Studies of aneugenic effects in mollusc tissues

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Exposure of the organism to any kind of industrial and municipal contamination may induce significant biological changes. The main aim of this study was to assess the aneugenic effects in molluscs from selected sites of the Neris River, differing by the ecotoxic exposure. Additionally, the genotoxic influence of the Vilnius city was studied by transferring molluscs from Verkiai, a comparatively non-polluted area, to a heavily polluted site below Vilnius at Gariūnai.

Somatic and gonadal cells of *Anodonta cygnea, Unio tumidus* and *Viviparus viviparus* were used for detection of aneugenic effects. It was found that the aneugenic effects in the tissues of molluscs sampled from the Neris below Vilnius was 1.5–4 times stronger than in samples from other sites studied. The predominant type of cytogenetic damage was cell hypodiploidy.

After transferring molluscs from Verkiai locality to the Neris River by Gariūnai, the aneugenic effects were significantly stronger ($P < 0.0001$). The cytogenetic damage (after caging of molluscs during 4 weeks) increased by 14.7% in *Anadonta cygnea* and by 18.7% in *Viviparus viviparus.*

Key words: cytogenetic damage, aneugenic effects, molluscs, caging, the Neris River

INTRODUCTION

Cytogenetic analysis has been considered an important tool for evaluation of the quality of aquatic environment and for ecological risk assesment. Pollutants that enter aquatic systems are absorbed into suspended particles and subsequently accumulate in surficial sediments. Aquatic organisms can accumulate harmful substances and achieve very high concentrations in their tissues. Exposure of the organism to industrial and municipal contamination may induce significant biological changes. The major part of pollution intake into the Neris River is from the Vilnius city. Ecogeochemical studies in Vilnius showed that the highest level of heavy metals (Hg, Pb, Zn, Cu, Cd, Cr, Ni, V, As) was in soils of territories of industrial enterprises and near the highways [19]. Comparatively high concentrations of Pb, Cr, Ni, Co and V were detected in the Neris River sediments by Vilnius [14]. It should be also taken into account that certain pollutants such as metals, are essential elements, and often their toxicity depends not only on the dose, but also on critical features such as solubility, oxidation state, and complexation with ligands. Furthermore, inhibitors of mutagenesis and carcinogenesis, exerting protective effects by a variety of mechanisms, occur in the aquatic organisms [10].

Molluscs, often used as a sentinel organism – they are long-living, sedentary, widely distributed, very abundant, relatively sensitive to environmental contamination. They are filters or detritus-feeding organisms.

The main aim of this study was to examine the aneugenic effects in the tissues of molluscs inhabiting various sites of the Neris River, differing by ecotoxic exposure. In order to demonstrate the genotoxic impact of Vilnius industrial and municipal activities, additionally molluscs from the Verkiai locality (comparatively non-polluted area) were transferred into a heavily polluted site below Vilnius at Gariūnai.

MATERIALS AND METHODS

Material for evaluation of aneugenic effects was collected from somatic and gonadal cells of molluscs inhabiting different zones of the Neris River (Fig. 1, Table). Sampling places at Nemenčinė and Verkiai are characterized as comparatively unpolluted, while the Neris below Vilnius city at Gariūnai is heavily polluted by municipal and industrial contaminants [18]. A locality at Raudondvaris served as a reference site. Twenty specimens of *Anodonta cygnea* and 30 specimens of *V. viviparus* were transferred from Verkiai into a heavily contaminated site by Gariūnai. Caging of molluscs was done during four weeks in August 1999.

Chromosome set disturbances were counted in the cells of gill and gonad tissues of the bivalve

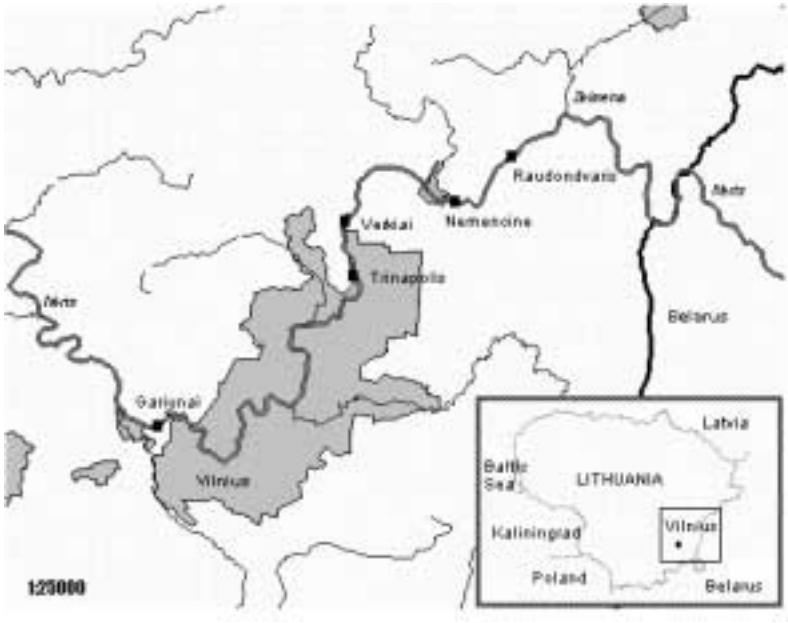

Fig. 1. Map of the sampling localities

Table. **Material for cytogenetical studies of molluscs**

Mollusc species	Raudondvaris	Verkiai	Gariūnai
Anodonta cygnea	10	11	12
Unio tumidus	4	10	4
Viviparus viviparus	12	24	13

mollusc *Anadonta cygnea Unio tumidus* and gastropods *Viviparus viviparus*. Pieces of these tissues were dissected from molluscs and prepared according to modified methods previously used in karyological studies of trematodes [1] and in cytogenetic studies of molluscs [6]. Blocking somatic (including embryonic) and gonadal cell divisions at metaphase was achieved by injection of 0.1–0.2% aqueous soluion of colchicine into large snails using *ca*. 1 ml per 100 g of mollusc weight, 4–6 h before dissection. Hypotinization of mollusc tissues was done 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 cells were smeared on slides, slighty heated up to human body temperature on a flame. The slides were stained with Giemsa for 30–50 min using 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:

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

a is the abnormal / normal cell number; x is all examined cells.

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

$$SD = (p(100–p)/\Sigma x)^{1/2}.$$

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

RESULTS

The diploid chromosome number of *A. cygnea* equals to 38. In the tissues of these clams, 38 chromosomes were found in 51.5–81.2% of cells. The highest level of cytogenetic damage was detected in cells of *A. cygnea* inhabiting the Neris below Vilnius by Gariūnai (Fig. 2). 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. Significant differences in cytogenetic damage occurred in molluscs inhabiting the Neris upstream the sites and below Vilnius ($P < 0.0001$).

The numerical changes in chromosome sets of *U. tumidus* species were studied in somatic metaphase and meiotic nuclei of gonads. Diploid chromosome complement consists of 38 chromosomes. This number of chromosomes was detected in 69–83% of cells. Cell hypodiploidy (cells consisting of one or more chromosome less than in diploid sets) was a predominant type of cytogenetic damage in *U. tumdus* (Fig. 3). The highest level of chromosome set injures was observed in molluscs

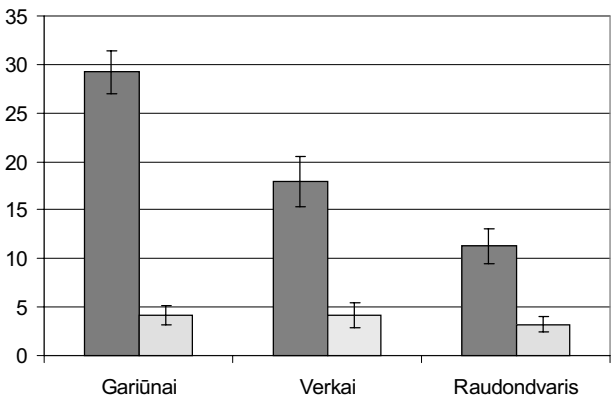

Fig. 2. Aneugenic effects in tissues of *Anodonta cygnea*
■ Hypodiploidy, □ Polyploidy

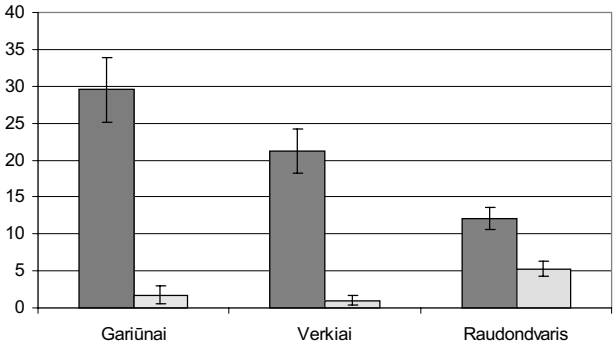

Fig. 3. Aneugenic effects in tissues of *Unio tumidus*
Hypodiploidy, Polyploidy

from Gariūnai. Fisher's exact and Chi-square statistical tests showed, that there were significant differences ($P < 0.0001$) between cytogenetic damage in the cells of *U. tumidus* from the Neris below Vilnius at Gariūnai and all other studied sites. Not significant differences were observed between populations of molluscs inhabiting comparatively uncontaminated sites of the Neris river upstream from Vilnius (at Verkiai and Raudondvaris).

Numerical changes of chromosomes were also investigated in the gastropod snail *V. viviparus* sampled from three zones of the Neris River. A diploid chromosome complement was detected in 40–88% of cells. The highest amount of hypodiploidy (42%) and polyploidy (18%) was determined in snails sampled from the site below Vilnius. Cytogenetic damage in viviparids from this site was significantly different from those in other two localities ($P < 0.0001$). The results of environmental genotoxicity in the Verkiai and Raudondvaris sites indicated that molluscs here had more convenient ecological conditions and cytogenetic damage in their tissues was at the level of spontaneous changes (Fig. 4). The level of aneugenic effects detected in embryonic organisms inhabiting the Neris River below Vilnius was higher (51.9%) than in adult males (28.6%), whilst the count of polyploidy disturbances was higher in adult viviparids (30.3%) than in embryonic organisms (7.8%).

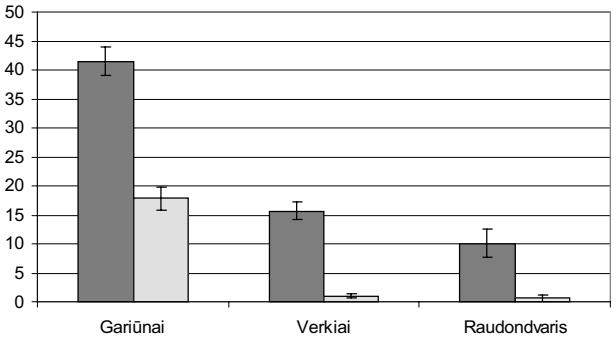

Fig. 4. Aneugenic effects in tissues of *Viviparus viviparus*
Hypodiploidy, Polyploidy

Molluscs (*A. cygnea* and *V. viviparus*) from the Neris at Verkiai above Vilnius were transferred into another site in the same river below Vilnius (at Gariūnai). The cytogenetic damage after 4 weeks of exposure increased up to 36.7% in *A. cygnea* and up to 36.0% in *V. viviparus* (Figs. 5, 6). According to Chi-square and Fisher's exact tests, differences in the level of cytogenetic changes in both species were significant ($P < 0.0001$). The prevalence of hypodiploid cells was observed in somatic and gonadal cells of both species studied. In comparison to indigenous molluscs inhabiting the heavily polluted site below Vilnius, the level of cytogenetic disturbances in transferred molluscs was lower in tissues of *V. viviparus* ($P < 0.0001$) and slightly but not significanly higher in *A. cygnea* (Figs. 5, 6).

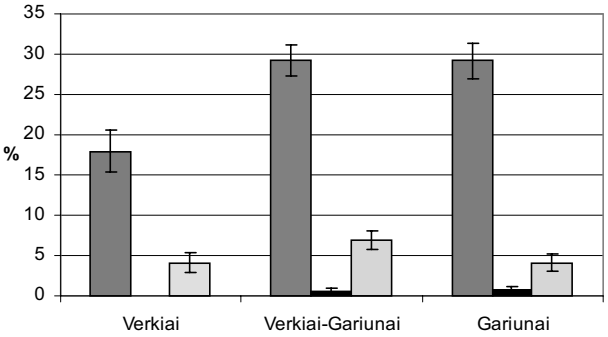

Fig. 5. Amount of aneuploid cells in tissues of *Anodonta cygnea* from the Neris River at Verkiai and Gariūnai, and after 4 weeks of exposure in Gariūnai locality
Hypodiploidy, Hyperdiploid, Polyploidy

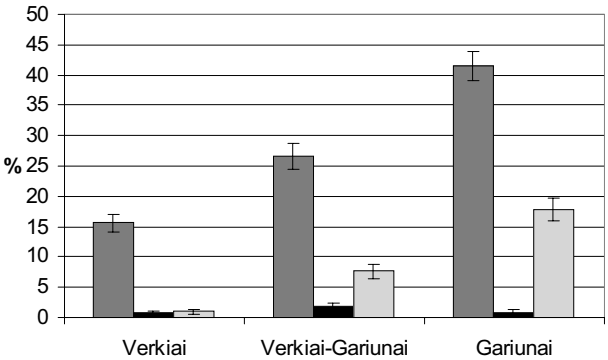

Fig. 6. Amount of aneuploid cells in tissues of *Viviparus viviparus* from the Neris River at Verkiai and Gariūnai, and after 4 weeks of exposure in Gariūnai locality
Hypodiploidy, Hyperdiploid, Polyploidy

DISCUSSION

Exposure of the organism to any kind of environmental contamination may induce significant biologi-

cal changes. Populations living in a contaminated environment often exhibit significant changes in their genetic structure compared to conntrol populations from reference sites. Genotoxic damage in cells can initiate cancerogenesis in various tissues and organs. The concentrations of agents that cause genetic effects may be present at a very low or sublethal level. Changes caused by environmental contaminants may result in direct alterations in genes and gene expression or selective effects of pollutants on gene frequencies.

This study shows that environmental genotoxic effects are exhibited in the tissues of molluscs inhabiting different sites of the Neris River. The aneugenic influence in this river below Vilnius was 1.5–4 times higher than in other studied sites upstream from the Vilnius city. Hypodiploidy of cells was the main type of aneugenic influence in the tissues of molluscs. The amount of hypodiploid cells in tissues of *V. viviparus* collected from the river below Vilnius reached 42%. The highest frequency of polyploid cells (17.8%) in tissues of bivalves was determined in Gariūnai locality, too. This means that the pollutants accumulated in tissues of molluscs originate mainly from the Vilnius city.

Significant differences of the values of cytogenetic injuries were found in tissues of indigenous molluscs from Verkiai and Gariūnai sites. It worth noting that a significant increase of aneugenic effects was observed in molluscs from Verkiai, caged during 4 weeks in a site of the Neris below Vilnius at Gariūnai.

The level of aneugenic effects in tissues of filter-feeding bivalves was slightly higher than in indigenous specimens inhabiting Gariūnai locality, whilst *V. viviparus* after caging didn't reach the level of aneugenic effects which wa observed in indigenous specimens from the highly contaminated area below Vilnius. These differences can arise because of different types of feeding and possibilities to accumulate genotoxic compounds in gastropod snails and bivalve clams.

Heavy metals As and Hg are mitotic spindle poisons and can induce aneugenic effects as a result of desegregation of chromosomes [15]. Compounds of Cr, Ni, Co, Cd and Fe are genotoxic to organisms [12]. These heavy metals were detected in tissues of molluscs inhabiting different sites of the Neris River [7], while in molluscs from Raudondvaris, which was selected as a reference site, comparatively high concentrations of Cd and Ni were determined. Taking into consideration that the Neris downstream from Raudondvaris to Nemenčinė is stretching mainly across a forestry area, there is increasing evidence that an intake of these heavy metals occurs from the Žeimena River (perhaps from the former military areas) or from the territory of Belarus [7]. A significant increase of cytogenetic damage in polluted, in comparison with unpolluted, areas has been determined in *Chironomus riparius* from the Po River in Italy. In sediments of contaminated sites of this river higher concentrations of Cr, Pb, Cd and Cu were observed [16].

Heavy metals can cause an oxidative DNA damage, interfere with the DNA repair and replication process [12]. The formation of reactive oxygen species leads to oxidative DNA damage and induction of DNA strand breaks, DNA–protein cross-links, and therefore chromosomal aberrations are formed. In different aquatic habitats of Lithuania, polluted by heavy metals, PAHs, PCBs and in the areas of the Chernobyl fallout spots, cytogenetic damage in molluscs was higher than in snails from unpolluted areas. It was found that frequency of polyploid cells, chromosome sets instability and neoplastic lesions were higher in molluscs inhabiting areas polluted by both chemicals and radionuclides, which shows the synergistic effects of those pollutants [2–5, 9].

Taking into consideration the complexity of environmental contamination there are studies on other chemical compounds, which affect chromosome distribution, induce aneuploidy and structural aberations. It was found that aneugenic effects were directly related to the level of carcinogenic compounds [11]. Polycyclic aromatic hydrocarbons are well known genotoxins and may cause genetic damage in aquatic organisms [20]. Ionising radiation is well known as inducing mainly structural aberrations of chromosomes [13]. Polychlorinated biphenyl's are stable pollutants which can be found in most aquatic ecosystems. Clams *Mya arenaria* inhabiting heavily polluted with PCBs areas showed a significantly higher amount of micronuclei. Genotoxic effects in the tissues of *Anodonta cygnea* were determined after chlorination of the drinking water [17].

Future studies of environmental genotoxicity in the Neris River must concentrate on determination of relationships between chemical stressors and aneugenic inpact, taking into acount adaptation and elimination mechanisms tolerated by organisms. In this direction one of the very important tool is translocation of organisms in heavily polluted sites and then caging them back to the native places. From the other hand the exsperiments with heavy metals in laboratory conditions will give as some answers about certain toxicant dose providing early warning signs of adverse long-term effects in the populations and communities organisms.

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ANEUGENINIŲ EFEKTŲ TYRIMAI MOLIUSKŲ AUDINIUOSE

S a n t r a u k a

Pagrindinis šio darbo tikslas – įvertinti aneugeninius efektus moliuskuose, gyvenančiuose skirtingo užterštumo Neries zonose. Taip pat nustatyti iš Vilniaus miesto patenkančių teršalų genotoksinę įtaką moliuskams, perkeliant juos iš sąlyginai neužterštos Neries zonos prie Verkių į stipriai užterštą upės zoną ties Gariūnais.

Citogenetinė analizė buvo atliekama *Anodonta cygnea Unio tumidus* ir *Viviparus viviparus* moliuskų somatinėse bei gonadinėse ląstelėse. Atlikus tyrimą paaiškėjo, kad moliuskuose, gyvenančiuose žemiau Vilniaus miesto, aneugeninių efektų skaičius buvo 1,5–4 kartus didesnis nei kitose tirtose Neries zonose. Vyraujantis citogenetinių pažaidų tipas buvo ląselių hipodiploidija. Perkėlus moliuskus iš Verkių zonos į Nerį ties Gariūnais, aneugeninių efektų skaičius patikimai padidėjo ($P < 0,0001$). Citogenetinių pažaidų (po 4 savaičių ekspozicijos) *Anodonta cygnea* moliuskuose padidėjo 14,7%, *Viviparus viviparus* moliuskuose – 18,7%.