių moliuskų audiniuose.