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**ENVIRONMENTAL STUDIES  
IN THE  
NEMUNAS RIVER BASIN, LITHUANIA**

Edited by

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## FOREWORD

Scientists from Lithuania and the United States have been involved in a joint research program since 1988 on the fate and effects of pollutants in the environment. Cooperators on the Lithuanian side have included scientists from the Lithuania Environmental Protection Department, the University of Vilnius, and several Institutes within the Lithuania Academy of Sciences. The U.S. scientists were from the Environmental Research Laboratory, Athens, Georgia (ERL-Athens), U.S. Environmental Protection Agency, and the Fisheries Bioassay Laboratory at Montana State University (MSU), Bozeman, Montana.

Research under this cooperative effort has developed in five technical areas: (1) Identification of organic and inorganic chemical pollutants and monitoring of chemical and biological parameters in Lithuanian surface and ground waters; (2) Ecological and water quality modeling, including evaluation of assumptions for modeling fate and effects of pollutants; (3) Measurement of transformation and equilibrium constants for predicting fate of pollutants; (4) Investigation of microbiological transformation processes; (5) Toxicity testing of identified pollutants in biological species indigenous to the Baltic Republics.

Since the initial establishment of goals for this joint research effort in 1988, six scientists from ERL-Athens and MSU have visited Lithuania for 1-4 weeks on ten occasions, and six Lithuanian scientists have visited the U.S. for 2-18 weeks for training at MSU and ERL-Athens. During their trips to Lithuania, U.S. scientists have had the opportunity to work and exchange ideas with several Lithuanian scientists involved in closely related areas of environmental research. The present report results from this association. It contains 17 papers authored by 56 scientists who share a joint concern for the ecological health of this Baltic Republic that has only recently been able to begin to deal with the consequences of the chemical insults to its environment over the past several decades.

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## ABSTRACT

Selected research results from a joint environmental studies project that began in 1988 between Lithuania and the United States of America are presented. Chemical and hydrological studies address hazardous chemicals, including trace metals, in surface waters of Lithuania; methods for determining heavy metals (an interlaboratory comparison); identification of chemicals at pesticide storage sites in Lithuania; and monitoring pollutant loads in the Nemunas River. Biological studies examine biodegraders associated with hydrobionts; toxicity of acetanilide herbicides; water quality in Kuršių Marios Lagoon; cytogenetic changes in freshwater bivalve molluscs; morphological changes of bivalve molluscs under heavy metal bioaccumulation; carcinogenesis research on fishes; toxicity of wastewaters and heavy metals to rainbow trout; and fish avoidance reactions to pollutants. Radionuclide studies investigate strontium 90 concentrations in the Kuršių Marios Lagoon ecosystem and thyroid diseases in Lithuania. Baltic Sea studies examine the mechanism of hydrogen sulfide oxidation and biological consequences of deep water stagnation.



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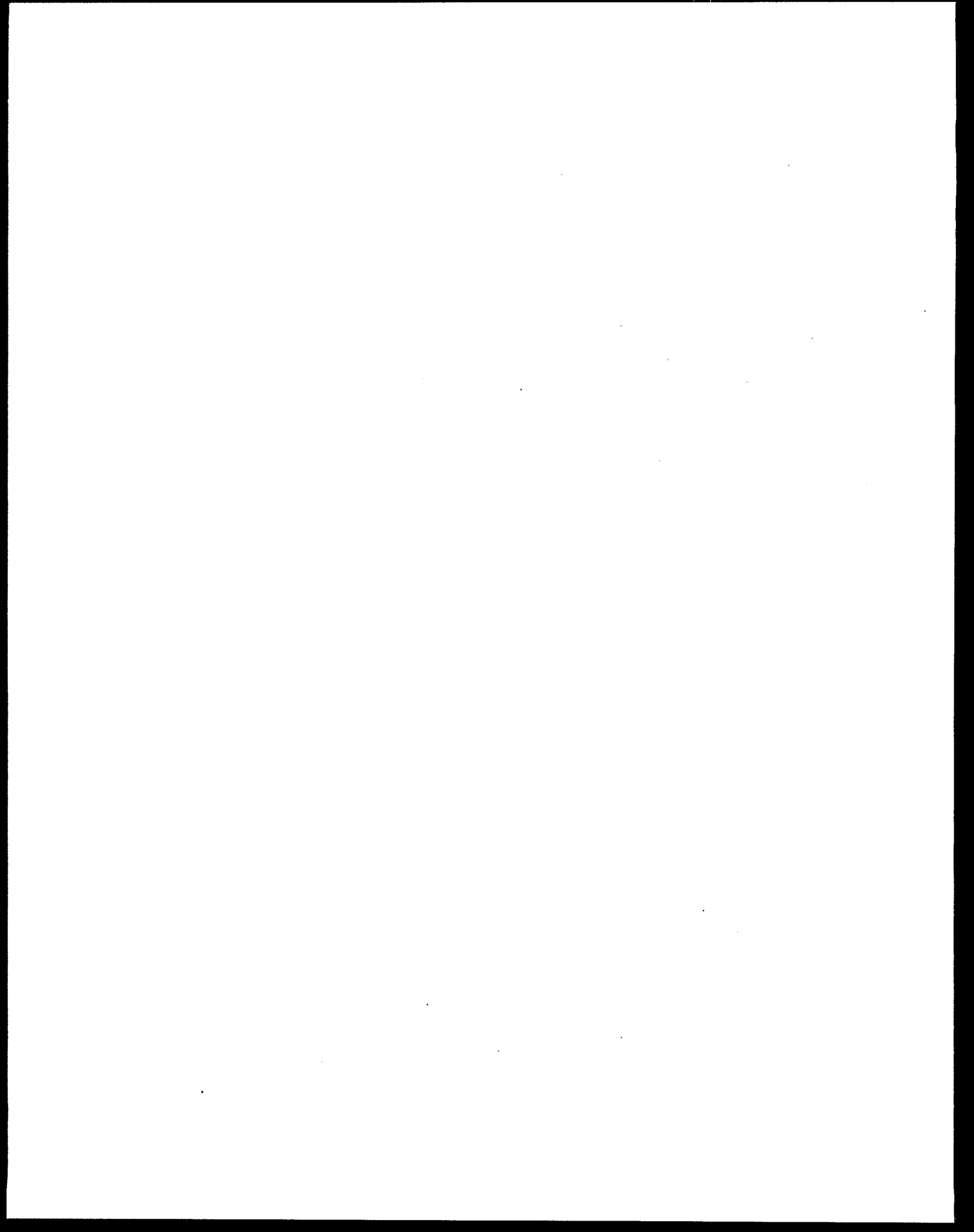
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# HAZARDOUS CHEMICALS IN SURFACE WATERS OF LITHUANIA, 1990-1993

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J. Šukytė<sup>3</sup>, and K. Kvietkus<sup>4</sup>

## ABSTRACT

Water samples were collected from 45 locations throughout Lithuania between 1990 and 1993 as part of a study to determine the distribution and nature of hazardous chemicals in Lithuanian surface waters. Organic chemicals were extracted from the water samples by use of solid phase extraction cartridges or disks in Lithuania, and the cartridges and disks were taken to the United States for elution and analysis by means of gas chromatography and infrared/mass spectrometry. The herbicide, alachlor, was identified in a sample taken from Klaipėda Harbor, and 2-(methylthio)benzothiazole and a dialkylthiophene were identified in a sample taken from the Nemunas River at Grigiškės, in addition to the expected hydrocarbons and fatty acids detected at several locations. Five hydrological water quality variables (pH, temperature, dissolved oxygen, conductivity, and redox potential) were measured at most of the sampling sites. Several water bodies were identified as being in a state of decline.

## INTRODUCTION

A field study on the quality of surface waters in the Republic of Lithuania was initiated in 1990 under the sponsorship of the risk assessment program of the United States Environmental Protection Agency (U.S. EPA), Environmental Research Laboratory, Athens, Georgia (ERL-Athens). During six separate visits in December 1990, July 1991, June and July 1992, and June and October 1993, water samples were collected from 45 locations and sediment samples from two locations throughout Lithuania. These visits were conducted in cooperation with scientists from the Lithuania Environmental Protection Department, the Lithuania Academy of Sciences, and Vilnius University. Sampling sites were located on the Nemunas River and five of its tributaries, Kaunas Reservoir, Kuršių Marios Lagoon, Klaipėda Harbor, the Baltic Sea coast, Lake Dusia, Lake Drūkšiai, and the Kulpė River (Figure 1, Appendix A). Organic chemicals were extracted from the water samples by use of solid phase extraction cartridges or disks and these cartridges and disks were taken back to ERL-Athens for elution and analysis by means of gas chromatography and infrared/mass spectrometry.

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During the 1990-1992 expeditions, hydrological water quality variables were also measured at each site using a remote sampling probe. Water samples were also collected from monitoring wells at a controlled landfill for pesticide wastes at Zigmantiškės and from runoff water at an agrochemicals storage facility at Utena; results of analysis of those samples are reported separately (Ellington *et al.* 1994).

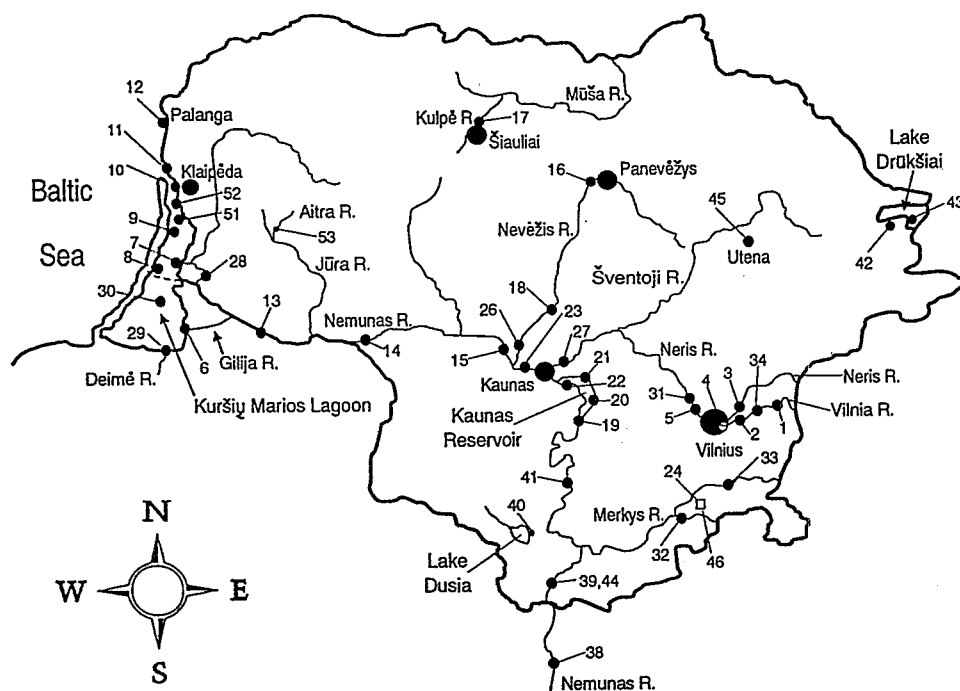


Figure 1. Field study water sampling stations.

## METHODS

### Hydrochemical and Physical Water Quality Variables

On-site water quality measurements in 1990 and 1991 were made with a Hydrolab Surveyor 2, and in 1992 with a Surveyor 3 (Hydrolab Corporation, Austin, Texas, USA), equipped with pH, temperature, dissolved oxygen (DO), conductance, redox potential, and depth sensors. The pH electrode (accuracy  $\pm 0.2$  units, resolution  $\pm 0.01$  unit) was calibrated with buffer solutions at pH 3, 7, and 10. The temperature sensor (accuracy  $\pm 0.2^\circ\text{C}$ , resolution  $\pm 0.01$ ) was verified by simultaneous emersion in a flask of water with a thermometer certified by the U.S. National Institute of Standards and Technology.

The DO sensor (accuracy  $\pm 2\%$ , resolution 0.01 mg/L) was calibrated by the saturated air method as described in the instrument manual. The conductance sensor (accuracy  $\pm 1\%$  of range, resolution 4 digits) was calibrated with solutions of potassium chloride prepared with distilled, deionized water. The redox potential sensor (accuracy  $\pm 20$  mV, resolution 1 mV) was calibrated with quinhydrone dissolved in pH 4 and pH 7 buffer solutions. The stated accuracies are the variations anticipated by the manufacturer if measurements are made under ideal conditions.

### Blanks and Filter Preparation for Collection of Organic Chemicals

The adsorption media for concentration of organics from the water samples were of three types: Bakerbond™ SPE cartridges (6 ml) that contained 500 mg of octylsilane (C-8) or octadecylsilane (C-18), Burdick and Jackson cartridges (6 ml) that contained 500 or 1000 mg of C-8 or C-18, and Empore™ extraction disks (47 mm) consisting of 500 mg of C-8 or C-18 suspended in a Teflon fibril network. The Bakerbond™ cartridges were precleaned at ERL-Athens by removing lettering on the cartridges with a tissue soaked in hexane and extracting the cartridges in a Soxhlet apparatus overnight with hexane:acetone (9:1). The Burdick and Jackson cartridges and the Empore™ disks were precleaned by applying vacuum and pulling through the sorbent bed, in sequence, two 5-ml portions of the final extraction solvent, methanol, and organic-free distilled, deionized water. The Bakerbond™ and Burdick and Jackson cartridges were capped with solvent-cleaned aluminum foil and stored in screw-cap glass vials until sample collection. The Empore™ disks were wrapped flat in solvent-cleaned aluminum foil and stored and transferred in petri dishes. Two blanks of each solid phase sorbent were left refrigerated at ERL-Athens during each sampling trip and two were taken on the sampling trip where they were kept with the solid phase sorbent containers and returned to ERL-Athens for extraction and analysis together with the samples. Silicone tubing (Masterflex™) was used for pumping water during some sampling regimes, and this was checked for leachable organic contaminants by slicing a 2-cm portion of the tubing into cross sections approximately 3 mm thick, and extracting these by immersion under ethyl ether with frequent stirring for 6 hours. Organic chemicals in the ethyl ether extract were identified before and after treatment with diazomethane. Low levels of silica-containing compounds, hydrocarbons, and alkyl fatty acids were detected in the extract.

### Sampling Techniques in the Field for Organic Chemicals

Water samples were filtered through borosilicate microfiber prefilters (Micro Filtration Systems), 47 mm diameter, to remove particles  $> 2.7 \mu\text{m}$  at time of collection, or within 24 hours thereafter. The extraction cartridges and disks were activated with methanol according to supplier specifications prior to concentration of the organic chemicals. The C-18 cartridges were used to concentrate nonpolar organic chemicals from the water samples. Carboxylic acids and phenols were extracted from water samples by adjusting the pH of the sample  $\leq 2$  to protonate the anions before passage of the water through the prefilter and the C-8 cartridge or disk.

A hand pump (Masterflex<sup>TM</sup>) was used in the field to force the water samples through the prefilter contained in an in-line 47-mm filter holder, then through the SPE cartridges at the exit end of the silicone tubing. When laboratory facilities were available, water samples were pumped through the prefilters at the sampling site and then transported to the laboratory where they were pulled through the cartridges and disks by vacuum supplied by water aspiration or mechanical pump. The amount of water sampled at each location varied from 0.2 to 1.5 L, but generally water was pumped or pulled through the SPE cartridges or disks until flow decreased to a few ml/minute. Upon return to ERL-Athens, the SPE cartridges and disks were stored at 4°C until elution and analysis.

### **Extraction and Derivatization of Organic Chemicals**

Prior to eluting the sorbed organics from the SPE cartridges with solvent, excess water was removed by applying vacuum. Carboxylic acids and phenols were eluted from the C-8 cartridges with 5 ml methanol/acetone 9:1. Neutrals were similarly eluted from the C-18 cartridges with 5 ml hexane/acetone 9:1. The disks were placed in a test tube with 5 ml of hexane/acetone 9:1 and sonicated for 2 minutes, and after standing for 5 minutes the solvent was transferred to another tube and the extraction repeated. In 1992 the extraction sequence for the disks was changed to a sequence of 5 ml of ethyl acetate, 5 ml of methylene chloride, and finally 5 ml of ethyl acetate/methylene chloride (1:1) (U.S. EPA 1991). Extracts were reduced to a final volume of 1 ml. Methyl esters of carboxylic acids were formed in the extract from the C-8 cartridges, which were those used for extraction of the pH 2 water samples, by bubbling gaseous diazomethane through the final volume.

The sediment samples collected for analysis of organic chemicals were extracted according to U.S. EPA Method 8151 for the extraction of herbicides and U.S. EPA Method 3550A for the extraction of base/neutrals (U.S. EPA 1992). The appropriate surrogate and matrix spikes and method blanks were performed as prescribed.

### **Gas Chromatography (GC) Analysis**

High resolution GC analysis of sample extracts was performed with a Hewlett-Packard model 5890 GC equipped with both flame ionization (FID) and electron capture (ECD) detectors, splitless inlet, and autosampler (7673A). Separation of sample components was achieved using a 30 m x 0.32 mm i.d. x 0.25  $\mu$ m film DB-5 capillary column operated initially at 40°C for 2 minutes, then temperature-programmed to 290°C at 8°/minute. Helium carrier gas was used at a flow rate of 30 cm/second. Detector temperatures were set at 300°C (FID) and 350°C (ECD). Injections of 1  $\mu$ L were made at an inlet temperature of 250°C.



## **GC/Mass Spectrometry (GC/MS) and GC/Fourier Transform-Infrared Spectrometry/Mass Spectrometry (GC/FT-IR/MS) Analysis**

Low resolution electron-impact (EI) GC/MS was performed on either a Finnigan 4500 (Finnigan MAT, San Jose, California), or a Hewlett-Packard (Palo Alto, California) mass selective detector (MSD). Chemical ionization (CI) mass spectrometry with methane gas was performed on the Finnigan 4500 system. High resolution EI GC/MS analyses of sample extracts were performed on a VG 70-SEQ high resolution hybrid mass spectrometer (VG Instruments, Manchester, United Kingdom). The GC/FT-IR/MS analyses were performed on a Hewlett-Packard system equipped with a 5965B infrared detector and a 5971 series mass selective detector. The gas chromatographs attached to the mass spectrometers were equipped with capillary columns similar in dimensions, liquid phases, and flows to the one described above; the temperature program was also the same.

## **SAMPLING PROGRAM, RESULTS, AND DISCUSSION**

### **Hydrochemical and Physical Water Quality**

In December 1990, the measured pH values ranged from a low of 7.5 in the Kulpė River at Šiauliai (LT-17) to a high of 8.7 at Nida (LT-08) (Appendix B). The high temperature, low DO, and high conductivity (1.55 mS/cm) at Šiauliai were due to the high volume of industrial effluent released into the Kulpė River as it flows through an industrial section of Šiauliai. The other values reported in Appendix B are within the ranges expected for the time of year and the high volumes of surface water caused by heavy rainfall.

In July 1991, the surface DO levels in Kaunas Reservoir were higher at the sampling sites closer to the dam and above deeper water (LT-19A, -19B, -20, -21, and -22) (Appendix C). The DO levels in the Nemunas River below Kaunas Reservoir and continuing into Kuršių Marios Lagoon were also lower than in other surface waters. The Kulpė River at Šiauliai (LT-17) again had a high conductivity value (2.14 mS/cm) for surface water and extremely low DO (0.53 mg/L).

In June 1992, the measured pH and DO values were higher than those measured in July 1991 at all but two sampling sites, Melnragė (LT-11) and Rasytė (LT-30) (Appendix D). The pH value of 9.5 and DO value of 17 mg/L recorded at Lake Drūkšiai (LT-43) are the result of extensive photosynthetic activity by the dense plant growth in the lake, both standing and planktonic. This photosynthetic activity, while raising the level of molecular oxygen, also increases the hydroxyl ion load with a corresponding increase in pH.

## Organic Chemicals in Water

In December 1990, water measurements were made and samples collected on SPE cartridges from 24 sites in Lithuania, including the Vilnia, Neris, Nevėžis, Kulpė, and Nemunas Rivers, Kaunas Reservoir, and Kuršių Marios Lagoon (Appendix B). Upon return to ERL-Athens, the sorbed organics were eluted and GC-FID and GC-ECD chromatograms were obtained. The retention times of all the major peaks in the chromatograms from the surface water samples were identical. GC/MS analysis of the 1990 surface water extracts identified the major peaks as several dialkyl phthalates, silica-containing components, saturated hydrocarbons, and some unidentified peaks with the same mass fragmentation pattern that appeared in every extract. Dialkyl phthalates are commonly found in environmental samples and stringent precautions are necessary to assure their presence in the collected sample is not due to accidental contamination. Hydrocarbons, phthalates, and fatty acids were found in a few of the method and field blanks but at lower levels than in the samples; the presence of phthalates in the samples may be real. Absence of man-made chemicals other than possibly phthalates in the surface water samples was surprising, but could have been caused by the ambient water temperature (4°C) at this time of year, and the high levels and flows of water from recent heavy rainfall. Both of these conditions would lower the concentration of organic chemicals in the surface water, possibly below the level of detection for the volume of water sampled. Organic chemicals identified in water samples collected during this and subsequent expeditions are listed in Table 1.

Table 1. Hazardous chemicals identified in surface water samples.

Chemical	Grigiškės (LT-31)	Kaunas Reservoir (LT-19-22)	Green Harbor (LT-52)
Alachlor <sup>1</sup>			x
Alkyl hydrocarbons <sup>2</sup>	x	x	
Aromatic hydrocarbons <sup>2</sup>	x		
Dialkylthiophene <sup>2</sup>	x		
2,4-Dichlorobenzoic acid <sup>1</sup>	x	x	
Fatty acids <sup>2</sup>	x	x	x
2-(Methylthio) benzothiazole <sup>2</sup>	x		
Phenanthrene carboxylic acid <sup>2</sup>	x	x	
Phthalates <sup>1,3</sup>	x	x	x
Silicon organics <sup>2</sup>	x	x	x
Tetrachlorinated unknowns <sup>2</sup>	x	x	

<sup>1</sup>Identified by GC/FT-IR/MS and confirmed with a standard

<sup>2</sup>Identified by GC/FT-IR/MS but not confirmed with standards

<sup>3</sup>Diisooctyl-, Butylbenzyl-, Di-n-octyl-, and Di-n-butylphthalates were observed the most frequently

In July 1991, 15 of the former sites were sampled, as were eight new locations including the Šalčia River (LT-32) downgradient from the long-term pesticide storage site at Zigmantiškes (LT-24) (Ellington *et al.* 1994), the Neris River (LT-31) downstream from Vilnius below a paper manufacturing plant, and the Merkys River (LT-33) downstream from a combined poultry growing and processing facility (Appendix C). Low resolution GC/MS indicated the presence of three tetrachlorinated isomers in the diazomethane-treated sample extracts from the Neris River and Kaunas Reservoir (LT-20) (Figure 2).

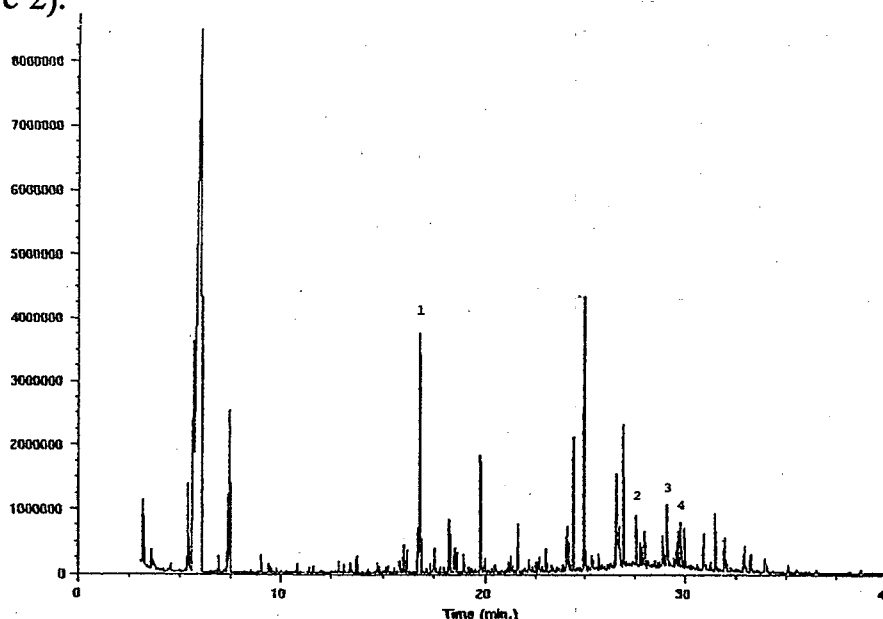


Figure 2. Total ion mass chromatogram of the solid phase extraction eluant from Kaunas Reservoir. GC/MS Peak 1 = 2,4-dichlorobenzoic acid methyl ester. GC/MS Peaks 2, 3, 4 = unknown tetrachlorinated isomers.

High resolution EI GC/MS of the three tetrachlorinated peaks in the Kaunas Reservoir water sample extract yielded best fit empirical formulae of either  $C_7H_{13}Cl_4O_5P$  or  $C_{14}H_8Cl_4O_2$ . A tetrachlorinated trialkyl phosphate fits the former formula while tetrachlorinated aromatic esters and other structures containing carbonyl groups can be drawn for the latter. Both formulae are similar to those of compounds commonly used as electrical insulators and flame retardants. The compound containing phosphorus was eliminated as a possibility by further GC analysis of the sample using nitrogen and nitrogen-phosphorus specific detectors, respectively. The presence of the carbonyl functional group in the three unknowns was confirmed by GC/FT-IR. The loss of the  $m/z$  31 ion (loss of  $-OCH_3$ ) during electron impact GC/MS argued for the presence of a methoxy functional group, possibly a methyl ester. However, the absence of a strong peak in the  $1250-1310\text{ cm}^{-1}$  region of the IR spectrum and the broad carbonyl peaks argued against the ester functionality. Detection of the unknowns only after treatment of the extract with diazomethane is evidence for a reactive hydrogen needed to form a methoxy group. A tetrachlorinated aromatic ring system containing a carbonyl group,

methoxy group, and differing chlorine substitution is the most likely structure for the three unknown isomers. The surface water samples collected at Kaunas Reservoir and the Neris River at Grigiškės (LT-31) also contained 2,4-dichlorobenzoic acid in addition to the three tetrachlorinated unknowns. Fatty acids, identified in the Kaunas Reservoir and Grigiškės samples as their methyl esters, could be an indication of contamination by municipal sewage.

In June 1992, samples were collected at some of the sites previously sampled and new sampling sites were added at Lake Dusia, Lake Drūkšiai, and three locations on the Nemunas River, two of these upstream from Kaunas Reservoir, and one downriver from Gardinas in Belarus (Appendix D). Lake Drūkšiai is both the source of and receiving body for the cooling water for the Ignalina nuclear power facility. Chemicals tentatively identified in surface water samples, but not confirmed with standards, included 2,2-dimethoxy-1,2-diphenyl ethanone, dialkylthiophene, and 2-(methylthio)thiophene.

During June 1993, water samples were collected at Juodkrantė, Klaipėda Harbor, and at three new sampling sites: Amber Cove (LT-51) and Green Harbor (LT-52) in the Klaipėda Harbor area of Kuršių Marios Lagoon, and the Aitra River (LT-53). Organics were extracted with Empore<sup>TM</sup> disks from the Klaipėda Harbor water samples, but only the Green Harbor sample was analyzed by GC/FT-IR/MS. This site is located at the mouth of a channel that is used for drydock-ship repair, and sewage from the Klaipėda municipal treatment facility is discharged into this channel. In addition to the expected fatty acids, hydrocarbons, and phthalates tentatively identified by interpretation of MS and IR spectra, an herbicide (alachlor) was identified and confirmed with a standard. The alachlor was present at approximately 3 ppb. The source of the alachlor remains unknown and it is uncertain if this concentration of alachlor poses a threat to aquatic organisms. The only compounds detected in the Aitra River sample were sulfur, trace amounts of phthalates and hydrocarbons, and low molecular weight carboxylic acids in the 10-40 ppb concentration range.

### Organic Chemicals in Sediments

In June 1992, two sediment samples were collected for analysis for organic chemicals. One of these samples was collected off the mouth of the Gilija River (LT-06) as it flows into Kuršių Marios Lagoon, and the other from the Neris River at Grigiškės (LT-31), downstream from Vilnius. After extraction of the organics, the resulting acid/herbicide and base/neutral extracts were analyzed by GC/FT-IR/MS. Fatty acid methyl esters, hydrocarbons and the methyl ester of phenanthrene carboxylic acid were the only compounds identified with a high degree of confidence using both the spectral matching library and manual interpretation. The presence of multichlorine substitution was indicated in the mass spectra of several peaks in each extract but tentative identifications were not possible. The only compounds other than hydrocarbons identified in the base/neutral extracts were cholesterol in the Grigiškės sample and the octyl ester of phosphoric acid in the Gilija sample. Alkyl phosphate esters are commonly used in lubricating greases, and this is one possible source of the octyl ester.

## CONCLUSIONS AND RECOMMENDATIONS

The majority of the population and industry of Lithuania are located in the Nemunas River basin which includes the Neris River. Anthropogenic chemicals were detected in only a few of the surface water samples, and we cannot explain the reasons for this. For the December 1990 samples, this might have been at least in part because of a combination of the low water temperatures and the dilution of surface waters from heavy rainfall during that season of the year. Failure to detect a larger number of chemicals in the samples collected during the summers of 1991-1993 might have been partially the result of a national economic recession, with commensurate decrease in industrial activity. The low levels of DO ( $<5$  mg/L) in the Kaunas Reservoir in July 1991 is an indication of a water system in decline, as are the extremely high DO (17 mg/L) and pH (9.5) measurements in Lake Drūkšiai.

Other sites of concern are the Kulpė River in Šiauliai, outside the Nemunas River basin, which receives the majority of its total flow within the city limits from industrial and municipal sources, and the Deimė River flowing from Kaliningrad into the southern end of Kuršių Marios Lagoon. Analysis of sediment samples from rivers and lakes should be given a high priority, inasmuch as sediments contain the historical burden of pollution and are one of the eventual sinks for pollutants.

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## Appendix A. Field study water sampling stations

Number	Name	Description
LT-01	Lavoriškės	Vilnia River, ca 40 km above Vilnius at hay field
LT-02	Pavilnys	Vilnia River, ca 8 km above Vilnius at dam
LT-03A	Pioneer Camp	Neris River, ca 16 km above Vilnius at Young Pioneer Camp
LT-03B	Turniškės	Neris River, ca 1-2 km below Pioneer Camp, and 102 km above Turniškės
LT-04	Gediminas Castle	Vilnia River, ca 200 m above confluence with Neris River
LT-05	Gariūnai	Neris River, ca 2 km below Vilnius wastewater treatment facility, near field with abandoned Soviet tanks
LT-06	Gilija	Kuršių Marios Lagoon off the mouth of the Gilija River (Matrosovo Kanalas)
LT-07	Uostadvaris	Kuršių Marios Lagoon, ca 10 m offshore at Uostadvaris
LT-08	Nida Station	Kuršių Marios Lagoon, ca 200 m offshore at Nida
LT-09	Juodkrantė	Kuršių Marios Lagoon, at Juodkrantė from the shore at boat beaching area
LT-10A-B	Klaipėda Harbor	Klaipėda Harbor, east side, from the dock, north side of Danė River mouth
LT-11A-D	Melnragė-A-D	Baltic Sea, at Melnragė, (A) from the end of breakwater, near grounded freighter, "Rudolph Breitscheid", (B) from boat 100 m west of "Rudolph Breitscheid", (C) from boat immediately inside mouth of breakwater, (D) from boat inside mouth of breakwater, opposite stone tower and ca 100 m downstream from museum
LT-12	Palanga	Baltic Sea, at Palanga from the pier 50 m from shore
LT-13-1,2	Sovetskask	Nemunas River, At Sovetskask, west of bridge, ca 3 km below one paper plant and ca 11 km below another, (1) from north bank, (2) from boat at river center
LT-14	Smalininkai	Nemunas River, at Smalininkai from the north bank near houses
LT-15A-C	Kulautuva-A-C	Nemunas River, ca 13 km below Nemunas-Nevėžis confluence, (A) from north bank, (B) south bank at Zapyškis, (C) north bank ca 1 km below Zapyškis
LT-16	Panevėžys	Nevėžis River, ca 1 km below discharge from Panevėžys wastewater treatment facility, south bank ca 200 m from abandoned church
LT-17	Šiauliai	Kulpė River, ca 100 m below abattoir and ca 1 km downriver from Šiauliai
LT-18	Kėdainiai	Nevėžis River, ca 1.5 km below wastewater treatment facility, from south bank at collective farm

(Continued)

## Appendix A. Continued

Number	Name	Description
LT-19A	Nemunas, II Cut	Kaunas Reservoir, upper end inside Nemunas River, before discharging into the reservoir
LT-19B	Lapaine	Kaunas Reservoir, upper end even farther inside Nemunas River
LT-20	Kaišiadorių	Kaunas Reservoir at Hydroaccumulation Electric Power (HAEP), near middle, 200 m offshore
LT-21	Dambrava, IV Cut	Kaunas Reservoir, near sauna, 200 m offshore
LT-22	Pažaislis, VII Cut	Kaunas Reservoir, near dam, 400 m off Pažaislis Monastery
LT-23A	Sand Beach	Nemunas River, below mouth of Neris River and above mouth of Nevėžis River, on south bank across from heavy industry
LT-23B	Lampedžiai	Nemunas River, below mouth of Neris River and above mouth of Nevėžis River, on north bank ca 500 m below LT-23A
LT-24A-F	Pesticide Storage Site	Ministry of Agriculture Pesticide Storage Site southwest of Vilnius, ca 3 km north of Šalčia River; Monitoring Wells #1-6 inside the site
LT-26A-B	Raudondvaris	Nevėžis River, (A) ca 2.5 km above confluence with Nemunas, from west bank, (B) ca 1 km above confluence with Nemunas, from west bank
LT-27	Neris at Kaunas	Neris River at last bridge confluence with Nemunas River, from north bank
LT-28A	Rusnė-A	Nemunas River at bridge, ca 10 km before Kuršių Marios Lagoon
LT-28B	Rusnė-B	Nemunas River, ca 2 km above bridge
LT-29	Deimė	Deimė River, immediately inside mouth before flowing into southernmost section of Kuršių Marios Lagoon
LT-30	Rasytė	Kuršių Marios Lagoon, approximate center
LT-31	Grigiskės	Neris River, ca 17 km west of Vilnius, from south bank
LT-32	Zigmantiškės	Šalčia River, ca 3 km south of Pesticide Disposal Site
LT-33	Jašiūnai	Merkys River, ca 25 km south of Vilnius
LT-34	New Vilnia	Vilnia River, ca 20 km above Vilnius and just above New Vilnia
LT-38	Sredny Gonkolsky	Nemunas River, east bank ca 5 km below Gardinas city limits (in Belarus)
LT-39	Liškiava	Nemunas River, west bank, ca 10 km below Druskininkai
Lt-40	Lake Dusia	Lake Dusia, east side, ca 40 km northwest of Druskininkai
LT-41	Alytus	Nemunas River, ca 3 km below Alytus water treatment facility

(Continued)



## Appendix A. Continued

Number	Name	Description
LT-42	Visaginas	Wastewater treatment facility outflow at Visaginas
LT-43	Lake Drūkšiai	Lake Drūkšiai, south shore of west arm
LT-44	Crusader Creek	Mouth of creek before flowing into Nemunas River, ca 40 m below LT-39
LT-45A-E	Utena Storage	Utena warehouse toxic chemical storage location: A - access hole to drainage pipe from under warehouse and water catchment area B - water catchment under warehouse, east side C - storage tank inside door on right side D - storage tank inside door on left side E - storage tanks LT-45C and LT-45D combined and treated
LT-46A-D	Pesticide Storage Site	Adjacent to the Ministry of Agriculture Pesticide Storage Site southwest of Vilnius, Monitoring Wells #1-4 outside the fence
LT-51	Amber Cove	Kuršių Marios Lagoon, 1 km north of Juodkrantė, ca 400 m offshore
LT-52	Green Harbor	Klaipėda Harbor, ca 200 m offshore from discharge of Klaipėda municipal wastewater treatment facility
LT-53	Girėnai	Aitra River, east of Girėnai, ca 10 km above confluence with Jūra River

# Appendix B. Hydrochemical and physical water quality data, November-December 1990

Station Number	Station Name	Depth <sup>1</sup> (m)	Temp. <sup>2</sup> (C)	Dissolved Oxygen <sup>3</sup> (mg/L)	pH <sup>4</sup>	Conductivity <sup>5</sup> (mS/cm)	Salinity (ppt)	Redox Potential <sup>6</sup> (mV)
Kulpe River								
LT-17	Šiauliai	Sur	11.17	4.66	7.50	1.55	0.3	41
Vilnia River								
LT-01	Lavoriškės	0.6	5.22	10.28	7.89	0.444	0.0	91
LT-02	Pavilnys	Sur	4.79	9.70	7.79	0.463	0.0	95
Neris River								
LT-03A	Pioneer Camp-A	Sur	3.27	11.74	7.98	0.415	0.0	97
LT-04	Gediminas Castle	Sur	4.90	11.78	8.14	0.472	0.0	111
LT-05	Gariūnai	Sur	3.45	11.25	8.13	4.23	0.0	90
Nevėžis River								
LT-16	Panevėžys	0.8	2.68	11.95	7.86	0.798	0.0	105
LT-18	Kėdainiai	0.6	2.62	12.41	8.04	0.876	0.0	127
Kaunas Reservoir								
LT-19A	Nemunas-II	3.3	2.66	11.61	8.09	0.484	0.0	121
LT-20	Kaišiadoryų	1.0	2.53	11.55	8.06	0.484	0.0	138
LT-21	Dambrava-IV	0.9	2.82	11.30	8.10	0.486	0.0	148
LT-22	Pašaislis-VII	0.9	2.98	11.15	8.11	0.487	0.0	148
Nemunas River								
LT-23A	Sand Beach-A	0.7	3.14	10.97	8.12	0.512	0.0	145
LT-15A	Kulautuva-A	0.7	1.60	10.89	8.06	0.784	0.0	157
LT-14	Smalininkai	0.8	2.38	11.01	8.05	0.570	0.0	166
LT-13A-1	Sovetskask-A	0.7	2.62	7.40	7.62	0.635	0.0	120
Kuršių Marios Lagoon								
LT-06	Gilija	Sur	3.77	10.43	7.89	0.546	0.0	97
LT-07	Uostadvaris	Sur	3.60	11.06	8.02	0.524	0.0	112
LT-08	Nida Station	Sur	3.95	11.77	8.27	0.467	0.0	106
LT-09	Juodkrantė	0.9	3.13	12.00	8.10	0.480	0.0	39
LT-10A	Klaipėda Harbor-A	0.7	5.24	11.34	7.58	6.44	3.2	95
Baltic Sea Coast								
LT-11A	Melnragė-A	0.7	6.29	11.43	7.79	11.02	5.9	101
LT-12	Palanga	0.7	5.59	11.71	7.92	9.29	3.8	160

<sup>1</sup>Depth probe accuracy  $\pm 0.45$  m, Sur = approximately 0.3-0.5 m below surface <sup>2</sup>Temperature accuracy  $\pm 0.2^\circ$  <sup>3</sup>DO accuracy  $\pm 2\%$  <sup>4</sup>pH accuracy  $\pm 0.2$  units <sup>5</sup>Conductivity accuracy  $\pm 1\%$  of range, resolution 4 digits <sup>6</sup>Redox accuracy  $\pm 20$  mV

# Appendix C. Hydrochemical and physical water quality data, July 1991

Station Number	Station Name	Depth <sup>1</sup> (m)	Temp. <sup>2</sup> (C)	Dissolved Oxygen <sup>3</sup> (mg/L)	pH <sup>4</sup>	Conductivity <sup>5</sup> (mS/cm)	Salinity (ppt)	Redox Potential <sup>6</sup> (mV)
Kulpė River								
LT-17	Šiauliai	Sur	21.80	0.53	7.69	2.14	0.6	- 95
Neris River								
LT-31	Grigiškės	Sur	18.53	8.30	8.10	0.476	0.0	61
LT-27	Neris at Kaunas	Sur	21.06	14.51	8.58	0.453	0.0	83
Šalčia River								
LT-32	Zigmantiškes	Sur	15.82	8.46	7.72	0.452	0.0	94
Merkys River								
LT-33	Jašiūnai	Sur	14.73	9.32	7.91	0.458	0.0	102
Nevėžis River								
LT-16	Panevėžys	Sur	19.14	6.85	7.82	0.728	0.0	76
LT-18	Kėdainiai	Sur	21.20	7.20	7.84	0.808	0.0	61
LT-26A	Raudondvaris	Sur	22.25	5.40	7.55	0.829	0.0	106
Kaunas Reservoir								
LT-19A	Nemunas-II	0.5	22.65	1.45	7.56	0.478	0.0	92
"	"	2.0	22.71	1.42	7.62	0.479	0.0	83
"	"	4.0	22.54	0.58	7.58	0.478	0.0	85
"	"	6.0	22.58	0.26	7.60	0.477	0.0	76
"	"	8.0	22.58	0.19	7.62	0.477	0.0	70
LT-19B	Lapaine	0.5	22.74	2.58	7.83	0.486	0.0	53
"	"	1.0	22.71	2.54	7.83	0.486	0.0	51
"	"	2.0	22.61	2.21	7.81	0.487	0.0	50
"	"	3.0	22.60	2.16	7.80	0.486	0.0	49
"	"	4.0	22.60	2.11	7.80	0.486	0.0	47
"	"	5.0	22.59	2.16	7.81	0.485	0.0	45
"	"	6.0	22.58	2.09	7.80	0.485	0.0	43
"	"	7.0	22.54	1.73	7.78	0.486	0.0	41
"	"	8.0	22.51	1.66	7.77	0.486	0.0	39
"	"	9.0	22.5	1.49	7.74	0.487	0.0	37
"	"	10.0	22.5	1.22	7.72	0.487	0.0	33
"	"	11.0	22.5	1.42	7.70	0.489	0.0	30

(Continued)

# Appendix C. Continued

Station Number	Station Name	Depth <sup>1</sup> (m)	Temp. <sup>2</sup> (C)	Dissolved Oxygen <sup>3</sup> (mg/L)	pH <sup>4</sup>	Conductivity <sup>5</sup> (mS/cm)	Salinity (ppt)	Redox Potential <sup>6</sup> (mV)
Kaunas Reservoir								
LT-20	Kaišiadorių	0.5	22.81	1.13	7.60	0.476	0.0	114
"	"	2.0	22.85	1.13	7.62	0.477	0.0	109
"	"	3.0	22.85	1.05	7.64	0.477	0.0	104
"	"	4.0	22.85	1.03	7.65	0.477	0.0	101
"	"	6.0	22.73	0.91	7.63	0.478	0.0	122
"	"	8.0	22.71	0.65	7.63	0.477	0.0	116
"	"	10.0	22.30	0.54	7.60	0.490	0.0	115
"	"	12.0	22.09	0.55	7.58	0.496	0.0	113
LT-21	Dambava-IV	0.5	22.48	4.76	7.72	0.461	0.0	117
"	"	2.0	22.51	4.91	7.74	0.459	0.0	115
"	"	4.0	22.50	4.99	7.81	0.458	0.0	113
"	"	6.0	22.44	5.56	7.83	0.456	0.0	112
"	"	8.0	22.30	4.49	7.70	0.456	0.0	115
"	"	10.0	22.17	3.14	7.61	0.456	0.0	116
"	"	12.0	22.15	2.74	7.59	0.456	0.0	115
"	"	14.0	22.17	2.71	7.59	0.453	0.0	99
LT-22	Pažaislis-VII	0.5	22.22	8.44	7.93	0.443	0.0	141
"	"	1.0	22.05	5.22	7.81	0.446	0.0	140
"	"	2.0	21.59	3.45	7.62	0.447	0.0	146
"	"	4.0	21.45	3.25	7.55	0.488	0.0	145
"	"	6.0	21.37	2.98	7.53	0.448	0.0	145
"	"	8.0	21.28	2.84	7.52	0.449	0.0	144
"	"	10.0	21.18	2.54	7.50	0.447	0.0	142
"	"	12.0	21.12	2.31	7.49	0.449	0.0	141
"	"	14.0	20.75	1.45	7.46	0.450	0.0	142
"	"	16.0	20.66	1.21	7.44	0.450	0.0	141
"	"	18.0	20.67	1.31	7.45	0.449	0.0	139
"	"	20.0	20.65	1.23	7.45	0.450	0.0	138
Nemunas River								
LT-23B	Lampedžiai-B	Sur	20.97	12.02	8.52	0.466	0.0	73
LT-15B	Zapyškis-B	Sur	20.56	5.14	7.47	0.464	0.0	93
LT-14	Smalininkai	Sur	20.25	11.88	8.42	0.462	0.0	74
LT-13-1	Sovetskask	Sur	20.55	10.28	7.88	0.516	0.0	75
LT-28A	Rusnė-A	Sur	20.72	11.70	8.21	0.484	0.0	82

(Continued)

# Appendix C. Continued

Station Number	Station Name	Depth <sup>1</sup> (m)	Temp. <sup>2</sup> (C)	Dissolved Oxygen <sup>3</sup> (mg/L)	pH <sup>4</sup>	Conductivity <sup>5</sup> (mS/cm)	Salinity (ppt)	Redox Potential <sup>6</sup> (mV)
Kursių Maršas Lagoon								
LT-29	Deimė	0.5	21.40	3.88	7.48	0.593	0.0	86
"	"	2.0	21.21	3.76	7.50	0.593	0.0	83
"	"	4.0	20.90	2.89	7.47	0.592	0.0	83
"	"	6.0	20.85	2.75	7.46	0.590	0.0	81
"	"	8.0	20.84	2.77	7.44	0.590	0.0	75
"	"	10.0	20.84	2.66	7.43	0.590	0.0	75
LT-06	Gilija	0.5	20.93	8.68	7.75	0.495	0.0	105
"	"	1.5	20.29	9.35	8.01	0.481	0.0	97
LT-30	Rasytė	0.5	19.72	9.71	8.37	0.475	0.0	88
"	"	2.0	19.69	9.60	8.33	0.475	0.0	87
"	"	4.0	19.17	7.28	8.02	0.480	0.0	95
LT-07	Uostadvaris	0.5	20.18	11.03	8.47	0.467	0.0	57
"	"	2.0	20.14	10.96	8.45	0.468	0.0	53
"	"	4.0	20.12	10.75	8.40	0.467	0.0	52
"	"	6.0	20.13	10.77	8.36	0.467	0.0	50
LT-8	Nida Station	0.5	19.83	7.17	8.08	0.476	0.0	106
"	"	2.0	19.70	7.20	8.03	0.477	0.0	108
LT-10A	Klaipėda Harbor-A	Sur	19.27	9.22	8.34	5.30	2.4	115
Baltic Sea Coast								
LT-11A	Melnragė-A	Sur	18.84	8.45	8.18	11.72	6.4	78
LT-12	Palanga	Sur	19.78	8.57	8.48	10.25	5.5	78
"	"	1.5	19.73	8.40	8.50	10.26	5.5	72

<sup>1</sup>Depth probe not operating - actual depth measurements reported. Sur = approximately 0.3-0.5 m below surface

<sup>2</sup>Temperature accuracy  $\pm 0.2^\circ$  <sup>3</sup>DO accuracy  $\pm 2\%$  <sup>4</sup>pH accuracy  $\pm 0.2$  units

<sup>5</sup>Conductivity accuracy  $\pm 1\%$  of range, resolution 4 digits <sup>6</sup>Redox accuracy  $\pm 20$  mV

# Appendix D. Hydrochemical and physical water quality data, June 1992

Station Number	Station Name	Depth <sup>1</sup> (m)	Temp. <sup>2</sup> (C)	Dissolved Oxygen <sup>3</sup> (mg/L)	pH <sup>4</sup>	Conductivity <sup>5</sup> (mS/cm)	Salinity (ppt)	Redox Potential <sup>6</sup> (mV)
<b>Lakes</b>								
LT-40	Lake Dusia	Sur	19.81	9.32	8.46	0.327	0.2	302
LT-43	Lake Drūkšiai	Sur	22.14	17.15	9.49	0.320	0.2	264
<b>Vilnia River</b>								
LT-34	New Vilnia	Sur	15.80	12.18	8.08	--	--	297
LT-04	Gediminas Castle	Sur	17.64	11.44	8.17	0.509	0.3	298
<b>Neris River</b>								
LT-03B	Turnišķės-B	Sur	19.23	13.59	8.62	--	--	279
LT-31	Grigiskės	Sur	19.29	13.20	8.55	--	--	276
<b>Nevėžis River</b>								
LT-26B	Raudondvaris	Sur	19.41	10.52	8.52	0.421	0.2	333
<b>Nemunas River</b>								
LT-38	Sredny Gonkolsky	Sur	21.64	9.78	8.51	0.377	0.2	284
LT-39	Liškiava	Sur	20.16	7.99	8.31	0.366	0.2	305
LT-44	Crusader Creek	Sur	11.71	9.73	8.10	0.460	0.2	311
LT-41	Alytus	Sur	21.11	9.76	8.55	0.399	0.2	303
LT-15C	Kulautuva-C	Sur	19.53	10.42	8.53	0.431	0.2	322
LT-13-2	Sovetskask	Sur	21.45	14.98	8.97	0.405	0.2	286
LT-28B-2	Rusnė-B	Sur	21.98	14.36	8.94	0.400	0.2	276
<b>Kuršių Maros Lagoon</b>								
LT-29	Deimė	Sur	22.10	6.07	8.10	0.558	0.3	330
"	"	5	21.93	5.15	7.99	0.560	0.3	331
"	"	10	21.65	4.11	7.89	0.558	0.3	334
"	"	12	26.64	3.95	7.86	0.556	0.3	334
LT-06	Gilija	Sur	20.16	11.07	8.95	0.424	0.2	292
"	"	2.5	19.98	10.37	8.95	0.428	0.2	317
LT-30	Rasytė	0.7	20.46	8.79	8.69	0.441	0.2	319
"	"	2.2	20.47	8.83	8.75	0.442	0.2	314
LT-07	Uostadvaris	Sur	22.08	13.34	8.84	0.411	0.2	309
"	"	4.5	21.50	9.89	8.57	0.424	0.2	322
LT-08	Nida Station	Sur	20.50	7.99	8.57	0.429	0.2	310
<b>Baltic Sea Coast</b>								
LT-11B	Melnragė-B	0.6	7.63	7.81	7.61	12.56	7.2	392
"	"	8	6.59	7.25	7.57	12.62	7.2	389
LT-11C-2	Melnragė-C	Sur	6.83	7.33	7.31	12.54	7.2	409
"	"	10	6.58	6.95	7.48	12.57	7.2	396
LT-11D-2	Melnragė-D	Sur	7.06	6.77	7.58	12.66	7.3	420
"	"	10	6.89	6.88	7.59	12.69	7.3	425

<sup>1</sup>Depth probe accuracy  $\pm 0.45$  m, Sur = approximately 0.3-0.5 m below surface <sup>2</sup>Temperature accuracy  $\pm 0.2^\circ$  <sup>3</sup>DO accuracy  $\pm 2\%$  <sup>4</sup>pH accuracy  $\pm 0.2$  units <sup>5</sup>Conductivity accuracy  $\pm 1\%$  of range, resolution 4 digits <sup>6</sup>Redox accuracy  $\pm 20$  mV

# TRACE METAL CONCENTRATIONS IN NATURAL WATERS IN LITHUANIA, 1991-1992

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## ABSTRACT

The concentrations of ten metals in natural waters in Lithuania were measured in 1991 and 1992 to understand the influence of industrial wastes on the quality of those waters. The analyses were performed by atomic absorption spectroscopy. Samples from the Kulpė River at Šiauliai and from the Baltic Sea near Klaipėda Harbor had high trace metal concentrations. Hydrological parameters such as pH, dissolved oxygen, conductivity, redox potential, and salinity were also measured; these showed significant differences among the sampling sites.

## INTRODUCTION

Low mineral levels and simple chemical composition are general characteristics of Lithuanian rivers (Garunkštis 1988). The anthropogenic chemical composition of small rivers in Lithuania is usually determined by the chemicals used in agriculture, and the composition of large rivers is influenced mostly by sewage from industrial enterprises and big cities. Lithuanian rivers are generally alkaline because dissolved calcium, magnesium, and carbonate ions comprise up to 85% of all dissolved substances. The abundance of these ions is caused by carbonated sediments and by climatic conditions that stimulate the leaching of carbonates. The lowest levels of carbonates are found in the small rivers, flowing from marsh waters (Garunkštis 1988).

Of the area of Lithuania, 70 percent (46,600 km<sup>2</sup>) falls within the Nemunas River basin, so the water quality of most rivers in Lithuania influence the quality of the Nemunas River and Kuršių Marios Lagoon, into which the Nemunas River flows. Kuršių Marios Lagoon (1,610 km<sup>2</sup>) is the largest reservoir of fresh water in Lithuania. In addition to Kuršių Marios Lagoon being the recipient of pollutants discharged from the Nemunas River, other major sources of pollution to the lagoon are the discharge of the Deimė River from Kaliningrad and several wastewater outfalls from the City of Klaipėda into Klaipėda Harbor, a major shipping port. Approximately 26 factories discharge their contaminated waters into the northern part of the lagoon (Griškevičius 1987).

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The number of analyses performed on river waters to measure trace metals has greatly increased in recent years due to improvements in analytical techniques used for geoexploration and environmental management (Salomons and Förstner 1984). There are very significant regional differences with respect to various processes occurring in rivers and other natural water basins, to concentrations of different elements, and to compounds and poisonous effects which they induce (Anikiev *et al.* 1990, Nimmo *et al.* 1989). Natural water, especially river water, has a direct influence on groundwater, which is used as drinking water (Pelig-Ba *et al.* 1991), with resultant effects on humans (McClain and Becker 1975). Until 1990, there were no detailed investigations of heavy metals in natural waters in Lithuania, mainly because of the lack of adequate instrumentation.

The present study was undertaken during 1991-1992 to assess the distribution of trace metals in Lithuanian surface waters. Water samples were collected and analyzed for 10 trace metals at 23 sites on five rivers, Kaunas Reservoir, Kuršių Marios Lagoon, and the Baltic Sea at the mouth of Klaipėda Harbor (Figure 1, Table 1). Twenty of these sites were sampled during July 1991, a representative climate year, and 15 sites were sampled during June 1992, following an especially dry spring. Eight of the sites were sampled both years, enabling comparison between the representative and dry periods.

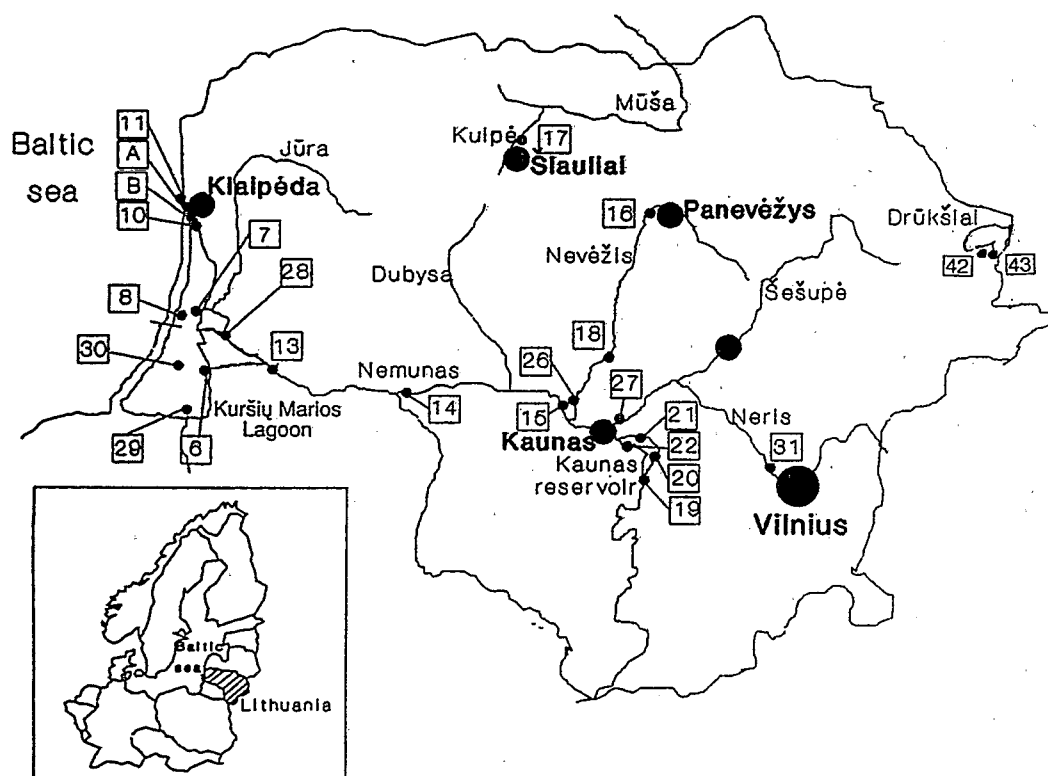


Figure 1. Map of surface water sampling sites.



**Table 1. Description of surface water sampling sites.**

<b>Number</b>	<b>Name</b>	<b>Description</b>
LT-06	Gilija	Kuršių Marios Lagoon at mouth of Gilija River
LT-07	Uostadvaris	Kuršių Marios Lagoon from the Neringa Spit 17 km east of Nida
LT-08	Nida Station	Kuršių Marios Lagoon 1.5 km off the dock at Nida
LT-10A-B	Klaipėda Harbor	Klaipėda Harbor (A) north side of mouth of Danė River, (B) middle of channel off Danė River
LT-11A-B	Melnragė	Entrance to Klaipėda Harbor at the Baltic Sea, (A) east side of east breakwater, (B) west side of east breakwater
LT-13-1-3	Sovetskask	Nemunas River at Sovetskask, (1) south bank, (2) middle of channel, (3) north bank
LT-14	Smalininkai	Nemunas River north bank, ca 50 km upstream from Sovetskask
LT-15B-C	Kulautuva	Nemunas River north bank, 20 km downstream from Kaunas and 13 m downstream from Nevėžis River-Nemunas River confluence, (B) south bank at Zapyškis, (C) north bank ca 1 km below Zapyškis
LT-16	Panevėžys	Nevėžis River 11 km downstream from Panevėžys and 1 km below the discharge pipe of wastewater treatment facility
LT-17	Šiauliai	Kulpė River at Šiauliai
LT-18	Kėdainiai	Nevėžis River, east bank, 1.5 m below wastewater treatment facility
LT-19A	Nemūnas, II Cut	Nemunas River just before emptying into Kaunas Reservoir
LT-20	Kaišiadory	Kaunas Reservoir, 300 m offshore from HydroAccumulation Electric Power facility (HAEP)
LT-21	Dambrava, IV Cut	Kaunas Reservoir below HAEP
LT-22	Pažaislis, VII Cut	Kaunas Reservoir at dam, within sight of Pažaislis Monastery
LT-26B	Raudondvaris	Nevėžis River, west bank, 10 km west of Kaunas
LT-27	Neris at Kaunas	Neris River immediately above bridge before confluence with Nemunas River
LT-28A-B	Rusnė	Nemunas River, delta region near Rusnė, (A) 10 km upstream from Kuršių Marios Lagoon, (B) ca 12 km upstream from Kuršių Marios Lagoon
LT-29	Deimė	Kuršių Marios Lagoon, southernmost section in the mouth of Deimė River
LT-30	Rasytė	Kuršių Marios Lagoon, in the "center"
LT-31	Grigiskės	Neris River, 17 km west of Vilnius
LT-42	Visaginas	Sewer line from Ignalina NPP, near Lake Drūkšiai
LT-43	Drūkšiai	Lake Drūkšiai, south shore of west arm

## METHODS

All samples were collected at a depth of approximately 0.5 m. In 1991, samples were stored in nitric acid-washed 50-ml plastic bottles to which one drop of metal-free nitric acid had been added, and then stored refrigerated until analyzed within 4 months of collection. Sampling and analysis in 1992 was identical to 1991, with the exception that glass bottles were used instead of plastic bottles. One sample from each location was analyzed by a graphite furnace atomic absorption spectrophotometer Zeeman/3030, Perkin-Elmer, USA. Ten trace metals--cadmium, chromium, cobalt, copper, iron, lead, manganese, nickel, vanadium, and zinc--were analyzed, and six hydrological parameters--pH, dissolved oxygen, conductivity, redox potential, salinity, and temperature--were measured. Detection limits and calibration concentration ranges of the instrument for different elements are reported in Table 2. These were determined by methods similar to those prescribed in the instrument operating manual. No matrix modifiers were used. Operational conditions are reported by Kvietskus *et al.* (1994).

Table 2. Detection limits and calibration concentration ranges of the instrument (AAS).

Metal	Calibration concentration ranges ( $\mu\text{g/L}$ )	Detection limit ( $\mu\text{g/L}$ )	Sampling quantity ( $\mu\text{L}$ )
Pb	10-50	0.5	50
Cu	10-50	0.5	40
Ni	10-50	0.8	80
Co	10-50	0.9	60
V	10-50	1.0	100
Cr	10-50	0.5	20
Zn	10-50	0.3	5
Fe	20-100	1.0	20
Cd	1-5	0.2	40
Mn	10-50	0.3	20

At the same time water samples were collected for trace metal analysis, on-site water quality measurements were made at most stations using a Hydrolab<sup>TM</sup> Surveyor 2 (Hydrolab Corporation, Austin, Texas, USA) equipped with pH, temperature, dissolved oxygen (DO), conductivity, redox potential, and depth sensors. The pH electrode (accuracy  $\pm 0.2$  units, resolution  $\pm 0.1$  unit) was calibrated with pH 3, 7, and 10 buffer

solutions. The temperature sensor (accuracy  $\pm 0.2^{\circ}\text{C}$ , resolution  $\pm 0.01$ ) was verified by simultaneous immersion in a flask of water with a thermometer certified by the U.S. National Institute of Standards and Technology. The DO sensor (accuracy  $\pm 2\%$ , resolution 0.01 mg/L) was calibrated by the air saturation method. The conductivity sensor (accuracy  $\pm 1\%$  of range, resolution 4 digits) was calibrated with solutions of potassium chloride prepared with distilled, deionized water. The redox potential sensor (accuracy  $\pm 20$  mV) was calibrated with aqueous solutions of quinhydrone.

## RESULTS AND DISCUSSION

### Trace Metals

With the exception of zinc, the measured concentrations of metals (Table 3) at stations where water samples were taken in both 1991 and 1992 varied by a factor of five or less. The concentrations of zinc in the 1992 samples were an order of magnitude or more greater than in samples collected at corresponding locations in 1991. We believe that in 1992 there was an unidentified error affecting the measurement of zinc, possibly contamination related to preservation and/or storage of samples prior to analysis. Accordingly, although we are reporting the zinc values obtained for 1992, they will not be considered further in the discussion.

Freshwater chronic toxicity criteria (FCT) values recommended by the U.S. EPA (1992) for six of the metals (cadmium, chromium, copper, lead, nickel, and zinc) are listed in Table 3 Footnote 1; the maximum contaminant levels (MCL) used in the former USSR (Bespamiatnov and Krotov 1985) for cobalt, iron, vanadium and manganese are listed in Footnote 2.

In 1991, out of 200 individual measurements, 18 of these exceeded either the FCT or MCL criteria, and of these, seven measurements exceeded these criteria by more than two-fold. In 1992, 150 measurements were made. Excluding zinc, out of the remaining 135 measurements none exceeded MCL criteria, and although 11 exceeded FCT criteria, only two of these, both for lead, exceeded the criteria more than two-fold. Although the number of values exceeding FCT and MCL criteria were fewer in 1992 than 1991, part of the reason for this is that in 1992 we did not resample some of the sites found to be most polluted based on the 1991 sampling.

Of all metals measured, iron most often exceeded the MCL criterion; this happened in 13 out of 35 samples. The only location at which iron was markedly higher than the criterion, however, was the Nemunas River at Rusnė (LT-28). We sampled the Kulpė River at Šiauliai (LT-17) in 1991 and obtained measurements for lead, copper, and chromium that exceeded FCT criteria values by factors of two, three, and 50 respectively. Cadmium, copper, and lead measurements at Melnragė (LT-11), also made in 1991, exceeded FCT criteria by two-, five-, and 50-fold. Klaipėda Harbor (LT-10) was sampled in both 1991 and 1992, and although the measurement for lead in the single sample collected in 1991 was below the detection limit, lead in samples collected in 1992 at two separate locations were five- and ten-fold the FCT criterion.

Table 3. Trace metal concentrations in natural waters in Lithuania (values expressed in  $\mu\text{g/L}$ ).

Site number	Pb <sup>1</sup>		Cu <sup>1</sup>		Ni <sup>1</sup>		Co <sup>2</sup>		V <sup>2</sup>	
	1991	1992	1991	1992	1991	1992	1991	1992	1991	1992
LT-06	BD <sup>3</sup>	BD	2.7	5.7	1.8	0.8	0.9	BD	2.4	1.1
LT-07	BD	BD	1.7	3.6	1.2	2.0	BD	1.7	1.3	BD
LT-08	0.5	0.9	2.9	4.5	0.9	1.1	BD	BD	1.8	1.0
LT-10A	BD	59.0	2.7	2.9	1.2	5.5	3.0	61.0	BD	6.1
LT-10B	-- <sup>4</sup>	16.0	--	3.0	--	3.5	--	160	--	5.1
LT-11A	152	--	66.0	--	29.5	--	29.0	--	10.9	--
LT-13-1	BD	BD	6.3	7.4	2.2	1.6	BD	0.9	1.5	1.5
LT-13-2	--	BD	--	2.6	--	0.8	--	1.0	--	1.0
LT-13-3	--	BD	--	2.4	--	1.0	--	1.7	--	1.4
LT-14	1.8	--	10.4	--	2.8	--	BD	--	1.2	--
LT-15B	BD	--	0.8	--	1.3	--	BD	--	BD	--
LT-15C	--	BD	--	6.3	--	2.1	--	1.3	--	1.9
LT-16	4.7	--	14.3	--	18.0	--	BD	--	1.2	--
LT-17	7.9	--	32.0	--	35.0	--	1.0	--	1.9	--
LT-18	BD	--	3.7	--	6.6	--	0.9	--	7.7	--
LT-19A	BD	--	19.6	--	2.8	--	BD	--	1.2	--
LT-20	BD	--	5.7	--	1.0	--	BD	--	BD	--
LT-21	BD	--	1.6	--	1.4	--	BD	--	1.0	--
LT-22	0.8	--	3.1	--	1.3	--	BD	--	1.1	--
LT-26B	--	0.5	--	3.2	--	BD	--	BD	--	BD
LT-27	BD	--	4.6	--	1.6	--	BD	--	BD	--
LT-28A	0.8	--	3.2	--	2.6	--	1.7	--	2.2	--
LT-28B	--	BD	--	4.9	--	1.4	--	1.7	--	1.8
LT-29	BD	0.6	0.7	3.8	0.8	0.8	0.8	0.9	1.1	0.8
LT-30	0.6	0.9	1.8	66	1.5	2.0	BD	BD	2.1	1.4
LT-31	0.6	--	6.0	--	2.3	--	BD	--	BD	--
LT-42	--	0.5	--	0.42	--	1.1	--	BD	--	BD
LT-43	--	BD	--	8.4	--	BD	--	BD	--	BD

<sup>1</sup>U.S. EPA FCT criteria values (see text) in  $\mu\text{g/L}$  are Pb 3.2, Cu 12\*, Ni 160\*, CrVI 11, Cd 1.1\*, Zn 110\* (\* = hardness dependent criteria, 100 mg/L as  $\text{CaCO}_3$ )

<sup>2</sup>Former USSR standards (see text) in  $\mu\text{g/L}$  are Co 100, V 100, Mn 10,000, Fe 500

Table 3. Continued

Site number	Cr <sup>1</sup>		Mn <sup>2</sup>		Fe <sup>2</sup>		Cd <sup>1</sup>		Zn <sup>1</sup>	
	1991	1992	1991	1992	1991	1992	1991	1992	1991	1992
LT-06	0.6	1.1	120	163	730	276	BD	0.23	3.5	320
LT-07	0.5	1.0	128	206	450	191	BD	0.20	2.7	23.0
LT-08	0.6	1.5	148	170	750	781	BD	0.20	4.8	740
LT-10A	BD	BD	120	75	120	133	BD	2.0	152	360
LT-10B	--	BD	--	68	--	112	--	1.7	--	290
LT-11A	BD	--	33	--	250	--	2.4	--	4.0	--
LT-13-1	1.6	1.4	202	240	780	642	BD	BD	12.9	570
LT-13-2	--	1.2	--	350	--	426	--	2.0	--	250
LT-13-3	--	0.9	--	2120	--	380	--	BD	--	380
LT-14	1.2	--	158	--	450	--	BD	--	6.6	--
LT-15B	BD	--	157	--	310	--	BD	--	2.7	--
LT-15C	--	1.6	--	270	--	508	--	BD	--	17.7
LT-16	1.3	--	66	--	270	--	0.30	--	7.2	--
LT-17	520	--	81	--	820	--	0.45	--	43.0	--
LT-18	1.5	--	76	--	110	--	0.40	--	3.3	--
LT-19A	BD	--	175	--	350	--	BD	--	10.4	--
LT-20	BD	--	178	--	280	--	BD	--	5.0	--
LT-21	BD	--	120	--	300	--	BD	--	1.7	--
LT-22	BD	--	60	--	30	--	BD	--	2.9	--
LT-26B	--	0.9	--	131	--	170	--	0.24	--	13.5
LT-27	0.9	--	127	--	530	--	BD	--	6.7	--
LT-28A	2.7	--	207	--	2430	--	BD	--	7.2	--
LT-28B	--	1.7	--	306	--	707	--	0.22	--	60.4
LT-29	0.7	0.6	156	163	560	276	BD	0.21	3.3	13.7
LT-30	1.2	2.2	102	172	510	688	0.27	0.34	90	570
LT-31	3.7	--	103	--	290	--	0.80	--	14.5	--
LT-42	--	BD	--	34	--	140	--	BD	--	63.1
LT-43	--	BD	--	30	--	20	--	0.26	--	440

<sup>3</sup>BD = Below detection limit<sup>4</sup>-- = Not sampled

## Hydrological Parameters

The hydrological parameters measured in 1991 and 1992 at the stations at which water samples were taken for trace metal analyses are reported in Tables 4 and 5. Metal speciation in aquatic solution is dependent upon such factors as temperature, pH, redox, alkalinity, and concentration of the cations present.

Table 4. Hydrological parameters of natural waters in Lithuania, 1991.

Site No.		Temp. <sup>1</sup> °C	pH <sup>2</sup>	DO <sup>3</sup> mg/L	Conductivity <sup>4</sup> mS/cm	Redox <sup>5</sup> mV	Salinity ppt
LT-06	Gilija <sup>6</sup>	20.9	7.75	8.7	0.50	105	0.0
LT-07	Uostadvaris <sup>6</sup>	20.2	8.47	11.0	0.47	57	0.0
LT-08	Nida Station <sup>6</sup>	19.8	8.08	7.2	0.48	106	0.0
LT-10A	Klaipėda Harbor-A	19.3	8.34	9.2	5.30	115	2.4
LT-11A	Melnragė-A <sup>6</sup>	18.8	8.18	8.5	11.7	78	6.4
LT-13-1	Sovetskias-A <sup>6</sup>	20.6	7.88	10.3	0.52	75	0.0
LT-14	Smalininkai	20.3	8.42	11.9	0.46	74	0.0
LT-15B	Zapyškis <sup>6</sup>	20.6	7.47	5.1	0.46	93	0.0
LT-16	Panevėžys	19.1	7.82	6.9	0.73	76	0.0
LT-17	Šiauliai	21.8	7.69	0.5	2.14	-95	0.6
LT-18	Kėdainiai	21.2	7.84	7.2	0.81	61	0.0
LT-19A	Nemunas, II Cut	22.7	7.56	1.5	0.48	92	0.0
LT-20	Kaišiadoryų	22.8	7.60	1.1	0.48	114	0.0
LT-21	Dambrava, IV Cut	22.5	7.72	4.8	0.46	117	0.0
LT-22	Pažaislis, VII Cut	22.2	7.93	8.4	0.44	141	0.0
LT-27	Neris at Kaunas	21.1	8.58	14.5	0.45	83	0.0
LT-28A	Rusnė-A <sup>6</sup>	20.7	8.21	11.7	0.48	82	0.0
LT-29	Deimė <sup>6</sup>	21.4	7.48	3.9	0.59	86	0.0
LT-30	Rasytė	19.7	8.37	9.7	0.48	88	0.0
LT-31	Grigiškės	18.5	8.10	8.3	0.48	61	0.0

<sup>1</sup>Temperature probe accurate to  $\pm 0.2^\circ\text{C}$

<sup>2</sup>pH probe accurate to  $\pm 0.2$  units

<sup>3</sup>DO probe accurate to 2%

<sup>4</sup>Conductivity sensor  $\pm 1\%$  of range, resolution 4 digits

<sup>5</sup>Redox probe accurate to  $\pm 20$  mV

<sup>6</sup>Locations resampled in 1992

Table 5. Hydrological parameters of natural waters in Lithuania, 1992.

Site number	Name	Temp. <sup>1</sup> °C	pH <sup>2</sup>	DO <sup>3</sup> mg/L	Conductivity <sup>4</sup> mS/cm	Redox <sup>5</sup> mV	Salinity ppt
LT-06	Gilija <sup>6</sup>	20.2	8.95	11.1	0.4	292	0.2
LT-07	Uostadvaris <sup>6</sup>	22.1	8.84	13.3	0.4	309	0.2
LT-08	Nida Station <sup>6</sup>	20.5	8.57	8.0	0.4	310	0.2
LT-11B	Melnragė-B <sup>6</sup>	7.63	7.61	7.8	12.6	392	7.2
LT-13-2	Sovetskask-A <sup>6</sup>	21.5	8.97	15.0	0.4	286	0.2
LT-15C	Kulautuva <sup>6</sup>	19.5	8.53	10.4	0.4	322	0.2
LT-26B	Raudondvaris	19.4	8.52	10.5	0.4	333	0.2
LT-28B-2	Rusnė-B <sup>6</sup>	22.0	8.94	14.4	0.4	276	0.2
LT-29	Deimė <sup>6</sup>	22.1	8.10	6.1	0.6	330	0.3
LT-30	Rasytė	20.5	8.69	8.8	0.4	319	0.2
LT-43	Drūkšiai	22.1	9.49	17.2	0.3	264	0.2

<sup>1</sup>Temperature probe accurate to  $\pm 0.2^\circ\text{C}$    <sup>2</sup>pH probe accurate to  $\pm 0.2$  units   <sup>3</sup>DO probe accurate to 2%

<sup>4</sup>Conductivity sensor  $\pm 1\%$  of range, resolution 4 digits   <sup>5</sup>Redox probe accurate to  $\pm 20$  mV

<sup>6</sup>Locations samples in 1991

The pH values of natural waters from which we collected samples for metal analyses in 1991 were found to be very similar throughout, and were within a range of 7.5-8.6 (Table 4). In 1992, a slightly lower value (7.3) was found in the Baltic Sea at Melnragė, at the mouth of Klaipėda Harbor (Table 5). According to theoretical calculations, it has been suggested that the heavy metals in Lithuanian natural waters are in a carbonated form (Turner *et al.* 1981). This is supported also by the fact that natural waters of Lithuania are generally alkaline (Garunkštis 1988).

Dissolved oxygen in natural waters is of great importance because it represents the vital capacity of water. Very low values were found in some parts of the Kaunas Reservoir (1.1-1.5 mg/L) and Kuršių Marios Lagoon (3.9 mg/L) in 1991, and an extremely low value of 0.53 mg/L was found in the Kulpė River near Šiauliai, where wastewater discharges from an industrial complex are discharged directly into the river. In 1992, DO values ranged from 6 to 17 mg/L (Table 5), and at all seven of the freshwater locations sampled both years the DO values ranged from 1 to 5 mg/L higher in 1992 than in 1991. Dissolved oxygen values measured at Melnragė, in the Baltic Sea at the mouth of Klaipėda Harbor, were as much as 1 mg/L lower. It has been suggested that these higher values in 1992 may be related to a decrease in industrial production because of the current economic crisis, with corresponding decreases in wastewater discharges into freshwater bodies.

During 1991 and 1992, the conductivity of the water in the Neris and Nemunas Rivers, Kaunas Reservoir, and the freshwater area of Kuršių Marios Lagoon ranged from 0.44 to 0.59 mS/cm. Slightly higher values for conductivity, relative to other surface waters, were observed in the Nevėžis River at Panevėžys, Kėdainiai, and Raudondvaris (0.73-0.81 mS/cm), and an extremely high value for freshwater of 2.14 mS/cm was measured in the Kulpė River at Šiauliai. Elevated conductivity values were also found in other more polluted rivers, and inside the mouth of Klaipėda Harbor where high conductivity values are normal due to infusion of ions and salts from the Baltic Sea. Salinity at Melnragė on the Baltic Sea was approximately 6 ppt. Speciation of metals in seawater can be different from that in freshwater, where anions other than carbonates may prevail (Turner *et al.* 1981): toxicity to aquatic animals of heavy metals can depend upon that speciation. This has been detailed for copper by Pagenkopf *et al.* 1974 and Chakoumakos *et al.* 1979.

Temperature, salinity, and conductivity values at all sites were within ranges considered to be normal, except for the Kulpė River at Šiauliai. The negative redox value measured in 1991 in the Kulpė River at Šiauliai (Table 4) is indicative of reducing conditions and very low DO levels. The products of contaminant degradation under the reducing conditions at Šiauliai would differ from other surface waters where oxidative conditions exist and positive redox values were obtained.

## CONCLUSIONS AND RECOMMENDATIONS

The number of sampling sites visited and samples analyzed for this study were restricted due to time constraints; but even so, information obtained was sufficient to confirm two "hot spots," namely Klaipėda Harbor and the Kulpė River at Šiauliai. Except for the chromium concentration in the Kulpė River sample and the high lead and copper concentrations measured at Melnragė (Klaipėda Harbor), the other metal concentrations ranged from below detection limits to a factor of 2-3 higher than criteria values recommended by the U.S. EPA (1992). Because only about one-third of wastewaters discharged in Lithuania are treated to meet any established standards, future metal analyses should be focused on the sites downstream from cities and industries involved in metal plating and other activities that use metals. Detailed investigations are needed on trace metal concentrations in the rivers flowing into Kuršių Marios Lagoon. In addition, a study is needed on pollution sources discharging into the Kulpė River at Šiauliai.

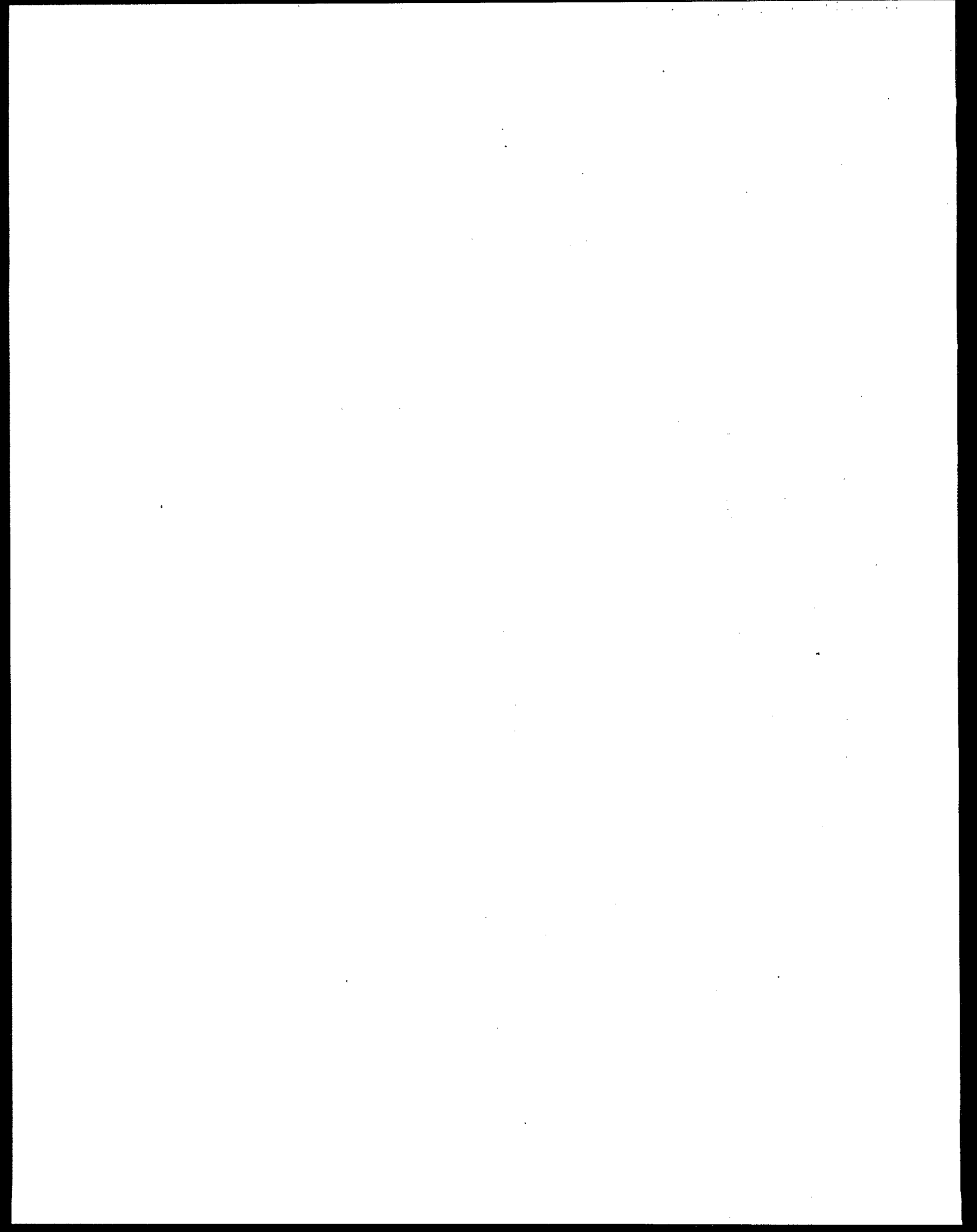
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# INTER-LABORATORY COMPARISON OF METHODS FOR THE DETERMINATION OF HEAVY METALS IN NATURAL WATERS

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## ABSTRACT

An inter-laboratory study was conducted by the Ecological Spectroscopy Laboratory (ESL), Institute of Physics of the Lithuania Academy of Sciences, and the Environmental Research Laboratory, Athens, Georgia (ERL-A), of the U.S. Environmental Protection Agency, to compare the analytical methods used by each laboratory to measure trace metals in natural waters. Duplicate water samples were collected at 19 locations throughout Lithuania in July 1991, and each laboratory analyzed these samples for eight metals: cadmium, cobalt, chromium, copper, lead, manganese, nickel, and zinc. Analyses were performed by graphite furnace atomic absorption spectroscopy (AAS). Some samples required dilution before analysis in order to reduce absorbance to levels where accurate background correction and quantitation could be achieved using the Zeeman effect. Although eight metals were measured, a correlation equation was determined for only six of these because of considerable differences reported for zinc, and because of the failure to detect cobalt in any of the samples analyzed at ERL-A. The regression line plot that was calculated for the six metals had a slope of 0.957 and a coefficient of determination of 0.829.

## INTRODUCTION

Until 1990, no detailed investigations of heavy metals in natural waters had been performed in Lithuania, mainly because of a lack of adequate instrumentation. Also, nitric acid with sub parts-per-billion metal content for sample preservation was not available, and usually only glass containers were available for sample collection and storage. Bottles made of glass often contain appreciable amounts of metals in their walls, and these metals can easily dissolve and contaminate the sample (Ross 1986). To avoid such contamination, bottles must be washed in strong acid to leach the metals from the container wall. Polyethylene or teflon bottles are preferred for storing samples that contain trace amounts of metals because, after acid washing, these containers are least likely to contaminate the water samples (Moody and Lindstrom 1977).

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An additional problem, associated with water analysis for metals, is that metal concentrations in the bulk aqueous phase can alter during storage due to sorption--commonly referred to as the "container wall effect." This effect is minimized by acidifying the samples with nitric acid. Another factor that affects the measured concentration of metals in water samples is the analytical method, e.g., the conditions of atomization of the sample and the use of matrix modifiers to minimize interferences and improve quantitation.

As part of a joint US/Lithuania cooperative research program, water samples were collected in duplicate for analysis of heavy metals at both ESL and ERL-A. This inter-laboratory comparison would be useful for two reasons: (1) as a background from which to discuss and compare the instrument variables and the techniques employed by each laboratory, (2) as a database through which trends or possible problems associated with the measurement of some of the metals could be revealed.

## MATERIALS AND METHODS

Samples were collected in 50-ml plastic bottles, precleaned at ERL-A by washing with 3% nitric acid (Fisher Scientific Co., Trace Metal Grade) and rinsing with organic-free deionized water. Nitric acid (300  $\mu$ L) was then added immediately after cleaning to lower the pH of the water to  $\leq 2$  to serve as a sample preservative and to minimize sorption to the wall of the container. The samples were stored under refrigeration and analyzed (within 4 months of collection) without filtration. All glass and plastic used in the analyses were acid-washed and rinsed with metals-free water prior to use. The metals for which analyses were performed were cadmium, cobalt, copper, chromium, lead, manganese, nickel, and zinc.

Analyses at ESL were performed on a Perkin-Elmer Zeeman 3030 atomic absorption spectrophotometer (AAS) equipped with an AS-60 autosampler, and a HGA-600 graphite furnace with a L'vov platform. The AAS system was controlled by an internal microcomputer using a keyboard and video monitor. The internal gas flow rate was 300 mL/min. ERL-A used a Perkin-Elmer Zeeman 5100 PC AAS, equipped with an AS-60 autosampler, an HGA-600 graphite furnace with a L'vov platform, and an IBM PS/2 50Z computer with graphics-based application software. The internal gas flow rate was 300 mL/min, except during atomization when it was 0 mL/min (Tables 1 and 2).

At each laboratory, calibration standards (1 to 40  $\mu$ g/L) were prepared at the time of analysis by a series of dilutions using organics-free deionized water that was made 0.2% in nitric acid. ESL stock solutions (1,000 mg/L) were obtained from the Physics/Chemistry Institute of the Ukrainian Academy of Sciences, and ERL-A stock solutions of each metal (1,000 mg/L) were obtained from Fisher Scientific Co. At ESL, a programmed non-linear calibration method based on a two-coefficient equation, part of the 3030 software, was used. At ERL-A, non-linear calibration curves that satisfied correlation coefficients of at least 0.995 were used.

Table 1. Ramp and hold times for furnace condition (in seconds).

Step	Ramp		Hold	
	ESL	ERL-A	ESL	ERL-A
Dry	2	1	10-50	50
Char	2	1	10	30
Cool	--	1	--	15
Atomize	0	0	3	5
Clean	1	1	3	5

Table 2. Furnace temperature and instrument operating conditions.

Element	Temperature (°C)					Conditions (nm)	
	Dry	Char	Cool	Atomize	Clean	Wavelength	Slit
ESL							
Cadmium	110	250	--	1600	2650	228.8	0.7
Chromium	110	1200	--	2300	2650	357.9	0.7
Cobalt	110	1000	--	2200	2650	242.5	0.2
Copper	110	900	--	2000	2650	324.8	0.7
Lead	110	500	--	1800	2650	283.3	0.7
Manganese	110	1000	--	1900	2650	279.5	0.2
Nickel	110	1300	--	2300	2650	232.0	0.2
Zinc	110	400	--	1600	2650	213.9	0.7
ERL-A							
Cadmium	120	850	20	1650	2600	228.8	0.7
Chromium	120	1650	20	2500	2600	357.9	0.7
Cobalt	120	1400	20	2500	2600	242.5	0.7
Copper	120	1300	20	2500	2600	324.8	0.7
Lead	120	850	20	1800	2600	283.3	0.7
Manganese	120	1400	20	2200	2600	279.5	0.7
Nickel	120	1400	20	2500	2600	232.0	0.7
Zinc	120	700	20	1800	2600	213.9	0.7

Nitric acid/deionized water blanks were prepared at both ESL and ERL-A by adding purified water ( $18 \text{ M}\Omega \text{ cm}^{-1}$ ) to 50-ml plastic bottles that contained nitric acid. Metal impurities were not detected in ESL and ERL-A blanks. Matrix modifiers were not available for use in the analysis of samples at ESL; magnesium nitrate was used as a matrix modifier at ERL-A for each metal except nickel, which was analyzed without the addition of a modifier.

By agreement, each laboratory analyzed samples using instrument conditions and sampling techniques that had been used historically at that laboratory. The operating conditions in Tables 1 and 2 are reflective of the use of matrix modifiers at ERL-A that allowed higher char temperature, faster ramps, and longer hold times. Higher temperatures enhance removal of compounds from the matrix that could interfere with the analysis. The modifiers also shorten the time required for the furnace wall and the vapor to attain a stable temperature, an essential characteristic of the L'vov platform technique. A modification of the method described in the instrument operating manual (Perkin-Elmer 1982) was used to determine the instrument detection limits for each metal (Table 3).

Table 3. Instrument detection limits.

Element	ESL		ERL-A	
	Detection Limit ( $\mu\text{g/L}$ )	Sample Amount ( $\mu\text{L}$ )	Detection Limit ( $\mu\text{g/L}$ )	Sample Amount ( $\mu\text{L}$ )
Cadmium	0.3	20	0.3	20
Chromium	0.5	20	0.5	20
Cobalt	0.9	60	0.7	20
Copper	0.5	40	0.6	20
Lead	0.5	50	0.9	20
Manganese	0.3	20	0.9	20
Nickel	0.8	80	1.2	20
Zinc	0.3	5	0.6	20

## RESULTS AND DISCUSSION

Each laboratory analyzed for eight metals in water samples collected simultaneously from 19 sampling stations; this totalled 152 measurements at each laboratory, and a combined total of 304 measurements (Table 4). Of these 304 measurements, 119 were reported as below detection limits, 44 by ESL and 75 by ERL-A. Most of the measurements at ERL-A for cadmium, cobalt, lead, and nickel were below detection limits; cobalt was not found in any sample, and nickel in only three. However, at ESL nickel was found in all 19 samples, although cadmium, cobalt, and lead were detected in half or fewer. Both laboratories reported chromium, copper, manganese, and zinc in most or all of the samples.

There were two areas where the results at ESL and ERL-A differed significantly. Measurements at ERL-A for zinc in five of the samples were five to 35 times higher than at ESL, while measurements for zinc at ESL for two of the samples were five and 10 times higher than at ERL-A. In the case of the seawater samples (LT-11A), seven of the metals measured considerably higher at ESL than at ERL-A. Reasons for both of these large discrepancies will need further study. Elimination of the zinc data, the seawater sample data, and those data pairs where either of the two laboratories reported a concentration below detection, left 52 data pairs for six of the eight metals. A log-log regression plot of these 52 data pairs was calculated using Slide Write Plus<sup>TM</sup> software (Figure 1).

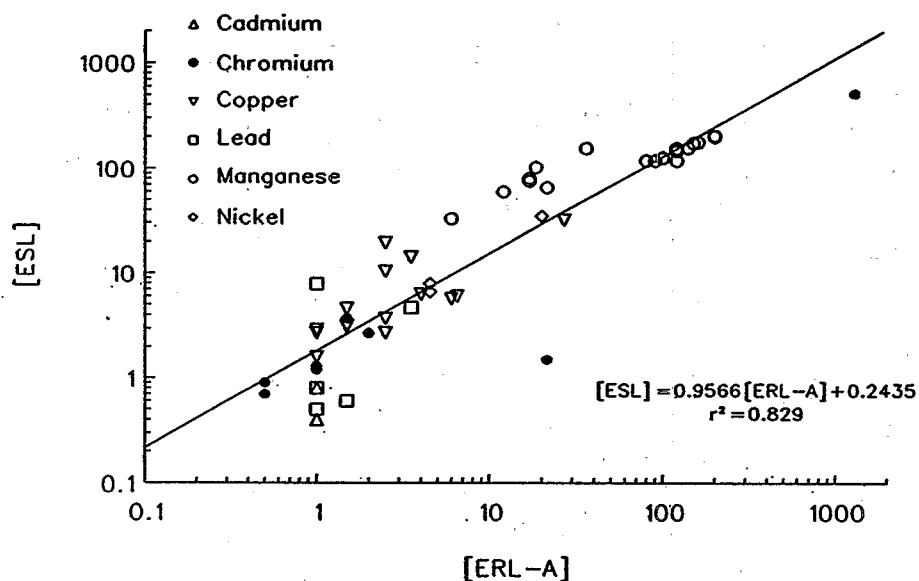


Figure 1. Regression line for metals analyses at Ecological Spectroscopy Laboratory and Environmental Research Laboratory-Athens.

Table 4. Results of water analyses by element<sup>1</sup> (concentrations reported in  $\mu\text{g/L}$ ).

Sample	Cadmium		Chromium		Copper	
	ESL	ERL-A	ESL	ERL-A	ESL	ERL-A
LT-06	BD <sup>3</sup>	BD	0.6 $\pm$ 0.1	BD	2.7 $\pm$ 0.1	2.5 $\pm$ 0.5
LT-07	BD	BD	0.5 $\pm$ 0.1	BD	1.7 $\pm$ 0.1	BD
LT-08	BD	BD	0.6 $\pm$ 0.1	BD	2.9 $\pm$ 0.5	1.0 $\pm$ 0.2
LT-10A	BD	BD	BD	19.0 $\pm$ 2.0	2.7 $\pm$ 0.1	1.0 $\pm$ 0.2
LT-11A	2.4 $\pm$ 1.0	BD	BD	BD	66.0 $\pm$ 1.0	BD
LT-13-1	BD	BD	1.6 $\pm$ 0.1	BD	6.3 $\pm$ 0.1	4.0 $\pm$ 1.0
LT-14	BD	BD	1.2 $\pm$ 0.2	1.0 $\pm$ 1.0	10.4 $\pm$ 0.1	2.5 $\pm$ 0.5
LT-15B	BD	BD	BD	BD	0.8 $\pm$ 0.1	BD
LT-16	0.3 $\pm$ 0.1	BD	1.3 $\pm$ 0.3	1.0 $\pm$ 0.5	14.3 $\pm$ 0.1	3.5 $\pm$ 0.5
LT-17	0.45 $\pm$ 0.05	BD	520 $\pm$ 60	1300 $\pm$ 80	32.0 $\pm$ 1.0	27.0 $\pm$ 0.2
LT-18	0.4 $\pm$ 0.1	1.0 $\pm$ 0.3	1.5 $\pm$ 0.3	21.5 $\pm$ 3.5	3.7 $\pm$ 0.1	2.5 $\pm$ 0.5
LT-19A	BD	BD	BD	9.5 $\pm$ 0.5	19.6 $\pm$ 0.1	2.5 $\pm$ 1.0
LT-20	BD	BD	BD	4.0 $\pm$ 2.0	5.7 $\pm$ 0.1	6.0 $\pm$ 1.0
LT-21	BD	BD	BD	3.0 $\pm$ 2.0	1.6 $\pm$ 0.1	1.0 $\pm$ 0.2
LT-22	BD	BD	BD	2.0 $\pm$ 1.0	3.1 $\pm$ 0.1	1.5 $\pm$ 0.5
LT-27	BD	BD	0.9 $\pm$ 0.1	0.5 $\pm$ 0.5	4.6 $\pm$ 0.1	1.5 $\pm$ 0.5
LT-28A	BD	BD	2.7 $\pm$ 0.2	2.0 $\pm$ 1.0	3.2 $\pm$ 0.2	1.5 $\pm$ 0.5
LT-29	BD	BD	0.7 $\pm$ 0.1	0.5 $\pm$ 0.5	0.7 $\pm$ 0.2	BD
LT-31	0.8 $\pm$ 0.3	1.0 $\pm$ 0.3	3.7 $\pm$ 0.2	1.5 $\pm$ 0.5	6.0 $\pm$ 0.1	6.5 $\pm$ 0.5

<sup>1</sup>Cobalt omitted from table because all measurements at ERL-A were below detection limit. Values for cobalt at ESL were: LT-06, 0.9 $\pm$ 2; LT-10A, 3.0 $\pm$ 1.5; LT-11A, 29.0 $\pm$ 5.0; LT-17, 1.0 $\pm$ 0.1; LT-18, 0.9 $\pm$ 0.1; LT-28A, 1.7 $\pm$ 0.3; LT-29, 0.8 $\pm$ 0.1



Table 4. Continued.

Lead		Manganese		Nickel		Zinc <sup>2</sup>	
ESL	ERL-A	ESL	ERL-A	ESL	ERL-A	ESL	ERL-A
BD	BD	120±1.0	120±3.0	1.8±0.4	BD	3.5±0.1	9.0±1.0
BD	BD	128±2.0	100±3.0	1.2±0.1	BD	2.7±0.1	2.0±0.2
0.5±0.5	1.0±0.3	148±2.0	120±3.0	0.9±0.1	BD	4.8±0.4	3.0±0.2
BD	BD	120±1.0	80.0±4.5	1.2±0.4	BD	152±30.0	15.0±1.2
152±5.0	BD	33.0±1.0	6.0±0.9	29.5±3.0	BD	4.0±0.2	25.0±2.0
BD	BD	202±2.0	200±3.0	2.2±0.1	BD	12.9±0.4	460±20
1.8±0.3	BD	158±4.0	140±3.0	2.8±0.1	BD	6.6±0.1	150±10.0
BD	BD	157±2.0	120±3.0	1.3±0.1	BD	2.7±0.4	3.0±0.2
4.7±0.3	3.5±0.5	66.0±1.0	21.5±0.5	8.0±0.1	4.5±0.5	7.2±0.3	9.0±0.2
7.9±0.3	1.0±0.3	81.0±1.0	17.0±1.5	35.0±0.1	20.0±0.4	43.0±3.0	7.5±2.5
BD	BD	76.0±2.0	17.0±1.0	6.6±0.1	4.5±0.5	3.3±0.3	7.0±0.2
BD	BD	175±3.0	150±10	2.8±0.1	BD	10.4±1.3	20.0±2.0
BD	BD	178±4.0	160±3.0	1.0±0.1	BD	5.0±0.5	25.0±2.0
BD	BD	120±2.0	90.0±10	1.4±0.1	BD	1.7±0.2	2.0±0.2
0.8±0.5	BD	60.1±1.0	12.0±0.9	1.3±0.1	BD	2.9±0.4	3.5±0.5
BD	BD	127±2.0	100±9.0	1.6±0.1	BD	6.7±0.8	5.5±0.5
0.8±0.3	1.0±0.3	207±5.0	200±9.0	2.6±0.1	BD	7.2±0.1	6.0±0.2
BD	BD	156±1.0	36.0±1.0	0.8±0.2	BD	2.3±0.3	2.0±0.2
0.6±0.2	1.5±0.5	103±1.0	18.5±0.5	2.3±0.1	BD	14.5±0.1	200±6.0

<sup>2</sup>Zinc data were not used in calculating the regression line (Figure 1).

<sup>3</sup>BD Below detection limit of the instrument used.

The coefficient of determination ( $r^2$ ) of 0.829 and slope of 0.957 are an indication that the results of the measurement processes in each laboratory are similar. Of the 52 data pairs, ESL reported higher values for 43, which is reflected in the +0.244 offset of the plot. We cannot determine from the data available whether the frequently higher values reported by ESL are the result of an unknown background source of metals or some other cause at either ESL or ERL-A.

### CONCLUSION

This inter-laboratory comparison study provided the desired interaction between chemists at ESL and ERL-A and identified six metals that can be analyzed with confidence in results. The analysis for zinc presents a problem that must be addressed, and the analysis for cobalt will require a concentration step to attain easily quantifiable concentrations. Additional work will be needed to determine reasons for differences in the results of analyses for metals in seawater. The slope and correlation coefficient for data on six of the metals measured indicate the data are similar, with a bias toward frequently higher concentrations at ESL.

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# CHEMICALS IDENTIFIED AT TWO PESTICIDE STORAGE SITES IN LITHUANIA, 1990-1993

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## ABSTRACT

Water samples were collected from two sites in Lithuania being used to store hazardous chemicals, and these samples were analyzed to determine what chemicals might migrate from these sites into the surrounding environment. One of these sites, at Zigmantiškės, is a controlled landfill for banned, unsuitable, and unknown pesticides. Water samples were collected from wells inside the boundary of this landfill on five occasions during the period 1990-1993, and on two occasions during 1993 immediately outside the landfill boundary. Chemicals considered to be mobile in underground water, e.g., s-triazine herbicides, were identified in water samples from inside the boundary but were below the level of detection in samples from outside the boundary. The second site is a warehouse near Utena that is being used as a storage facility for toxic chemicals. A fire occurred at the warehouse in April 1993, and water used to extinguish the fire seeped into the warehouse basement and its drainage system. Water was subsequently pumped from the basement into temporary storage tanks to reduce the amount of contaminated water seeping into the local aquifer. In June and October 1993 water samples were collected for analysis from the basement, the drainage system, and the storage tanks. Atrazine and several chlorinated carboxylic acids, including 2,4-D, were identified in all of these samples.

## INTRODUCTION

A field study on the quality of surface waters in the Republic of Lithuania was initiated in 1990 under the sponsorship of the risk assessment program of the United States Environmental Protection Agency (U.S. EPA), Environmental Research Laboratory, Athens, Georgia (ERL-Athens). During the course of this study, two sites that did not involve surface waters were also investigated, one at Zigmantiškės and the other at Utena.

The Zigmantiškės site is a controlled landfill established by the Ministry of Agriculture of Lithuania for the storage of banned, unsuitable, and unknown pesticides and related wastes; it is located 40 km southwest of Vilnius, between the Merkys and Šalčia Rivers. The perimeter of the landfill is enclosed by a fence and monitoring wells

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have been drilled both inside and outside the perimeter (Figure 1). The second site is a warehouse near Utena that is now being used as an agrochemical storage facility. It is located 115 km north of Vilnius, in the Šventoji River watershed. Approximately 32,000 kg of pesticides and unknown powders had been collected and placed in temporary storage at the warehouse when, in April 1993, a fire started somewhere within the mass of the stored pesticides. Water, used to extinguish the flames, drained into the basement of the warehouse, and although much of this runoff water was collected by pumping into holding tanks, some of it reached an underground drainage system that empties to a nearby stream.

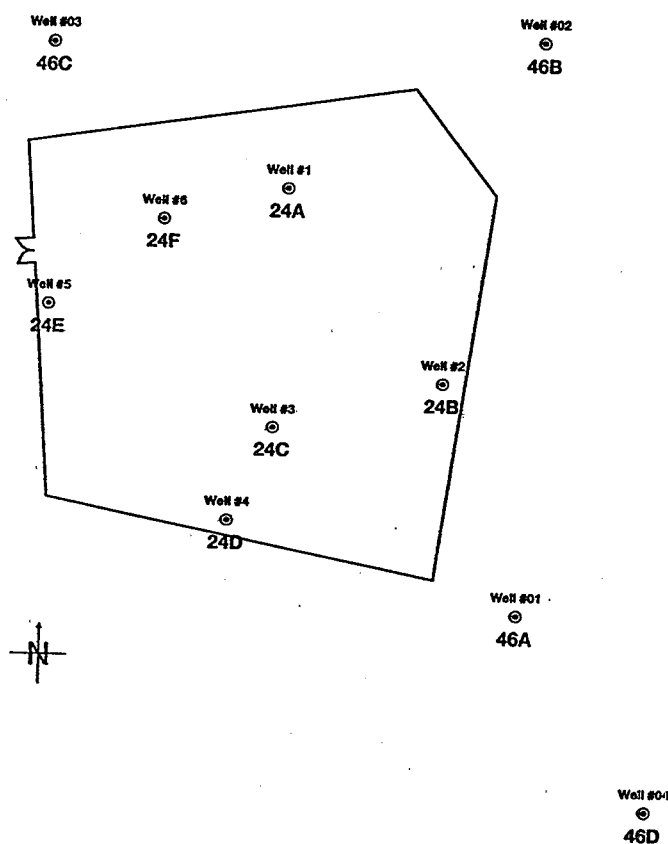


Figure 1. Zigmantiškės Pesticide Storage Site.

Water samples were collected from the Zigmantiškės site on five occasions between 1990 and 1993, and from the Utena site on two occasions in 1993. These water samples were passed through cartridges or disks containing sorbent media to extract the organic chemicals, and the cartridges and disks were taken to ERL-Athens for elution and identification of the sorbed organics. This manuscript presents the results of these analyses.

## METHODS

Water samples were collected from monitoring wells inside the fenced boundary at the Zigmantiškės site in December 1990, July 1991, June 1992, June 1993, and October 1993. In May 1993, monitoring wells were installed outside the fenced boundary by direction of the Environmental Protection Department (EPD), and in June and October 1993 samples were also collected from these monitoring wells. Water samples were collected from three locations at the Utena site in June and October 1993.

The cartridges and disks used for extraction of organic chemicals from water samples in this study were pre-cleaned at ERL-Athens, and activated in Lithuania. After water samples were collected, those to be later analyzed for organic acids were adjusted to pH 2 with sulfuric acid before passage through a cartridge or disk. After return to ERL-Athens, the cartridges and disks were eluted with organic solvent. Those eluants to be analyzed for organic acids were treated with diazomethane before chromatographic/spectral analysis. Gas chromatographs (GC) were used to separate the analytes in the extracts. These were then identified using low and high resolution mass spectrometers (MS) and Fourier transform-infrared spectrometers (FT-IR) interfaced to the GC. The methods used for the preparation and activation of the cartridges and disks, collection of samples, extraction of the organic chemicals, and identification of the extracted organics has been reported by Ellington *et al.* (1994).

## RESULTS

### Zigmantiškės Pesticide Storage Site

Organic chemicals identified from water samples collected at the Zigmantiškės storage site are listed in Table 1. Dialkyl phthalates are commonly found in environmental samples, so stringent precautions are necessary to assure their presence in the collected samples is not due to accidental contamination. Hydrocarbons, phthalates, and fatty acids were found in a few of the method and field blanks, although at lower levels than in the groundwater samples. At the present time, it is not known whether the phthalates identified in the groundwater samples are representative of the ground water from which the samples were taken, or whether they resulted from contamination during collection and/or sample preparation in the field prior to analysis.

Analysis of the extract of a groundwater sample taken from monitoring well #6 (LT-24F) during December 1990 revealed the presence of dimethyl sulfone, caprolactam, 2,4-dichlorobenzoic acid (derivatized to form the methyl ester), simazine, atrazine, and propazine. The latter three compounds are s-triazine herbicides that have been detected worldwide in ground and surface waters. Atrazine is one of the most heavily used herbicides worldwide, with global release in 1980 estimated at 90,000 tons (Rippen 1987). The identities of the s-triazines and the dichlorobenzoic acid were confirmed with standards. We believe the 2,4-dichlorobenzoic acid is most likely a product of degradation of a more complex chemical. The 2,4-dichlorobenzene fragment is part of

the structure of several agricultural and/or industrial chemicals including chlorfenvinphos, propiconazole, and polychlorinated biphenyls, whose degradation in the environment would yield 2,4-dichlorobenzoic acid. Caprolactam is a monomer used in the synthesis of high molecular weight polymers including Nylon 6, and dimethyl sulfone is also a solvent for high temperature reactions.

Table 1. Anthropogenic chemicals identified in water samples from monitoring wells at the Ministry of Agriculture Pesticide Storage Site, Zigmantiškės (LT-24).

Alkyl hydrocarbons <sup>1</sup>	2,2-Dimethoxy-1,2-diphenyl ethanone <sup>1</sup>	Pristane <sup>2</sup>
Atrazine <sup>2</sup>	Dimethyl sulfone <sup>1</sup>	Prometryn <sup>2</sup>
Benzophenone <sup>1</sup>	Fatty acids <sup>1</sup>	Propazine <sup>2</sup>
Caprolactam <sup>1</sup>	Lenacil <sup>2</sup>	Pyridine <sup>1</sup>
Desmetryn <sup>2</sup>	Lindane <sup>2</sup>	Silicon organics <sup>1</sup>
Dialkylthiophene <sup>1</sup>	2-(Methylthio) benzothiazole <sup>1</sup>	Simazine <sup>2</sup>
2,4-Dichlorobenzoic acid <sup>2</sup>	Phthalates <sup>2,3</sup>	Tetrachlorinated unknowns <sup>1</sup>
Dichlorprop <sup>1</sup>	Phytane <sup>2</sup>	

<sup>1</sup>Identified by GC/FT-IR/MS but not confirmed with standards

<sup>2</sup>Identified by GC/FT-IR/MS and confirmed with a standard

<sup>3</sup>Diisooctyl-, Butylbenzyl-, Di-n-octyl-, and Di-n-butylphthalates were observed the most frequently

In July 1991, new compounds identified in water samples taken from monitoring well #6 were benzophenone, pyridine, pristane, and phytane. Three compounds that contained four chlorine atoms each were detected in the acidified sample from well #6 after elution of organics from a solid phase extraction (SPE) cartridge and treatment of the concentrated eluant with diazomethane. A molecular weight of 348 was determined by chemical ionization GC/MS for all three compounds, indicating they were isomers. These isomers were also found in samples from the Neris River and Kaunas Reservoir (Ellington *et al.* 1994). Best fit empirical formulae of  $C_7H_{13}Cl_4O_5P$  and  $C_{14}H_8Cl_4O_2$  were determined by high resolution GC/MS. A tetrachlorinated trialkyl phosphate fits the former formula while tetrachlorinated aromatic esters and other structures containing carbonyl groups fit the latter. Both formulae are similar to those of compounds commonly used as electrical insulators and flame retardants. The compound containing phosphorus was eliminated as a possibility by further GC analysis using nitrogen and nitrogen-phosphorus specific detectors. The presence of the carbonyl functional group in the three unknowns was confirmed by GC/FT-IR. The loss of the  $m/z$  31 ion (loss of  $-OCH_3$ ) during low resolution electron impact GC/MS argued for the presence of a methoxy functional group, possibly a methyl ester. However, the absence of a strong peak in the  $1250-1310\text{ cm}^{-1}$  region of the IR spectrum and the broad carbonyl peaks argued against the ester functionality. Detection of the unknowns only after treatment of the extract with diazomethane is evidence that they contained a reactive hydrogen. A tetrachlorinated aromatic ring system containing a carbonyl group, methoxy group, and differing chlorine substitution is the most likely structure for the

three unknown isomers. The presence of pristane and phytane could be an indication of contamination by crude oil, while benzophenone and pyridine are used in the synthesis of organics.

In June 1992, samples were also taken from wells #1, 2, 3, and 5 (LT-24A-C,E) which had not been sampled during previous trips. Chemicals in a sample from well #5 (LT-24E), first tentatively identified by GC/MS and/or GC/FT-IR/MS, and subsequently confirmed with standards, were: atrazine, desmetryn, lenacil, lindane, prometryn, propachlor, propazine, and simazine. Chemicals tentatively identified in this same sample, but not confirmed with standards, included 2,2-dimethoxy-1,2-diphenyl ethanone, dialkylthiophene, and 2-(methylthio)thiophene.

During June and October 1993, water samples were collected from three of the four monitoring wells (LT-46A-D) installed outside the boundary fence of the landfill in May 1993. Chemicals tentatively identified by GC/FT-IR/MS in extracts of samples from wells #01 and 02 (LT-46A and B), downgradient from the underground water flow from the landfill, were sulfur, dibutyl- and dioctylphthalates, hydrocarbons, and carboxylic acids. These chemicals were almost identical to those found in the water taken from well #4 (LT-24D) located 1.5 m inside the boundary and upgradient from well #01 outside the boundary. Except for phthalates and hydrocarbons, none of the chemicals identified in samples from wells #1, 2, 3, 5, or 6 inside the boundary appeared in samples from well #4 also inside the boundary, or wells #01 or 02 outside the boundary. Phthalates and hydrocarbons were detected in trace amounts in the majority of samples, and we believe their presence was due to contamination from equipment during sampling procedures. The absence in wells outside the boundary of other chemicals found in wells inside the boundary, especially the extremely mobile chemical atrazine, is evidence that chemicals at the Zigmantiškės site have not migrated beyond the landfill boundary at this time.

#### Utena Pesticide Storage Warehouse

The chemicals stored in the warehouse at Utena at the time of the fire in April 1993 had been inventoried by the Lithuania EPD and the Ministry of Agriculture. The inventory showed 31,586 kg of pesticides and unknown powders, with the unknown powders (23,730 kg) and unknown solids (1,610 kg) comprising the majority of that inventory. The remaining pesticides consisted of fentiuramas (3,458 kg), 2% ethylmercury chloride (190 kg), ziramias (970 kg), nitrophen (1,070 kg), and DNOC (2-methyl-4,6-dinitrophenol, 558 kg) (LEPD 1993). After the fire, runoff water was pumped from the basement of the warehouse into two 50,000-L tanks (LT-45C,D) to prevent environmental contamination from water seeping into the drainage system and into a nearby creek. The volume of water in both tanks was approximately 26,000 L. In June 1993 a 100 ml sample of water from LT-45D was extracted by EPA Method 505 (base/neutrals) and EPA method 515.1 (chlorinated acids), and the extracts were analyzed by GC/MS (U.S. EPA 1991). Compounds identified from this sample are listed in Table 2.

Table 2. Anthropogenic chemicals<sup>1</sup> identified in water samples collected at the Toxic Chemical Storage Warehouse, Utena (LT-45). (Detected concentrations were in the ppb range.)

Chemicals by class <sup>2</sup>	LT-45A	LT-45D	LT-45E
<b>Carboxylic acids</b>			
Chlorobutenoic acid		x	
Chloropropanoic acid		x	
Dichloroacetic acid		x	
Dichlorobutenoic acid		x	
Dichloropropanoic acid		x	
Trichloropropanoic acid		x	
Trichloroacetic acid	x	x	
<b>Phenols</b>			
Chloromethylphenol		x	
Dichlorophenol		x	
Dimethylphenol		x	
Methylphenol		x	
Trichlorophenol		x	x
<b>Phenoxy-carboxylic acids</b>			
Chloromethylphenoxyacetic acid	x	x	
Chloromethylphenoxybutanoic acid		x	
Dichlorophenoxyacetic acid	x	x	x
Dichloromethylphenoxyacetic acid		x	x
Dichlorophenoxybutanoic acid		x	
Dimethylphenoxyacetic acid		x	
Dimethylchlorophenoxyacetic acid		x	
N,N-Dimethyl-2-(chloromethylphenoxy)-acetamide		x	
Methylphenoxyacetic acid		x	
Trichloromethylphenoxyacetic acid	x	x	x
<b>s-Triazine/phosphate pesticides</b>			
Atrazine	x	x	
Propazine	x	x	
Simazine	x	x	
Vapona		x	
<b>Ureas and thiooxamides</b>			
Lenacil		x	
Tetramethylurea	x	x	x
Tetramethylthiourea		x	
N,N,N',N'-Tetramethylthiooxamide		x	
N,N,N',N'-Tetramethyldithiooxamide		x	

<sup>1</sup>The EPA/NIH Mass Spectral Library was used for tentative identification of the chemicals identified by "x". Atrazine, simazine, propazine, and lenacil were confirmed with standards.

<sup>2</sup>High resolution GC/MS was used to determine the molecular ions and empirical formulas. Identical empirical formulas for some peaks indicated isomers but the structures of the individual isomers could not be determined from the spectral information.



Mercury was suspected to be in the water in the holding tanks, so in the summer of 1993 the Lithuania EPD attempted to precipitate this mercury by the addition of ferric chloride. In addition, calcium hypochlorite was added to degrade other pesticides. Water in LT-45C was transferred to LT-45D, and the combined contents were treated with  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  and  $\text{Ca}(\text{OCl})_2$ . In October 1993, samples of water from the ferric chloride-treated water (LT-45E) and from the underground drainage system (LT-45A) were extracted and the resulting base/neutral and acid fractions were analyzed by GC/MS. The compounds identified in these two samples are also listed in Table 2.

Three chemicals, namely, tetramethylurea, and dichloro- and trichloromethylphenoxyacetic acids, were detected in all three samples. The ferric chloride/calcium hypochlorite treatment had reduced the concentrations of many chemicals below their detection level, and the chemicals that were detected in that sample were factors of 2-8 below the levels previously determined in the untreated runoff water (LT-45D). The concentration of isomers of hexachlorocyclohexane (HCH), namely  $\gamma$ -HCH (lindane), and  $\alpha$ -HCH, was reduced 50- to 200-fold by the ferric chloride/calcium hypochlorite treatment from initial concentrations down to concentrations of 0.06-0.11 ppb. The treatment also reduced the concentrations of dichlorophenoxyacetic acids from 250 down to 30 ppb, and tetramethylurea was reduced from 600 to 200 ppb. The corresponding concentrations for these two chemicals in the drainage system water (LT-45A) were 12 ppb and 30 ppb, respectively. The s-triazine herbicide concentrations ranged from 250 ppb in the holding tank water to 10 ppb in the drainage system water, and was not detected in the treated water.

## CONCLUSIONS AND RECOMMENDATIONS

The Zigmantiškės Pesticide Storage Site is only one of several possible groundwater pollution sources in Lithuania. The ferric chloride/calcium hypochlorite treatment of the runoff water in the tanks at Utena substantially lowered the concentrations of many of the chemicals, some to below their detection limits, but the toxicity of the treated water remains unknown. Chemicals in groundwater have the potential to migrate and contaminate off-site water supplies, and for this reason monitoring should be continued in the area of the Zigmantiškės site and should be expanded to include other such facilities as they are identified. The monitoring at the Zigmantiškės site, now that several of the chemicals in the underground water have been identified, could be based on an immunoassay test for the most mobile chemical. Atrazine, because of its water solubility and low partitioning to organic carbon, is one of the more mobile chemicals identified in samples from both Zigmantiškės and Utena. Commercial immunoassay test kits are available for atrazine and could be used to monitor its movement in underground water at both sites. These kits are relatively inexpensive, their use does not require extensive technical training, and the results can be provided on location within minutes.

## ACKNOWLEDGEMENTS

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# PROBLEMATIC ASPECTS OF NEMUNAS RIVER LOAD MONITORING

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## ABSTRACT

Problematic aspects of monitoring are analyzed on the basis of microscopical and chemical analysis of material composition of water, suspensions, suspended load, bed load, and bottom core sediments of the Nemunas River during 1990-1991. These include organic matter, trace metals, and hydrocarbons. Taking into account saturation of different transport media with anthropogenic ingredients and their spatial/temporal fluctuations, we have concluded that it is groundless to use only water for monitoring and assessment purposes. We consider comprehensive knowledge about recent sedimentation processes to be most important in assessing pollutant trends. We recommend that some of the transport media, such as bottom sediments and, in certain cases, water, should be used for identification of local pollution, and others, particularly sediment load, for assessment of changes of chemical and physical state. Analysis of suspended load material, collected by long-term sampling methods which integrate short-term and accidental fluctuations, is indispensable in the monitoring of dynamic trends of environmental pollution.

## INTRODUCTION

Advances in physical and chemical analytical methods and technical measurements, as well as an increase of information about contamination of water bodies, have not only failed to bring the implementation of objective monitoring closer, but in some cases have confused our ability to assess realistically the state of an aquatic system at a given time. Objective monitoring is impeded by limited understanding of the ecological-philosophical approach even among researchers themselves or, at best, failure to recognize how it may be realized. Furthermore, the concept of the water basin as a system with an undefined number of controlling variables does not exist. The absence of a search for causality of an observed state in certain investigations (or self-limitation by empirical explanations) along with the increase of non-representative measurements not only fails to help solve the questions of objective monitoring and assessment of ecological state, but is even harmful as a stereotype.

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The frequent non-representative character of pollution measurements is predetermined by an abundance of localized point-sources, the especially complex structure of pollutant transport, and ignorance about the complexity of natural processes taking place in aquatic systems. All this forces us to doubt the correctness of available data and the validity of its interpretation. Technological pollution must be investigated against the background of natural equilibria and regularities and not *vice versa*.

The speculations presented here concern all larger water bodies including Kuršių Marios Lagoon, the Baltic Sea, and the Nemunas River, which, integrating and carrying the largest part of Lithuanian total sediment load, which should obtain priority in the national monitoring program. Based on the data collected in 1990-1991 from this main Lithuanian artery, we shall attempt to define the problematic aspects of the Nemunas River system monitoring.

Natural and technogenic substances carried by the Nemunas River take part in the sedimentation cycle (inflow-migration-transformation-precipitation-accumulation on the bottom), where they change phases and composition, fractionate, and undergo physical-chemical reactions and related changes of pollutant toxicity. The results of discrete investigations of only one medium may be biased by random observation of one link of the sedimentation cycle without knowing whether the point of observation was in a zone of transition, partial discharge, or rapid sedimentation. Different media and migration forms of a substance are not equally informative. Some are helpful in identifying aspects of local pollution, while others are more helpful in characterizing the state of the aquatic system. Elaboration of the concept of Nemunas River monitoring demands a preliminary analysis of the character, direction, and intensity of predominant processes. This prompted us to carry out a qualitative and quantitative analysis of the interrelation between priority pollutants in different media and migration forms at different cross-section locations along the river.

## MATERIALS AND METHODS

Samples of water, suspensions, suspended load, bed load, and bottom sediments from cores were taken for chemical and microscopical investigations during April to October 1990 and 1991 in the lower part of the Nemunas River (Figure 1). Material composition and particle size of the samples, hydrocarbon content, nature of the material, and trace metal concentrations (iron, manganese, copper, zinc, cadmium, lead, and chromium) were determined. Water and samples of suspended matter were taken from the surface (0-20 cm) and near-bottom (50-100 cm above the bottom) layers in three to five points of river transverse profile. Suspended load material was collected with traps (vessels with 0.3 L capacity and 6 radially spread out holes with 0.7 cm diameter) in the central channel of the river at distances of 0.2, 0.4, 0.6, 1.0, and 1.5 m above the bottom. Bed load was caught with traps of original construction. Exposition time of the traps was 1-2 days. Bottom sediments were taken with a vacuum cylinder (length 1 m, diameter ~5 cm) at five to eight locations along a cross-section of the river profile.

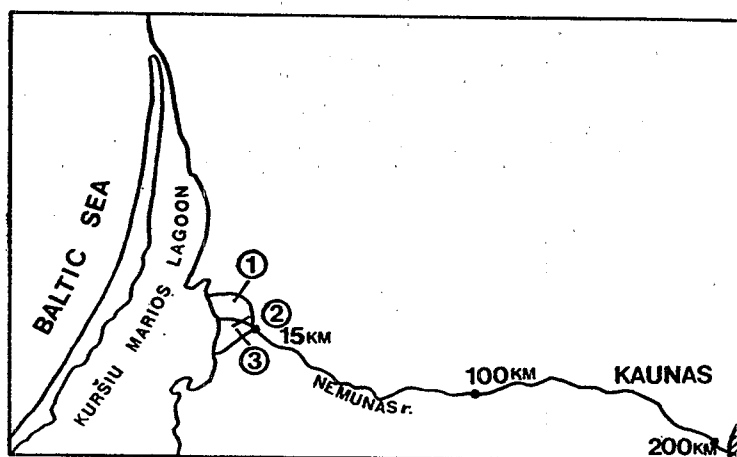


Figure 1. Situation scheme: Station 1-Atmata, Station 2-Pakalnė, Station 3-Skirvytė.

Suspensions were collected with the aid of filters containing  $0.45 \mu\text{m}$  diameter pores. Suspensions, suspended load material, and bed load samples were prepared for examination according to uniform methods (Lisitsin 1956, Bogdanov and Lisitsin 1968), using dyes and clarifying with Canada balsam. Particles under the microscope were counted separately for the following fractions:  $>0.05$ ,  $0.05-0.01$ ,  $0.01-0.005$ ,  $0.005-0.0025$  and  $<0.0025 \text{ mm}$ . Depending upon concentration, particles were counted in the 2-5th microscope fields.

For hydrocarbon analysis in different media, we applied our own methods (Zareckas 1989). In water, suspension, bed load, and bottom sample analyses the same solvent was used, seeking to avoid methodical complications, and allowing comparison of results from different media. Hydrocarbon analyses were performed by gas chromatography (Chrom-5<sup>TM</sup>).

Metals in the water column were investigated in two phases: soluble and suspended. To distinguish between them, water samples were filtered through Dubna<sup>TM</sup> lavsan filters with a pore diameter of  $0.45 \mu\text{m}$ . The suspended matter that accumulated on the filters was dissolved in acid mixture of  $\text{HNO}_3$  and  $\text{HClO}_4$  in teflon crucibles. The soluble metals were concentrated and precipitated with Tioxine<sup>TM</sup> (Vircaus *et al.* 1984), and the accumulated tioxinates were filtered through Dubna<sup>TM</sup>; these also were decomposed with an acid mixture of  $\text{HNO}_3$  and  $\text{HClO}_4$ . Metals from sediment traps, bed load, and bottom sediment samples were each also similarly dissolved in acid mixture. In the resulting five concentrates, the content of metals was determined by atomic absorption spectroscopy (Perkin-Elmer Model 403).

## RESULTS AND DISCUSSION

The concentration of soluble trace metals and oil hydrocarbons carried by the Nemunas River water is not high. Hydrocarbon content is 0.6 to 52  $\mu\text{g/L}$ , which is close to or below the maximum permissible limit of 50  $\mu\text{g/L}$ . (Sachaev and Scherbitsky 1986). The spatial distribution of oil products in water is especially complex. Lateral and vertical variations of hydrocarbon within a cross-section (Smalininkai-112 km, Rusnė-15 km) can vary as much as eight times, which is almost the same variation as for the whole Nemunas River (Figure 2). In addition, occasional variations in the cross-sectional concentrations of trace metals in the Nemunas River, which can vary as much as nine-fold, are caused by point-source pollution. There are also marked variations in metal concentrations in the Nemunas River profile attributable to sorption onto suspended matter (Figure 3). The concentration of total suspended material may reach as much as 40 mg/L toward the lower Nemunas River and, as a consequence, the total load of metals transported on particulates also increases. Because of abrupt and frequent changes in trace metal concentrations, especially cadmium, lead, zinc, and copper which can have 20-fold variations, this eliminates either suspended or soluble trace metal forms as optimum media for continuous monitoring.

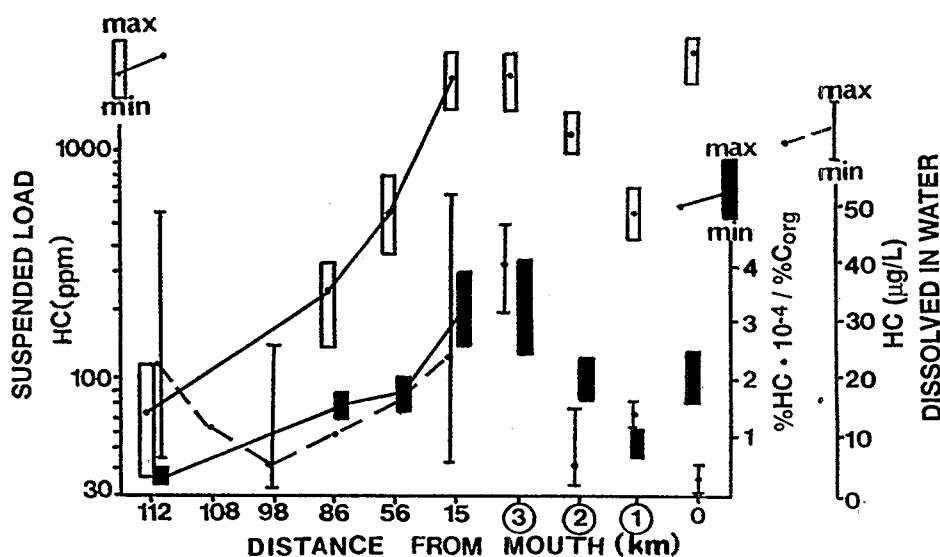


Figure 2. Concentration of hydrocarbons, suspended load ( $\text{HC}_{\text{ppm}}$ ), and changes of technogenization degree (percent  $\text{HC} \cdot 10^{-4}$ /percent  $\text{C}_{\text{org}}$ ) in a longitudinal profile of the Nemunas River.

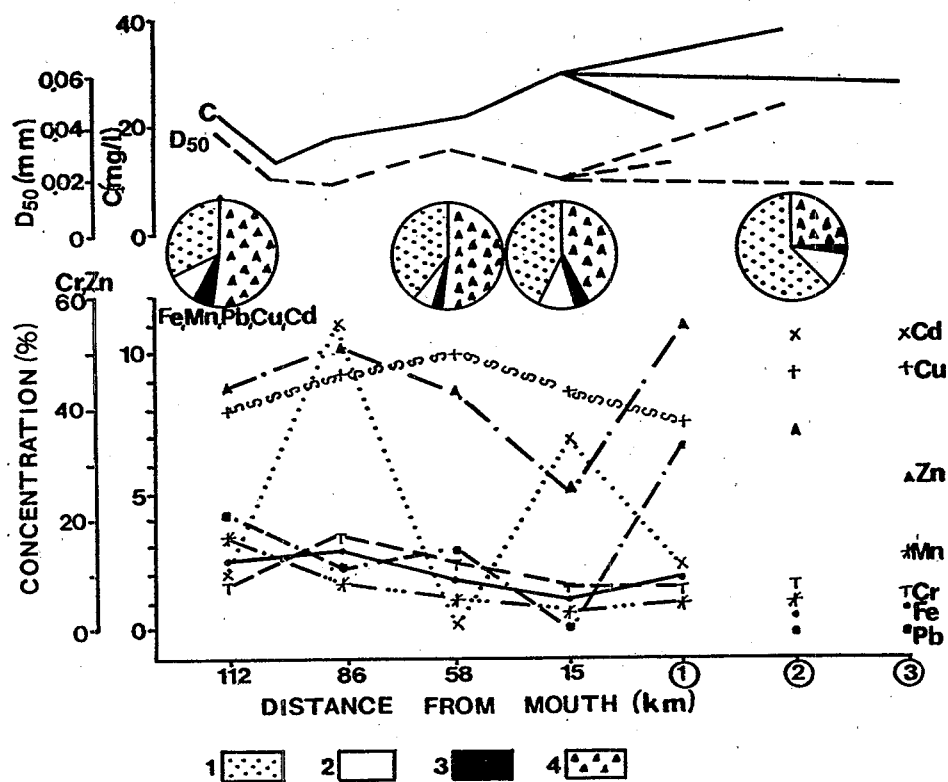


Figure 3. Substantial and microelemental composition of normally suspended microparticles (suspensions). Substantial composition percent: (1) mineralogenic, (2) autochthonous detritus, (3) allochthonous detritus, (4) phytoplankton skeletons. Chemical composition percent (ppm =  $10^{-4}$  %): Fe x 1; Mn, Pb x  $10^{-2}$ ; Cu, Zn, Cr x  $10^{-3}$ ; Cd x  $10^{-4}$ .  $D_{50}$  = medial diameter of transported substance. C = concentration of suspensions.

Suspended matter collected in the long-term sampling traps may not be representative of the river runoff suspended matter as a whole because those particles likely possess higher sinking rates resulting from different granulometric and substance composition. Nonetheless, the trap material integrates short-term fluctuations of pollution over a long period of time and permits observation of the dynamics of transported pollutants and concomitant changes of river "health."

With decreasing water current speed and diminishing median diameter ( $D_{50}$ ) of transported particles, as well as with mechanical fractioning of suspended material in which mineralogenic particles predominate, there is an increase in the concentrations of the trapped trace metals and hydrocarbon material (Figures 2, 4). As the solids suspended in the Nemunas River begin to deposit in the Nemunas River delta (Figure 1), the river becomes less polluted (Table 1). The degree of oil pollution changes correspondingly. Hydrocarbon, expressed as percent of organic carbon ( $C_{org}$ ), has been calculated at 4% at Rusne (Figure 2), 15 Km from the river mouth. This parameter is about 0.05% for natural organic matter in the river. In transported sedimentary matter of the Nemunas River delta, biogenic hydrocarbons do not exceed 3% of the total of all hydrocarbon.

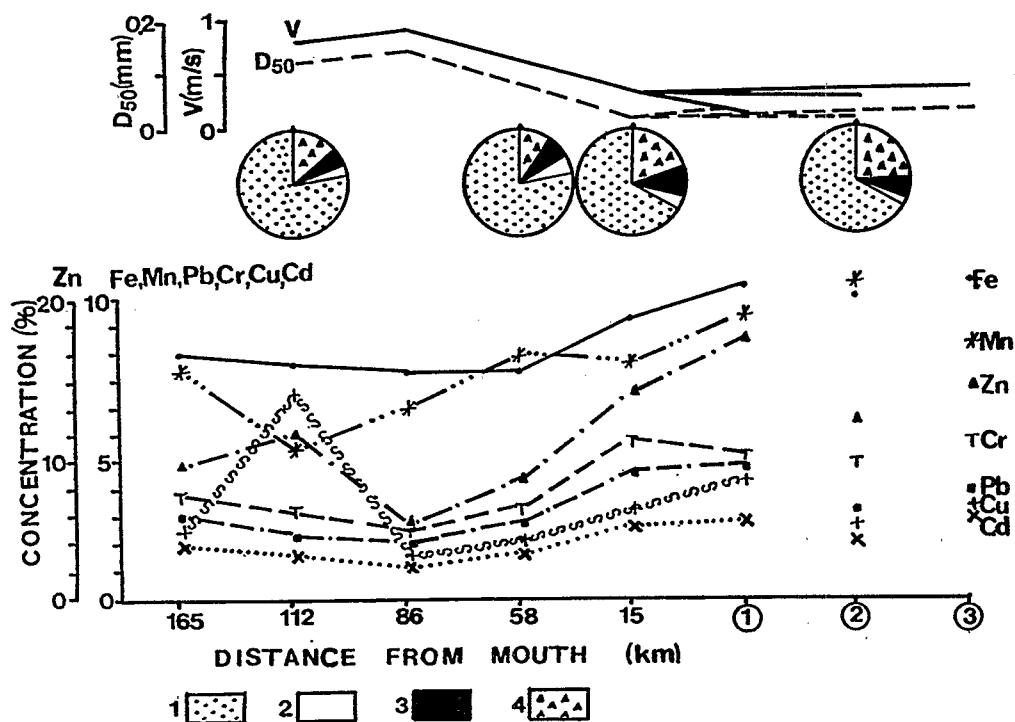


Figure 4. Substantial and microelemental composition of suspended load (settleable solids). Substantial composition percent: (1) mineralogenic, (2) autochthonous detritus, (3) allochthonous detritus, (4) phytoplankton skeletons. Chemical composition percent (ppm =  $10^{-4}$  %): Fe x 1; Mn x  $10^{-2}$ ; Cu, Zn, Pb x  $10^{-3}$ ; Cd, Cr x  $10^{-4}$ .  $D_{50}$  = median diameter of transported substance. C=concentration of suspensions. V=current velocity.



Table 1. Suspended matter runoff.

Section (Distance from mouth)	Q (m <sup>3</sup> /s)	R (kg/s)	Substance Groups of Detritus (kg/s)			
			Biogenic			Mineral- ogenic
			Autoch- thonous	Alloch- thonous	Phyto- plankton	
Smalininkai (112 km)	515	12.23	0.61	0.49	7.34	3.79
Smalininkai -bed load material		0.0184				
Rusnė (15 km)	370	11.19	1.23	0.28	5.09	4.59
Atmata (Delta)	176	3.87	0.13	0.17	2.09	1.48
Skirvytė (Delta)	161	5.96	0.21	0.15	1.55	4.05
Pakalnė (Delta)	35	0.98	0.054	0.005	0.627	0.29
Pakalnė (Delta) -bed load material		0.00013				

As a result of sorption of oil products, the amount of transported hydrocarbon in suspended form regularly increases towards the lower Nemunas River when compared with the soluble form. In the Nemunas River mouth the ratio of suspended to dissolved hydrocarbons reaches 25. Similar changes in trace metal forms occur (Figure 5).

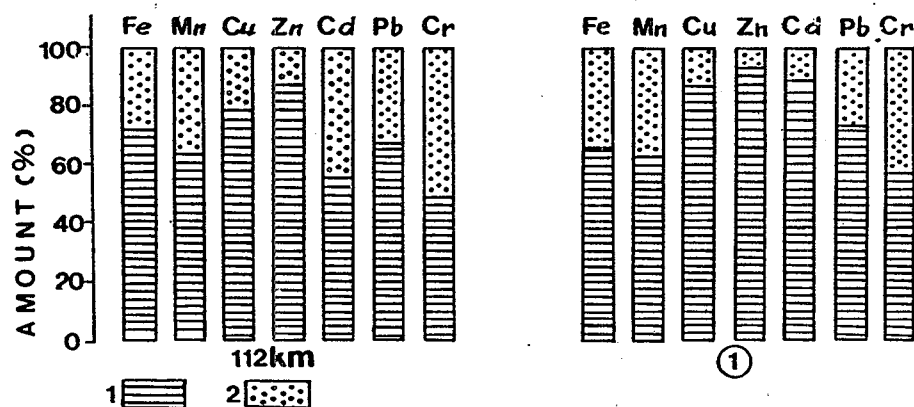


Figure 5. Change in metal forms in the lower course of the Nemunas River:  
(1) metals in suspension, (2) metals in soluble form.

On average, 90% of the zinc, 86% of the copper, 81% of the cadmium, and 67% of the lead are carried in suspended form by the Nemunas River (Table 2). As background values constantly change, these must be borne in mind when evaluating the health of an aquatic system.

Table 2. Total chemical runoff (g/s). Percent suspended shown in parentheses.

Section	Fe	Mn	Cu	Zn	Cd	Pb	Cr	HC
Smalininkai (112 km)	496 (73)	7.05 (66)	1.31 (79)	6.43 (86)	0.050 (60)	8.20 (71)	3.12 (51)	12.01 (7.50)
Smalininkai -bed load material ( $10^{-5}$ g/s)	8830	773	13.80	20.10	1.310	20.10	0.92	1.64
Rusnė (15 km)	263 (65)	3.20 (35)	1.21 (88)	3.35 (90)	0.084 (95)	2.23 (45)	1.4 (59)	33.68 (68)
Atmata	125 (72)	1.30 (45)	0.32 (97)	2.60 (88)	0.025 (20)	3.29 (76)	0.80 (49)	4.78 (47)
Skirvytė	156 (64)	3.03 (75)	1.11 (94)	2.07 (90)	0.072 (97)	1.16 (61)	0.75 (61)	19.45 (66)
Pakalnė	21 (70)	0.23 (52)	0.12 (92)	0.42 (93)	0.013 (77)	0.21 (62)	0.14 (71)	1.42 (87)
Pakalnė -bed load material ( $10^{-5}$ g/s)	145	18.10	0.36	1.66	0.030	0.36	0.54	2.89

The longitudinal distribution of pollutants in the surface layer of bottom sediments of the Nemunas River is much more complex, generally decreasing towards the delta, even though there may be occasional maxima (Figures 6 and 7). Fluctuations of hydrocarbon concentrations can be 1,000-fold, thereby altering the typical exponential dependence of trace metals and hydrocarbon content upon median diameter (Figure 8). The anomalies are related to intensive accumulation of nonhomogeneous sedimentary matter below the larger sources of pollution entering the Nemunas River between Kaunas and the river mouth at 199, 165, 112, and 56-58 km. The concentration of metals in bottom sediments is directly related to organic substances (Figure 8) which have good sorptive capacity and transport pollutants to the bottom. An important role in hydrocarbon accumulation and transportation is played by phytoplankton, the skeletons of which control the concentration of oil products in the transported sedimentary matter and bottom sediments (Figure 6).

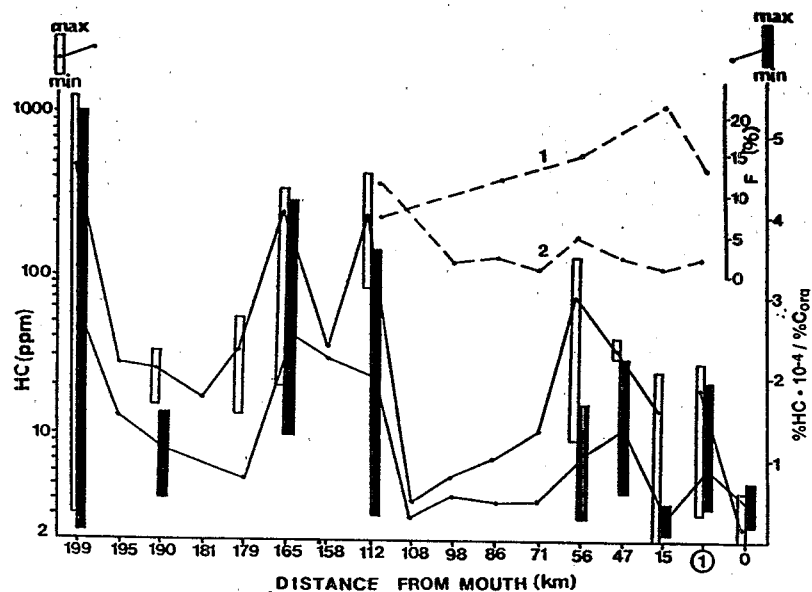


Figure 6. Changes of the content of hydrocarbons ( $HC_{ppm}$ ) and degree of technogenization (percent  $HC \cdot 10^{-4} / \text{percent } C_{org}$ ) in the bottom sediments of longitudinal profile of the Nemunas River. F = amount of phytoplankton skeletons (percent) in (1) suspended load, (2) and bottom sediments.

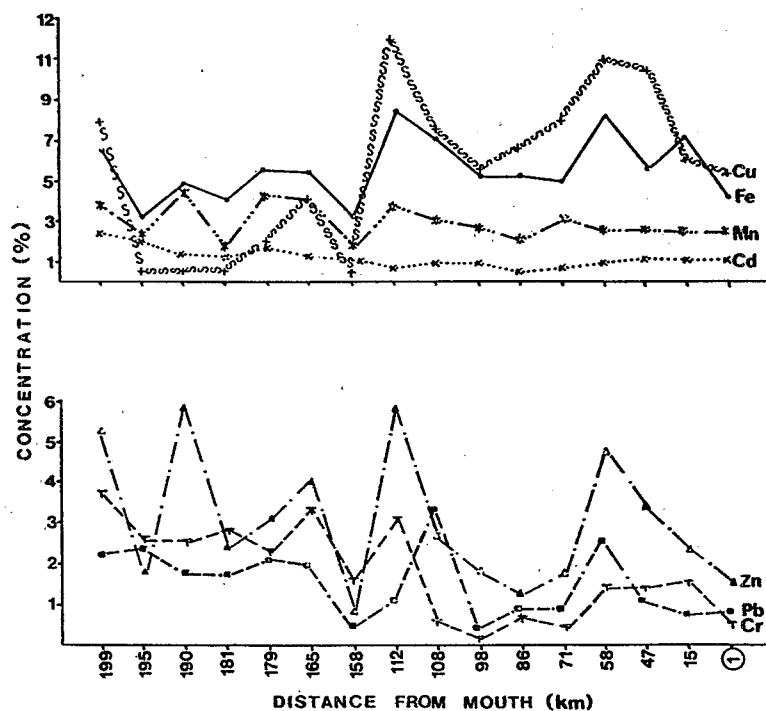


Figure 7. Metals in the surface layer of bottom sediments percent (ppm =  $10^{-4}\%$ ): Fe x 10; Mn x  $10^{-2}$ ; Zn, Pb, Cr x  $10^{-3}$ ; Cu, Cd x  $10^{-4}$ .

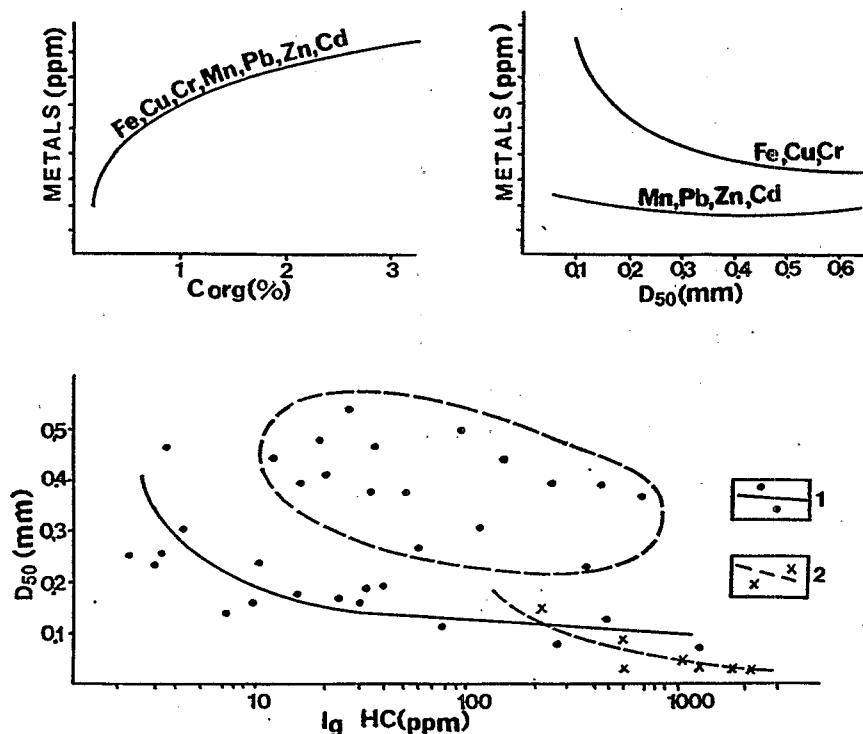


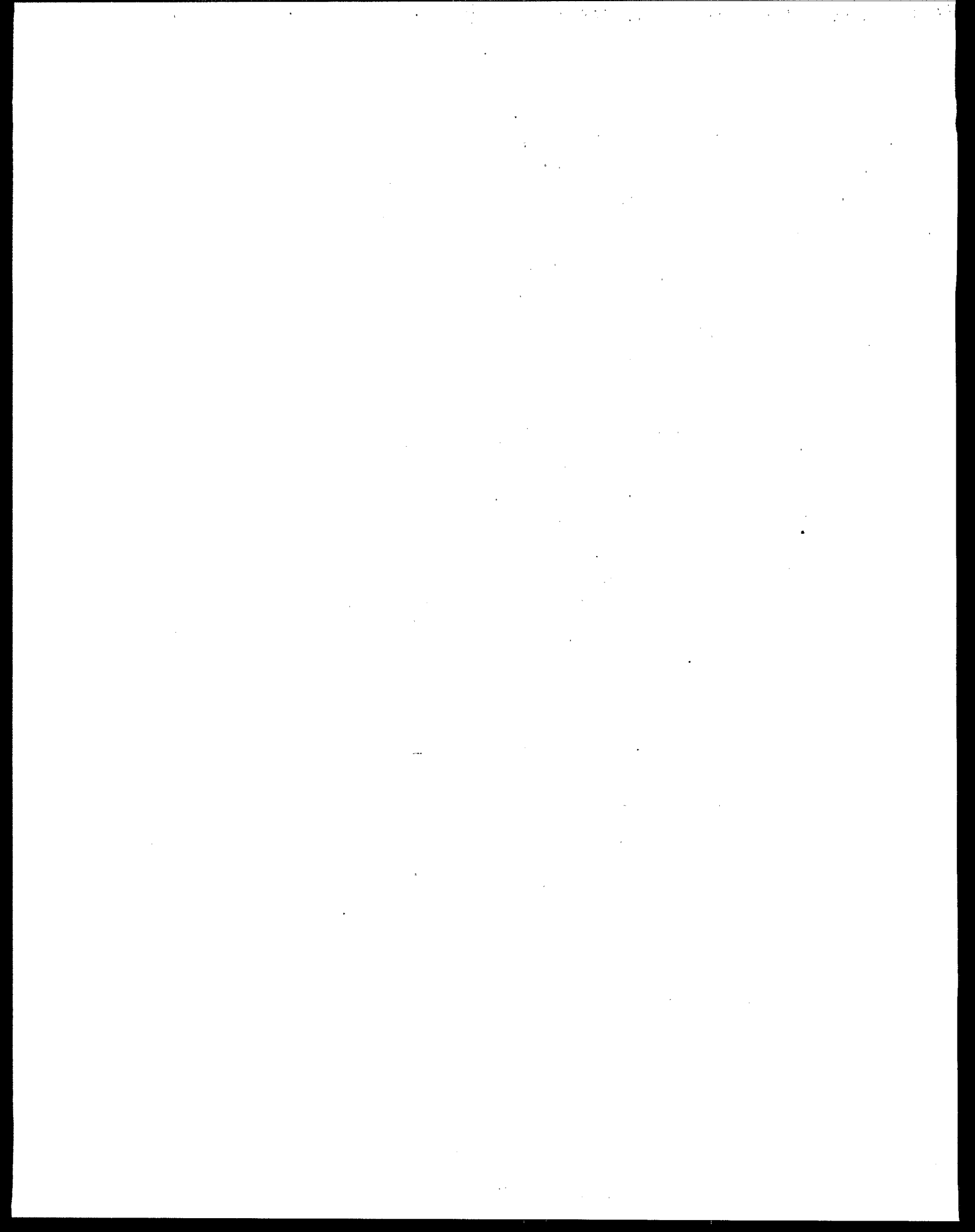
Figure 8. Metals concentration dependence in bottom sediments on organic matter ( $C_{org}$ ) and median diameter ( $D_{50}$ ), and hydrocarbons content dependence on  $D_{50}$  in (1) bottom sediments, and (2) suspended load. (Circled region contains anthropogenically polluted samples.)

## CONCLUSIONS

Distribution of priority pollutants in different media in the Nemunas River, and the tendencies to change both within and among these media, lead us to conclude that a better way to monitor trace metals and hydrocarbons in the Nemunas River would be to analyze sedimentary matter collected from long-term sampling traps. Contamination intensity must be evaluated in the context of natural variation and trends, and in the context of types of samples being studied and how the sample types vary in respect to one another. Furthermore, to be able to assess the condition of those sections of the river below larger pollution sources, locally accumulating bottom sediments should also be monitored.

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# INVESTIGATION OF MICROORGANISMS: BIODEGRADERS ASSOCIATED WITH HYDROBIONTS

J. Šyvokienė and L. Mickėnienė<sup>1</sup>

## ABSTRACT

The ability to decompose oil products is characteristic of the microorganisms in the digestive tract of molluscs. Molluscs were collected from the Nemunas River and Kuršių Marios Lagoon in 1991, and their activity is related to using hydrocarbons as a source of carbon in the processes of water purification. The largest number of oil-degrading bacteria was obtained from digestive organs of specimen #708

*Anodonta piscinalis*, collected upstream from Smalininkai. The number of oil-degrading bacteria exceeded that of saprophytic bacteria in the digestive tract of investigated molluscs in most cases. Enzymatic systems of microorganisms from the digestive tract of molluscs take part in an inactivation process of biologically active materials. *A. piscinalis* can be used as a biofilter in ecologically polluted waters. Determination of microorganisms in the digestive tract of molluscs or other hydrobionts which take part in degradation of oil-products, will strengthen the knowledge base of ecosystem processes which aid in self-purification.

## INTRODUCTION

An increase in environmental pollution undoubtedly has a strong influence on functional activity and properties of microorganisms of hydrobionts. The effects include the localization and specialization of microflora inhabiting the digestive tract. Because of changes in the living conditions of hydrobionts, alterations in the community structure of intestinal microorganisms occur, as do changes in the regularities of functioning of some populations. Any pollutant entering the environment has a potential negative effect on water quality, and tends to impact intestinal microorganisms of hydrobiont populations. Data on microorganisms, located in the digestive tract of molluscs taking part in decomposition of hydrocarbons, are scarce.

The purpose of this research was to show the direct participation of microorganisms associated with individual molluscs in the decomposition of pollutants of organic origin. These investigations are important for two reasons: determination of common biological regularities, and application of the results in predicting the response of natural communities of hydrobionts to anthropogenic impact. Participation of microflora of hydrobionts in water purification processes is not well understood, and has not been widely studied. Natural water purification through the activities of hydrobionts may be a very important ecological process.

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## MATERIALS AND METHODS

Two species of molluscs (*Unio pictorum* L., *Anadonta piscinalis* Nilsson) were collected for microbiological investigations in 1991 from the Nemunas River at Smalininkai and from Kuršių Marios Lagoon at Ventės Cape. Microorganisms isolated from the intestine, crystalline style, and coelomic fluid of these molluscs were evaluated according to their ability to grow on agar media containing hydrocarbon. Samples were taken from the digestive system of every specimen from Smalininkai and of 3-5 specimens of molluscs from the Ventės Cape. The digestive systems were investigated according to methods described by Mattheis (1964). The ability of microorganisms to degrade black oil and diesel oil hydrocarbons was investigated by measuring growth based on colony-forming counts on agar media (Gerhardt 1983), where the hydrocarbon served as the only source of carbon. Other microbiological indices were evaluated according to methods of Rodina (1965) and Romanenko (1985).

## RESULTS

The data show that microorganisms of molluscs are possibly capable of using hydrocarbons as the only source of carbon and of growing in such media (Table 1). The concentration of oil-degrading bacteria was different in separate organs, with the highest concentration being found in the intestine and crystalline style. The number of microorganisms in coelomic fluid was less than in the intestine by a factor of 10 or more (Figure 1), and even fewer were found in water (from  $11 \times 10^3$  to  $19 \times 10^3$  cells/ml) and in soil ( $25 \times 10^5$  cells/g) (Jankevičius *et al.* 1991).

Table 1. Number of microorganisms in the content of intestine (I), crystalline style (CS), and coelomic fluid (CF) of molluscs from two sampling sites in Kuršių Marios Lagoon (I and CS, millions/ml; CF, millions/g).

Specimen number and species		Oil degrading bacteria						Saprophytic bacteria		
		With black oil			With diesel oil					
		I	CS	CF	I	CS	CF	I	CS	CF
Smalininkai										
537	<i>Unio pictorum</i>	0.20	1.20	0.02	0.40	0.00	0.04	2.10	0.10	0.05
538	"	5.20	0.30	0.10	1.40	0.20	0.20	1.40	0.06	0.03
707	<i>Anadonta piscinalis</i>	3.40	0.10	0.09	1.50	0.01	0.01	2.70	0.30	0.07
708	"	3.40	3.80	1.20	5.60	8.20	0.10	6.80	0.01	0.10
Ventės Cape										
	<i>U. pictorum</i>	4.10	1.00	0.08	1.20	0.30	0.18	2.00	0.15	0.02
	<i>A. piscinalis</i>	3.50	3.10	1.40	4.80	6.80	0.08	4.70	0.22	0.06



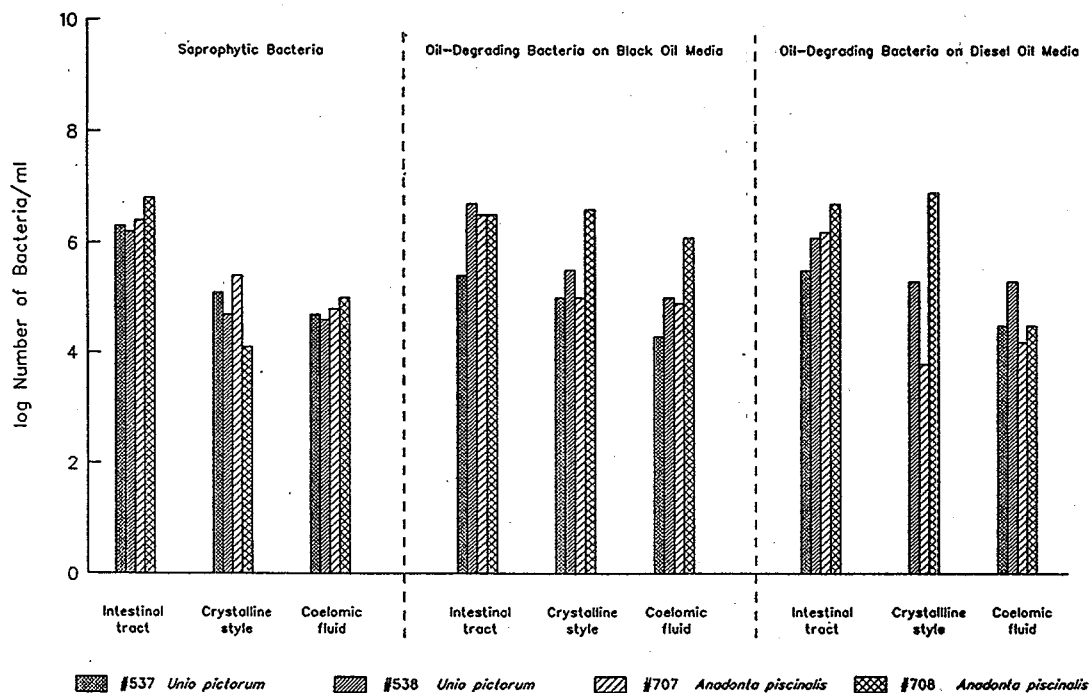


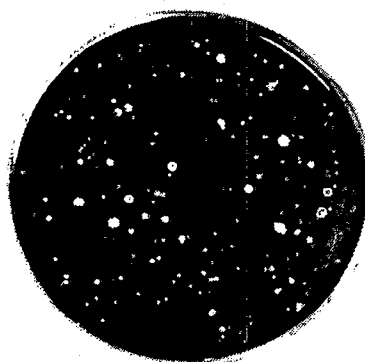
Figure 1. Bacteria counts in intestinal tract, crystalline style, and coelomic fluid of *Unio pictorum* and *Anadonta piscinalis*.

Results indicate that microorganisms from the digestive tract of molluscs might grow by degrading oil pollutants. In agar containing black oil (Figure 2) and diesel oil (Figure 3), degradation occurred based on increases in the number of microbial cells. In contrast, on media containing no black or diesel oil (Figure 4), microbial growth was negligible.

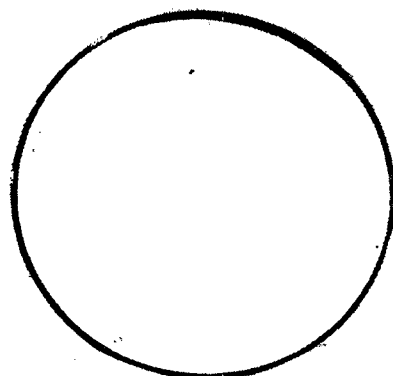
In all the individuals studied saprophytic bacteria were uniformly distributed, however, their concentration fluctuated and the highest concentration observed was in the intestine and the lowest in coelomic fluid (Table 1). Data of Zandman (1984) indicate that water pollution may be evaluated by determining the ratio of oil-degrading to saprophytic bacteria. Our investigations confirmed the findings of Zandman, although we also found that in the presence of both black and diesel oil the oil-degrading bacterial counts were high and rivaled the saprophytic bacterial counts.



**Figure 2.** Growth of bacteria isolated from the intestine of molluscs and incubated on agar media with black oil.



**Figure 3.** Growth of bacteria isolated from the intestine of molluscs and incubated on agar media with diesel oil.



**Figure 4.** Growth of bacteria isolated from the intestine of molluscs and incubated on agar media without either black or diesel oil (control).

## DISCUSSION

Literature dealing with research on oil degradation by microbial hydrobionts in molluscs is virtually nonexistent. There is some information about aquatic microorganisms and intestinal microflora of mussels (*Mytilus*) taking part in decomposition of hydrocarbons in fresh water (Mironow 1985), and in brackish water (Puchenkova 1988a, Gunkel and Dahlman 1986, Shamshoom *et al.* 1990). There are several opinions concerning the authenticity of oil-degrading bacteria (Prieur 1982, Minet *et al.* 1987, Ledo *et al.* 1983, Puchenkova 1988b). The large concentration of microorganisms found reveal that hydrocarbon utilizers are inhabitants of the intestine of molluscs. It should be noted that enzymatic systems of microorganisms from the digestive tract of molluscs may play an important role in transformation processes of biologically active combinations of black oils.

Intestinal microflora react to changes in environmental conditions. When microbiological systems of organisms are disturbed, biosynthetic activity of microorganisms appear affected, with a weakening of enzymatic functions. Microorganisms, however, utilize pollutants and convert them into organic acids, amino acids, and other products, which serve as a source of energy. Hydrocarbon degradation was characteristic of microorganisms associated with the content of intestinal tracts of molluscs collected at Ventès Cape, and in three of the four molluscs collected at Smalininkai. The functional activity of hydrocarbon-degrading microorganisms is related to availability of a metabolizable carbon source (oil product), and the concentration of microorganisms taking part in self-purification processes.

Identification of oil-degrading bacteria in the digestive tract of bivalve molluscs assists in the evaluation of the health of ecological systems. This study is intended to be helpful in future monitoring of oil pollution levels. Research should be expanded on oil-degrading bacteria in the digestive tracts of other species of hydrobionts, *i.e.*, in fishes such as roach (*Rutilus rutilus*) and stickleback (*Gasterosteus aculeatus*).

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# TOXICITY OF ACETANILIDE HERBICIDES AND THEIR BIODEGRADATION IN PURE *PSEUDOMONAS* CULTURES

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## ABSTRACT

The toxicity of acetanilide herbicides was determined using a *Photobacterium* bioluminescence quenching method. It has been shown that both propanil and its primary degradation product, 3,4-dichloraniline, are more toxic, especially at lower concentrations, than propachlor and its degradation product, aniline. Biodegradation kinetics for two acetanilide herbicides, propanil and propachlor, were measured using pure *Pseudomonas* bacterial cultures grown on either glucose or sodium dodecylsulfate (SDS) as a sole carbon source. Second-order degradation rate constants were calculated for primary degradation of both propanil and propachlor. Degradation of propanil was approximately a factor of 2 higher as compared to propachlor. *Pseudomonas* strains grown on SDS as a sole carbon source exhibited a higher primary degradation rate for propanil than those strains grown on glucose. Differences in propachlor degradation in cultures grown on SDS or glucose were negligible.

## INTRODUCTION

The class of acetanilide herbicides represents a set of compounds possessing an amide type bond (NH-CO) and the side groups of more complex or simple structure (Fedtke 1986). Parallel to symtriazine herbicides, acetanilides such as propachlor or pronamide are still used in Lithuanian agriculture (Šukytė 1990). Residue analyses of symtriazine and acetanilide herbicides were performed at the Lithuanian Agriculture Institute, Voke Branch (Kavoliunaite 1990). Biodegradation of these herbicides was also investigated in surface water samples (Steen and Collette 1989, Vasiljeva *et al.* 1989). Some information is available on the biodegradation of acetanilide herbicides using a pure culture of fungi (Rotmistrov *et al.* 1975). Toxicity studies in conjunction with biodegradation experiments are anticipated to provide a more complete picture and elucidation of biodegradation mechanisms. The objective of this research was to investigate both the toxicity and biodegradation kinetics of acetanilide herbicides using pure cultures of *Pseudomonas*.

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Experimentally a high priority was set for assessment of degradation kinetics and the reliability of the second-order reaction equation for describing microbial transformation (Paris *et al.* 1981):

$$-\frac{dc}{dt} = K_b \cdot B \cdot C$$

where  $K_b$  is a second-order reaction rate constant,  $B$  is bacterial biomass,  $C$  is concentration of the test herbicide,  $t$  is time, and  $K_b \cdot B = K$ , *i.e.*, first-order rate constant  $K$  for herbicide disappearance and is linearly dependent upon microbial biomass concentration.

This equation describes the dependence of xenobiotic biodegradation rate on both herbicide concentration and bacterial biomass, and likewise provides some insight into the effects of the chemical structure of xenobiotics when compared across structures within the same chemical class (Paris *et al.* 1981). The second-order rate constants for biodegradation and chemical degradation of xenobiotics (alkaline hydrolysis) can be used to describe the fate of chemical substances in aquatic environments, *i.e.*, transformation, degradation, and sorption using the Exposure Analysis Modeling System (EXAMS II).

## MATERIALS AND METHODS

The herbicides propanil and propachlor (99.9% purity, reference standards, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina, were used in this study as well as 3,4-dichloraniline (U.S. EPA, technical grade), and acetonitrile (HPLC grade, Merck, Germany). For microbiological analysis, nutrient agar (USSR) containing 17 g/L sprat hydrolysate, 11.2 g/L marine agar, 5.9 g/L sodium chloride, pH 7.4 to 7.7, and glucose (sterile ampoules, 40%) or sodium dodecylsulphate (SDS, Merck, Germany) were used to provide a final concentration of 0.1% as a sole carbon source.

Bacterial strains used in this work were: *Pseudomonas sp.* 332 (obtained from Institute of Applied Enzymology, Vilnius, Lithuania), *P. aeruginosa* 1C (obtained from I. A. Krivetc, Institute of Colloidal Chemistry and Water Chemistry, Kiev, Ukraine), *Photobacterium phosphoreum* (obtained from of A. Ruzgene, Faculty of Nature Sciences, Vilnius University).

Samples of pure bacterial cultures for biodegradation experiments were prepared as follows: (1) *Pseudomonas* cultures were grown on minimal salt medium ( $\text{KH}_2\text{PO}_4$ , 3 g/L;  $\text{K}_2\text{PO}_4$ , 7 g/L;  $\text{NH}_4\text{NO}_3$ , 1 g/L;  $\text{KCl}$ , 0.5 g/L;  $\text{MgCl}_2$ , 0.1 g/L; pH 7.3) with glucose or SDS (0.1 percent) as a sole carbon source until stationary growth was observed ( $\text{OD}_{590 \text{ nm}} = 0.5-0.9$ ); (2) the bacterial suspension was centrifuged at 3000 rpm for 30 minutes with aseptic precaution, and re-suspended in the same sterile medium to  $\text{OD}_{590 \text{ nm}} = 0.1$  and volume 50 ml; (3) control samples were autoclaved and after cooling the herbicides were added to all samples; (4) sample flasks were incubated in the heated water bath shaker (Poland) at 28°C, and shaken at 150 rpm.

Bacterial concentration (microbial biomass in number of cells/L), was determined by direct plate counting, using sprat-hydrolysate/agar medium (35 g/L, pH 7.2).

Disappearance of herbicide in bacterial suspensions was followed by HPLC analysis following centrifugation at 12,000 rpm for 15 minutes to separate bacteria from the supernatant. The resulting supernatant was analyzed for both residual herbicide and/or product.

Conditions for HPLC analysis were: (1) reversed phase (C<sub>18</sub>) Ultrasphere ODS<sup>TM</sup> column 4.6 mm x 25 cm (Beckman Instruments); (2) UV detector with absorption  $\lambda_{\max}$  = 225 nm for propanil and 3,4-dichloraniline, and  $\lambda_{\max}$  = 238 nm for propachlor; (3) sensitivity of UV detector 0.05 RF for propanil and 0.2 RF for propachlor; (4) ratio of solvents acetonitrile:water = 75:25 for propanil and 80:20 for propachlor; (5) flow rate 1 ml/minute.

Bacterial toxicity of the herbicides was determined using a *P. phosphoreum* luminescence quenching method (Microtox<sup>TM</sup> prototype) (Gil *et al.* 1988). *Photobacterium* were grown on sterile sprat hydrolysate/agar medium, pH 7.2, containing 3% NaCl for 12 to 15 hours at 28°C and transferred from the agar medium surface to 3% NaCl solution and mixed carefully. A Luminometer<sup>TM</sup> Model 1250 (LKB-Wallac, Sweden) was used for bioluminescence quenching measurements. Luminescence in samples of *Photobacterium* suspensions with herbicide and appropriate controls was measured at 1 minute and at 2 and 20 hours.

Statistical analysis of the data on biodegradation experiments was based on the linear least square regression. The half-life for herbicide biodegradation reaction and first-order reaction rate constants were calculated according to the equations of Metzler (1980). The statistical and regression parameters, offered by scientists of the U.S. EPA Environmental Research Laboratory in Athens, Georgia, were used in this work (Paris *et al.* 1981, Steen and Collette 1989, Vasiljeva *et al.* 1989).

## RESULTS AND DISCUSSION

Following 20 hours incubation, cells with propachlor at 10 ppm were observed to yield diminished bacterial luminescence, an indication of toxicity (Figure 1A). Toxicity to the enterobacteria species *P. phosphoreum* was clearly exhibited at the 10 ppm propachlor concentration. A decrease in luminescence was not observed during the 20 hours incubation at the 5 ppm propachlor concentration, although toxicity might have been observed had there been a more prolonged incubation period.

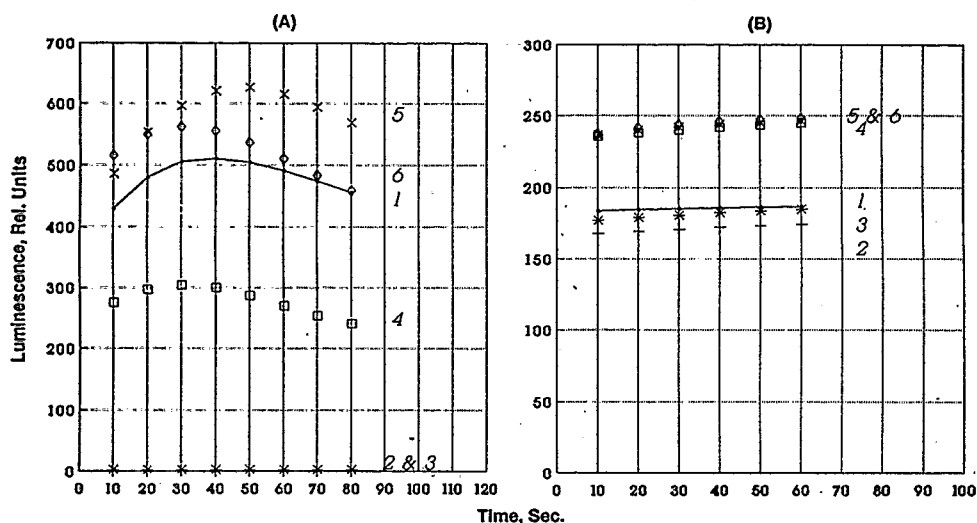


Figure 1. Inhibition of *Photobacterium phosphoreum* bioluminescence by (A) propachlor, and (B) aniline after 20 hours of incubation in 3% NaCl. Concentrations of propachlor: 1 - 0 ppm (Control), 2 - 100 ppm, 3 - 50 ppm, 4 - 10 ppm, 5 - 5 ppm, 6 - 1 ppm. Concentrations of aniline: 1 - 0 ppm (Control), 2 - 100 ppm, 3 - 50 ppm, 4 - 10 ppm, 5 - 5 ppm, 6 - 1 ppm.

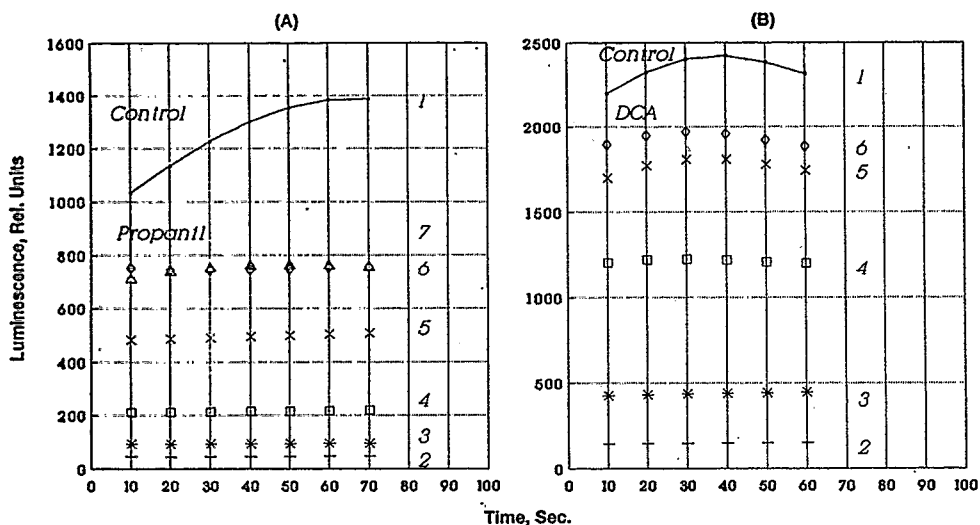


Figure 2. Inhibition of *Photobacterium phosphoreum* bioluminescence by (A) propanil, and (B) 3,4-dichloraniline after 2 hours incubation in 3% NaCl. Concentrations of propanil: 1 - 0 ppm (Control), 2 - 100 ppm, 3 - 50 ppm, 4 - 20 ppm, 5 - 10 ppm, 6 - 5 ppm, 7 - 1 ppm. Concentrations of 3,4-dichloraniline: 1 - 0 ppm (Control), 2 - 100 ppm, 3 - 25 ppm, 4 - 10 ppm, 5 - 5 ppm, 6 - 1 ppm.



Aniline, a degradation product of propachlor, exhibited lower membrane toxicity for enterobacteria; inhibiting *Photobacterium* bioluminescence only at the 25 ppm exposure concentration (Figure 1B). On the other hand, the results of our experiment showed that propanil had a toxic effect on *P. phosphoreum*. Following 2 hours of incubation of cells at 1 ppm propanil, a resultant luminescence quenching (Figure 2A) was observed. The 3,4-dichloroaniline, a primary degradation product of propanil, exhibited similar membrane toxicity. A slight luminescence quenching was observed at the 1 ppm and 5 ppm concentrations following a 2-hour incubation (Figure 2B).

Experimental data from biodegradation experiments with acetanilide herbicides, using pure *Pseudomonas* cultures, *Pseudomonas* sp. 332 and *P. aeruginosa* 1C, are presented in Table 1 and in Figures 3 and 4.

*Pseudomonas* cultures grown on glucose and on SDS as sole carbon sources were used in biodegradation experiments. The objective of these investigations was to compare biodegradation rates of cultures grown on different carbon sources and differences in the degradation of aromatic compounds of the same structural class.

Propachlor degradation investigations were carried out at an initial concentration of propachlor at 10 ppm. The total number of bacteria, *Pseudomonas* sp., remained relatively constant, ( $2.5 \times 10^{11}$  cells/L), reflecting a negligible toxicity effect from propachlor following treatment for 1000 hours.

Cultures of *Pseudomonas* sp. 332 cells grown on SDS exhibited a greater biodegradation rate for propachlor than those cells grown on glucose. The mean degradation half-lives ( $t_{1/2}$ ) were 900 hours on SDS and 1480 hours on glucose (Table 1). On the other hand, the mean degradation half-lives for propachlor in cultures of *P. aeruginosa* 1C grown on SDS or glucose were 1910 hours and 1340 hours.

In experiments at an initial propanil concentration of 4.2 ppm, both *Pseudomonas* 332 and *P. aeruginosa* 1C exhibited higher propanil degradation rates when grown on SDS than when grown on glucose as a sole carbon source, possibly reflecting the well known phenomenon of glucose catabolic repression. The arithmetic means of the degradation half-lives for propanil in glucose-grown and SDS-grown *Pseudomonas* sp. 332 cultures were 444 hours and 199 hours, respectively. In the culture grown on SDS, the biodegradation reaction was 2.23 times greater. Mean degradation half-lives for propanil disappearance for *P. aeruginosa* 1C cultures grown on glucose and SDS were 893 hours and 318 hours, respectively. Total bacterial concentration in experiments with propanil ranged from  $2.4 \times 10^{11}$  cells/L during a 600-hour incubation period.

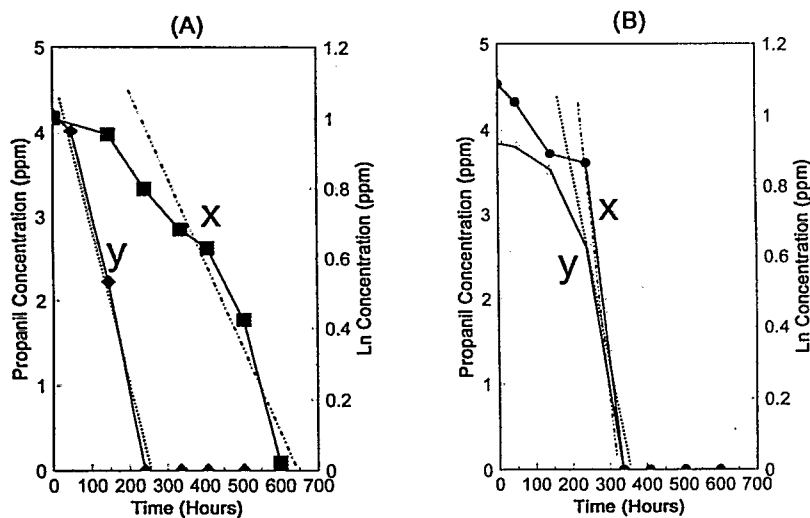


Figure 3. Propanil biodegradation of (A) *Pseudomonas* species 332 and (B) *Pseudomonas aeruginosa* 1C cultures, grown on (X) glucose, or (Y) sodium dodecylsulphate. (Dotted line is least square fit.)

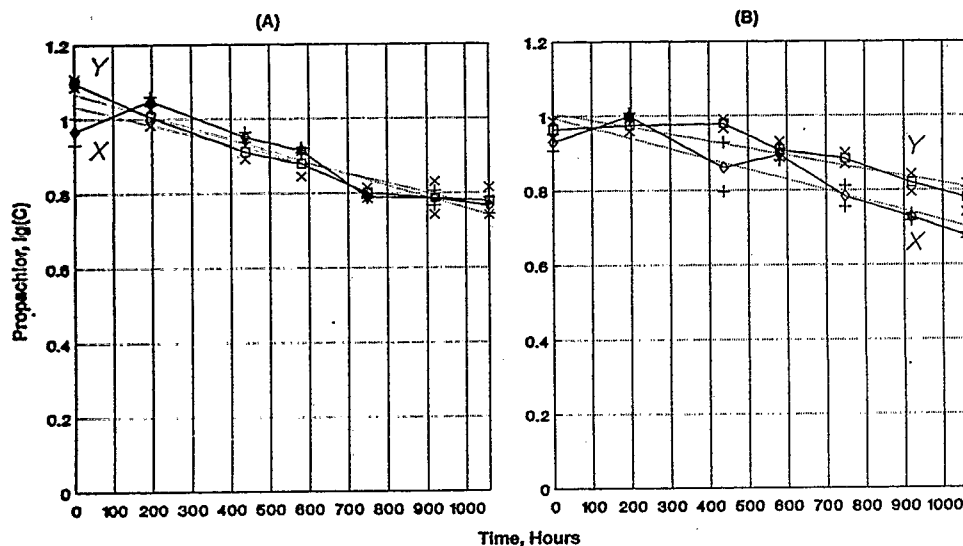


Figure 4. Propachlor biodegradation in (A) *Pseudomonas* species 332 and (B) *Pseudomonas aeruginosa* 1C cultures, grown on (X) glucose or (Y) sodium dodecylsulphate. (Dotted line is least square fit.)

**Table 1. Kinetic parameters of propachlor and propanil biodegradation in pure *Pseudomonas* bacterial cultures.**

No.	<i>Pseudomonas</i> bacterial culture	Initial concentration (ppm)	Degradation half-life (hours)	1st order degradation rate constant $\times 10^{-3}$	Bacterial number, N/L (average) $\times 10^{11}$	2nd order degradation rate constant $\times 10^{-15}$
<b>Propachlor</b>						
1.	P.sp. 332, glc, [S1]	10.05	1170	0.593	2.82	2.10
2.	P.sp. 332, glc, [S2]	8.53	1800	0.386	2.78	1.39
3.	P.aer.1C, glc, [S1]	8.09	1530	0.453	2.98	1.52
4.	P.aer.1C, glc, [S2]	8.96	1150	0.605	2.94	2.06
5.	P.sp. 332, SDS, [S1]	12.85	753	0.921	2.93	3.14
6.	P.sp. 332, SDS, [S2]	12.25	1050	0.661	2.93	2.26
7.	P.aer.1C, SDS, [S1]	8.76	1850	0.376	2.35	1.60
8.	P.aer.1C, SDS, [S2]	9.31	1980	0.350	2.38	1.54
<b>Propanil</b>						
9.	P.sp. 332, glc, [S1]	4.17	255	2.715	3.61	7.52
10.	P.sp. 332, glc, [S2]	4.13	632	1.097	4.13	2.66
11.	P.aer.1C, glc, [S1]	4.32	710	0.976	5.62	1.74
12.	P.aer.1C, glc, [S2]	4.25	1080	0.644	4.90	1.31
13.	P.sp. 332, SDS, [S1]	4.21	163	4.253	3.61	11.8
14.	P.sp. 332, SDS, [S2]	3.85	235	2.948	2.88	10.2
15.	P.aer.1C, SDS, [S1]	4.24	165	4.203	4.16	10.1
16.	P.aer.1C, SDS, [S2]	3.84	472	1.470	3.30	4.45

Abbreviations: P.sp. = *Pseudomonas* species, P.aer. = *P. aeruginosa*, [S1] = Sample 1, [S2] = Sample 2

Results of this work indicated a faster primary degradation of propanil as compared to propachlor in pure bacterial cultures. During other research in our laboratory, propachlor degradation occurred, slower than that of propanil, in surface water samples collected from the Neris and Vilnelė Rivers (Unpublished data). Enhanced biodegradation of acetanilide herbicides *Pseudomonas* by cultures grown in the presence of SDS was observed. Data also indicate differences in the membrane toxicity within *Photobacterium* cultures exposed to acetanilide herbicides and their degradation products. Propanil was found to be more toxic to the gram-negative enterobacterium *Photobacterium* as compared to propachlor. Toxicities to propanil based on bioluminescence quenching were more pronounced at the 1-5 ppm level than that observed for propachlor at 10 ppm. Similarly, toxicity to the degradation products 3,4-dichloroaniline and aniline were significantly different.

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# HYDROBIOLOGICAL CONDITION AND WATER QUALITY ESTIMATION OF KURŠIŲ MARIOS LAGOON, 1991

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## ABSTRACT

During 1991, the water pollution level in Kuršių Marios Lagoon, as indicated by the amount of phytoplankton biomass, reached 6 and 7 based on a scale of 9 (most polluted). The biomass quantity in the investigated zones amounted to 10.8-48.8 and 58.0-63.5 mg/L. The most eutrophic part of the lagoon was observed in the areas of Ventės Cape and the mouth of the Nemunas River. The gross primary production and organic matter destruction ratio in the observed region amounted to 9.2-14.2. According to the amount of chlorophyll "a", trophic index, and algotest biomass in certain zones of the lagoon, a hypertrophic level was observed during summer and autumn. Sixty-two taxons of protozooplankton were registered in the lagoon, 29 of which were saprophytes. In certain cases the saprobic index was as high as 3. The bacterioplankton and bacteriobenthos quantity in the lagoon fluctuated from  $0.88 \times 10^6$  to  $8.6 \times 10^6$  cells/ml in the water, and from  $45 \times 10^6$  to  $1600 \times 10^6$  cells/g in the bottom sediments. The mineralization intensity of organic matter in the middle part of the lagoon reached 745 mg dry substance/m<sup>3</sup> water/day. An anaerobic organic matter destruction process, sulfate-reduction, is characteristic of the lagoon. The highest rates of sulfate-reduction ( $6.1 \text{ mg S}^{2-}/\text{dm}^3/\text{day}$ ) were measured at Klaipėda Harbor during the summer. Compared with the data obtained in 1990, higher amounts of pyridoxine and vitamin B<sub>12</sub> were registered in the lagoon water; nicotinic acid, and thiamine, in addition to pyridoxine and vitamin B<sub>12</sub>, were also higher in bottom sediments. The most intensive organic matter assimilation ( $V_{\max}$ ) in the lagoon was observed in July; it amounted to about 20 μg/L per hour. The turn-over time (T) for natural substrate consumption ranged from 7 to 37 hours, which is normal for hypereutrophic water bodies. The main sources of the lagoon contamination are industrial effluents and municipal wastewaters. Therefore, improvement of Kuršių Marios Lagoon water quality is directly related to treatment of these industrial and municipal wastewaters.

## INTRODUCTION

Most of the Republic of Lithuania is located within the Nemunas River basin. The Nemunas River flows into Kuršių Marios Lagoon, which has a major economic and recreative significance to the Republic including its significance as a major fisheries resource. Thus, it is very important to know how and at what rate changes occur in water quality in the Nemunas River basin, and in this connection it is necessary to carry out direct investigations in Kuršių Marios Lagoon.

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Since 1950 comprehensive investigations have been carried out in the lagoon (Anonymous 1959). In 1991 specialists from the Laboratory of Hydrobotany of the Institute of Botany took part in several investigations measuring the following environment variables: macrophyte condition, composition and quantitative structure of phytoplankton and bottom sediments diatoms, photosynthetic pigment and biomass, and B-group vitamins, as well as kinetics of organic matter assimilation, amount of bacterioplankton and bacteriobenthos, and sulfate-reduction.

## MATERIALS AND METHODS

Test samples were collected in northern and central parts of Kuršių Marios Lagoon in 1991 (Figure 1). Water and bottom sediment samples were taken from seven stations: Station 2, Kopgalis; Station 3, at the International Ferry Station; Station 3a, by a cellulose cardboard factory; Station 10, by the town of Nida; Station 11, midway between Nida and Ventės Cape; Station 12, by Ventės Cape; and Station 12a, the outflow of the Atmata River. Samples were taken June 7, July 17, and October 1 and 2.

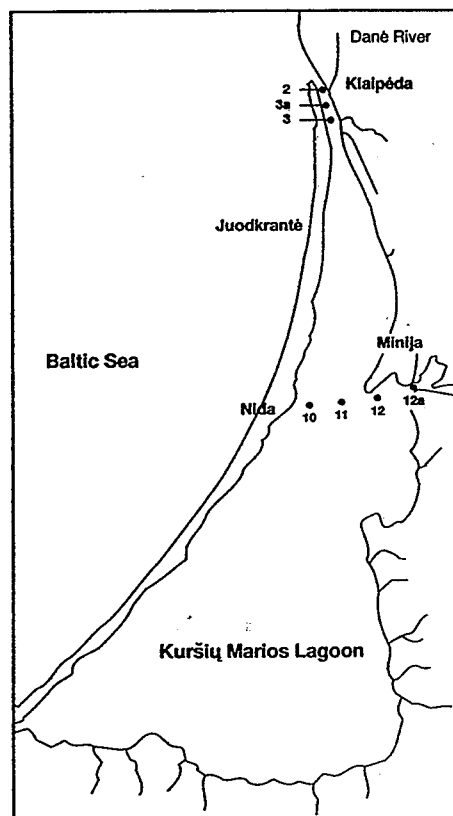


Figure 1. Location of water and sediment sampling stations in the northern and central parts of Kuršių Marios Lagoon.

Macrophytes were sampled and identified according to the methods of Raspopov (1985). Phytoplankton and diatoms were investigated according to ecological, physiological, cytological, and morphological methods of Kiselev (1969), Anonymous (1975a,b), and Davydova (1985, 1987). Water trophic potential was evaluated by means of algotests, SCOR-UNESCO (1966), Toom and Leis (1982), and Bednarz (1984). Primary production and destruction according to Steiman-Nielsen (1952), Vinberg (1960), and Buljon (1983). Meta- and protozooplankton was investigated according to Ćorik (1968), Kiselev (1969), and Mažeikaitė (1969). B group vitamins, as the indicators of the lagoon trophic level, were analyzed according to Odincova (1959), Kuceva (1961), and Žukova and Odincova (1967). Organic matter assimilation kinetics were determined according to Starikova and Koržikova (1972), and Romanenko and Kuznetsov (1974). Bacterial flora, as a water pollution indicator, was investigated according to Razumov (1947), Mačys and Jankevičius (1971), and Rodina (1965). The intensity of the sulfate-reduction process was measured according to Kuznetsov and Dubinina (1989), Sorokin (1982), and Ivanov (1983).

## RESULTS

### Macrophytes

The principal producers of organic matter in Kuršių Marios Lagoon are macrophytes and phytoplankton. Over 20 species of macrophytes are usually found in the northern part of the lagoon and in the Nemunas River delta, dominated by *Phragmites australis*, *Schoenoplectus lacustris*, and *Potamogeton perfoliatus*.

### Microalgae

The greatest variety of microalgae species were determined in June by the Atmata River outflow. Phytoplankton biomass was determined for the large form diatom genera *Thalassiosira*, *Stephanodiscus*, and *Cyclotella*. Their total biomass amounted to 25.9-36.2 mg/L.

In July phytoplankton qualitative and quantitative structure was determined by the blue-green algae *Aphanizomenon flos-aquae* which dominated in Kleipeda Harbor and at Ventės Cape. The biomass of algae in Kleipeda Harbor at Stations 2, 3, and 3a was 1.6, 29.2, and 29.5 mg/L. The highest phytoplankton biomass registered during July, near the Atmata River outflow, was 63.5 mg/L. The biomass at the International Ferry was 59.1 mg/L, and by the cellulose cardboard factory it was 58.0 mg/L.

In October diatoms and blue-green algae dominated in the phytoplankton. The genera *Aphanizomenon*, *Thalassiosira*, *Stephanodiscus*, and *Cyclotella* prevailed. The highest phytoplankton biomass was observed at the Ferry Station, at Nida, and at Ventės Cape. The biomass in these areas fluctuated within the limits 24.5-34.0 mg/L.

The dominating group of saprobity indicators in Kuršių Marios Lagoon were  $\beta$ -mesosaprobites (about 80%) and  $\alpha$ -mesosaprobites (about 13%). Research on water and bottom sediments diatoms was conducted, and 183 species were determined (Table 1). Seventy-three species of water saprobity indicator diatoms were observed. The saprobic index ranged between 1.98 and 3.1 (Table 2). These values were consistently high, indicating a fairly steady degree of pollution at the sites studied in the lagoon.

Table 1. The distribution of diatom algae in plankton in Kuršių Marios Lagoon, 1991.

Sampling stations	Water layer	Diatoms cells density ( $10^6/L$ )		
		06.17	07.17	10.01
2	surface	—	—	3.10
	above the sediments	—	—	2.14
3	surface	—	3.38	3.82
	above the sediments	—	—	5.01
3a	surface	—	2.43	3.42
	above the sediments	—	—	3.51
10	surface	12.45	3.93	—
11	surface	7.43	—	4.71
12	surface	11.04	29.61	43.38
12a	surface	19.15	36.47	12.17
	above the sediments	14.80	24.30	46.04

Table 2. Saprobic indices according to diatom algae data in water samples from Kuršių Marios Lagoon, 1991.

Sampling stations	Water layer	Saprobic index (S)		
		06.17	07.17	10.01
2	surface	—	—	2.24
	above the sediments	—	—	2.1
3	surface	—	2.02	2.4
	above the sediments	—	—	2.05
3a	surface	—	2.44	2.3
	above the sediments	—	—	2.06
10	surface	1.9	2.07	—
11	surface	3.1	—	2.1
12	surface	2.57	2.03	2.62
12a	surface	2.47	2.12	2.13
	above the sediments	2.45	2.03	1.98



## Trophic Index of Water

The amount of chlorophyll "a" in the middle part of the lagoon in June ranged between 31.2 to 117  $\mu\text{g/L}$ . Water trophic index according to amount of chlorophyll "a" was 69.6-88.7. The eutrophication level in the area between Nida and the Atmata River outflow increased in the direction of the Atmata River. However, on the basis of pigment index, carotenoid and chlorophyll ratio, and assimilation number, phytoplankton photosynthetic activity decreased in the direction of the Atmata River outflow.

## Pigments

In July the amount of pigments in Kuršių Marios Lagoon increased, pigment index and day assimilation numbers decreased, while the chlorophyll "a" trophic index was 75.0-95.8. The eutrophication level at the Ferry Station was high; chlorophyll "a" amount made up 192.6  $\mu\text{g/L}$ . In October the amount of pigment in the lagoon water decreased; however, the potential productivity remained high; algotest biomass amounted to 53 mg/L.

Phytoplankton photosynthesis rate in the surface water layer in Klaipėda Strait at Stations 2, 3, and 3a was similar in both June and October, 300-400 mg C/m<sup>3</sup> (Figure 2). In the Nida-Atmata River region this process was the most intensive in June (from 600 to 1200 mg C/m<sup>3</sup> per day). This process decreased to 300-600 mg C/m<sup>3</sup> per day in July, while in October it amounted only to 80-150 mg C/m<sup>3</sup> per day, with the exception of the stations at Ventės Cape and the Atmata River outflow. A relatively lower average day production during the summer season in Klaipėda Strait is related to sea water influence and pollution effect (Šulijienė and Jankevičius 1978).

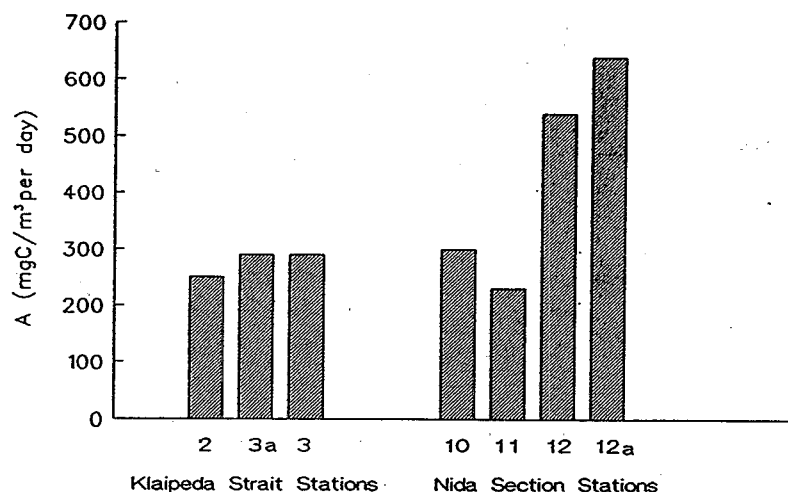


Figure 2. Mean phytoplankton daily primary production (mg C/m<sup>3</sup>/day) in the surface water (0.0-0.5 m) of Kuršių Marios Lagoon, 1991.

Gross primary production ( $A_t$ ) and organic matter destruction ( $R$ ) ratio in Kuršių Marios Lagoon water indicate that organic matter is synthesized by phytoplankton (autochthonous) and is transported from the surrounding environment (allochthonous). The ratio of these two was determined for the surface and trophogenic water layers. On the surface layer phytoplankton photosynthesis is very intensive and the  $A_t/R$  ratio was often higher than 1 (in Klaipėda Strait – 0.8-3.0; in the Ventės Cape and the Atmata River outflow – 9.2-14.2). However, in the whole trophogenic layer the  $A_t/R$  ratio was different; in Klaipėda Strait it was about 0.2, between Nida and Ventės Cape it was 0.6, and at the Atmata River outflow it was 1.4 (Figure 3).

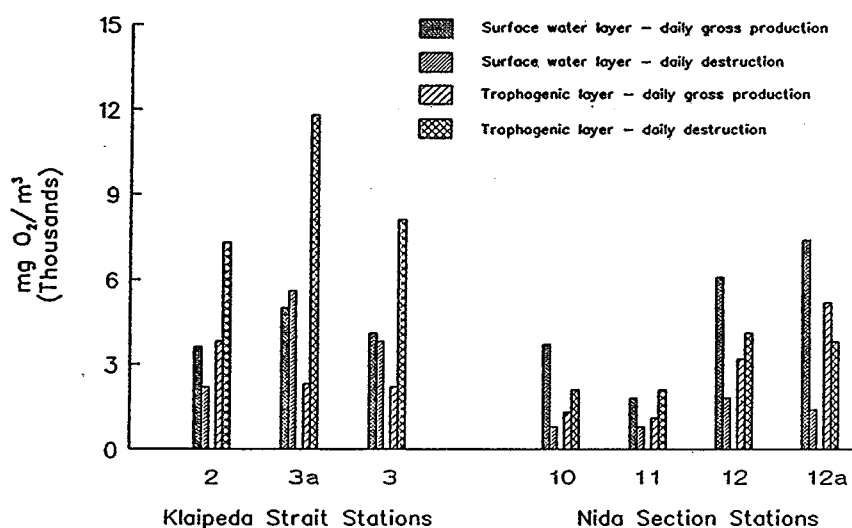


Figure 3. Mean daily gross production and daily destruction of organic matter in the surface water and trophogenic layers of Kuršių Marios Lagoon, Summer 1991.

### Protozooplankton

There were 62 taxons in protozooplankton observed in Kuršių Marios Lagoon, of which 29 were saprobic indicators. *Holotricha* and *Spirotricha* dominated, and small amounts of infusoria subclasses *Peritricha* and *Suctorina* were observed. The data on the total protozooplankton quantity are presented in Table 3. The highest amount of these organisms was observed in the summer in the Atmata River outflow. Thus, water from the Atmata River is very eutrophic. The saprobic index in June in the middle part of the lagoon was 1.80-2.75. In July in the middle part of the lagoon it was 2.4-2.7, and in the northern part it was 2.20-2.67. In October the saprobic index in the middle part of the lagoon was 1.93-3.00 and in the northern part it was 2.56-2.92. A saprobic index over 2 indicates pollution (Table 4).

Table 3. Common quantity of protozooplankton (cell/L) in Kuršių Marios Lagoon, 1991.

Date 1991	Stations						
	2	3a	3	10	11	12	12a
June 7	–	–	–	2640	2620	13918	49600
July 17	5396	3120	5510	2546	–	10880	13981
October 1	211418	64168	48800	2448	3504	22260	17520

Table 4. Saprobic index, according to protozooplankton data, in Kuršių Marios Lagoon, 1991.

Date 1991	Stations						
	2	3a	3	10	11	12	12a
June 7	–	–	–	2.50	2.75	1.82	2.50
July 17	2.21	2.67	2.62	2.41	–	2.56	2.70
October 1	2.56	2.92	2.77	2.80	3.00	2.18	1.93

## Vitamins

Microalgae form certain structures of vitamins which, in turn, determine structural succession of phytoplankton. Our investigations on vitamins in Kuršių Marios Lagoon indicated that diatoms and green algae both synthesize and isolate B-group vitamins into the environment in larger amounts than do blue algae or pyrophytic algae. Larger amounts of pyridoxine and vitamin B<sub>12</sub> were registered in 1990 in the lagoon water, and nicotinic acid, thiamine, as well as pyridoxine and vitamin B<sub>12</sub> were registered in bottom sediments. These B group vitamin amounts and dynamics determine quality and quantitative changes as well as physiological activity of the planktonic organisms (phytoplankton and bacterioplankton).

## Kinetics of Organic Matter Assimilation

The data on organic matter assimilation present information about water self-purification possibilities; this information also enables evaluation of water quality. The assimilation intensity of the microorganisms in the surface water layer was investigated by means of <sup>14</sup>C-labelled protein hydrolyzate. The highest rates of assimilation were

registered in July (Figure 4); average uptake was  $20.2 \mu\text{g/L}$  per hour. In early summer and in autumn the process decreased 1.5-fold. The shortest period (7 hours) was observed in July at the Atmata River outflow, but in autumn this period was lengthened to 35 hours. Such circulation time is characteristic to hypereutrophic water bodies (Seki 1986). In similar media, like Kuršių Marios Lagoon, microorganisms assimilate  $2.7\text{--}23.7 \mu\text{g/L}$  per hour of organic matter due to their active transport system (Antanynienė, Unpublished data).

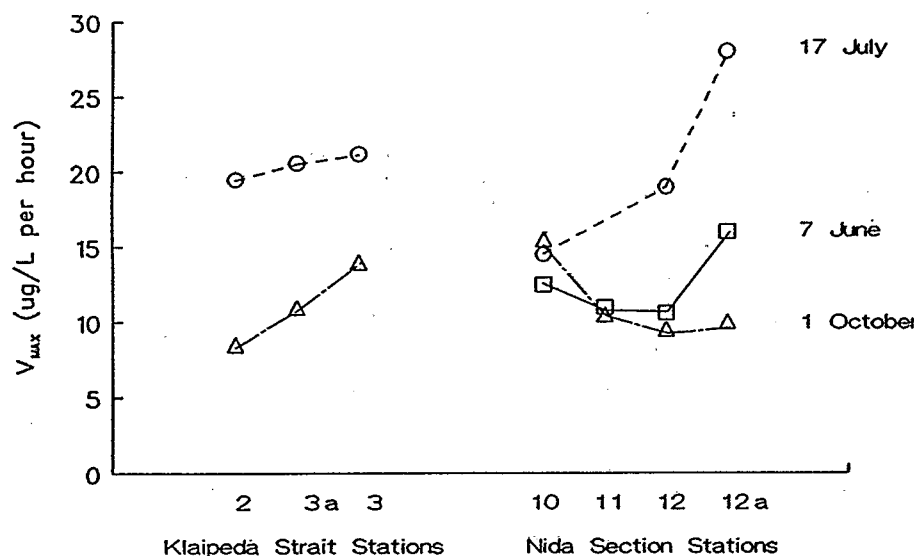


Figure 4. Changes of organic substances assimilation intensity in the water of Kuršių Marios Lagoon, 1991.

### Bacterial Flora

Bacterial flora, participating in the organic matter mineralization processes, is an excellent water pollution indicator. The amount of bacterioplankton and bacteriobenthos fluctuated within wide limits from  $0.8 \times 10^6$  to  $8.6 \times 10^6$  cell/ml in the water and from  $44.8 \times 10^6$  to  $1600 \times 10^6$  cells/g in bottom sediments. The bacteria amount in the bottom sediments exceeded water bacteria as much as 250 times. The spatial distribution of bacteria (their concentration) in Kuršių Marios Lagoon characterizes pollution of certain zones by organic matter from industrial and municipal waste waters. The maximum hydrobacteria concentration was determined in the northern part of the lagoon. Data obtained concerning protein mineralizing bacteria ( $21 \times 10^3$  cells/ml), oil oxidizing bacteria ( $17 \times 10^3$  cells/ml), and yeast ( $8.8 \times 10^3$  cells/ml) were all similar. Intensity of organic matter mineralization processes in the middle area of the lagoon in the summer amounted to  $745 \text{ mg dry material/m}^3$  per day. This process is intensive, however, it is theoretically insufficient to mineralize organic pollutants in water.

## Sulfate Reduction

Sulfate reduction indicates a high concentration of organic matter and its degradation under anaerobic conditions. The most intensive sulfate-reduction process was determined during the summer in the northern part of the lagoon in the region of the International Ferry,  $6.1 \text{ mg S}^{2-}/\text{dm}^3$  per day. The highest concentrations of hydrogen sulfide and acid soluble sulfides were measured at the International Ferry and cardboard factory stations. In the bottom sediments at these stations the concentration of sulfides amount to 760 and  $532 \text{ mg}/\text{dm}^3$ , accordingly. Analysis of the sulfate-reduction data indicate that water pollution by industrial and municipal organic wastes determine the intensive sulfate-reduction process and accumulation of hydrogen sulfide and other sulfides in the bottom sediments of the polluted zone. Because of the resultant high concentrations of sulfides, considerable damage is caused to the benthic fauna.

## RECOMMENDATIONS

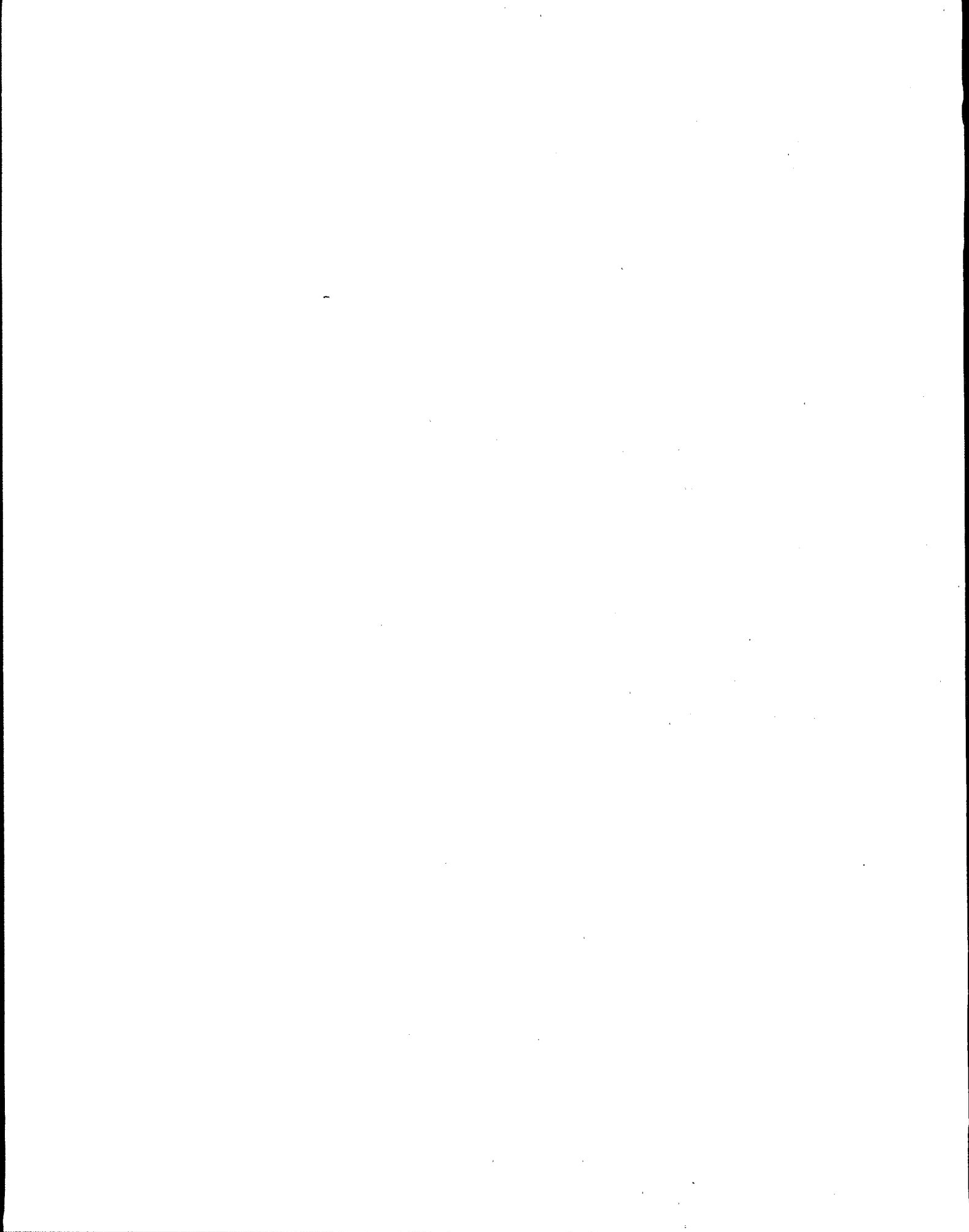
Kuršių Marios Lagoon water protection measures have a direct reference to industrial and municipal wastewater treatment in Klaipėda. Construction of wastewater treatment facilities, improvement of production technologies, and organization of circulating water treatment systems must be carried out without delay. In order to decrease pollution in the Nemunas River system, constant monitoring of surface water quality is needed.

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# CYTOGENETIC CHANGES IN FRESHWATER BIVALVE MOLLUSCS FROM THE NEMUNAS RIVER AND KURŠIŲ MARIOS LAGOON

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## ABSTRACT

A study has been conducted on cytogenetic disturbances of eight species of bivalve molluscs collected from the estuaries of the Šventoji River, the Kuršių Marios Lagoon near Dreverna and by Ventė Cape, and the Nemunas River. The data show that the highest level of changes in chromosome sets was present in biotopes from the Šventoji River estuaries and the Nemunas River. Forty percent of the clams examined from the estuaries and 50% of those from the Nemunas River had such a high level of chromosome set disturbances that they were sterile. There was a strong genome polyploidization trend (42% of bivalves studied) in the Nemunas River population. In the tissues of polyploid specimens, there was a dangerously high number of transformed cells. A significant instability of chromosome sets in snails from Kuršių Marios Lagoon by Ventė Cape was also detected. We presume an ecologo-genetical risk threshold has been crossed when there are cytogenetical alterations in more than 50% of the cells of molluscs. Existence of polyploid, mosaic, and hermaphroditic specimens of clams, as well as presence of high numbers of transformed cells and mitotic suppression in their tissues, suggest the existence of ecologically dangerous zones in the "Nemunas River – Kuršių Marios Lagoon – Baltic Sea" hydrosystem.

## INTRODUCTION

Anthropogenic pollutants have contaminated many ecosystems in Lithuania, and for this reason it is important to evaluate the state of the gene pool balance in the biocenoses, the tendencies for and mechanisms of biological changes, and the consequences of these for reproduction. It has been shown that effects of pollutants are usually displayed first at the biochemical, molecular, or genetic level (Veldhuizen-Tsoerkan *et al.* 1991). Ecologists must find a suitable species for estimating ecological risk levels in aquatic systems. Molluscs, especially bivalves, may be useful as a biomarker for regional risk assessment. Different species of *Anadonta*, *Unio*, and other genera of Unionidae are very suitable for studies of bioaccumulation, biodegradation, and other effects of pollutants because of their distribution, size, sedentary life, and filtration activity. They are also suitable for studies of relationships between aneuploidy, growth rate, reproductive peculiarities, and cytogenetic effects of biologically active pollutants. In the present study, freshwater clams were collected from highly polluted

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areas of the Nemunas River, Kuršių Marios Lagoon, and estuaries of the Šventoji River. The specimens collected were examined for cytogenetic damage, for bioaccumulation of pollutants, and, in some cases, for biodegradation of xenobiotics.

## MATERIALS AND METHODS

Karyological analysis was performed on specimens of eight species of clams, *Crassiana crassa*, *Unio tumidus*, *U. pictorum*, *U. longirostris*, *Anadonta cygnea*, *A. piscinalis*, *A. subcircularis* and *Pseudoanadonta anatina*, collected during the summer months of 1989-1991 (Table 1). Tissues were obtained according to the methods of Baršienė and Grabda-Kazubská (1988). Preparations were produced from cellular suspensions, stained with 4% Giemsa solution prepared in phosphate buffer (pH 6.8), and analyzed by means of a Jena Med<sup>TM</sup> cytology microscope.

Table 1. Numbers and collection location of mollusc specimens studied.

Species	Sampling sites			
	Estuaries of Šventoji River	Kuršių Marios Lagoon by Dreverna	Kuršių Marios Lagoon by Ventė Cape	Nemunas River above Smalininkai
<i>Crassiana crassa</i>	14			
<i>Unio tumidus</i>	5	3	10	2
<i>U. pictorum</i>			6	13
<i>U. longirostris</i>				4
<i>Anadonta cygnea</i>	17	14		
<i>A. piscinalis</i>	6		28	2
<i>A. subcircularis</i>	3			
<i>Pseudoanadonta anatina</i>	2			

## RESULTS

Analysis revealed a diploid number of 38 chromosomes in somatic cells (Figure 1), although some individuals possessed other modal chromosome sets, i.e.,  $2n=32, 36$ , or  $37$ ;  $3n=57$ ;  $4n=76$  (Figure 2). A high frequency of chromosome set abnormalities was detected in tissues of specimens collected from the Šventoji River estuary. Only three of 23 individuals studied had modal chromosome numbers, which is well outside the normal range for molluscs from clean water; normally 80-85% have the modal chromosome number. In half of the clams studied, only 51-84% of the cells were

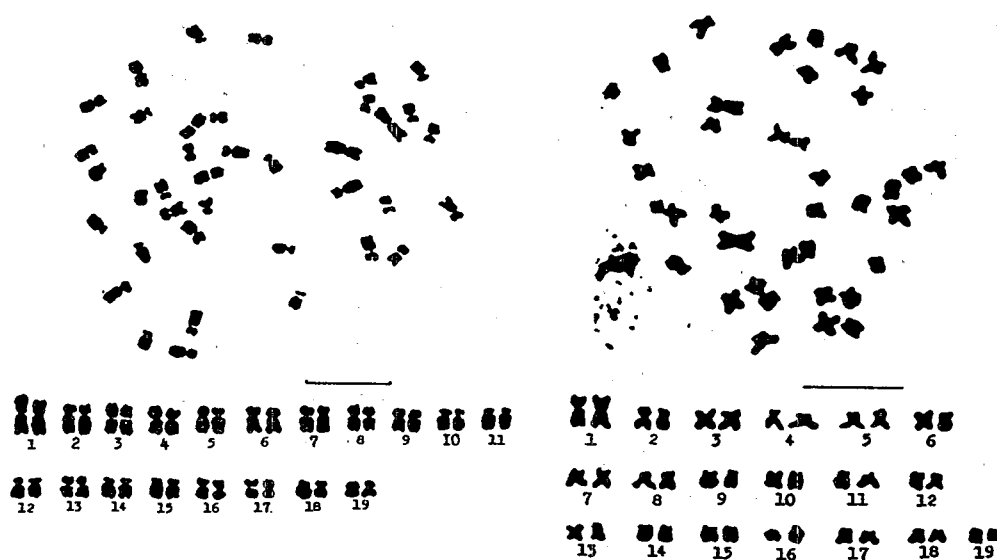


Figure 1. Metaphase chromosomes and normal diploid karyotypes of:  
A-621 *Anadonta cygnea*, B-444 *Unio tumidus* (scale 10 $\mu$ m).



Figure 2. Aneuploid chromosome set of 622 *Anadonta cygnea* (scale 10  $\mu$ m).

normal, while in one-third of the clams as few as 7-50% of the cells were normal (Table 2). Thirty-two chromosomes predominated in the nuclei of specimen #673 *Anadonta cygnea*, 36 chromosomes in #521 *C. crassa*, 37 chromosomes in #686 *A. cygnea* and #683 *A. subcircularis*, and tetraploid sets occurred in #685 *A. cygnea*. Most of polyploid nuclei of #685 *A. cygnea* have been associated with the presence of visible tumors; this included fragmented nuclei and nuclei with highly irregular shapes.

Table 2. Percentage of cells according to number of chromosomes of molluscs collected from the Šventoji River estuary.

Specimen number and species	Number of chromosomes											
	32	33	34	35	36	37	38	39	54	74	76	~150
507 <i>Crassiana crassa</i>					14.3	14.3	71.4					
509 "		5.0			4.8	5.2	85.0					
511 "			4.2		20.8	4.2	66.6		4.2			
518 "					16.6	16.6	66.8					
521 "					75.0		25.0					
670 <i>Pseudoanadont</i>			5.3		6.6	4.0	84.2					
671 <i>Anadonta piscinalis</i>			2.2		10.9	10.9	73.8			2.2		
675 "					16.2	3.8	80.0					
678 "		4.4		37.5	4.2	12.3	41.6					
695 "					14.8	3.8	65.6	7.9		7.9		
672 <i>A. cygnea</i>				7.7	11.5	11.5	69.2					
673 "	60.0					9.8	30.2					
674 "			4.3			16.7	72.7	6.3				
676 "			11.9		7.1	4.8	76.2					
677 "		7.3	7.3	7.3	19.5	14.6	41.6	2.4				
684 "	25.0	12.5			12.5		50.0					
785 "						6.6	13.3			18.9	50.4	10.8
686 "		6.2	13.4			67.0	6.7					6.7
687 "				5.8	12.2	16.3	65.7					
689 "			2.4		18.2	8.8	61.8	8.8				
679 <i>A. subcircularis</i>		8.7	4.3	8.7	15.0	8.7	52.3	4.3				
680 "							66.7				33.3	
683 "				10.0	10.0	60.2	19.8					

During the summer of 1990, a large *A. cygnea* population was found in Kuršių Marios Lagoon by Dreverna. Disturbances in chromosomes were detected in 30-50% of cells (Table 3). By the summer of 1991 there was only a great number of empty shells at this same location, and surviving clams had no mitosis.

Table 3. Percentage of cells according to number of chromosomes of molluscs collected from Kuršių Marios Lagoon near Dreverna.

Specimen number and species		Number of chromosomes								
		<35	35	36	37	38	39-48	54-57	60-76	90-150
621	<i>Anadonta cygnea</i>	6.6	3.2	3.2	14.0	72.0	1.0			
622	"	4.5	3.3	6.8	50.0	33.6	1.6			
623	"	6.8	4.5	2.3	2.3	72.7	11.4			
624	"	9.5	15.9	6.3	9.5	50.8	8.0			
626	"	2.6	2.6	7.9	7.9	73.8	2.6	2.6		
628	"	2.7	3.5	9.8	8.0	72.4	1.8		1.8	
629	"	3.3		9.8	5.4	71.6	5.4		3.3	1.1
630	"	4.5	4.5	17.6	3.2	64.2	3.6		1.0	1.4
631	"	3.1	9.4	15.6	12.5	56.3	3.1			
632	"	6.8	6.8	13.7	9.1	52.3	9.0			2.3
633	"		4.5	4.5		82.0	9.0			
704	"	14.3		28.6		57.1				
536	<i>Unio tumidus</i>			20.0		80.0				

A similar situation was noted in tissues of clams which inhabited the Kuršių Marios Lagoon biotope by Ventė Cape. Only 20% of individuals examined had 38 chromosomes in 80-91% of nuclei. However, 25% of snails had modal amount of chromosomes in only 25-40% of cells. We noticed chromosome sets changes in 20-40% of cells in the tissues of all remaining molluscs. Some of the bivalves from Ventė Cape were mosaic, for example, specimens #442 and #524 *U. tumidus*, #690 and #694 *A. piscinalis* (Table 4). Also, there were two hermaphroditic clams studied, #442 *U. tumidus* and #699 *A. piscinalis*. The normal diploid number of chromosomes of #442 *U. tumidus* was present in only 35.1% of the cells examined, while only 25% of the nuclei of #699 *A. piscinalis* were normal.

Table 4. Percentage of cells according to numbers of chromosomes of molluscs collected from Kuršių Marios Lagoon by Ventė Cape.

Specimen number, sex, and species		Number of chromosomes								
		<35	35	36	37	38	39-48	54-57	60-76	90-150
441 F	<i>Unio tumidus</i>		2.9	11.4	11.4	68.6	5.8			
442 FM	"	5.4	5.4	5.4		35.1	5.4		32.4	10.9
443 F	"	23.6		5.9	17.6	52.9				
444 M	"	10.1	8.8	22.5	7.4	37.4	1.3		12.5	
445 F	"	10.7	7.1	3.6	3.6	67.8	3.6	3.6		
523 F	"		4.0		16.0	64.0	16.0			
524 M	"			33.3		66.7				
525 F	"			8.3	8.3	83.3				
528 F	<i>U. pictorum</i>	7.1	7.1	14.3		71.5				
620 M	<i>Anadonta piscinalis</i>		5.0	5.0	5.0	65.0	5.0		15.0	
688 F	"	10.0				60.0				30.0
689 F	"			14.3		85.7				
690 M	"	27.3		18.2		36.4	18.2			
691 M	"			9.1		90.9				
693 M	"	10.7	3.6	10.7	3.6	67.9	3.6			
694 F	"	6.0		47.0		47.0				
695 M	"	3.0		12.1	6.0	78.8				
696 M	"	25.0		16.7		50.0			8.3	
698 F	"			4.2	8.3	83.3	2.1		2.1	
699 FM	"	25.0		50.0		25.0				

Different tendencies of disturbances of chromosome sets in tissues of bivalves inhabiting the Nemunas River above Smalininkai were determined. Five out of 12 individuals examined were polyploid. There were also carcinous cells in their tissues. We counted only 32 chromosomes in 57% of the cells of #544 *U. pictorum* (Table 5). Consequently, 50% of the bivalves studied had strong genetic changes. We noticed significant meiotic anomalies in the formation process of sexual cells. It would appear that they frequently were unable to take part in reproduction processes and are being eliminated from biocenoses.

Table 5. Percentage of cells according to number of chromosomes of molluscs collected from the Nemunas River above Smalininkai.

Specimen number and species		Number of chromosomes							
		<35	36	37	38	39-48	51-57	60-80	90-200
438	<i>Unio tumidus</i>		12.5	6.2	62.5			18.8	
439	<i>U. longirostris</i>		13.6	4.5	77.4	4.5			
440	"	3.3			13.3	6.7	76.7		
437	"					17.8		82.2	
533	"	6.4	10.6		23.5	4.3	6.4	19.0	29.8
537	"	5.7	7.5	1.9	43.4	5.7	3.8	10.6	21.4
541	"	20.0			80.0				
544	"	57.1	28.6		14.3				
545	"	25.0		25.0	50.0				
547	"		20.0		80.0				
548	"	7.7	15.4		15.4	15.4		30.8	15.4
549	"	10.0	12.5	2.5	17.5	2.5		30.0	25.0

We found normal karyotypes in only 25% of the individuals among molluscs of Smalininkai biotopes. Bivalves that inhabited the Nemunas River by Vilkija (below Kaunas) and the Nėris River by Lazdėnai (below Vilnius) had no mitosis.

## DISCUSSION

Chemical and physical biologically active agents can produce genetic alterations at subtoxic concentrations (Landolt and Kocan 1984). It is possible for heavy metals to damage DNA (Choi and Simpkins 1986). Walton *et al.* (1988) were able to determinate that PAHs caused a considerable number of different chromosomal aberrations in fish tissues. Exposure of killifish (*Fundulus heteroclitus*) embryos to methylmercury decreased mitotic counts 1.5-5-fold, increased chromosomal aberrations 2-2.5-fold, and retarded development of embryos (Perry *et al.* 1988). Mitotic inhibition was marked in our karyological studies of molluscs. This phenomenon was significant in the areas of highest pollution, namely the Nėris River below Vilnius, the Nemunas River below Kaunas and Tilžė (Sovietskai), and Kuršių Marios Lagoon near Klaipėda (Baršienė 1991). We did not observe any mitotic activity in tissues of 75-95% of the specimens we examined. In 25-40% of specimens examined, polyploid interphase nuclei predominated. It was presumed that appearance of polyploid cells was a result of pollutant effects on microtubules and mitotic spindle fibers of nuclei of clams.

The greatest abundance of molluscs with damaged chromosome sets was found in the estuary of the Šventoji River and in the Nemunas River just above Smalininkai. One of the most significant findings from our study is the correlation between occurrence of polyploidy in clams and of cancerous cells. There was a notable predominance of cancerous cells in specimen #685 *A. cygnea*, and occasionally these cells were found in polyploid clams from the Nemunas River above Smalininkai. Polyploid hepatopancreas cells had indications of typical tumor cells. In addition, in tissues of most specimens studied there were remarkable numbers of aneuploid cells. Earlier it was stated that chromosomal lesion in mussels might lead to the DNA replication errors and appearance of polyploid cells (Elston *et al.* 1990). Consequently, chromosome lesions, genome polyploidization, and carcinogenesis in molluscs might be in close relationships. It would be interesting to study preneoplastic or neoplastic processes of aneuploid bivalves specimens.

There is sufficient evidence that chromosomal damages can be the initiating event in carcinogenesis (Yunis 1981). The progressive nature of hemic neoplasia in *Mytilus edulis* was connected with remarkable increases of polyploid cell population and decreases of diploid cells. In the terminal stage of hemic neoplasia, the neoplastic cells predominate and fragmentation of giant nuclei (about 10n) is common (Elston *et al.* 1990). In the case of sarcomatoid proliferative disease of *M. edulis*, aneuploidy as well as polyploidy in atypical cell populations has been described (Farley 1969).

The majority of autosomal aneuploids result in a lethal event or in reduction of viability (Bond and Chandler 1983). Nevertheless, some of the bivalve molluscs that we studied possessed one to six chromosome lesions and survived in the most polluted waters. The karyotypes of about 20% of Unionidae clams from the cooling basin of the Lithuanian Hydroelectric Power Station (HEPS) on the shores of the Kaunas Reservoir consisted of 32, 36, or 37 chromosomes (Baršienė and Petkevičiūtė 1988). All these hypoploid specimens were found in the cold basins zone near the estuaries of the Strėva River which flows into the reservoir. These were the most polluted areas of the basin because of atmospheric input from the HEPS and input from the Strėva River. A similar pattern of aneuploidy in clams was found in the Šventoji River estuary. These anomalies have influence to some degree on the growth rate and viability of mussels (Thiriot-Quievreux *et al.* 1988).

There is considerable evidence that high quantities of polyploid-aneuploid cells in mollusc tissues, as well as polyploid, aneuploid, mosaic or hermaphroditic specimens of molluscs exist in ecologically dangerous zones. In our opinion, 12-25% of bivalves specimens possessing normal diploid non-altered karyotypes in more than 85% of cells could not maintain a normal population through auto-reproduction. We believe the clam populations we studied have passed beyond an ecologo-genetic threshold, especially those from the Šventoji River estuaries and the Smalininkai biotopes.



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# MORPHOLOGICAL CHANGES IN BIVALVE MOLLUSCS UNDER DIFFERENT LEVELS OF HEAVY METAL BIOACCUMULATION

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## INTRODUCTION

Many water bodies in Lithuania are currently in a critical state because their ecological balance has been destroyed and intense industrial activity has impaired their potential for natural biological self-cleaning. Because they are exclusive filterers, molluscs play an extremely important role in the process of self-cleaning and self-regulation of natural communities. They are widely used as biological indicators in studies of ecological effects of pollutants and for estimation of the environment, in particular hydroecosystems. The filtering and bioaccumulative capabilities of individual molluscs depends upon their general morphophysiological state, such as sex, age, size, and growth rate, as well as on their environment, including hydrochemical and geochemical conditions (Phillips 1988).

The largest river in Lithuania is the Nemunas, which flows into south-central Lithuania from Belarus. Its watershed covers 70% of the territory of the republic, thereby accumulating most of its water outflow with its dissolved components. Except for some natural water quality recovery taking place along tributaries before they flow into the Nemunas River, this river reflects the cumulative pollution level of much of Lithuania as well as some parts of Belarus. The Neris River is the second largest river in Lithuania; it flows through Vilnius, which is also a significant source of pollution because it is the country's largest city. Over 100 km downstream from Vilnius the Neris River joins the Nemunas River at Kaunas, the second largest city in Lithuania, and one that does not have a water purification system. The Nemunas flows farther westward, collecting on its way the water from most of central Lithuania brought by a few other major tributaries, and finally reaches Kuršių Marios Lagoon.

Kuršių Marios Lagoon is triangular-shaped and has only a narrow channel at the northern end connecting it with the Baltic Sea. The lagoon has water of low salinity brought by the rivers flowing into it, although some of the higher salinity water at the Baltic Sea flows into the lagoon through the narrows at Kleipėda Harbor. The ratio of flow from Kuršių Marios Lagoon into the sea is approximately three to one. Because of the relatively long residence time of water in the lagoon, it serves as a cumulative reservoir for waste products, not only from Lithuania, but also from Belarus and, especially, from the Kaliningrad region of Russia. Thus, a pollution gradient occurs along the Neris-Nemunas-Kuršių Marios Lagoon aquatic system, and our sampling stations were distributed along this gradient.

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## MATERIALS AND METHODS

Mollusc samples for evaluating the extent of pollution were taken at the following stations: the Cape of Ventès and Dreverna (Kuršių Marios Lagoon), Smalininkai and Vilkija (Nemunas River), and Verkiai (Neris River). The Verkiai station might be taken for a control; it is located slightly upstream from Vilnius and because it is devoid of major pollution sources, water from the Verkiai station can be considered comparatively clean when compared to the other stations.

The following species of molluscs were selected for our investigation, and collected in the littoral zone at each station: *Unio pictorum*, *U. tumidus*, and *Anadonta piscinalis*. The parameters measured were: total weight; the weight, length, width and height of shell; and the weight of branchiae and liver. Modifications and deformations of the shell or soft parts of the body, damages to the nacreous layer, its granular structure, spot patterns and color changes were all evaluated. Attention was also given to the age and sex structure of the populations and to the state and abundance of individuals. Spectral analysis was applied to measure the quantities of heavy metals, and this method has been described in Lazauskienė *et al.* (1991a). Data of the investigation of benthic communities carried out in 1951-1990 (*Ibid*) were used for comparison.

## RESULTS AND DISCUSSION

Molluscs have played an important role in the benthic communities of Kuršių Marios Lagoon. Some 10-15 years ago 22 species of Mollusca were found in the Cape of Ventès area, and their total biomass reached as much as 5 kg/m<sup>2</sup> (Gasiūnas 1952, 1972). At present, communities of molluscs and other aquatic organisms have become impoverished, and their species diversity and biomass has decreased four- or five-fold since the early 1950s (Table 1). Peleophylic (mud-affiliated) communities with their

Table 1. Dynamics of the yearly averages of benthic biomass in Kuršių Marios Lagoon.

Year	Average of benthic biomass, (g/m <sup>2</sup> )	Percent molluscs
1951	27.1	80
1954	16.6	80
1955	18.1	80
1957	16.2	80
1973	15.1	55
1989-90	6.5	20

Source: Lazauskienė *et al.* 1991a

characteristic complex of aquatic species have increased their habitat area by 20%. The occurrence of so-called "dead zones", where no aquatic life is possible, has also been noticed because of increases in water and bottom sediment pollution in Kuršių Marios Lagoon. Some 150 various chemical compounds have already been identified in Kuršių Marios Lagoon (Anonymous 1992). Lazauskienė *et al.* (1991a) reported that sediment concentrations of heavy metals such as manganese, titanium, vanadium, chromium, and cobalt exceeded those found in the macroscopic zoobenthos, but the concentrations of cadmium, nickel, copper, lead, and mercury had an opposite distribution pattern, being in the higher aquatic organisms, including Mollusca.

Mercury concentrations in biotic and abiotic components of the northern Kuršių Marios Lagoon ecosystem have also been reported (Table 2).

Table 2. Mercury content in various objects from the northern Kuršių Marios Lagoon ecosystem at Dreverna.

Objects	Mercury content (mg/kg)	
	Mean	Range
Water	0.25	0.01-0.68
Bottom sediments	0.10	0.01-0.27
Oligochaeta	0.13	0.04-0.41
Polychaeta	0.08	0.08-0.19
Chironomidae	0.08	0.02-0.20
Mollusca	0.11	0.01-0.27
Gammaridae	0.90	0.02-1.80
Mysidae	0.70	0.09-0.91

Source: Lazauskienė *et al.* 1991a

Mercury has a high toxicity level, easily accumulates, and is highly persistent in organisms. Direct interdependence between the mercury levels in the ground, water, and macroscopic zoobenthos was established. The highest mercury levels were found in Gammaridae, Mysidae, and Mollusca. Accumulation of chemical compounds containing heavy metals in molluscs, regardless of species, is much more intense in the soft parts of the body than in the shell (Table 3).

Table 3. Accumulation of chemical elements in the soft tissues and shell of molluscs collected at Dreverna. Averages in mg/kg of ash for each fraction are presented.

Analyzed portion	Chemical elements										
	Mn	Ti	V	Cr	Co	Ni	Cu	Pb	Ag	Sr	Ba
Soft tissues	2400	1000	36	12	6.5	8.4	80	18	1	380	120
Shell	600	100	3	5	2	20	20	5	0.1	330	30

Source: Lazauskienė *et al.* 1991b

Chronic exposure to pollutants kills aquatic organisms gradually, impairing their normal biological functions. Heavy metals enter the tissues of organisms as macroscopic, microscopic, and ultramicroscopic compounds or in simple elemental form, and thereby accumulate in the environment. Organisms can acquire resistance to a certain extent and adjust themselves to the modified environment, due to the processes of detoxication and adaptation. Yet the adaptation mechanisms have their limits, and when these limits have been reached various changes occur. Ecological crisis of an organism starts when morphophysiological and biochemical declinations from the normal state can be detected. The weight and size parameters of molluscs of the Unionidae family in the Nemunas River and Kuršių Marios Lagoon indicate that growing conditions are unfavorable (Figures 1-3).

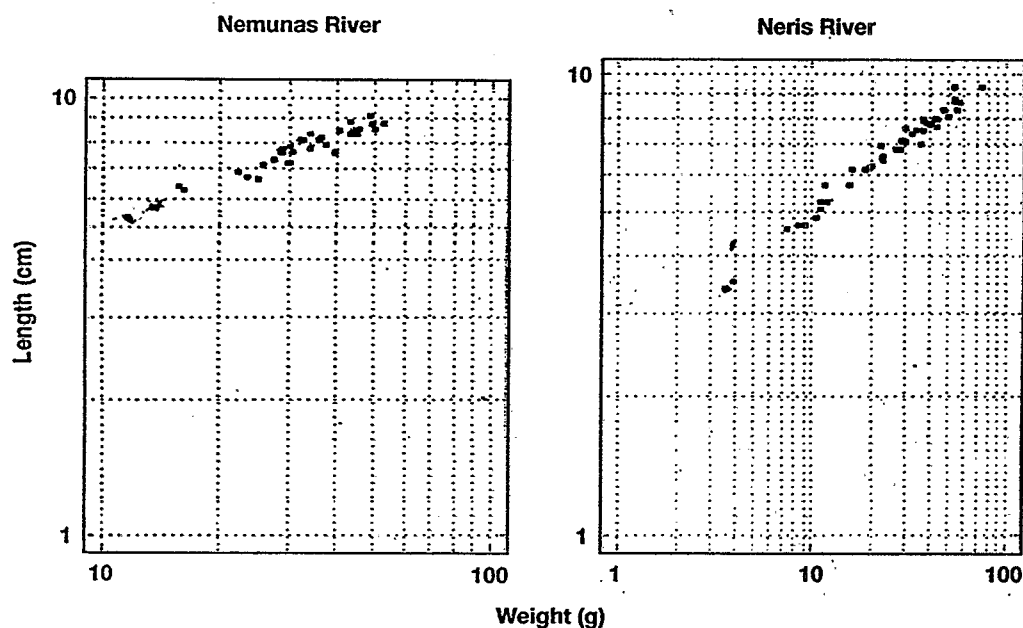


Figure 1. Comparison of weight and length of *Unio pictorum* sampled in the Nemunas River (at Smalininkai) and Neris River (Control, at Verkiai).

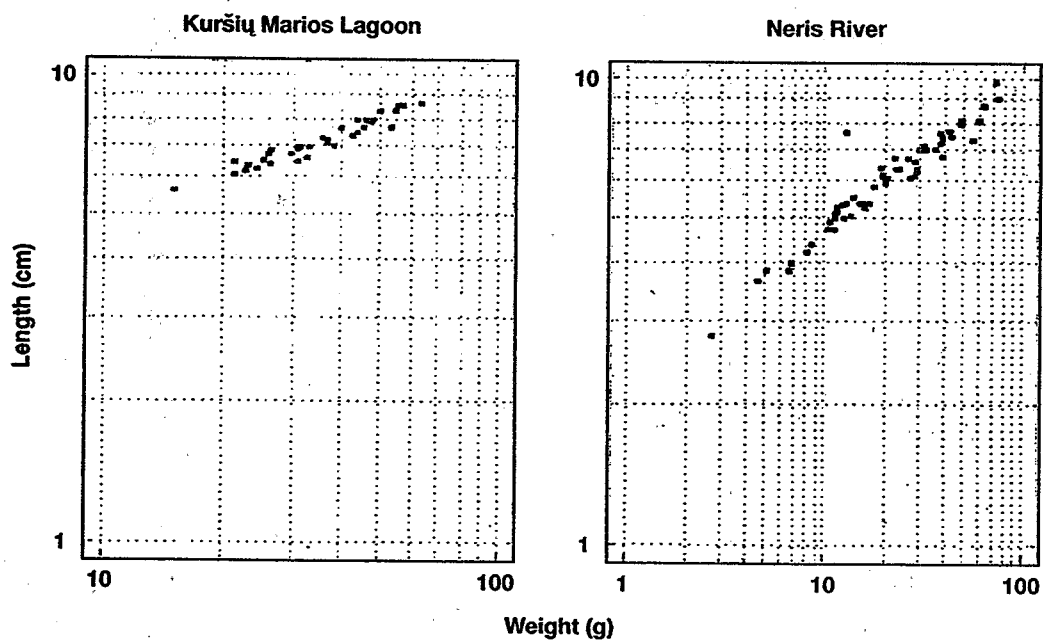


Figure 2. Comparison of weight and length of *Unio timidus* sampled in Kuršių Marios Lagoon (at Cape of Ventès) and in the Neris River (Control, at Verkiai).

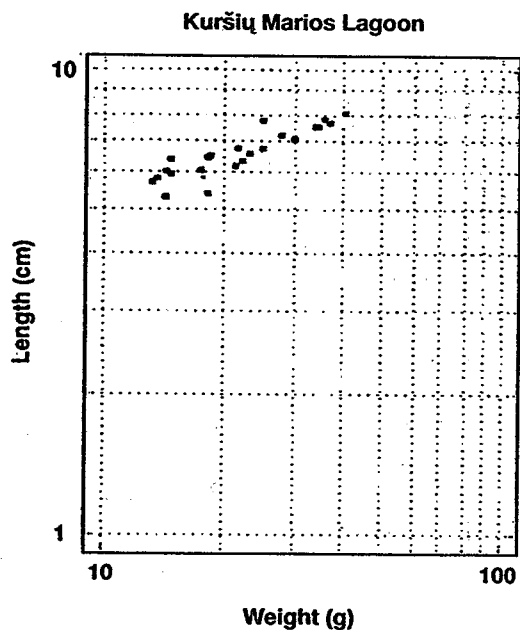


Figure 3. Comparison of weight and length of *Anadonta piscinalis* sampled in Kuršių Marios Lagoon (at Cape of Ventès).

Three species of molluscs were distinguished according to their age structure, with individuals of the age of 3 and 4 years being the most numerous. There was a distinct difference in numbers between males and females; the proportion of females at the Cape of Ventès area was almost twice as great as that of males. Damage of various kinds was found in 94% of molluscs sampled at the Cape of Ventès (Table 4). The damage consisted of obvious shell deformations, many swellings, and spots on the nacreous layer of shell, and the liver of some individuals was considerably enlarged.

Table 4. Morphophysiological changes in shell and soft tissues of two *Unio* species.

Sampling location	Species	Percent damage
Smalininkai (Nemunas River)	<i>U. pictorum</i>	40
Verkiai (Neris River)	"	54
Verkiai (Neris River)	<i>U. tumidus</i>	41
Cape of Ventès (Kuršių Marios Lagoon)	"	94

Mollusc communities in the Nemunas and Neris Rivers in 1951-1980 were previously more dense and diverse; as many as 32 species of molluscs were once reported, with total biomass up to 8 kg/m<sup>2</sup> (Gasiūnas 1972). At present, the communities are affected by increased water and sediment pollution, and total biomass of molluscs has decreased to 0.2 kg/m<sup>2</sup> (Lazauskienė *et al.* 1991a). Many empty shells of dead molluscs have been found in the littoral zone. Currently the communities are predominated by *U. pictorum*. The male/female ratio was 1:1.4. Even more sharp distinction of senior age groups (5-6 years old) has been detected in length class analysis of the molluscs from the Nemunas River, compared to populations in Kuršių Marios Lagoon. Defects of shells and the nacreous layer have been found in all the age groups of molluscs from the Nemunas River, yet they were less severe than those found in molluscs from Kuršių Marios Lagoon. Defective individuals comprised 40% of the total in the Nemunas River mollusca communities at Vilkija and Smalininkai, which were located closer to the sewer water discharge from Kaunas. The Neris River, generally less polluted than the Nemunas River (Lazauskienė and Kirlys 1991), had higher species diversity and productivity of bivalves. Both young and mature individuals were found at the stations sampled, and their sex ratio was closer to 1:1 (Table 5). Defects of the molluscs of this area consisted mostly of shell deformations and there was less swelling in their nacreous layers.



Table 5. Sex composition of the bivalve populations of two *Unio* species.

Sampling location	Species	Percent Males	Percent Females
Cape of Ventės (Kuršių Marios Lagoon)	<i>U. tumidus</i>	35	65
Verkiai (Neris River)	"	46.5	53.5
Verkiai (Neris River)	<i>U. pictorum</i>	50	50
Smalininkai (Nemunas River)	"	40.6	59.4
Vilkija (Nemunas River)	"	44.4	55.6

### SUMMARY AND RECOMMENDATION

The results of the studies of bivalve communities in the Nemunas and the Neris Rivers indicate that productivity and species diversity of representatives of the family Unionidae have decreased significantly in the last decade, indicating severe environmental degradation. Increased pollution of these hydroecosystems, especially the accumulation of heavy metals, is having a negative impact on the population structure of the molluscs. Accumulation of heavy metals damages the vital functions of molluscs, including morphometric changes and shifts in sex and age structure of their populations. Based on these data, and the fact that young individuals are almost absent, we predict that the Kuršių Marios Lagoon population of Unionidae is doomed to extinction. This is confirmed by cytogenetic changes in these molluscs (Baršienė 1991). Analysis of the changes in sex structure of the populations suggests that females are more resistant to pollution than males, and may possess some additional mechanisms for ecological adaptation and compensation. To generalize, it can be predicted that the ecological situation in the Nemunas River–Kuršių Marios Lagoon–Baltic Sea aquatic ecosystem will get worse. To control this situation, we suggest introduction of conservation measures such as artificial reefs and biofilters (Lazauskienė and Kirllys 1991). Introduction of the artificial biotopes and new habitat substrata will enable increased filtering capacity of bivalves, and thereby soften the negative ecological effects of pollutants.

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# CARCINOGENESIS RESEARCH ON FISHES OF KURŠIŲ MARIOS LAGOON, 1991

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## ABSTRACT

Between May and October 1991 fishes were collected for tissue analysis from the Nemunas River near Vilkija, the Nemunas River delta, and Kuršių Marios Lagoon. We found changes in the microstructure of the liver of roach and bream, and we also found tumors in a crucian carp. We attribute these tissue alterations to environmental pollution by industrial effluents.

## INTRODUCTION

Contamination of water that disturbs the stable environment of hydrobionts can cause preneoplastic and neoplastic changes. Such changes occur especially in those water basins that receive industrial effluents (Sorensen 1980, Bogovskij and Kudolej 1987). In Lithuania such water basins are the Nemunas River and Kuršių Marios Lagoon. In this paper we will report additional research on a study started in 1990 on preneoplastic and neoplastic changes in fishes.

## MATERIALS AND METHODS

Fishes for this study were caught from May to October 1991 in the Nemunas River near Vilkija, the Nemunas River delta, and Kuršių Marios Lagoon near Ventė Cape. Histologic preparations of the livers of 60 breams (*Abramis brama*) and 50 roaches (*Rutilus rutilus*) were prepared for the study. Two breams, in which visual preneoplastic changes of the bowels could be observed, were used for the scientific research. The breams and one crucian carp (*Carassius auratus*) were caught in Kuršių Marios Lagoon. Over 3,000 breams were caught, of which 111 were used for this study.

Liver tissues for histologic study were prepared as follows: pieces approximately 2-3 cm<sup>3</sup> were excised and fixed in 15-20 times that volume of 10% neutral formalin solution. These pieces were then washed in running water for 24 hours, and then dehydrated by placing them progressively in solutions of 70%, 80%, 96%, and twice in 100% ethanol, each for 24 hours. Samples were then fixed in paraffin by immersing in 50/50 ethanol/o-xylene solution for 3 hours, o-xylene for 3 hours, 50/50 o-xylene/paraffin at 37°C for 3 hours, and finally, paraffin at 57°C for 2-3 hours. Samples were cooled and then cut to a thickness of 5-7μ with a rotary microtome. The cuts were glued on

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clean objective glass-bits with the help of an egg-white and glycerine mixture (1:1). Before staining, the paraffin was removed from the cuts by dipping the samples into o-xylene for 3-5 minutes, into ethanol at 96°C for 2-5 minutes, into ethanol at 75°C for 2-5 minutes, and finally into distilled water for 30 minutes. Samples were stained with Erlich hematoxyline and eosine, and then clarified with a xylene-carbolic acid mixture. Cover glass-bits were glued with Canada balsam. Preparations were marked with India ink, examined under a microscope, and photomicrographs were taken.

## RESULTS

The liver of roaches partially encases the duodenum. Although generally the livers were dark red in color, they sometimes were light brown. We did not find any macromorphological or micromorphological changes in the roach livers we studied. There were no distinct lobules. The secreting lamellae were made from cellular tissues. Hepatocytes were small, oblong, and had anomalous forms when compared with those of a variety of other fishes. The ratio of sizes of the nucleus and cytoplasm was 1 to 4. Cytoplasm was granular, filled up the volume of the cell, and contained very little fat. Liver parenchyma was furrowed with blood capillaries but these were not abundant. Ducts of the bile branched out between the lamellae. Bile secretion was of medium activity. We found a triad made from artery, vein, and bile. The roaches which were caught at various locations did not exhibit any differences in micromorphological structure (Figure 1).

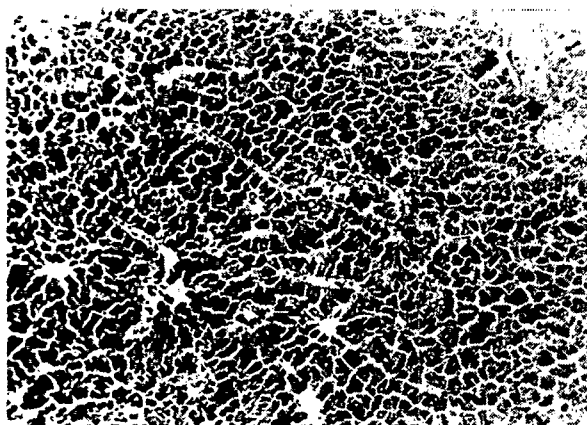


Figure 1. Photomicrograph of a liver of a roach.

The livers of the bream were of a dark red color, with little variation among specimens. The liver was compact. Hepatocytes of the bream liver were bigger when compared to the roach liver hepatocytes. Between the lamellae (which consisted of some cells) there were several capillaries that were filled with formal blood elements and bile ducts, but bile secretion was almost invisible. The micromorphology of the bream

livers differed in cases where these fish were caught at different locations. The liver of the breams from the Nemunas River delta had evenly distributed hepatocyte cytoplasm and vacuolization was rare.

Vacuolization of hepatocytes was very distinct in the livers of breams caught in Kuršių Marios Lagoon near Ventė Cape (Figure 2). All the hepatocytes had vacuoles, occupying 50% or more of the cell, and as a consequence the cells contained a considerable amount of fat. Cytoplasm of hepatocytes was around the nucleus. The nuclei were distinct, and not contracted. Such fatty tissue was perhaps caused by the intoxication influence of the fermentative system of the liver cells. Decomposed lipoprotein was apparent in the wounded hepatocytes. The microstructure of the breams from the Nemunas River near Vilkija appeared unstable because we saw the vacuolization of most hepatocytes. The nuclei were distinct with micronuclei in them. There were no vacuoles in some hepatocyte cytoplasm, or they occupied only a very small part of the cell.

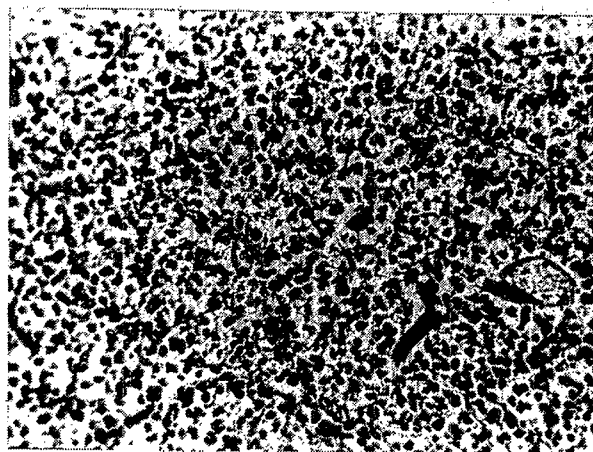


Figure 2. Photomicrograph of a liver of a bream.

In October 1991 in the Kuršių Marios Lagoon near Ventė Cape we caught two breams, in the liver of which distinct morphological changes were seen. After making micropreparation of the liver we established that general view was typical. The functional triads looked like the cavities of the large blood vessels that were located side by side. In most hepatocytes a distinct vacuolization was seen. Cytoplasm could be seen only around the nucleus.

In many places it was not possible to establish boundaries between the hepatocytes. Parenchyma cells seemed disordered, and the nuclei were not seen. Caryolysis and cytolysis had already begun. In the large bile duct we could see bile secretion. Necrotic areas were dispersed, and not focused in any single location. In the liver of one of these breams, hepatocytoma had begun to form.

The crucian carp caught in Kuršių Marios Lagoon near Ventė Cape had a large tumor on its gills. Microscopic analysis showed that this tumor formed from reticular cells which produce reticular fibres that were weaved among the tumor cells. The tumor was many-lobuled, with necrosis in the center of these lobules. Between the tumor cells there were masses of amorphous connective tissue which were formed after cariorexis, cariolysis, and cytolysis (Figure 3). These symptoms allowed us to characterize the changes which formed on the gills of the crucian carp as a diffusive malignant histiocytic type of reticulosarcoma.



Figure 3. Photomicrograph of a reticulosarcoma in the gills of a crucian carp.

In conclusion, our histological sampling of fishes of the Nemunas River, its delta, and Kuršių Marios Lagoon suggests that fishes in these waters may have received environmental insults. Additional histological examinations could form the basis for a baseline that could be used to monitor progress toward bioremediation of these waters.

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# TOXICITY OF FACTORY WASTEWATERS AND HEAVY METAL SOLUTIONS TO RAINBOW TROUT

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## ABSTRACT

A study has been undertaken on the lethal effects to rainbow trout (*Salmo gairdneri*) of galvanic wastewaters of some Vilnius factories, and of two solutions of heavy metals representative of the heavy metals in these wastewaters. Composition of these model solutions was based on those of wastewater discharges from Vilnius and Kaunas. The effects of the wastewaters and the model solutions on feeding, behavior, and respiratory indices have been estimated. The relationship between the toxicity of the wastewaters and of the model solutions has been compared. Suggested safe concentrations for fish of the model mixtures have been calculated.

## INTRODUCTION

Studies of toxic effects of industrial wastewaters in Lithuania have mostly been limited to research into hydrobiont communities and their quantitative and qualitative changes in a given water body. The most complete among such was conducted in Kuršių Marios Lagoon and in the Nemunas River (Gerulaitis and Valušienė 1991, Jokšas 1991, Lazauskienė *et al.* 1991). It was found that due to industrial and agricultural activities there has been an increase of chemical pollution in Kuršių Marios Lagoon. Oil products, heavy metals, and fertilizers have all contributed to an increase in the proportion of fishes of low commercial value, and a decrease in the harvest of more commercially desirable species. Because the pollution is multicomponent containing heavy metals, oil products, and other organic substances, it is very difficult to estimate the toxic effect of specific pollutant groups on the ichthyofauna. Special studies on the toxicity to fishes of single pollutants and their mixtures are needed to do this, as well as to set effluent discharge limits.

The first studies to estimate toxic effects of heavy metal mixtures on fishes were started at the Lithuania Academy of Sciences, Institute of Ecology in 1991. Heavy metals (zinc, copper, nickel, chromium, iron) are lost in electrodeposition processes, and if discharge purification systems at electrodeposition facilities do not work effectively, these metals can escape into wastewaters. These metals are often found in rivers downstream from many of the larger cities of Lithuania.

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The purpose of the present study was to examine the toxicity to rainbow trout of wastewaters from some Vilnius factories where galvanic processes are common, and to compare the data obtained to those on the toxicity of two different model solutions of five heavy metals, representative of those metals in industrial discharges.

## MATERIALS AND METHODS

The tests were conducted in July, September, and October, 1991, and performed in duplicate. The fish under study were juvenile rainbow trout (*Salmo gairdneri*), total length 2.5-3.5 cm in July and 7-8 cm in October; the weight ranges were 0.5-2.5 g and 5-9 g, on those same dates. Trout were obtained from the Žeimena Hatchery, near Vilnius. They were kept in holding tanks of about 1000-L capacity supplied with aerated flow-through artesian water of average total hardness 250 mg/L  $\text{CaCO}_3$ , alkalinity approximately 200 mg/L  $\text{HCO}_3^-$ , and pH 7.2. The fish were transferred from holding tanks to 20-L aquaria for the experiments. They were kept until acclimated to the new medium, and until they started swimming freely and were feeding well. The water temperature during the experiments was 11-12° C, and dissolved oxygen was 8-10 mg/L. The effect of wastewater was observed over a 2-hour period after diluting it in proportion 1:1. After diluting in different proportions the effect was also observed at 24, 48, 72, and 96 hours. Every day the water was changed, the number of dead fish was recorded, both the feeding and behavior of live fish were estimated (in grades), and, if possible, respiratory indices such as gill ventilation frequency and "coughing" rate were recorded. Chemical analysis of wastewaters was performed by the Environment Protection Department.

The formulation of model mixtures was based on the available analytical data of the amounts and relative proportions of five common heavy metals (copper, nickel, chromium, zinc, and iron) in the discharges from the cities of Vilnius and Kaunas into the Neris and Nemunas Rivers (Table 1). Copper was in lowest concentration; proportions of all other metals were set in relation to copper. The following chemically pure substances were used in making the model solutions:  $\text{CuSO}_4$ ,  $\text{NiSO}_4$ ,  $\text{K}_2\text{CrO}_4$ ,  $\text{ZnSO}_4$ , and  $\text{FeSO}_4$ . The data from the acute tests were processed by regression analysis of log-probit transformation using the methods of Miller and Tainter referenced in Belenkij (1963).

## RESULTS AND DISCUSSION

### A Study of Wastewaters of Vilnius Factories

*Electrodeposition wastewater discharge of Vilnius Amalgamated Enterprise of Construction-Finishing Machines:* Wastewater for testing was diluted to 50, 12.5, 10, 7.5, 5, and 0.5 %. The concentration of wastewater that could cause a lethal effect was very low (7.5-12.5%). All test mortalities occurred within 48 hours, so the 48-hour and 96-hour  $\text{LC}_{50}$  values were both 9.2% (Table 2).



**Table 1. Amount, mean concentration, and proportion relative to copper of selected heavy metals discharged in wastewaters from Vilnius and Kaunas.**

Discharge source	Receiving river	Flow rate (m <sup>3</sup> /sec)	Heavy metal				
			Cu	Ni	Cr	Zn	Fe
Amount (tons/year)							
Vilnius (1989-90)	Neris	100	9.3	10.1	12.6	21.0	243
Kaunas (1989)	Neris	169	3.04	2.43	7.05	7.70	48.7
Kaunas	Nemunas	420	7.08	8.37	6.44	23.8	155
Mean concentration (mg/L)							
Vilnius (1989-90)	Neris		0.11	0.12	0.15	0.25	2.9
Kaunas (1989)	Neris		0.15	0.12	0.35	0.38	2.4
Kaunas	Nemunas		0.11	0.13	0.10	0.37	2.4
Ratio relative to copper							
Vilnius	Neris		1	1.1	1.4	2.3	26
Kaunas <sup>1</sup>	Neris and Nemunas		1	1.0	1.7	2.9	18

<sup>1</sup>Ratios reported for Kaunas are based on mean of concentrations reported for discharges into both the Neris and Nemunas Rivers.

**Table 2. Acute toxicity characteristics to rainbow trout of wastewaters from Vilnius factories.**

Factory wastewater source and type	LC <sub>50</sub> (percent of discharge)	
	48-hours	96-hours
Amalgamated Enterprise of Construction-Finishing Machines, Electrodeposition discharge	9.2	9.2
Amalgamated Enterprise of Construction-Finishing Machines, Surface run-off	38.8	38.8
Polishing Machine-Tool Plant, Surface run-off	62.5	46.9
Fuel Equipment	57.2	54.8

Changes in feeding pattern and behavior were observed at concentrations of 5-10%; the fish showed unrest, often rising to the surface. These respiratory disturbances were noticed as soon as the test water was introduced. Gill ventilation frequency increased from  $92.5 \pm 8.5$  to  $115 \pm 2.7$  minute. After 24 hours, however, fish ventilation frequencies did not differ considerably from controls.

*Surface run-off water from the grounds of Vilnius Amalgamated Enterprise of Construction-Finishing Machines:* Wastewater for testing was diluted to 45, 40, 32.5, and 10%. As in the case of the electrodeposition wastewater, the range of lethal concentrations was very small, and the toxic effect was very acute. All test mortalities occurred during the first 24 hours. At wastewater concentrations of 45-32.5% the feeding and behavior of the fish changed significantly. Within 1-2 hours after exposure to wastewater of acutely toxic concentrations, gill ventilation frequency increased to 120-150/minute and the "coughing" rate to 20-30/minute. After 24 hours, ventilation frequency of the two surviving fish decreased to  $77 \pm 2.5$ /minute and  $83 \pm 2.6$ /minute.

*Surface run-off water from Vilnius Polishing-Machine Tool Plant:* As the amount of wastewater available for study was small, we analyzed only three concentrations: 75, 50, and 25%. During 96 hours at 75% wastewater concentration, eight out of 10 fish died and at 50% concentration all the fish survived. However, the effect of 50% concentration was rather toxic because the fish were not feeding, and gill ventilation frequency after 24 hours exposure decreased significantly to  $81.0 \pm 3.2$ /minute (as compared to the control of  $101 \pm 3.0$ /minute).

*Wastewater of Vilnius Fuel Equipment Plant:* Because most of the fish died within 48 hours, the calculated 48- and 96-hour  $LC_{50}$  values were only slightly different, 57.2% and 54.8%. Fish at the next lower concentration, 40.45%, stopped feeding and gill ventilation frequency increased.

*Wastewater of "Sigma" Plant:* Wastewater was diluted as follows: 60, 50, 25, and 15%. No deaths of fishes were recorded. At concentrations of 50% and 60%, however, the fish stopped feeding, gill ventilation frequency was 135-142/minute, and at 24 and 48 hours ventilation frequency decreased to 110-115/minute. The "coughing" rate remained unchanged.

#### **A Study of the Effect of Model Solutions of Common Heavy Metals**

When studying the effect of both model solution No. 1 (Vilnius variant) and model solution No. 2 (Kaunas variant) on the survival of rainbow trout, a number of common relationships were found. After introducing the model solutions into the aquaria, the majority of fish died within 48 hours; the fish that survived recovered very soon and within 24 hours after exposure appeared to be in satisfactory condition. An obvious qualitative transformation of the solutions was discovered: after introducing the solutions to the test aquaria, the color of the water changed to brown, and in time a brown precipitate settled out, indicating the ratio of metal concentration in solution was

no longer that of the original stock solution. There was a sharp boundary between the lethal and sublethal concentration zones. No significant differences in rainbow trout survival were established between the effects of the two model solutions (Table 3).

Table 3. Acute toxicity characteristics of heavy metal model solutions to rainbow trout.

Exposure time (hours)	Effective concentration (percent solution)			R <sup>2</sup>
	LC <sub>0</sub>	LC <sub>50</sub>	LC <sub>100</sub>	
Model solution No. 1 - Vilnius variant				
48	57.8	70.0	84.7	0.95
96	57.2	69.2	83.7	0.93
Model solution No. 2 - Kaunas variant				
48	55.2	68.1	83.8	0.86
96	55.7	68.0	83.0	0.89

With regard to behavior, the locomotor activity of fish significantly increased after introducing the model solutions at lethal concentrations; random swimming, body tremors, and respiratory disorders were observed. When the solution concentration exceeded 70% the fish did not respond to food, but at concentrations of 55-65% the fish were feeding like those in the control group.

The effect of the Kaunas variant solution on respiratory indices of rainbow trout after exposure for 1 hour was studied. Lethal concentrations of the solution had an effect upon respiratory indices of the trout (Table 4). Gill ventilation frequency decreased as much as 26% while the "coughing" rate increased 10- to 15-fold.

Table 4. Respiratory indices of rainbow trout after 1 hour exposure to model solution No. 2 (Mean  $\pm$  SE, n = 10).

Concentration of model solution (percent)	Gill ventilation frequency (number/minute)	"Coughing" rate (number/minute)
Control	102.9 $\pm$ 1.4	1.5 $\pm$ 0.2
55	79.0 $\pm$ 1.4*	21.4 $\pm$ 0.6*
60	86.0 $\pm$ 1.6*	14.3 $\pm$ 0.2*
65	105.3 $\pm$ 2.8	14.0 $\pm$ 0.8*
80	96.0 $\pm$ 0.6*	13.0 $\pm$ 0.6*
85	76.5 $\pm$ 0.8*	15.5 $\pm$ 0.3*

\*Significant differences from control (P < 0.05).

Data on the chemical composition of the wastewaters prior to dilution, and the estimated concentrations of the wastewater components at the 96-hour  $LC_{50}$  values are presented in Table 5. The most toxic wastewater was from Vilnius Amalgamated Enterprise of Construction-Finishing Machines: the 96-hour  $LC_{50}$  was 9.2%. During the tests of the wastewater of the "Sigma" plant, although all fish survived the 96-hour test period, they were obviously intoxicated. The 96-hour  $LC_{50}$  of both model solutions were much alike: 69.2% (Vilnius variant) and 68.0% (Kaunas variant). The difference in chemical composition of wastewaters was distinct, but it is clear that the concentrations of the five heavy metals in the model solutions that caused 50% mortality of fish was considerably higher than those found in the industrial wastewaters. Based on the data obtained, one may suppose that the reason for deaths of fish in different wastewater concentrations is not the heavy metals alone. It is quite possible that the toxicity of wastewaters was due to high pH, or a quantity of unknown suspended substances that were not analyzed. It is also possible that the toxicity of wastewaters was caused by factors not established analytically.

Table 5. Chemical composition of wastewaters of Vilnius factories and toxicological characteristics of wastewaters and model solutions (concentrations in mg/L).

Percent wastewater or model solution	Suspended substances	pH	Cu	Ni	Cr	Zn	Fe
Electrodeposition wastewater of Vilnius Amalgamated Enterprise of Construction-Finishing Machines							
100	4.0	10.7	0.005	0.04	0.125	0.16	0.13
9.2*	0.37	-	0.0005	0.004	0.012	0.015	0.012
Rain sewerage wastewater of Vilnius Amalgamated Enterprise of Construction-Finishing Machines							
100	7.2	9.7	0.011	0.06	0.055	0.08	0.44
38.8*	2.8	-	0.004	0.023	0.02	0.03	0.17
Rain sewerage wastewater of Vilnius Polishing Machine-Tool Plant							
100	114.7	8.41	0.14	0.00	0.00	0.15	1.7
46.9*	53.6	-	0.065	0.00	0.00	0.07	0.8
Wastewater of Vilnius Fuel Equipment Plant							
100	77.3	8.53	0.45	0.49	0.31	1.3	7.0
54.8*	42.4	-	0.25	0.27	0.17	0.7	3.8
Model solution No. 1 (Vilnius variant)							
69.2*	7.2	-	0.69	0.76	0.97	1.59	18.3
Model solution No. 2 (Kaunas variant)							
68.0*	7.2	-	0.68	0.68	1.2	1.98	12.6

\*96-hour  $LC_{50}$

Based on data from the Lithuania Environmental Protection Department on the annual amounts of discharges of common heavy metals from the cities of Vilnius and Kaunas, and information on the average annual flow rates of the Nemunas and Neris Rivers (Table 6), theoretical concentrations of heavy metals in the rivers were calculated. Using the 96-hour  $LC_{50}$  values of the model solutions, the suggested safe concentrations were estimated at 0.01 times these values. Such concentrations would be between 0.1% and 0.6%, depending upon the kind of wastewater.

Table 6. Estimates of representative heavy metal concentrations in the Nemunas and Neris Rivers and suggested safe concentrations for fishes.

Location or solution	Cu	Ni	Cr	Zn	Fe
Estimated concentrations (mg/L)					
Nemunas River below Kaunas	0.0005	0.0006	0.0005	0.002	0.012
Neris River immediately above confluence with Nemunas River	0.0006	0.0005	0.001	0.001	0.009
Neris River below Vilnius	0.003	0.003	0.004	0.007	0.077
Suggested safe concentrations* (mg/L)					
Model solution No. 1 (Vilnius variant)	0.007	0.008	0.01	0.016	0.18
Model solution No. 2 (Kaunas variant)	0.007	0.007	0.012	0.02	0.13

\* 0.01 times the 96-hour  $LC_{50}$

The data above would imply that the concentrations of heavy metals that are suggested as safe are higher than the actual concentrations of these metals in the Neris and Nemunas Rivers. Such conclusions may be too hasty. It should be borne in mind that natural waters contain background levels of heavy metals and that river flow rates are not constant. Pollution is usually local and zones of more polluted water can be formed. To suggest more exact safe concentrations of wastewaters and their components, physiological and behavioral tests are needed. In addition to the data from such biological tests, one should have more exact information on the physical and chemical equilibria composition of the receiving water, as well as data on the degradation of the pollutants and their accumulation in hydrobionts (Anonymous 1990). To study the toxic effect of model mixtures on fishes further, it would be necessary to determine the toxicity of each single component, to establish the relative toxicity of the components, and to find interdependence of the components in the given mixture.

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# AVOIDANCE REACTION TO POLLUTANTS BY *VIMBA* UNDER LABORATORY AND FIELD CONDITIONS

G. Svecevičius<sup>1</sup>

## ABSTRACT

Avoidance reactions of adult and juvenile vimba, *Vimba vimba* (L.), to two heavy metals and three organic pollutants were studied under laboratory and field conditions. The metals were zinc and copper, and the organic pollutants were a commercial sulfonate detergent, phenol, and oil products. Although fish demonstrated significant avoidance responses to the sulphonate detergent and heavy metals, avoidance to phenol was less pronounced and there was no avoidance reaction to the oil products. The avoidance of the sulphonate detergent by vimba was very consistent and independent of testing conditions. Individual adult and juvenile fish avoided zinc and copper in the laboratory equally, although these thresholds were considerably lower when the fish were tested in groups. Avoidance thresholds were not related to pollutant toxicity and were generally at sublethal levels, except that the concentration range of effective avoidance of phenol was close to its acutely toxic concentration. From the point of view of ecological significance to habitat occupied by vimba, the pollution with sulfonate detergents would impose the greatest danger, and it may be that less danger is caused by zinc and copper pollution.

## INTRODUCTION

Avoidance reaction by fishes is one form of phenotypic adaptation to water pollution (Flerov 1989). An active retreat of fishes out of polluted areas, however, results in disturbance to their migration patterns and distribution. This is especially true in the case of anadromous fishes. Therefore, a reduction of their normal area of habitat, as well as their resources, can occur.

Great attention has been given to the study of avoidance reaction to pollutants by fishes during recent decades, and a considerable amount of experimental data based on laboratory experiments are available (Giattina and Garton 1983, Beitinger and Freeman 1983). Literature data, however, are often fragmented, contradictory, and difficult to compare. Although avoidance of pollutants by fishes has been observed in nature, it is supposed that threshold avoidance concentrations in nature are much higher and more variable than under laboratory conditions where direct or masking factors capable of effecting fish behavior are absent (Sprague 1971, Beitinger and Magnuson 1976, Weber *et al.* 1981, Giattina *et al.* 1981). That is why it is necessary to conduct field studies to compare with laboratory tests.

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Our study deals with vimba, an anadromous fish, and although it is one of the most valuable commercial species in Lithuania, its population is being reduced each year. Avoidance reactions to pollutants by vimba have not been studied. The aim of the present work is to study regularities of occurrence of avoidance reaction by this fish to two heavy metals, phenol, a commercial detergent, and a diesel fuel. These are representative of the most common pollutants in the rivers of Lithuania.

## MATERIAL AND METHODS

The tests were conducted at the Experimental Aquarium, Institute of Ecology, Lithuanian Academy of Sciences. Test fish were vimba of both sexes, 23-30 cm total length, collected in 1983-1988 by means of electrofishing during spawning season (May-June) in the Širvinta River, and juveniles 7.5-10 cm total length, collected during autumn by sweep net in the Neris River 13 km above Vilnius. The fish were acclimated and kept in the laboratory in tanks supplied with aerated artesian water of good quality with total hardness approximately 250 mg/L as  $\text{CaCO}_3$ , alkalinity approximately 200 mg/L  $\text{HCO}_3^-$ , and pH 7.2.

Acute toxicity tests were conducted on juvenile vimbas. The following effective concentrations were set for 48 hours exposure: maximum acceptable concentration ( $\text{LC}_0$ ), median lethal concentration ( $\text{LC}_{50}$ ), and absolutely lethal concentration ( $\text{LC}_{100}$ ), found by regression analysis of log-probit transformation applying the method of Miller and Tainter, referenced in Belenkij (1963).

The test chamber was similar to those described by Fava and Tsai (1976) and DeGraeve (1982). The gradient chamber was 1500 x 600 x 300 mm in size, and the total flowing capacity was 6 L/minute. Fifteen minutes after the introduction of test solution into one of the canals of the chamber, three zones formed: Zone A, control water; Zone B, solution; and Zone C, partial mixing zone.

The field experiments for determining avoidance reactions to pollutants were performed on groups of adult fishes consisting of ten individuals, half male and half female, in a stream where a working area with a gravel bottom about 12 m<sup>2</sup> was arranged (Svecevičius 1989); the depth was 15-20 cm with a flow rate of 16-17 L/second. The water quality was similar to the characteristics of non-polluted rivers of Lithuania with total hardness of approximately 150 mg/L as  $\text{CaCO}_3$ . Test solution was added so that in the stream two zones were formed, equal by area and volume: one polluted and one control. A filming camera was used to take a picture every 30 seconds to record the fish distribution in the stream. The ecological characteristics of the test area, such as temperature, substratum, and flowing rate, was close to natural for vimba at migratory/spawning season. The effects of the following substances were studied:  $\text{CuSO}_4$ ,  $\text{ZnSO}_4$ , phenol, Lotos<sup>TM</sup> (a sulfonate detergent 22% alkylsulfonate and alkylbenzosulfonate), and oil products (diesel fuel in emulsion and solution).



Estimation of avoidance reaction under laboratory conditions was conducted as follows: a single fish, or a group of 10 fish, was placed in a gradient chamber and acclimated for 0.5-1.5 hours, followed by a 10-minute control period during which the location of the test fish in one or another zone of the test chamber was recorded visually at 15-second intervals. The testing was performed during 10-minute periods at 30, 60, 90, and 120 minutes from the start of addition of the test solution. The nature and intensity of reaction were estimated by the index of reaction through the formula:

$$\text{Reaction index} = 50(2 - N_T/N_C),$$

where  $N_C$  is the number of recordings during the control period of a fish in Zone B divided by the total number of recordings in both Zone A and Zone B;  $N_T$  is the number of recordings during the test period of a fish in Zone B divided by the total number of recordings in both Zone A and Zone B. When testing was conducted on a group of fish, the numbers recorded were changed correspondingly.

The value of the index 100 denoted maximal avoidance, while 0 denoted maximal preference and 50 denoted indifference. The significance of reaction was estimated by Student's t test with  $P \leq 0.05$ .

## RESULTS AND DISCUSSION

An avoidance threshold concentration of phenol by adult vimba was estimated to be 2.8 mg/L, and by juveniles it was estimated to be 4.6-4.8 mg/L (Table 1). The response difference between adults and juveniles was not significant ( $P > 0.1$ ). The reaction index reached a maximum value at 50 mg/L (Figure 1), and variations in reaction intensity with time were not significant. The contact of vimba with phenol at 5 mg/L or more caused external symptoms of poisoning, namely sudden spurts of speed, body tremor, and increase in the rate of respiratory disturbances which manifests itself though a gill-cleaning reflex referred to as "coughing." Under field conditions, there was no apparent avoidance reaction to phenol by groups of adult vimba over the range 0.1-1 mg/L, although an increase in locomotor activity was noted at 1 mg/L. Tests at higher concentrations were not conducted.

The most intensive avoidance occurred for the detergent Lotos<sup>TM</sup>. A significant reaction was found in single adult fish under laboratory conditions within the range 0.1-1 mg/L, and in single juvenile fish within the range 0.5-10 mg/L. At 5 mg/L the detergent caused the strongest avoidance reaction in juvenile vimba, but the intensity of reaction decreased considerably at 10 mg/L ( $P < 0.001$ ). Variations of reaction intensity to the detergent over time were not significant. Groups of juveniles under laboratory conditions, and adult vimba in field conditions, also intensively avoided Lotos<sup>TM</sup>. A significant reaction was noted within the concentration range of 0.1-1 mg/L.

Table 1. Avoidance threshold concentrations of pollutants by vimba under different testing conditions (values reported in mg/L with 95% C.I.).

Pollutant	Laboratory tests			Field tests
	Single juveniles	Group of juveniles	Single adults	Groups of adults
Phenol	4.6 (1.8-8.4)	4.8 (2.2-9.7)	2.8 (1.8-3.7)	>1
Lotos™	0.061 (0.024-0.13)	0.037 (0.012-0.13)	0.05 (0.02-0.09)	0.047 (0.016-0.11)
Zinc	0.69 (0.34-2.0)	0.16 (0.084-0.34)	0.6 (0.1-1.8)	0.026 (0.0057-0.077)
Copper	0.12 (0.07-0.25)	0.044 (0.014-0.13)	0.1 (0.05-0.3)	0.005 (0.0024-0.0087)

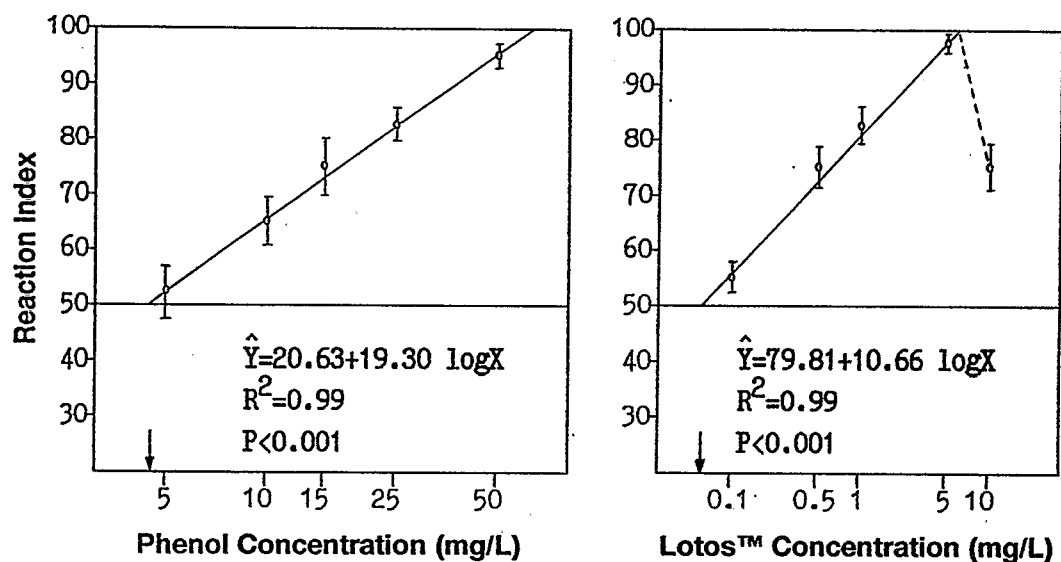


Figure 1. Avoidance reaction in juvenile vimba to phenol, and to the detergent Lotos™. Circles (o) indicate the mean values of the reaction of single fish. Vertical lines denote  $\pm 1$  SE. Threshold values are marked by arrows.

The intensity of avoidance reaction to zinc and copper by single adult and juvenile vimba in the laboratory was found to be significantly different over time. Long latent periods of reaction were noticed. Thus, adult vimba significantly avoided zinc at 2 and 4 mg/L and copper at 1 mg/L after 90 minutes into test, and copper at 0.5 mg/L after 120 minutes into test. The juvenile vimbas significantly avoided zinc at 2-4 mg/L after 90 minutes into test, and at 10 mg/L after 30 minutes into test. Copper at concentrations of 0.5-1 mg/L was also significantly avoided after 90 minutes into test, while at 1.5 mg/L the reaction occurred after 60 minutes. Groups of juveniles avoided zinc more strongly than did individuals. Zinc was significantly avoided even at concentration of 0.4 mg/L, and the reaction intensity was stable over time. Copper was avoided at 0.1 mg/L, although the latent period of avoidance was long. Under field conditions, adult vimba intensively responded to heavy metals: significant avoidance reaction to zinc was found at concentrations of 0.22, 0.4, and 2 mg/L, and to copper at 0.05, 0.1, and 0.5 mg/L.

The avoidance reaction to zinc and copper by vimba was strongly dependent on testing condition. Single adults and juveniles avoided zinc and copper equally. Under laboratory conditions, however, juveniles in groups avoided zinc and copper more intensely than did single fish, either adult or juvenile. Among the latter, the reaction to zinc and copper was notable for a long latent period. The results of field tests on groups of adult vimba were found to be greatly different from those in laboratory; avoidance thresholds were 0.026 mg/L for zinc and 0.005 mg/L for copper, an order of magnitude lower than in the laboratory.

It is known that vimba often form schools. The testing on single fish in the laboratory has shown, by external symptoms, that some are more sensitive than others to the same pollutant concentrations. It is very likely that in the avoidance reaction to metals by groups of vimba, some display of group behavior was taking place, i.e., the capability of less sensitive individuals to follow and imitate the behavior of more sensitive ones.

Our data obtained on the avoidance reaction of vimba to zinc and copper do not agree with data reported by other authors for Atlantic salmon (*Salmo salar*) (Sprague *et al.* 1965, Saunders and Sprague 1967). These authors report that salmon parr in the laboratory had a median avoidance concentration of 0.0024 mg/L for copper and 0.054 mg/L for zinc. Simultaneously abnormal slopes of adult salmon migrating for spawning were recorded as 0.017-0.021 mg/L for copper and 0.21-0.258 mg/L for zinc. These authors also reported that spawning migration was completely stopped at concentrations of copper over 0.038 mg/L and zinc at 0.48 mg/L. Consequently, under natural conditions the Atlantic salmon is less restrained by these metals, and this phenomenon was explained by the authors as a lack of strong motivation in the laboratory. The motivation to migrate was probably absent in our test fish, although the stretch of the stream used for field experiments corresponded in all ecological parameters (substratum, depth, and flowing rate) to the biotope, attractive for adult vimba during migratory/spawning season.

None of the fish tested, under either laboratory or field conditions, evidenced avoidance to oil products when these were added to water to make nominal concentrations within the range 0.1 to 100 mg/L. A longer contact of fish to oil products at 100 mg/L caused a number of external symptoms of poisoning. These were decrease of locomotor activity, respiratory disturbances evidenced by an increase in "coughing" rate, and, in some cases, loss of equilibrium.

The avoidance response of male and female adult vimbas under laboratory test conditions was equal; no differences between sexes in reaction insensitivity were noted ( $P > 0.1$ ). Avoidance reaction intensity to phenol and to Lotos<sup>TM</sup> by single juvenile vimba tested in the laboratory is illustrated in Figure 1. Regression analysis confirmed that a linearly proportional interdependence exists between concentration and avoidance reaction ( $P < 0.001$ ). The threshold concentration of avoidance is defined as that concentration value of the regression line at which the reaction index is 50 (Höglund 1961, Fava and Tsai 1976, Anestis and Neufeld 1986).

Data on acute toxicity of the pollutants studied in juvenile vimba were compared to threshold avoidance concentrations (Table 2). The data show that the avoidance thresholds of phenol are the closest of these to maximum acceptable concentration (68.2-71.6% of  $LC_0$ ). Thus the range of effective avoidance concentrations are close to acutely toxic levels of phenol. This agrees with the conclusions that some cyprinid fishes are capable of avoiding phenol only at lethal levels (Ishio 1965, 1969). Avoidance by vimba of the detergent Lotos<sup>TM</sup> occurs at low sublethal concentrations (0.3-0.5%  $LC_0$ ) as does avoidance of heavy metals. The data obtained indicate absence of correlation between toxicity of pollutant and intensity of its avoidance.

Table 2. Comparison of 48-hour acutely lethal concentrations to threshold avoidance concentrations in juvenile vimba (values reported in mg/L).

Pollutant	Lethal concentration			Threshold avoidance concentration	
	$LC_0$	$LC_{50}$	$LC_{100}$	Single	Group
Phenol	6.7	10.9	17.7	4.6	4.8
Lotos <sup>TM</sup>	12.2	19.2	30.1	0.061	0.037
Zinc	16.8	20.1	24.0	0.69	0.16
Copper	0.45	0.69	1.08	0.12	0.044
Oil products	9.6	14.9	23.0	absence of avoidance	

## SUMMARY

The present study enables us to estimate the ecological danger of pollution to vimba; one should be guided by the data of field tests as these best reflect possible reactions of fishes in natural media. Vimba avoided the detergent Lotos<sup>TM</sup> more than they avoided any of the other pollutants tested. The avoidance threshold to Lotos<sup>TM</sup> amounted to 0.037-0.061 mg/L and to 0.0081-0.013 mg/L as sulfonate. These are very low concentrations, and such concentrations may very well be in Lithuanian rivers along the migration routes of vimba. Even so, maximum permissible concentrations for alkylsulfonate and alkylbenzosulfonate detergents are as high as 0.5 mg/L (Anonymous 1975). Heavy metal concentrations in Lithuanian rivers vary within the range 0.001-0.01 mg/L, and may be much higher near pollution sources; zinc and copper can be considered ecologically potentially dangerous for vimba. Phenol was avoided by vimba at concentrations over 2.8 mg/L, a level seldom found in Lithuania under natural conditions, so from this point of view phenol is less harmful. Oil products, generally not avoided by vimba, may be harmful in those cases when a fish swims into oil solutions at concentrations high enough to cause unfavorable lethal or sublethal reactions.

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# TOTAL $\beta$ -ACTIVITY AND $^{90}\text{Sr}$ CONCENTRATION IN THE KURŠIŲ MARIOS LAGOON ECOSYSTEM

R. Dušauskienė-Duž<sup>1</sup>

## ABSTRACT

A great amount of radioactive matter was dispersed in the atmosphere after the accident in 1986 at the Chernobyl Atomic Power Station in Belarus. The area impacted included the Nemunas River basin and Kuršių Marios Lagoon ecosystem. There is a continued need to monitor the radioactive matter from Chernobyl to document its distribution in water basins, migration, bioaccumulation, transformation of chemical forms, and bottom deposition. The objective of this monitoring is to fix the beginning of global and technogenic influence and changes of natural systems caused by the accident. Our studies show a continued threat to benthic organisms by long-term pollution sources in the bottom sediments of Kuršių Marios Lagoon.

## INTRODUCTION

Fission products, together with wet and dry atmospheric deposition, accumulates on the earth's surface and in its aquatic systems. An interaction with the environment then begins, and radioactive matter is diluted and distributed among the main components of the ecosystem. Although Kuršių Marios Lagoon is a specific water basin, it is greatly influenced by water regime changes in the Baltic Sea, thereby influencing the aquatic life of the lagoon. At the same time, there is a great amount of suspended organic matter and nutrient material discharged into Kuršių Marios Lagoon from the Nemunas River, contributing to eutrophication of the lagoon.

Radioecological investigations in the Kuršių Marios Lagoon ecosystem began in 1967; prior to that no such work had been done on this water basin. The first step was to measure strontium-90 ( $^{90}\text{Sr}$ ) concentrations in water, bottom sediments, plants, and fish (Dušauskienė-Duž 1978). Later on, the influence of chemical composition factors, such as domestic and paper manufacturing wastewater, on the  $^{90}\text{Sr}$  concentrations in hydrobionts was estimated (Dušauskienė-Duž 1978). Still later, as eutrophication in the Kuršių Marios Lagoon became intense, measurement of lead-210 ( $^{210}\text{Pb}$ ) concentrations in water, biota and bottom sediments were included into the investigation program (Dušauskienė-Duž 1978, 1983). This enabled us to determine migration peculiarities, accumulation mechanisms, and the levels of two naturally different radionuclides. The marked  $^{210}\text{Pb}$  enabled us to date the bottom sediments of the lagoon and to establish sedimentation rates (Dušauskienė-Duž 1978). Total  $\beta$ -activity ( $\Sigma\beta$ ) allowed us to locate the most radionuclide-contaminated regions and their components, as well as to estimate the role of atmospheric fallout in forming the levels of radioactivity in the basin.

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## MATERIAL AND METHODS

The concentrations of  $^{90}\text{Sr}$  in water, biota, and bottom sediments was estimated according to the carbon method of Sokolova (1971), in which  $^{90}\text{Sr}$  is measured according to its daughter-product yttrium-90 which strikes a balance after 14 days. Chemical measurements of  $^{90}\text{Sr}$  made up 85-89% of total Sr. Radiometry was carried out by low intensity equipment (UMF-1500<sup>TM</sup>), with intensity of 3.5-4.0 cpm; measurement efficiency was 17-20%.

## RESULTS AND DISCUSSION

$\Sigma\beta$ -activity and  $^{90}\text{Sr}$  concentration was measured in the surface-water microlayer (SWML), 300-400  $\mu\text{m}$ , and surface-water layer, 0.5 m (Table 1). From this, one can draw a conclusion that the  $^{90}\text{Sr}$  concentration level in the Kuršių Marios Lagoon ecosystem is caused by atmospheric deposition.

Table 1. Total  $\Sigma\beta$ -activity and  $^{90}\text{Sr}$  concentration in rain, surface-water micro layer (SWML), and water samples (Mean values in  $\text{Bq/m}^3$ ).

	Rain	SWML (% rain)	Water (% SWML)
$\Sigma\beta$	1100	847 (77)	551 (65)
$^{90}\text{Sr}$	243	173 (71)	28 (16)

It was established that  $\Sigma\beta$ -activity in the SWML depends upon the time and place of sample collection. The  $\Sigma\beta$ -activity in SWML samples from the northern part of Kuršių Marios Lagoon (Figure 1) was lower than that in samples from the central part. There was a slight difference between filtered and unfiltered water. The  $\Sigma\beta$ -activity was slightly higher in unfiltered water samples collected from the central part of Kuršių Marios Lagoon during spring, while there was a significant difference between filtered and unfiltered samples collected in autumn because of a greater amount of suspended matter (Table 2).

Table 2. Mean (and range)  $\Sigma\beta$ -activity in the surface water micro layer ( $\text{Bq/m}^3$ ).

Station number	June 1991		October 1991	
	Unfiltered	Filtered	Unfiltered	Filtered
2,3,3 <sup>a</sup>	—	—	521 (471-555)	638 (617-700)
10,11, 12,12 <sup>a</sup>	743 (647-925)	666 (206-1200)	1010 (906-1430)	580 (370-755)



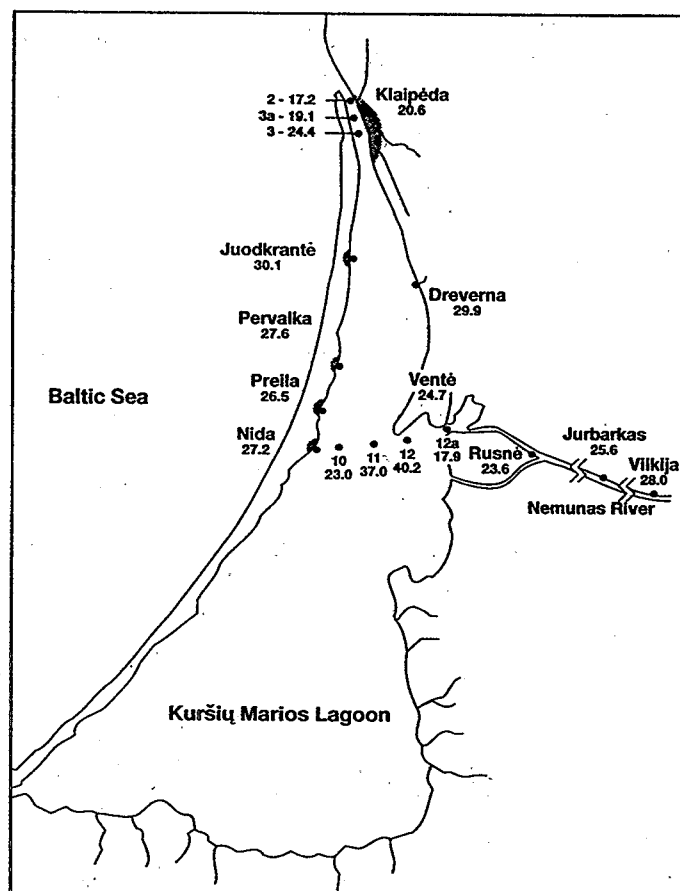


Figure 1. Mean concentration of  $^{90}\text{Sr}$  in Kuršių Marios Lagoon (Bq/m $^3$ ), 1978-1990.

Surface-water layer  $\Sigma\beta$ -activity was higher in filtered water from the northern part of Kuršių Marios Lagoon as well as in unfiltered water from the central part (Table 3). This can be explained by the fact that the northern part of the lagoon is under strong anthropogenic influence, which includes wastes from the Cellulose and Pasteboard Plant in Klaipėda (CPPK), the city's sewerage, and the Ship Repairs Plant. These activities cause changes in radionuclide chemical forms. This is evidenced by the prevalence of soluble-ionic radionuclide forms over nonsoluble-suspended forms, which poses great danger for living organisms because it increases migration properties of radionuclides. This conclusion is supported by the data of Jokšas (1991), indicating that concentrations of heavy metals in the waters of the northern part of Kuršių Marios Lagoon are in soluble form, not suspended particulate form.

Table 3. Mean (and range)  $\Sigma\beta$ -activity in surface-water layer (Bq/m<sup>3</sup>).

Station Number	June 1991		October 1991	
	Unfiltered	Filtered	Unfiltered	Filtered
2,3,3 <sup>a</sup>	—	—	647 (616-679)	987 (802-1170)
10,11, 12,12 <sup>a</sup>	477 (199-781)	304 (85.3-616)	786 (308-1110)	610 (370-780)

$\Sigma\beta$ -activity in the surface water of the northern part of Kuršių Marios Lagoon is higher than that in SWML because of the influence of surrounding factors. This indicates that the surface pellicle is destroyed under the influence of water streams in wastewater channels. In other water bodies, we have seen the same situation when  $\Sigma\beta$  of non-filtered water is higher than that of filtered water.

Considerable differences between the  $\Sigma\beta$ -activity in the bottom sediments of the northern and central parts of Kuršių Marios Lagoon was not established (Table 4). Knowing that conditions of hydrodynamic sedimentation are more favorable in the central part of Kuršių Marios Lagoon, one can assert that  $\Sigma\beta$ -activity is higher in the northern part. Thus, taking into account that the greatest concentration of suspended terrigenous matter lies in the northern part of the lagoon in the region of Klaipėda Harbor (Jokšas 1991), water mixing results in active coagulation, adsorption/absorption, and desorption processes. Very intensive degradation processes take place in this zone under the influence of anthropogenic factors. Small-dispersion muds of small particle size produce high rates of contamination accumulation. They can be found as soluble-migratory forms in waters of this part of the lagoon.

Table 4. Mean (and range)  $\Sigma\beta$ -activity in bottom sediment (Bq/kg).

Station Number	Concentration
2,3,3 <sup>a</sup>	581 (411-732)
10,11,12,12 <sup>a</sup>	533 (300-685)

Aquatic plants are of great importance when forming bottom sediment radioactivity. It has been established that phytoplankton, which frequently have great biomass, enrich themselves with heavy metals and their radionuclides (Jokšas 1991, Dušauskienė-Duž 1978). Phytoplankton  $\Sigma\beta$ -activity in the central part of the lagoon was in the 10-136 Bq/kg range, and varied depending upon its gathering place as well as on intercourse of its physiological groups.

Concentration of  $^{90}\text{Sr}$  in SWML  $\Sigma\beta$ -activity was 28.2% of the total (247/874 Bq/m<sup>3</sup>). Water surface pellicle is a short-term concentrated product, dispersing and diluting in the slightest wind or water movement, and only 11.3% of its primary activity was in the surface-water layer. Concentration of  $^{90}\text{Sr}$  in the near-shore water of Kuršių Marios Lagoon varies slightly because it is under the constant action of near-shore water currents; these flow from the Baltic Sea south into the west side of the lagoon and then go back to the Baltic Sea by flowing north along the east side.

Maximum concentration of  $^{90}\text{Sr}$  was measured in the middle part of the lagoon (39-50 Bq/m<sup>3</sup>) where influence of near-shore water currents is the lowest. Concentration of  $^{90}\text{Sr}$  in Kuršių Marios Lagoon undoubtedly depends on meteorological conditions such as amount of precipitation, prevailing wind direction, wind speed, and waving; this argument is supported by the seemingly active random measurements of  $^{90}\text{Sr}$  (Figure 1).

We obtained  $^{90}\text{Sr}$  concentrations in water from long-term measurements (data of 1978-1990) depending upon sampling place and time (Figure 1). This is illustrated by the results of one year of measurements of  $^{90}\text{Sr}$  in Kuršių Marios Lagoon (Table 5). It was established that the  $^{90}\text{Sr}$  concentrations measured in Kuršių Marios Lagoon are characteristic of other flowing type water basins, and were 2.5 times lower than those in secluded basins such as Drūkšiai Lake in northeast Lithuania (Dušauskienė-Duž 1992b).

Table 5. Concentration of  $^{90}\text{Sr}$  in Kuršių Marios Lagoon (Bq/m<sup>3</sup>) in 1990.

Sampling Station	Month								
	02	03	04	05	06	07	08	09	10
Smiltynė	-	18.2	28.4	29.3	-	22.9	25.9	24.4	28.5
Juodkrantė	40.7	31.0	30.4	34.9	27.5	37.0	-	19.9	25.8
Pervalka	-	31.0	29.0	37.0	18.8	31.8	26.6	-	-

The vitality of plankton and ability to accumulate  $^{90}\text{Sr}$  decreases in the northern part of the lagoon under the influence of salt water from the Baltic Sea. The  $^{90}\text{Sr}$  accumulation coefficient (AC), defined as the concentration of  $^{90}\text{Sr}$  in phytoplankton over the concentration in water, was 26 in the northern part of Kuršių Marios Lagoon

while in the central part it was 54. According to our data, in the northern part of Kuršių Marios Lagoon single-valent metal radionuclides are absorbed less than those of bi- or tri-valent metal radionuclides with characteristic high adsorption levels on suspended matters. The phytoplankton/water AC of  $^{90}\text{Sr}$  and  $^{210}\text{Pb}$  was 40 and 300, respectively.

Certain aquatic plants are active water quality stabilizers because they purify mechanically, biologically, and physically-chemically. *Nitellopsis obtusa*, *Potamogeton perfoliatus*, and *P. lucens* are significant in this purification process. Concentrations of  $^{90}\text{Sr}$  exceeded that in water 190 times for *Nitellopsis* and 240 times for *Potamogeton*. Alga are indicators of  $^{90}\text{Sr}$  contamination because they accumulate large amounts of easily exchangeable Ca, and duckweeds are able to form Ca inlays on leaves with  $^{90}\text{Sr}$  in their structure.

Concentrations of  $^{90}\text{Sr}$  in bottom sediment averaged 17 Bq/kg, and varied from 7 to 30 Bq/kg, mainly depending on bottom structure and morphology. Maximum rates were 15-30 Bq/kg in small-dispersed type bottom (mud), and 7-13 Bq/kg in sand type bottom. The most variable conditions for the accumulation of  $^{90}\text{Sr}$  in bottom sediment are found in zones with prevailing constant water currents, large vegetation association, and good sedimentation conditions.

Molluscs, which constitute an especially great biomass in Kuršių Marios Lagoon, are in close contact with bottom sediments. Because  $^{90}\text{Sr}$  is a calcium analogue, it can take place in Ca exchange and thus form shells. Concentrations of  $^{90}\text{Sr}$  in molluscs from Kuršių Marios Lagoon (Table 6) exceeded the concentrations of  $^{90}\text{Sr}$  in phytoplankton and in rooted aquatic plants.

Table 6. Concentration of  $^{90}\text{Sr}$  in molluscs from Kuršių Marios Lagoon (Bq/kg).

Species	Month		
	05	06	09
<i>Anadonta piscinalis</i>	31.1	11.1	12.0
<i>Dreissena polymorpha</i>	39.2	11.5	22.4

Concentrations of  $^{90}\text{Sr}$  in fish organs and tissues, independent of species and feeding type, indicate the analogous distribution character of this radionuclide (Table 7). Aquatic life accumulate  $^{90}\text{Sr}$  through water or with food. The data indicate that  $^{90}\text{Sr}$  accumulates in fish organisms, as well as in molluscs, mainly from the water. Experimental investigations indicated that only 30% of  $^{90}\text{Sr}$  in shells of molluscs was assimilated with radioactive food (Marčiulionienė *et al.* 1979). Investigations indicated that the ratio of  $^{90}\text{Sr}$  concentrations in fish and water was 10.6, while in fish and food it was 0.46. Thus, one can draw a conclusion that assurance of stability of water quality is the essence of normal ecosystem function.

Table 7. Distribution of  $^{90}\text{Sr}$  in fish organs and tissues (percent of total).

Organs, tissues	Pike-perch	Burbot
Backbones	19.9	35.1
Muscles	0.5	0.7
Head bones	15.9	31.8
Skin with scale	28.3	30.4
Fins	29.8	Not detected
Internal organs	0.4	2.0
Jaws	5.4	Not detected

It has been established that  $^{90}\text{Sr}$ , once in a water basin, distributes unequally among ecosystem components (Table 8). Its maximum level has been fixed in water where it is constantly replenished through atmospheric fallout, and in molluscs which use  $^{90}\text{Sr}$  as a calcium analogue in shell forming. Phytoplankton, as well as macrophytes which accumulate  $^{90}\text{Sr}$ , fall to the bottom when they finish their existence, and thereby take part in bottom sediment activity. Phytoplankton has limited capability to accumulate  $^{90}\text{Sr}$ , but because of its large biomass and short existence (5-6 days) it can contribute a significant portion of the radioactivity that is found in bottom sediments.

Table 8. Distribution of  $^{90}\text{Sr}$  in ecosystem components of Kuršių Marios Lagoon.

Component	Percent $^{90}\text{Sr}$ of total
Water	33.0
Molluscs	33.0
Bottom sediments	19.0
Water plants	10.6
Plankton	4.4
Fishes	1.0

Source: Dušauskienė-Duž 1978

Because of anthropogenic factors that cause degradation in water quality the physiological state of aquatic life deteriorates, and as a consequence the ratio between production and destruction processes is reduced. Thus, destruction processes begin to dominate and lead to new ecosystem equilibria and total disappearance of some of the original components. Mechanical sedimentation activity processes, rather than biological processes, are characteristic of the Kuršių Marios Lagoon ecosystem. This increases the accumulation of pollution in the bottom sediments, thereby causing formation of a second-rate long-term pollution source which is of great danger to benthic organisms.

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# INVESTIGATIONS OF THYROID DISEASES IN LITHUANIA IN RELATION TO RADIATION DOSES FROM CHERNOBYL

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## ABSTRACT

Iodine deficiency in the soil in some parts of Lithuania, especially the southern regions, contributes to thyroid abnormalities in these regions. The prevalence of thyroid problems in southern Lithuania was tremendously increased by the atmospheric distribution of iodine-131 as a consequence of the Chernobyl accident in 1986. Studies are now underway in Lithuania to expand knowledge of baseline formation and the extent of damage as a consequence of the Chernobyl accident.

## INTRODUCTION AND BACKGROUND

The epidemiology of thyroid diseases varies in different countries, and there are no precise data about this problem in Lithuania. According to Kiauleikienė and Kiauleikis (1989), 35-50% of children and teenagers examined have thyroid abnormalities. Investigations of the Institute of Endocrinology of Kaunas Medical Academy showed that 15% of school children in Kaunas have thyroid disease. The most frequent abnormalities encountered were hyperplasia of thyroid gland, autoimmune thyroiditis, and nodular goiter. It has been noted that thyroid disease in Lithuania is on the increase (Janulionytė *et al.* 1990, Nedveckaitė *et al.* in press). There are many factors that influence thyroid tissue and its functions. These factors include the radioactive iodine isotopes that comprised a large part of the atmospheric pollution that fell on Belarus, some regions of Ukraine and Russia, Poland, Sweden, and Lithuania after the accident at Chernobyl (Figure 1).

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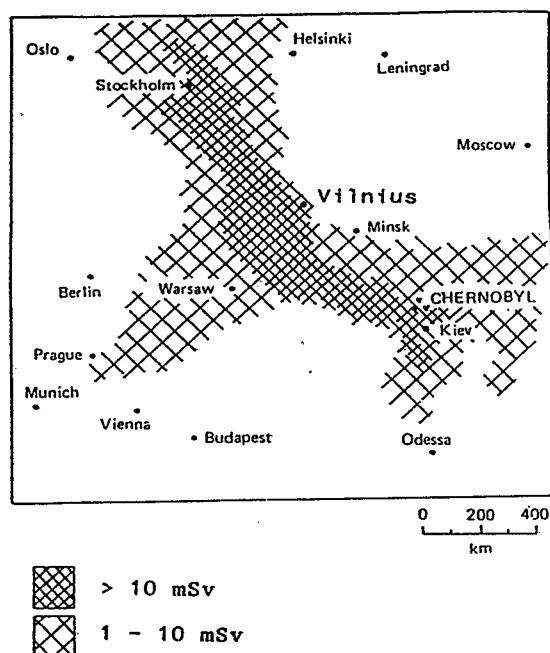


Figure 1. Predicted doses of  $^{131}\text{I}$  to adult thyroids accumulated from 26 April to 1 May 1986, by Lawrence Livermore National Laboratory.

Radionuclide uptake in thyroid is usually measured by special detectors placed directly over this tissue. Such measurements were not routinely performed in Lithuania after the Chernobyl accident for reasons beyond control of the experimenters. Instead, reconstruction of radioactive dose-equivalent in thyroid was performed by means of a three-compartmental mathematical model for the study of iodine transport kinetics in the human body. This involves a deterministic ICRP model (Figure 2) and dose factor values (Anonymous 1978) which convert measurable quantities of radioiodine activity in air and food (Styro *et al.* 1992) into an estimate of thyroid dose-equivalent.

The initial thyroid dose-equivalent assessment by this means for inhabitants of Lithuania was performed in May 1986. There are uncertainties associated with model predictions, such as errors in model structure and environmental variability, as well as uncertainties in parameter values (Werner and Ingbar 1978). A preliminary examination of radioiodine thyroid dose-equivalent to healthy adults was determined by a stochastic Monte Carlo approach having regard for the uncertainties mentioned above, and was  $9.8 \pm 1.0$  mSv. This preliminary analysis showed that estimates of dose may be made from various starting points. A purely deterministic approach does not make allowance for the magnitude of real doses. Results of a stochastic Monte Carlo-generated distribution predicted that 30% of the adult population received less than this value, and 5% of the adult inhabitants would have received thyroid doses ranging from 37 to 100 mSv (Figure 3). Infants and children were exposed to radiation doses up to 10 times higher.



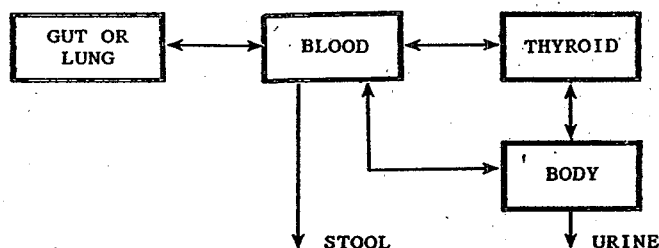


Figure 2. Model for the study of iodine transport kinetics in human body.

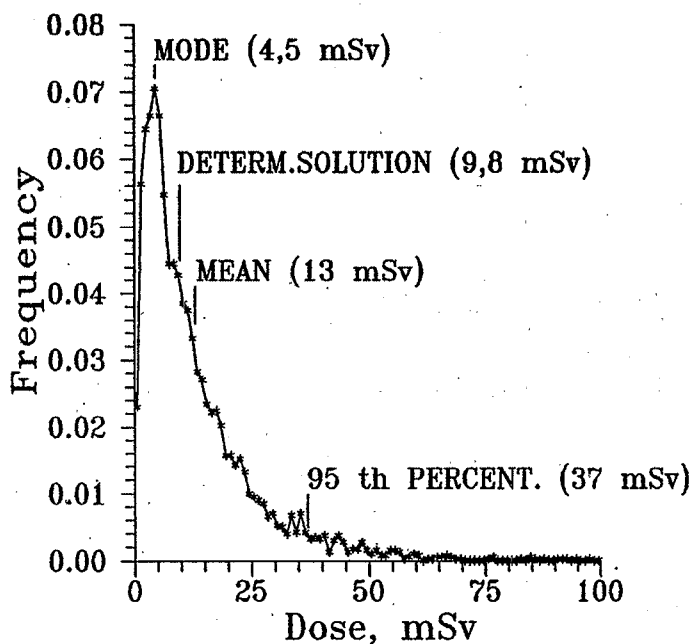


Figure 3. Monte Carlo generated frequency distribution of dose (mSv) to the thyroid gland of healthy adults from ingestion of milk products in southern part of Lithuania, May 1986.

According to Galle (1987) Lithuanian inhabitants received thyroid radiation doses which may have a negative influence on the development of thyroid cells, but not their function; under this influence, nodules and autoimmune alterations can appear. The ecological and economical situation in Lithuania, separate from the influence of the Chernobyl Accident, contribute to the frequency of thyroid pathology.

## RESEARCH PROGRAM AND OBJECTIVES

As a consequence of the thyroid diseases problem, especially among children, the Open Society Fund of Lithuania is supporting a Research Project "Relation between Radioactive Iodine Isotope Doses and Thyroid Diseases after the Chernobyl Accident (Epidemiological and Clinical Study in Lithuania)." In addition, the Lithuania Department of Health has initiated a program entitled "Investigation on Epidemiology of Thyroid Diseases in Lithuania," under the direction of the Kaunas Medical Academy Thyroid Laboratory of the Institute of Endocrinology, together with the Medical Faculty of Vilnius University and the Laboratory of Radiation Safety of the Institute of Physics. The program was initiated in collaboration with the Institute of Biomedical Investigations of Kaunas Medical Academy, location of the International Prophylactic Program of the World Health Organization (WHO).

The main purposes of these research projects and the overall program is to determine the incidences of thyroid diseases in the population of Lithuania, to investigate the relations between thyroid pathology and radioactive thyroid dose assessment, and the radioecological situation of the region. The main tasks are:

1. To investigate the child and adult populations from different regions and cities, evaluating their thyroid condition, diagnose disorders, and develop treatment strategies.
2. To evaluate the radioecological status throughout Lithuania.
3. To establish the relationship between incidence of thyroid diseases and exposure to radioiodine and other radionuclides.
4. To investigate iodine-deficient areas and evaluate the influence of this deficiency to thyroid pathology.
5. To map the incidence of thyroid pathology in Lithuania.
6. To confirm the findings and make recommendations to the Department of Health regarding preventive measures and treatment of thyroid disorders.

These research projects and program will permit partial estimation of the impact of the Chernobyl accident on Lithuania. Current understanding of the period of latency of thyroid diseases as a consequence of the influence of ionizing radiation varies between 5 and 30 years. It must be kept in mind, that at the present time in Belarus malignant thyroid diseases have undergone a rise, especially among infants and children (Polianskaja *et al.* 1991). Within the framework of these research projects and programs, investigation of thyroid burden in children, especially malignant diseases, will be carried out as a separate program.

The Thyroid Laboratory of Institute of Endocrinology has prepared a standard questionnaire; this information will be computerized according to the guidelines of WHO, "Investigations on Chernobyl Accident Medical Consequences." International collaboration will then be possible to compare the result of our research work with the results of others.

## PROGRESS TO DATE AND FUTURE PLANS

Some regions of Lithuania were visited in 1993, and result of examinations conducted are presented in Table 1. Ultrasound examinations were conducted using "real time" method which makes it possible to measure the size of thyroid by volumetry. These volumetric measurements were made using methods of the Belarus Radiation Medicine Institute; these are simple, although the equipment operator must be experienced. Instrumentation was made available through the Open Society Fund of Lithuania. Results are presented in Table 2. Control groups of healthy adults in each region have been selected and are now being studied to establish the median size of adult thyroid in Lithuania for comparison.

Table 1. Prevalence of thyroid gland pathology in some regions of Lithuania, 1993.

Region	Persons examined	Number	Abnormal thyroid, %	Nodules in thyroid, %	Increased ATMA, %
Kaišiadoryų	adult	300	22	2.5	15.1
Varėnos	adult	400	23	4	14.7
Kupiškio	adult	100	24	6	not tested
Kupiškis	students	372	47.5	12	not tested
Salčininkai	students	111	70	10	not tested
Zarasai	students	220	57	12.5	11.6

Table 2. Thyroid volumetric data from some regions of Lithuania, 1993.

Region	Thyroid volume, ml	
	Men	Women
Kaišiadoryų	16.3 ±1.0	15.3 ±0.8
Varėnos	20.8 ±3.6	17.0 ±3.4
Kupiškio	22.7 ±2.6	14.8 ±2.3

Collaboration with the Belarus Institute of Radiation Medicine helped us to carry out investigations on iodine concentration in urine. Twenty people from the Varėnos region were randomly selected for determination of the iodine excretion using the Cerium-Arsenite method. It was determined that the average iodine concentration in the urine was  $65.5 \pm 8.2 \mu\text{g/L}$ , as compared with a norm of more than  $100 \mu\text{g/L}$  (Šidlauskas, unpublished data). Concentrations from 50 to  $100 \mu\text{g/L}$  indicate mild iodine

deficiency. The result of these investigations show that in Lithuania there is a problem of iodine deficiency, separate from the problem of radioactive pollution. The turnover of the stable iodine will be investigated in other regions as well.

Future plans include of investigation of nitrite-nitrate concentration in ground water in some regions of Lithuania. There is some information in the literature suggesting that agricultural chemicals and other types of ecological contamination are instrumental in provocation of thyroid disorders.

#### ACKNOWLEDGEMENT

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# MECHANISM OF OXIDATION OF $H_2S$ IN THE BALTIC SEA

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## ABSTRACT

The mechanism of oxidation of  $H_2S$  in Baltic Sea water has been modelled in the laboratory based on our hydrochemical investigations data from the Baltic Sea. Our preliminary potentiometric and analytical investigations enable us to evaluate existing mechanisms of sulfide oxidation in seawater and to propose a new one through a complicated chain reaction caused by active forms of oxygen. The reaction enables formation of intermediate unstable sulfur compounds including  $HSO^-$  and  $HSO_2^-$ . These compounds in reaction with other products of a chain process, form the final products that have been analytically observed:  $HS_n^-$ ,  $S^0$ ,  $S_2O_3^{2-}$  and  $SO_4^{2-}$ .

## INTRODUCTION

At very irregular intervals, some parts of the bottom of the Baltic Sea may be entirely devoid of fauna for months or years as a result of oxygen deficiency and the subsequent presence of hydrogen sulfide. At its worst, these deserted areas may comprise up to 100,000 km<sup>2</sup>; equivalent to approximately 25% of the total surface area of the Baltic Sea. These major stress factors fluctuate intermittently and irregularly (Andersin *et al.* 1977, Voipio 1981). Hydrogen sulfide is oxidized in the sea water redox zone by microbiological as well as by chemical processes (Leonov and Aizatullin 1987). Using antiseptics suppressing microbiological activity, it has been determined that  $H_2S$  is oxidized by a chemical pathway (Sorokin 1970). The products of hydrogen sulfide oxidation by oxygen in sea water has been determined by analytical and indirect methods. The basic products are as follows: elemental sulfur ( $S^0$ ), polysulfides ( $S_n^{2-}$ ), sulfites ( $SO_3^{2-}$ ), thiosulfates ( $S_2O_3^{2-}$ ), sulfates ( $SO_4^{2-}$ ). Dithionite ( $S_2O_4^{2-}$ ), dithionate ( $S_2O_6^{2-}$ ), and tetrathionate ( $S_4O_6^{2-}$ ), this last in acidic media at pH 7, have also been reported (Leonov and Aizatullin 1987).

From the variety of oxidation products, an assumption could be made that the mechanism of oxidation is very complicated and still not fully understood. It is considered that hydrogen sulfide oxidation is going on in accordance with a chain reaction mechanism (autocatalytic reaction) and polysulfide ions  $S_n^{2-}$  serve as catalysts (Bowers *et al.* 1966, Chen and Morris 1971). However, other oxidation reaction products, such sulfate and thiosulfate ions, act as inhibitors and slow the reaction.

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Oxidation reactions of hydrogen sulfide are slowed down or speeded up by catalysts, such as calcium or magnesium ions, heavy metals, phosphates, pH, contact surfaces, and organic substances. Such a great sensitivity of reaction kinetics to various admixtures and mediums makes it difficult to assess the theoretical hydrogen sulfide oxidation rate in seawater based on studies under natural conditions. The most reliable factor here would be analytical data on seawater, as well as on laboratory experiments modelling natural conditions. For this purpose, H<sub>2</sub>S oxidation has been modelled in water of the Baltic Sea under laboratory conditions.

## MATERIALS AND METHODS

### Hydrochemical Investigations

Anaerobic processes in the Baltic Sea bottom have been investigated by seasonal expeditions carried out in 1981-1991 by the Klaipėda Hydrometeorological Observatory, Lithuania Environmental Protection Department (Figure 1).

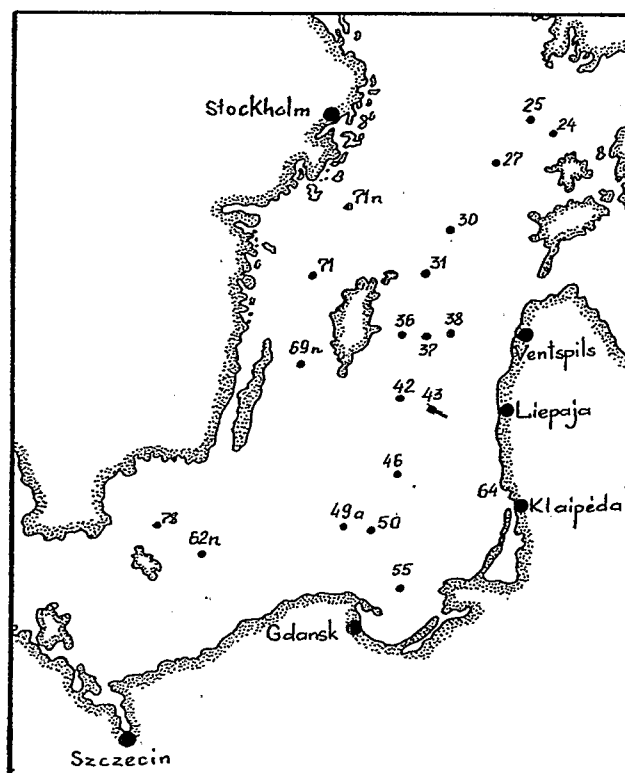


Figure 1. Location of sampling stations in the Baltic Sea.

Determinations of  $\text{H}_2\text{S}$  by a colorimetric method with p-phenylenediamine-dihydrochloride (Fonselius 1976) were carried out almost 20 years ago (Anonymous 1976-1991). Since 1981  $\text{H}_2\text{S}$  has been analyzed continuously at a depth of 80 m and deeper under the National Baltic Sea Monitoring Programme.

### Modelling of Hydrogen Sulfide Oxidation Reactions

Modelling of  $\text{H}_2\text{S}$  oxidation was carried out in samples of water from the Baltic Sea under laboratory conditions. Recrystallized  $\text{Na}_2\text{S}$  was used to prepare a 0.2 M solution in freshly boiled distilled water and kept isolated from air. In the absence of dissolved oxygen, the mean  $\text{H}_2\text{S}$  concentration in the Baltic Sea is about 0.1 mM. The solubility of oxygen at a temperature of 5°C is 55 ml/L or 1.5 mM. Thus, in order to have sufficient amount of dissolved oxygen for oxidation, 0.1-1 mM concentrations of  $\text{H}_2\text{S}$  solution should be used for laboratory testing.

Stock solutions of sodium sulfide were dissolved in distilled water or seawater and stored in flasks with corks slightly loose so that oxygen might penetrate. After dissolving  $\text{Na}_2\text{S}$  in distilled water, the pH of the resultant solution was 8.5; it was reduced to the seawater pH level of 7.5 by adding a drop of 0.1 M HCl. After dissolving  $\text{Na}_2\text{S}$  in the seawater, the pH level was unchanged, apparently due to the buffering capability of the seawater. The tests have been carried out at a temperature of 20°C. Periodically the samples were taken and titrated by iodine solution in alkaline medium in order to determine the amount of  $\text{H}_2\text{S}$  which had not been oxidized by oxygen. A platinum electrode together with a saturated calomel electrode as a reference electrode was used to indicate the end-point.

## RESULTS

### Formation and Change of Hydrogen Sulfide Zones

The redox conditions in the bottom water and sediment surface have fluctuated widely during the last 400 years (Hallberg 1974). During this century the oxygen deficiency as well as the formation of hydrogen sulfide in water layers below the salinity halocline at a depth of 70-90 m and in isolated basins was observed over 60 years ago (Granqvist 1931). The interchange of irregular aerobic and anaerobic periods are presumably determined by the intensity of the inflow of the North Sea waters together with an anthropogenic impact (Fonselius 1962, 1969).

The present regime of oxygen in the deep bottoms could be dated to 1980. According to Nehring and Matthäus (1990), there has been no dissolved oxygen detected in the Gotland Deep at a depth of 248 m since 1980, and at a depth of 200 m since 1983. According to our data of 1984-1991, dissolved oxygen has never been observed in the Gotland Deep below 200 m. During the same period in the Gdansk Deep at a depth of 100 m and below, hydrogen sulfide was observed to be occasionally formed and

this hydrogen sulfide zone persisted for 4-5 months. In the Gotland Deep, however, a hydrogen sulfide zone was constantly being formed, with the upper boundary at a depth of 130-140 m.

During the period 1981-1991, slight decreases in hydrogen sulfide concentration have been observed in the bottom layer of the Gotland Deep, and for the most recent 3 years there is an apparent positive trend (Figure 2). An intermediate redox zone, where both dissolved oxygen and hydrogen sulfide are observed, exists in the deep layers of the Baltic Sea above the hydrogen sulfide zone (Table 1). Hydrogen sulfide oxidation in this redox zone is one of the main chemical processes upon which changes of other hydrochemical variables depend, as well as does the general condition in the bottom layers of the Baltic Sea.

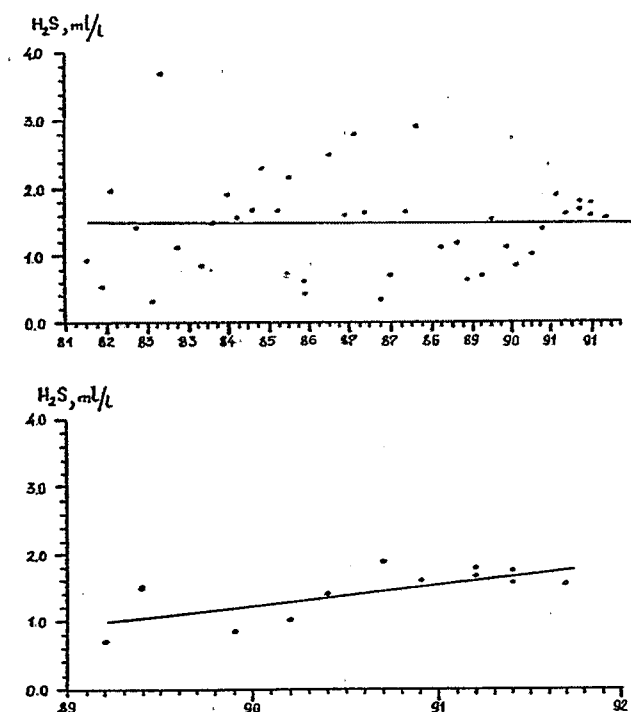


Figure 2. Long-term trends of annual means of  $H_2S$  in the bottom water (below 200 m) of the Gotland Deep (source Anonymous 1976-1991).

In the central part of the Baltic Sea, the redox zone is between 80-150 m below the surface. In the Landsort Deep (station 71n), the redox zone is between 140 and 250 m below the surface. Dissolved oxygen concentration in this zone was 0.0-1.0 ml/L, although occasionally as high as 5.5-6.0 ml/L, and the pH was 6.9-7.8. Relative alkalinity affects the oxidation process of hydrogen sulfide and the resulting sulfur forms in solution. The predominate form of  $H_2S$  at pH 7 is  $HS^-$ .



Table 1. Background ranges of pH, O<sub>2</sub>, and H<sub>2</sub>S in the redox layer of the Baltic Sea in 1981-1991.

Station	Depth (m)	pH	O <sub>2</sub> (ml/L)	H <sub>2</sub> S (ml/L)
24	100-105	7.32-7.36	1.04-1.71	0.06-0.18
25	80-83	7.56-7.88	3.60-3.73	0.06-0.08
27	100-150	7.18-7.35	0.25-0.99	0.11-0.17
30	80-118	7.46-7.47	1.94-2.30	0.08-0.15
31	150	6.88-7.56	0.24-0.68	0.08-0.10
36	80-108	7.16-7.51	0.29-2.32	0.06-0.17
37	80-100	7.00-7.68	1.39-3.63	0.06-0.10
	150	7.19-7.51	0.09-0.91	0.07-0.70
38	80-100	7.17-7.86	0.54-3.49	0.08-0.14
	150-160	7.31-7.62	0.39-0.91	0.07-0.16
42	80-100	7.30-7.47	2.62-4.17	0.07-0.08
	150-158	7.18-7.67	0.23-0.51	0.16-0.17
43	80-100	7.27-7.50	0.81-5.55	0.06-0.19
	150	7.32-7.75	0.49-2.72	0.15-0.18
50	83	7.28	0.76	0.15
55	95-104	7.28-7.44	0.14-2.11	0.06-0.14
62n	80-88	7.36-7.72	0.13-2.58	0.09-0.16
78	78	7.21	1.08	0.06
69n	100-108	7.20-7.49	0.23-6.02	0.07-0.19
71	100	7.33	0.27	0.08
	150-195	6.96-7.36	0.12-1.08	0.06-0.18
71n	150	7.00-7.85	0.13-1.11	0.06-0.17
	200-249	7.21-7.43	0.15-0.73	0.05-0.15

Peculiarities of the oxygen and hydrogen sulfide concentrations in the bottom layers of the Black Sea (reported by Skopintsev 1975) and of the Baltic Sea are shown in Figure 3. The concentration of oxygen is higher at the Baltic Sea bottom: it depends, presumably, not only on water dynamics but also on physical processes such as temperature. At the temperatures in the Baltic Sea bottom layers (4.0-6.3°C) H<sub>2</sub>S oxidation processes occur more slowly (Figure 4). The rates of redox reactions are partially dependent upon concentrations, and the assumption is made that there are minimal biochemical processes occurring.

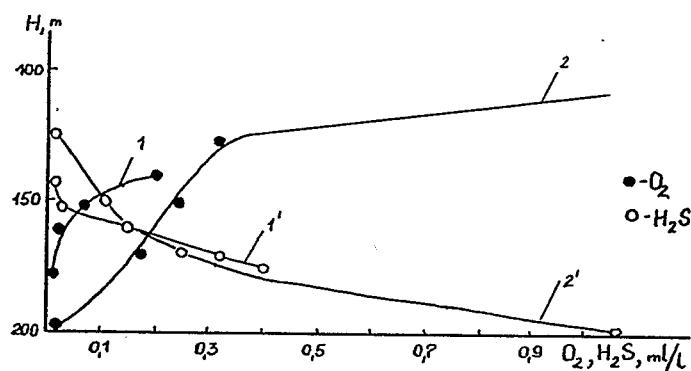


Figure 3. Changes in oxygen and hydrogen sulfide concentrations (ml/L) in the bottom layers of (1) the Black Sea (from Skopintsev 1975), and (2) the Baltic Sea (source Anonymous 1976-1991).

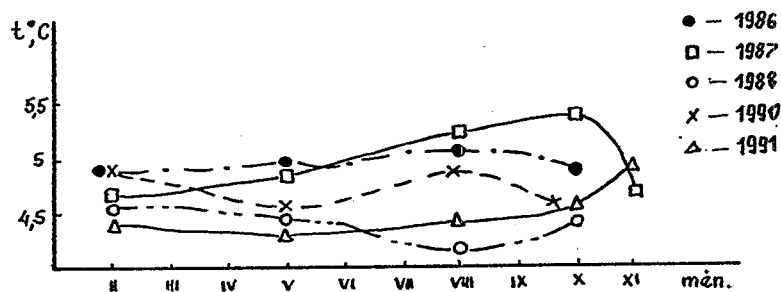


Figure 4. Temperature annual changes in the bottom layers of the Baltic Sea (source Anonymous 1976-1991).

Many scientists suggest models and empiric dependences for calculation of some redox zone indices (Stanev 1986, Leonov and Aizatullin 1987); however, standard expeditional investigations are not fully efficient. Besides, detailed investigations of vertical profiles of hydrogen sulfide and oxygen concentration distribution as well a modelling in the laboratory are required.

#### Modelling of Hydrogen Sulfide Oxidation

Curves of potentiometric titration (Figure 5) show that the redox potential increased from  $-350$  mV to  $+300$  mV during oxidation by iodine of a fresh  $\text{H}_2\text{S}$  solution. Two potential leaps could be observed in the titration curve. The first leap (6 eqv.  $\text{I}_2/\text{mol H}_2\text{S}$ ) should correspond to  $\text{HS}^-$  oxidation to  $\text{S}^{4+}$ , i.e., the sulfite ion:



The second leap (8 eqv.  $I_2$ /mol  $H_2S$ ) should correspond to full  $H_2S$  oxidation, according to the equation:



During the period of  $H_2S$  oxidation, the negative initial potential decreases, and after all  $HS^-$  has been transferred to polysulfides, it remains at  $-100 \pm 60$  mV (Figure 5).

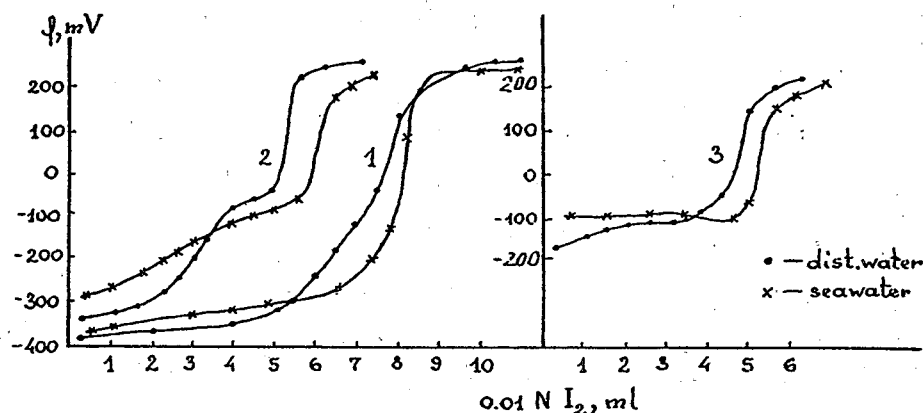


Figure 5. Potentiometric titration curves of 0.85 mM/L  $H_2S$  by iodine in alkaline solution: 1 – initial solution; 2 – after 20 hours oxidation; 3 – after 70 hours oxidation at 20°C.

Kinetic  $H_2S$  oxidation curves (Figure 6) show that  $H_2S$  oxidation in the sea water is going on a bit slower than in distilled water: it is conditioned by total impact of other ions existing in the sea water. According to our calculations, intermediate oxidation products account for 10% of the initial amount of hydrogen sulfide. The remainder is in the form of elemental crystallin sulfur ( $S_8$ ) which is deposited, and of sulfates, the origin of which is difficult to determine because of their great amount in seawater.

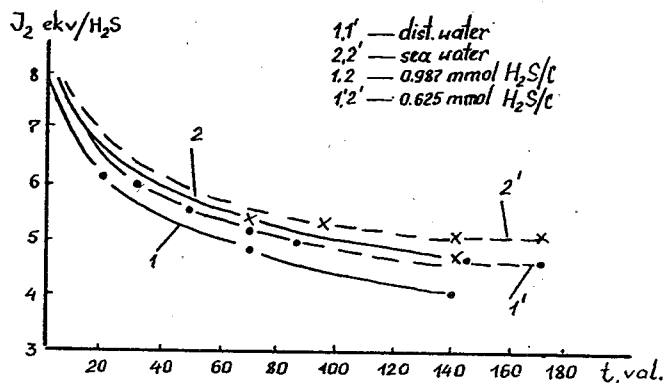


Figure 6. Kinetics curves for oxidation of  $H_2S$  (pH 7.5) at 20°C.

## DISCUSSION

### Mechanism of Oxidation of H<sub>2</sub>S

As it has been mentioned, the mechanism of oxidation of H<sub>2</sub>S by soluble oxygen is still not fully understood. Reactions presented in the literature, such as Leonov (1987), are as follows:



These reactions are not correct because the product is elemental sulfur which later reacts with hydrogen sulfide, to form polysulfides. Besides, this reaction is tri-molecular, and the occurrence of such reactions is rather doubtful.

Through investigation of thiosulfate acidic decomposition, H<sub>2</sub>S and SO<sub>2</sub> interaction in Wackenroder's liquid and other polythionate reactions, it has been determined that elemental sulfur appears in the form of S<sub>8</sub>. This molecule is formed through a number of intermediate products. For example, thiosulfate acid decomposition, expressed by the simple equation



is being carried out through the intermediate product sulfan-monosulfonates HS<sub>n</sub>SO<sub>3</sub>H where n = 1 to 8 (Yanitskij *et al.* 1971). Only after the product HS<sub>8</sub>SO<sub>3</sub>H with the greatest amount of sulfur has been formed does the molecule of S<sub>8</sub> appear, and the rest is sulfite acid. Intermediate products of this reaction, sulfan-monosulfonates, were isolated as complex nitron (Nt) salts (Yanitskij *et al.* 1971).

Our investigations conclude that the main product of hydrogen sulfide oxidation is molecular sulfur. It should be considered that molecular sulfur results from a number of already identified intermediate products, namely polysulfides. Isolation of molecular sulfur by reaction (2) is doubtful. There are data in the literature indicating that the initial hydrogen sulfide oxidation stage is a chain reaction, although radicals or ion radicals participating in the reaction are unknown. The most believable initiator of the chain reaction is active oxygen, which can exist in several forms.

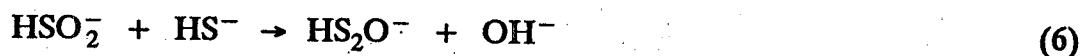
Thus, the first stage of the reaction would be the reaction with oxygen:



This hypothetical unstable compound could pose the further chain reaction:



The sulfoxilic acid anion  $\text{HSO}_2^-$  is isolated as a salt and is a very active compound. In the further interaction of  $\text{HSO}_2^-$  with hydrosulfide, polysulfides can be formed:



When the stage of  $\text{HS}_8\text{S}^-$  formation is reached, molecular sulfur ( $\text{S}_8$ ) is formed. However, sulfoxilate, because of its reactivity, can react with other substances participating in the reaction, resulting in hydrosulfite, thiosulfate, sulfate:



These reactions are much more probable since they are homogenic and bimolecular: similar mechanisms have been proven for many other reactions. These reactions also correspond to the contemporary attitude towards chemistry, and are confirmed by the variety of products found in hydrosulfide oxidation by oxygen.

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# SOME BIOLOGICAL CONSEQUENCES OF DEEP WATER STAGNATION IN THE EASTERN GOTLAND BASIN, BALTIC SEA IN 1980s

S. Olenin<sup>1</sup>

## ABSTRACT

The current stagnation period in the Eastern Gotland Basin of the Baltic Sea has resulted either in total destruction of the bottom communities and formation of so called benthic deserts, or in shifting of the dominant life forms of macrozoobenthos in the deep water areas. Benthic deserts now occupy the slope of the Eastern Gotland Basin below the primary halocline (80-85 m) in the northern part (near Saaremaa Island), below 100-110 m in the central part (Ventspils and Liepaja areas), and below 120 m in the southern part. In the southern areas the slowly moving infaunal deposit feeder *Scoloplos armiger* (Orbiniidae, Polychaeta) has been displaced by the active hemipelagic predator *Antinoella sarsi* (Harmothoe, Polynoidae, Polychaeta).

## INTRODUCTION

Strong deficiency of oxygen and almost constant presence of hydrogen sulfide in the deep-water layers have become one of the main features of the Baltic Sea ecosystem. The negative consequences of this phenomenon include destruction of bottom macrofauna and formation of large benthic deserts in all the Baltic deeps. The largest of benthic deserts in the Baltic Sea exists in the Eastern Gotland Basin, where the present stagnation period has lasted more than 15 years; this is the most serious stagnation event ever recorded in the Baltic Sea. The drastic changes in environmental conditions include mean decrease in salinity, temperature, and oxygen content, as well as an increase in hydrogen sulfide concentration (Kalejs and Ojaveer 1989, Nehring *et al.* 1989, HELCOM 1990, Matthäus 1991). This paper describes changes in macrozoobenthos as a consequence of the present stagnation process in the Eastern Gotland Basin.

## MATERIALS AND METHODS

Samples were collected in 1985-1989 in the northern, central, and southern parts of the basin along latitudinal (Sections 1-3, 5) and longitudinal (Section 4) directions starting from 70-90 m below the surface and at approximately every 10 m down to 110-140 m (Figure 1). At each station one to six bottom samples were taken with a 0.1 m<sup>2</sup> van Veen grab. Most samples were sieved through 0.5 mm meshes (a 1.0 mm mesh sieve was used in 1987 only) and preserved with 4% formalin neutralized with NaHCO<sub>3</sub>. A total of 338 samples were collected as follows: Section 1 - 107; Section 2 - 54; Section 3 - 111; Section 4 - 35; Section 5 - 31.

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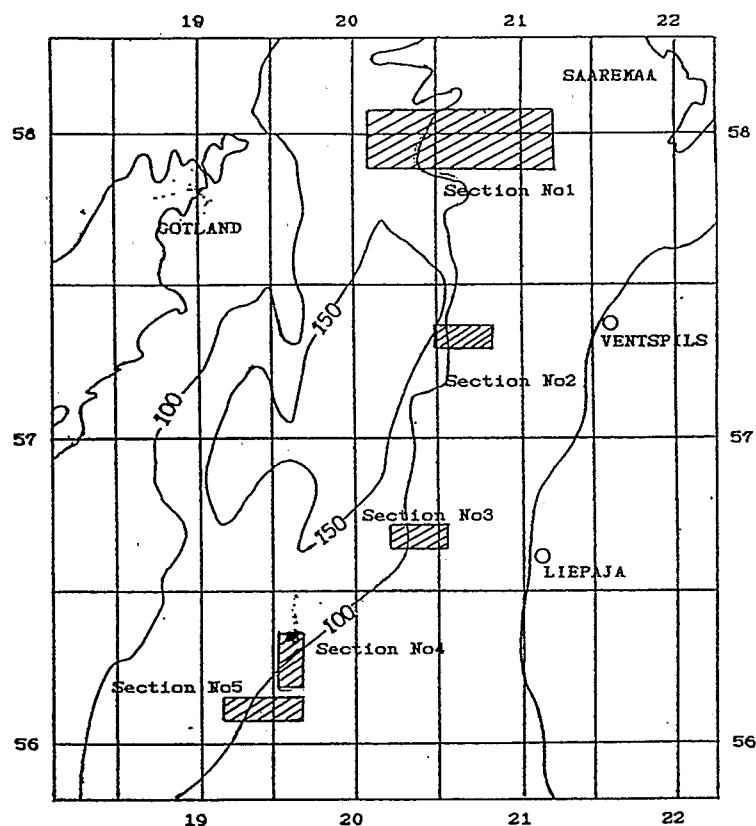


Figure 1. The zoobenthos sampling stations in the northern (Section 1), central (Section 2,3), and southern (Section 4,5) parts of the Eastern Gotland Basin in 1985-1989.

## RESULTS AND DISCUSSION

### Ecological Zones on the Slopes of the Eastern Gotland Basin

Due to strong vertical stratification of the water body in the area of the Eastern Gotland Basin, a clear ecological zonation of the sea bottom exists. Three different zones have been distinguished on the slopes of the Eastern Gotland Basin, based on literature data (Elmgren 1975, 1978, Andersin *et al.* 1978, Zmudzinski 1978, Jarvekulg 1979, Seire 1988) and our studies (Jarvekulg and Olenin 1989, Olenin 1989). Every zone has clear biological features, which in many respects are determined by properties of the different water layers (Figure 2). This scheme of vertical hydrological structure is compiled from papers by Kalejs (1984) and Kalejs and Tamsalu (1984).



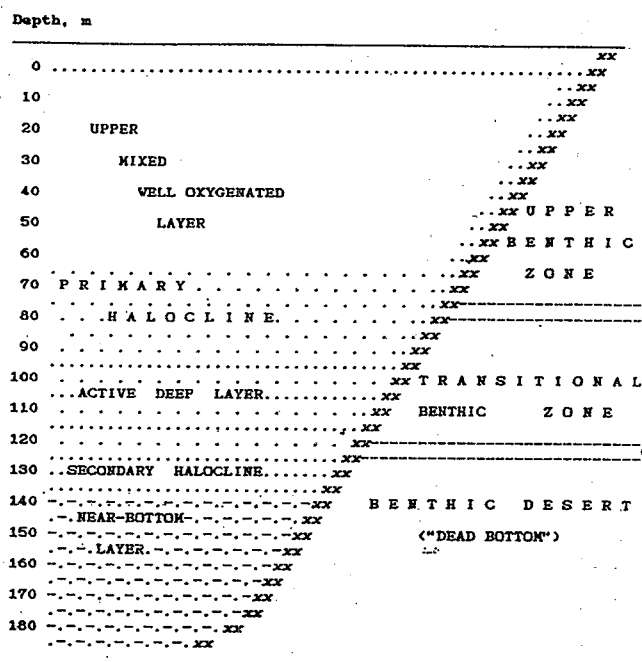


Figure 2. Ecological zonation of a sea bottom on the slopes of the Eastern Gotland Basin.

**Upper Benthic Zone.** This zone is situated in the well-oxygenated and mixed upper water layer. The lower border of this zone generally coincides with the lower border of the primary halocline (about 80-85 m). The macrofauna are comparatively diverse and abundant: the number of species is about eight to 12 per sample, total abundance fluctuates within the limits 1,000-10,000 individuals/m<sup>2</sup>, and biomass is between 10-100 g/m<sup>2</sup>. Commonly the benthic biomass is dominated by infaunal deposit and suspension feeding species with low mobility. The main biological feature to determine the border of the upper zone is the presence of a comparatively well-developed and mature bottom community, dominated by the bivalve mollusc *Macoma baltica*.

**Transition Benthic Zone.** Location of this zone generally depends on oxygen content in the active deep water layer which is situated below the primary halocline. Along this layer, which occupies the depths 100-130 m, influxes of saltwater from Skagerrack and Kattegat usually enter the eastern Baltic Sea (Kalejs and Tamsalu 1984). The transitional benthic zone is inhabited with a poor and sparse bottom community; the number of species is one to five per sample, total abundance usually is less than 10 individuals/m<sup>2</sup>, and total biomass usually varies within 0.001-1.00 g/m<sup>2</sup>. This community never reaches maturity because development is often interrupted by fluctuations of the water layer containing hydrogen sulfide. Only a few species resistant to low oxygen conditions are able to survive in this zone; these are hemipelagic

polychaete *Antinoella sarsi*, crustaceans *Saduria entomon*, *Diastylis rathkei* and *Pontoporeia femorata*. Usually only single specimens of the *A. sarsi* occur at the lower border. All species listed are able to settle in this zone at larval and/or adult stages, in the latter case migrating actively from the upper (cleaner) sections of the sea bottom.

**Benthic Desert.** This zone is located mainly in the near-bottom layer of the Gotland Basin which is oxygen-poor and continuously stagnant, and often contains hydrogen sulfide. The main biological feature of the benthic desert is absence of macrofauna, and synonyms for this zone are often seen in the literature as "lifeless zone," "azoic zone," and "dead bottom."

### Changes in Location of Ecological Zones in the Eastern Gotland Basin

**Northern Part of Eastern Gotland Basin (Section 1).** In the upper part of the slope (70-80 m), sampling was carried out at the beginning of the investigation period (May and August, 1985) and at the end (July, 1988; July, 1989). Only single specimens of *M. baltica* were found at 80 m in May, 1985 (24 individuals/m<sup>2</sup>, 10.56 g/m<sup>2</sup>) and a very poor community was detected at the same depth in August 1985, dominated by *P. femorata* (75 individuals/m<sup>2</sup>, 0.01 g/m<sup>2</sup>) (Figure 3). A mature "*Macoma baltica* community" (eight-ten species per sample; 1310-5880 individuals/m<sup>2</sup>, 21.6-118.4 g/m<sup>2</sup>) was noted at 75-79 m in 1988-1989. The critical depth for single adult specimens of *M. baltica* was 83 m. The border of the upper ecological zone was located approximately in the depth range of 75-80 m.

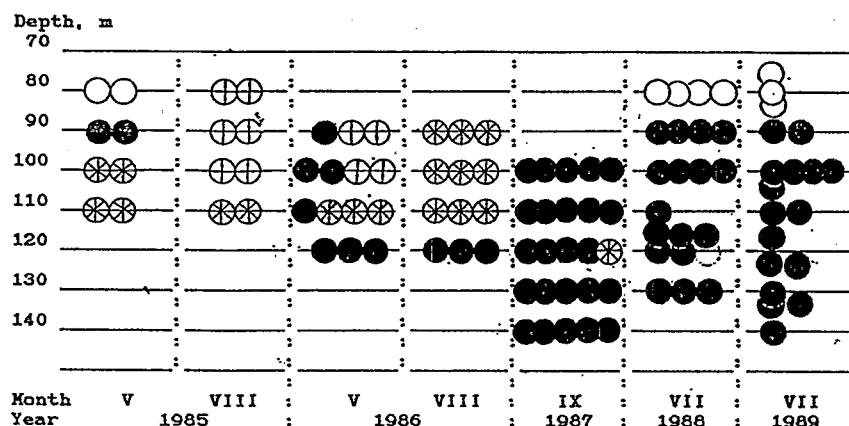


Figure 3. Vertical distribution of bottom macrofauna in the northern part of the Eastern Gotland Basin. One circle indicates one sample: open circle = "*Macoma baltica*" community; circle with a cross = community of the transitional zone consisting of few species; circle with a star = *Antinoella sarsi* only; filled circle = no macrofauna.

In the upper part of the transition zone (81-90 m) seven species were found. However, six of 16 samples taken at these depths during the study period were "empty" and in the other samples macrofauna were not abundant (10-140 individuals/m<sup>2</sup>; 0.05-1.83 g/m<sup>2</sup>). At 91-110 m, macrozoobenthos were represented by occasional specimens of *A. sarsi* until May 1986; only one crustacean, *D. rathkei*, was found (at 100 m). In September 1987 an expansion of the benthic desert was detected; only one specimen of *A. sarsi* was found in 25 samples taken at 100-140 m. In 1988-1989 no macrofauna were found in any sample below 85-89 m. Thus, in 1985-1986 the transitional zone covered the slope of the northern part of the basin approximately from the depth of 80 m down to at least 100 m. After 1987, this zone was completely displaced by the benthic desert.

*Central Part of the Eastern Gotland Basin in the Ventspils Area (Section 2).* In 1986, sampling was carried out between depths of 90-120 m, which was characterized as the transition zone. The bottom communities included two or three species per sample (*A. sarsi*, *S. entomon*, *P. femorata*, juveniles of *M. baltica*) at 90-110 m, and only one species (*A. sarsi*) at 120 m (Figure 4). The biomass reached 4.9-6.1 g/m<sup>2</sup> due to presence of big specimens of *S. entomon*; in the absence of that species the biomass

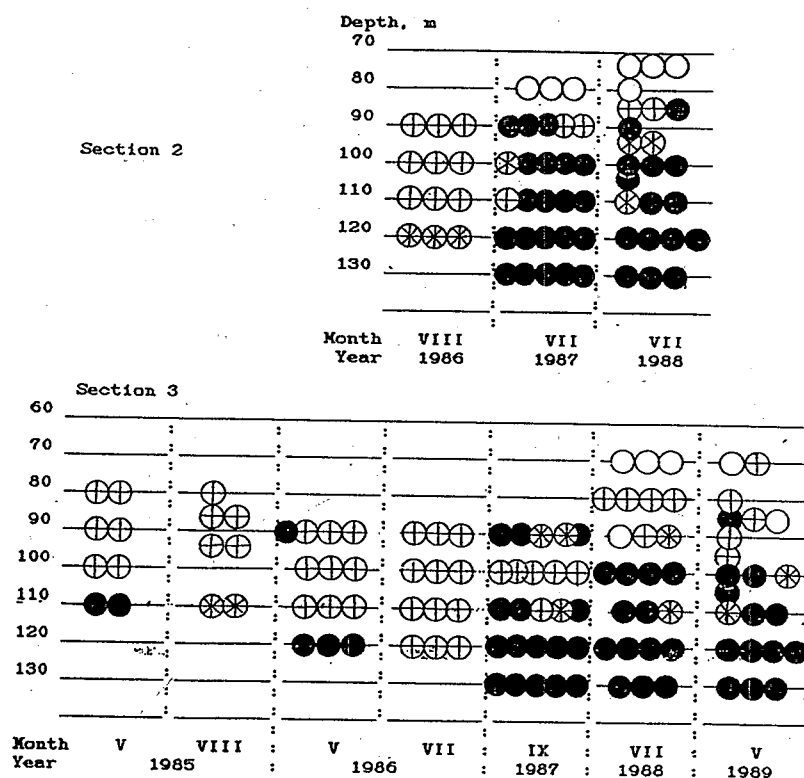


Figure 4. Vertical distribution of bottom macrofauna in the central part of the Eastern Gotland Basin in the vicinity of Ventspils (above) and in the vicinity of Liepaja (below). (Conventions see Figure 3)

did not exceed 0.01-0.06 g/m<sup>2</sup>. In 1988-1989 sampling carried out in the uppermost part of a slope showed that the mature "*Macoma baltica* community" occupied the bottom down to 80-81 m (four to nine species per sample; 50-1990 individuals/m<sup>2</sup>, 12.0-57.8 g/m<sup>2</sup>). The critical depth for a single adult specimen of *M. baltica* was 90 m.

Environmental conditions below 90 m became critical between 1986 and 1987: in 1986 macrofauna were found in all samples, however, in 1987-1988 27 of 34 samples taken at 90-120 m were "empty." The maximum depth at which macrofauna were noted was 109-110 m. Although expansion of the benthic desert took place in the central part of the Eastern Gotland Basin in 1987-1988, this expansion was not as abrupt as that in the northern area.

*Central Part of the Eastern Gotland Basin in the Liepaja Area (Section 3).* In May and July 1985, a bottom community consisting of six to nine species with low biomass (0.63-1.33 g/m<sup>2</sup>) was found in the upper part of the slope, at 80 m. There were few species characteristic of the upper benthic zone; these included the priapulid *Halicryptus spinulosus*, and the polychaetes *Pygospio elegans*, and *Terebellides stroemi*, but did not include adult *M. baltica* (Figure 4). In 1988-1989, the comparatively mature "*Macoma baltica* community," including six to nine species per sample, was found at 68-70 m (up to 2260 individuals/m<sup>2</sup>, and up to 20.97 g/m<sup>2</sup>). Furthermore, adult specimens of *M. baltica* were detected at 85 and 92 m. Concurrent with this, "empty" samples or samples without *M. baltica* were obtained at 70-92 m. Thus, the picture of ecological zonation in the upper part of the slope in the Liepaja area was not as clear as in Sections 1 or 2, apparently due to gentle sloping of the sea bottom in the upper part. Similar phenomena can be observed in littoral zones; the zonation is clearer on a steep rock shore than on a gentle beach.

The transition zone extended down at least 100-110 m in 1985 and 110-120 m in 1986. Unfortunately, due to lack of data at the lowermost depths, the border between this zone and the benthic desert could not be delineated more precisely. In 1987-1989, there were no benthic macroinvertebrates in 19 of 24 samples taken at 100-110 m; in one of the remaining five samples only *A. sarsi* and *Mysis oculata* were present, and in the other four samples only *A. sarsi*. At that time no macrofauna were found at 120 m and deeper. So, the border of benthic desert rose from approximately 120 m in 1986 to 100-110 m in 1987. On the whole, changes in 1986-1988 in the boundary of the benthic desert in the central part of the basin in the vicinity of Liepaja were similar to that in the Ventspils area.

*Southern Part of the Eastern Gotland Basin (Section 4).* In 1988 the macrofauna at 94-122 m was represented by *A. sarsi* with the exceptions of a single adult specimen of *M. baltica* at 97 m and of *P. elegans* at 108 m (Figure 5). There were no benthic macroinvertebrates at 130-140 m. In 1989 only *A. sarsi* and *Ostracoda* were detected between 99 and 118 m; four of seven samples taken at 110-120 m, and all the samples obtained at 125-138 m, were "empty." The border of the benthic desert was located approximately at 120-125 m.

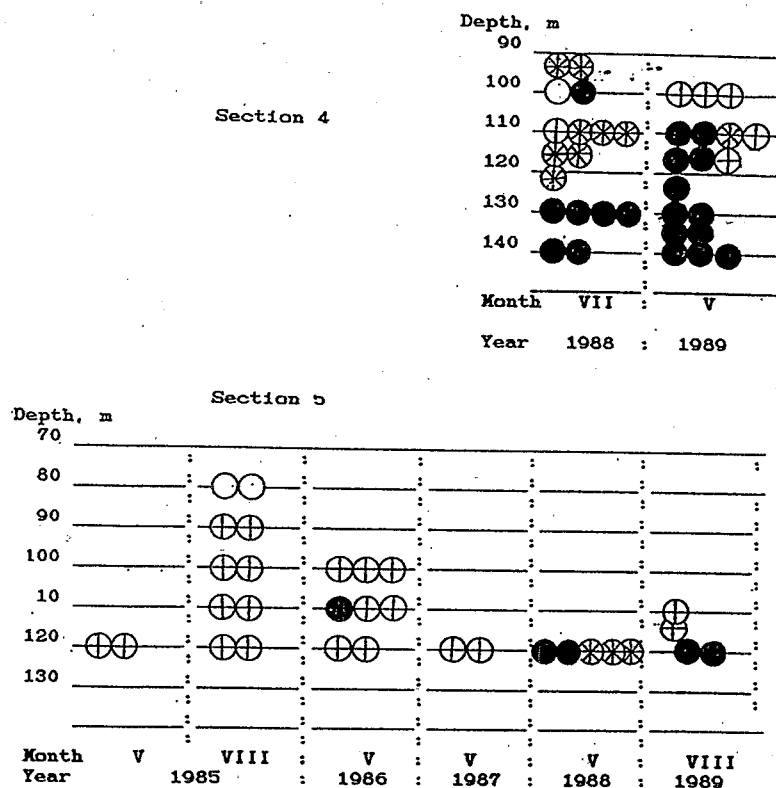


Figure 5. Vertical distribution of bottom macrofauna in the southern part of the Eastern Gotland Basin at meridional (above) and longitudinal (below) sections. (Conventions see Figure 3)

*Southern Part of the Eastern Gotland Basin (Section 5).* In 1985 the fairly diverse "*Macoma baltica* community" was found at 80 m (10-11 species per sample; 1100-1400 individuals/m<sup>2</sup>, 12.6-49.5 g/m<sup>2</sup>) (Figure 5). The macrofauna at the lower depths in 1985-1986 were also comparatively diverse; the bottom communities included immobile infaunal and epifaunal invertebrates (*H. spinulosus*, *P. elegans*, *Ampharete* sp., *Scoloplos armiger*) and "active swimmers" (*A. sarsi*, *Mysis* sp., *P. femorata*).

Deterioration was pronounced at the farthest west station of Section 5 at approximately 120 m. In 1985-1987, the bottom community consisted of two to five species with total biomass 2.48-6.63 g/m<sup>2</sup>, dominated by *S. armiger*. In 1988 only *A. sarsi* was noted, with biomass 0.15 g/m<sup>2</sup>, and in 1989 no macrofauna were found.

During the 1960s and 1970s the "*Scoloplos armiger* community" occupied the deepest sections, from 90 to about 140 m, of the slopes of the Northern and the Eastern Gotland Basins (Andersin *et al.* 1978, Jarvekulg 1979). During the 1980s complete deterioration occurred in the Northern and Central parts of the Baltic Sea, and at the present time the deep areas, represented earlier by the polychaete *S. armiger*, are void of infaunal deposit feeding animals. Because of this, there is no bioturbation of the sediments below the primary halocline, and organic matter, sinking from the upper layers of the water column, is no longer utilized as food for resident macrofauna. Several remarkable changes in the functioning of the Baltic Sea ecosystem have occurred as a consequence.

### CONCLUSIONS AND RECOMMENDATIONS

The benthic desert now occupies all the slopes below the primary halocline (80-85 m) in the northern part of the Eastern Gotland Basin, has reached depths of 100-110 m in the central part, and has reached 120 m in the southern part. Data on the collection of macrozoobenthos in the Eastern Gotland Basin clearly demonstrate that the benthic deserts has expanded in recent years.

Because of practical and scientific importance, regular information on changes in the bottom communities of the deep areas of the Baltic Sea are urgently needed. Benthic studies in the open Baltic Sea are the only investigations that clearly show biological long-term changes of the kind that may be expected due to eutrophication (Larsson *et al.* 1985). At the present time, almost all zoobenthos investigations in deep water areas are being conducted by different researchers operating independently within a framework of national programs; there are no regular sampling programs in the deep waters such as there are in inland waters. In addition, the grid of the HELCOM Baltic Monitoring Programme stations is too sparse for evaluation of ecological changes in the deep water areas of the Baltic Sea. Accordingly, in order to document the dynamics of the ecological zones in the Eastern Gotland Basin, annual observations should be continued.

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