VILNIUS UNIVERSITY

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INTEGRATED ASSESSMENT OF POLLUTION AND BIOMARKER RESPONSES IN THE BALTIC SEA

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Dedicated to the memory of
my husband Oleg Garnaga

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INTRODUCTION

The complexity and variability of aquatic systems, the influence of multiple environmental factors suggest that any of single measures is adequate for assessing the effects of multiple stressors on biota. There is no doubt that the result of chemical analyses is an important indication whether or not organisms are exposed to pollutants at unacceptable high levels (Den Besten, 1998). But the use of chemical criteria alone to assess the effects of water quality on ecosystems can be incomplete, because it does not include broader ecological view (Adams, 2005).

Biomarkers are useful tools, indicating bioavailability of contaminants and their effects at the same time (Den Besten, 1998). Biological-effect methods could be important elements in environmental monitoring programmes, because they can indicate links between contaminants and ecological responses (Thain et al., 2008).

In order to assess the effects of chemical contaminants and their mixtures in the Baltic Sea ecosystem more accurately, an integrated approach, i.e., monitoring the levels of hazardous substances in organisms or in the environment along with biological effects, is needed (Lehtonen, Schiedek, 2006b).

By adoption of the Baltic Sea Action Plan (BSAP) the HELCOM countries decided to cooperate in order to build up more information on the sources of the selected hazardous substances, the extent of their occurrence in the Baltic marine environment, as well as on their biological effects. HELCOM countries also agreed to develop a monitoring programme of biological effects which would facilitate a reliable ecosystem health assessment (HELCOM, 2007a).

The EU Marine Strategy Framework Directive (2008/56/EC) (MSFD) addresses hazardous substances, setting one of the qualitative descriptors for determining good environmental status as „Concentrations of contaminants are at levels not giving rise to pollution effects“. According to MSFD, the progress towards good environmental status will depend on whether pollution is

progressively being phased out, i.e. whether the presence of contaminants in the marine environment and their biological effects are kept within acceptable limits, so as to ensure that there are no significant impacts on or risk to the marine environment. And that is also the case where integrated view of the situation is needed.

Thus, integrated monitoring of hazardous substances and their effects is an important step forward to reach one of our future goals – Baltic Sea life undisturbed by hazardous substances.

Aim and objectives of the study

The aim of the study is an integrated assessment of the spread of contaminants and their biological effects in the Lithuanian zone of the Baltic Sea.

The main objectives are:

- to summarize the long-term monitoring data on contaminants in water, sediments and biota from the Lithuanian part of the Baltic Sea;
- to describe the peculiarities of distribution of contaminants in the Klaipėda harbour, Būtingė oil terminal, dredged sediments dumping site, adjacent area to the Russian D-6 oil platform and chemical munitions dumpsite;
- to evaluate the peculiarities of biomarker responses in marine organisms from the different areas of the southeastern Baltic Sea and the Curonian Lagoon;
- to assess the impact of contaminants on the biomarker response in mussels.

Novelty of the study

This study is the first attempt of an integrated assessment of environmental state of the marine environment in Lithuania using the data from national monitoring and other studies on specific contaminants and biomarker response data.

The long-term monitoring data on contaminants have been reviewed. A new set of statistical methods has been applied for this evaluation.

A laboratory experiment was performed in order to assess the combined effects of different PAH compounds (fluoranthene, pyrene and benzo(a)pyrene) and their mixtures on biomarker responses

(acetylcholinesterase (AChE), glutathione S-transferase (GST), catalase (CAT), glutathione reductase (GR), superoxide dismutase (SOD), lipid peroxidation (LPO), micronuclei (MN), nuclear buds (NB), fragmented-apoptotic (FA) and bi-nucleated (BN) cells) in *Mytilus edulis*.

The Curonian Lagoon and Klaipėda Strait species were not used for the biological effects studies before. Thus the zebra mussel *Dreissena polymorpha* from the Curonian Lagoon and polychaete *Nereis diversicolor* from the Klaipėda Strait were used in the biological effects studies. Due to the wide distribution of *D. polymorpha* in the central part of the Curonian Lagoon and *N. diversicolor* in the Lithuanian coastal area these species could be useful for monitoring of biological effects. The biochemical biomarker analysis can be applied in Lithuania in future.

As the first attempt of the integrated contamination assessment, the Pollution Index was applied for different areas within the Lithuanian Baltic Sea. Subsequently, the hazardous substances status of the Lithuanian Baltic Sea was assessed using HELCOM Chemical Status Assessment Tool (CHASE), which is a multimetric indicator-based tool developed for the integrated assessment of hazardous substances in the Baltic Sea.

Scientific and practical significance

The thesis reflects the overall goals of the hazardous substances segment of the HELCOM Baltic Sea Action Plan – to achieve a Baltic Sea with life undisturbed by hazardous substances and of the EU Marine Strategy Framework Directive – concentrations of contaminants are at levels not giving rise to pollution effects. The ongoing activities of HELCOM and EU are aimed at the integrated holistic assessments of the environmental state of the Baltic Sea. The step forward has been made by HELCOM since the combination of contaminant concentrations with the biological effects were used in the HELCOM Chemical Status Assessment Tool CHASE, which also has been applied for Lithuanian waters. Whereas more scientific evidences appear the biological effects become an important issue of the environmental research. In order to achieve the objectives set by EU and HELCOM Lithuania should also

look for the possibility to integrate biological effects to the Baltic Sea environmental monitoring programme.

Defensive statements

1. Long-term increasing and decreasing trends can be detected for certain contaminants in the Lithuanian part of the Baltic Sea.
2. Klaipėda harbour, Būtingė oil terminal, dredged sediments dumping site, Russian D-6 oil platform and chemical munitions dumping site are potential pollution sources in the Lithuanian part of the Baltic Sea.
3. Arsenic is an indicator of the leakage of chemical weapon substances at the chemical munitions dumpsite.
4. *Mytilus edulis* and *Macoma balthica*, as well as other species like *Dreissena polymorpha* and *Nereis diversicolor* can be used as sentinel species for the biological effects studies in the Lithuanian zone of the Baltic Sea.
5. Bivalve mussels are sensitive bioindicator organisms for the assessment of environmental genotoxicity and cytotoxicity in the Baltic Sea.
6. There are combined effects of exposure to different PAH compounds and their mixtures on the biomarker responses in *M. edulis*.
7. Integrated approach is an advantageous tool for the assessment of marine environment pollution.

Scientific approval and publications.

The results of the present study were published in 4 scientific papers in the international and Lithuanian peer-reviewed scientific journals and discussed during 7 presentations on international conferences and symposiums: EU-USA International Symposium „Integrated Ocean Observation Systems for Managing Global and Regional Ecosystems Using Marine research, Monitoring and Technologies“ (Klaipėda, 2006), Baltic SeaBreeze International Conference “Baltic Sea for future generations” (Palanga, 2007), Scientific conference “Biodegradation of oil and other environmental contaminants” (Vilnius, 2007), US/EU-Baltic International Symposium “Oceans observations, Ecosystem-based Management & Forecasting” (Tallinn,

2008), International seminar on sea-dumped chemical weapons. Perspectives of international cooperation (Vilnius, 2008), Regional conference "Marine and coastal research" (Nida, 2009), 15th International Symposium on Pollutant Responses in Marine Organisms, PRIMO 15 (Bordeaux, 2009).

Structure of the dissertation.

The dissertation manuscript is composed of the following chapters: Introduction, Literature Review, Material and Methods, Results, Discussion, Conclusions, References, List of author's publications, Annex. The material and results of the dissertation are presented in 202 pages. The text contains 63 figures and 17 tables. The list of references contains 244 sources. The list of author's publications comprises 4 publications. The dissertation is written in English with Lithuanian summary.

1. LITERATURE REVIEW

1.1 The Baltic Sea and its vulnerability

The Baltic Sea region is one of the largest brackish water areas in the world with a salinity varying from about 10 PSU in the south in the Baltic Proper, declining through the Bothnian Sea and reaching 2 PSU in the northern Bothnian Bay. The Baltic Sea is a very shallow water basin, with a mean depth of only 55 m. In spite of the comparatively small size of the sea, its various sub-basins have their own unique hydrographic characteristics. The Baltic Proper region of the sea is the largest and the deepest, with a maximum depth of 459 m. The catchment area of the Baltic Sea is approximately four times larger than the sea area itself (Fig. 1.1.1) (Kautsky, Kautsky, 2000; Myrberg, Andreev, 2003; HELCOM, 2003).

Fig. 1.1.1. The Baltic Sea and its drainage area divided into five larger sub-areas which are indicated by thick black lines. The major basins are the Bothnian Bay, the Bothnian Sea, the Baltic Proper, the Gulf of Finland and the Gulf of Riga (from Kautsky, Kautsky, 2000)

The Baltic Sea exhibits numerous important abiotic and biotic characteristics that make it exceedingly different from other European sea areas in regard to various ecological processes. The specific features of the Baltic Sea i.e. its semi-enclosed nature, brackish water, salinity stratification, partial ice cover in winter, and large drainage area make the Baltic Sea ecosystem particularly sensitive to natural and anthropogenic impacts (Dybern, Fonselius, 1981; HELCOM, 2003; Lehtonen, Schiedek, 2006a).

Narrow Danish straits and shallow sills (Darss Sill: 18 m depth; Drogden Sill: 7 m depth) limit the water exchange of the Baltic Sea. The turnover time for the surface water layer varies widely in the different coastal areas from less than 1 day at the open coasts to 12-25 years in the more enclosed archipelagos. Deep water in the central basins tends to stagnate for periods of several years. It is estimated that a renewal of the total water mass of the Baltic Sea would take about 25-35 years (Matthäus, Schinke, 1999; Kautsky, Kautsky, 2000). Moreover, the dominating water circulation in each of the three largest basins, i.e. the Bothnian Bay, Bothnian Sea and Baltic Proper, are anti-clockwise cells created by the Coriolis force due to the Earth rotation. Consequently, when a river enters the Baltic, the water turns to the right and follows the shore. This results in a longer residence time for the heavily polluted water from the large rivers entering in the south and east, which is thus kept in the Baltic Proper for a longer period than otherwise would have been the case (Kautsky, Kautsky, 2000). Thus, nutrients and hazardous substances, originated from marine and land-based sources, have a long residence time in the Baltic. Due to chemical properties of the contaminants most of them accumulate in sediments (Kennish, 1997; Ducrotoy, Elliott, 2008).

The positive water balance i.e. the surplus of freshwater supply (river runoff and precipitation) in comparison to evaporation, creates a steady outflow of brackish surface water from the Baltic. This outflow, in its turn, causes a reversal gradient current of saline water into the depth (Schulz, 1996). A permanent halocline at about 60-80 m depth prevents vertical circulation, and oxygenation of the deep water is limited to a few events of salt-water inflow.

About one third of the bottom area of the Baltic Proper is devoid of higher life due to low oxygen, therefore periodic large inflows of saline oxygen-rich water, which take place under specific weather conditions, are of vital importance for the Baltic Sea ecosystem (Ojaveer, Elken, 1997; Kautsky, Kautsky, 2000).

During the 10 000 years of the history of the Baltic, the fauna and flora have been subjected to major environmental changes several times, from fresh-water conditions to those of the *Yoldia* and *Littorina* Seas with higher salinity then the recent one, often referred to as the *Mya* Sea. After every disturbance, the succession had to start from almost zero, and the species composition has changed from predominantly marine to an almost completely freshwater one (Olenin, Leppäkoski, 1999). There are currently three main categories of organisms in the Baltic Sea: 1) species which belong to the North Sea ecosystems and penetrate into the Baltic Sea through the entrance sounds, 2) species from the neighboring fresh water ecosystems which invade the sea from the coastal areas, and 3) a small number of species which has survived from earlier stages of evolution of the Baltic Sea (Dybern, Fonselius, 1981).

Since the hydrographical conditions are very peculiar and even extreme with a series of gradients (salinity, temperature, gas content, turbidity, etc.), many marine species are excluded from the Baltic Sea entirely. Other species can penetrate only to certain zones, e.g. the north-south salinity gradient restricts the northward penetration of marine organisms. Distribution of species depends on their capacity to adjust to the prevailing conditions. A change in the environmental conditions may cause a species to withdraw from an area or to spread to new areas, depending on the character of the change (Dybern, Fonselius, 1981).

Since there is a lower genetic diversity in populations and ecosystems are simple with few species and few links between them, even a relatively small environmental change may cause severe imbalance in a whole ecosystem. The limited number of species involved in Baltic Sea food webs means that each individual species has a special importance in terms of the structure and

dynamics of the whole ecosystem. Since most organisms of this region already live under severe physiological stress, they are sensitive to pollution. Many pollutants carried out into the Baltic Sea accumulate in organisms sometimes reaching concentrations that may become damaging for this species, ecosystems or even humans (Dybern, Fonselius, 1981; Kautsky, Kautsky, 2000; HELCOM, 2003).

The Baltic Sea is surrounded by 14 densely populated and industrialized countries, where 85 million people live within the drainage area that adds a significant stress resulting from pollution. Nutrients and hazardous substances originating from cities, farmland, commercially managed forests, industrial and energy plants, transport and other human activities from the whole catchment area drain into the sea via rivers. Pollutants from an even larger area can enter the Baltic by the air transmission. Emissions and discharges from shipping and fish farms enter the sea directly. The response to nutrient enrichment results in eutrophication. The large inputs of nutrients, year after year, have resulted in increased concentrations of nitrogen and phosphorus in all basins. Nutrient enrichment may give rise to an increased rate of oxygen consumption leading to decreased oxygen concentrations and an increased frequency of oxygen depletion. Eutrophication favors growth of nuisance algae: harmful and toxic algal blooms have occurred annually in the Baltic Sea in recent years (Kautsky, Kautsky, 2000; Ærtebjerg et al., 2003; Olenina, 2003).

With its many sources of pollution and very slow water renewal the Baltic Sea is considered one of the worst polluted areas in the world (Kautsky, Kautsky, 2000; HELCOM, 2003).

1.2 Contaminants in the Baltic Sea

More than 40 years ago an American marine biologist Rachel Carson wrote a book “Silent Spring” (1962) where she turned her attention to conservation and the environmental problems caused by synthetic pesticides. It became apparent to the general public that the natural environment had been seriously

affected by anthropogenic contamination. In the Baltic Sea region, in Sweden, a pesticide debate started even before “Silent Spring”, after mercury-treated seed grain poisoned granivorous birds and their predators. A suspicion that such mercury might have leaked to watercourses led to the surprise discovery of high mercury concentrations in fish of inland and coastal waters. Most of this mercury was methylated, and accumulated in the food chain (Elmgren, 2001).

Since then, as a result of the prohibition and strict regulation of the use of DDT, PCB, mercury and other metals in the industrialized world, as well as reduced emissions of PAHs and dioxins from combustion plants, the levels of such substances in aquatic environments have decreased. Populations of some previously affected species have started to recover (Swedish EPA, 2000; Skei et al., 2000; Kautsky, Kautsky, 2000; Elmgren, 2001; Vallius, Leivuori, 2003; HELCOM, 2003, 2004b, 2008, 2009a). However, the fact that a certain substance is banned is no guarantee for its absence in the environment. Monitoring indicates that although the loads of some hazardous substances have been reduced considerably over the past 20–30 years, other problems still persist, and concentrations of some new substances in the marine environment have been increasing (HELCOM, 2007a). Long-range atmospheric transport of hazardous substances, unregistered use, release from imported goods, unintentional formation, former emissions of highly persistent substances as well as secondary sources like contaminated sediments, dump sites, contaminated land may all lead to contamination of the environment (Skei et al., 2000 Sternbeck et al., 2003). Another problem is that the degradation and transformation of some of these substances in the marine environment may change their structure and reactive properties. These unknown new substances could pose a considerable threat to the environment (HELCOM, 2003).

The present list of known contaminants is undoubtedly incomplete, principally due to the shortcomings in aquatic science. Thus, for example, polychlorinated biphenyls now thought to be of very considerable significance as a coastal and oceanic pollutant were not discovered in aquatic biota until 1966, some 37

years after their initial use by industry. And there is no doubt that new contaminants of concern will emerge as analytical techniques improve (Phillips, Rainbow, 1994). One of the examples would be the development of sensitive and reliable methods in 1970s which made it possible to measure levels of inorganic and organic pollutants in sediments, water and organisms in the Baltic region with comparatively great accuracy (Swedish EPA, 2000).

There is still too little comprehensive knowledge about the impact of the most widely used chemicals and their cocktail-like combinations on human health and the environment. Todau relatively few organic pollutants are fully understood or even identified (HELCOM, 2003). Their environmental risks depends on the speciation of contaminants and their association to media and matter and by that means affect exposure. Furthermore, the risk also depends on the mobility of the substances and their pathways in food chains (Skei et al., 2000).

Contaminants in the Baltic Sea include substances occurring at concentrations exceeding natural levels, including oil hydrocarbons, PAHs and heavy metals like lead, copper, cadmium, mercury and substances that do not occur naturally in the environment, i.e. organochlorine compounds like PCBs, DDTs, dioxins, organotin compounds, nonylphenolethoxylates (NP/NPE), short-chained chlorinated paraffins (SCCP), brominated flame retardants (PBDEs) and etc. (HELCOM, 2007a).

1.2.1. Heavy metals

Heavy metals comprise a group of elements that are potentially toxic to estuarine and marine organisms in concentrations above a threshold level. The term ‘heavy metal’ is used synonymously with ‘trace metal’ and includes both essential and non-essential trace metals (Rainbow, 1995; Kennish, 1997). Metals, at their natural concentration, play an essential role in many biochemical processes in organisms. Such concentration is called the background concentration. Any concentration lower or higher than this background can be toxic. Increased levels of metals are potentially serious,

since many metals can induce biological disturbances even at relatively low concentrations (Nemerow, 1991; Kersten et al., 1994; Swedish EPA, 2000).

Two subgroups are recognized: (1) transitional metals (e.g., copper, zinc) which are essential to metabolism at low concentrations but may be toxic at higher concentration, and (2) metalloids (e.g., arsenic, cadmium, lead, mercury, tin) which generally are not required for metabolic function and are toxic at low concentration (Kennish, 1997). If there is an excess of trace metals in the aquatic environment, then living organisms are forced to take part in the cycle of these metals (Kosior et al., 2002). Heavy metals can accumulate in the marine food web up to levels which are toxic to marine organisms, particularly predators, and via food web they may also represent a health risk for humans (HELCOM, 2007b).

Heavy metal levels in aquatic environment vary with a number of factors, including type of bedrock and sediment, mineralogy, organic content, redox conditions in water and sediments, water currents, salinity, etc. (Swedish EPA, 2000). The chemistry of heavy metals is also influenced by oxygen concentrations in the water. For example, a shortage of oxygen makes cadmium and copper precipitate as sulphide compounds, and they will subsequently be deposited in sediments in this form. This means the amounts of heavy metals in the water are directly linked to the oxygen depletion associated with eutrophication (HELCOM, 2003). Heavy metals may be either dissolved in the water or bound to particles. This affects the chances of organisms absorbing them from their surroundings (HELCOM, 2003).

When metals are trapped in the sediments, they become less biologically accessible and therefore less capable of affecting aquatic organisms. The accessible portion of metals varies considerably between different sediments, and may even vary within the same sediment due to fluctuations in redox conditions. The portion of the metals which is incorporated in minerals can be regarded as biologically inaccessible (Swedish EPA, 2000).

Heavy metals can be transported to the sea either via rivers, run-off in coastal areas, waterborne discharges of industrial and municipal waste or by wet and

dry atmospheric deposition. In the case of airborne loads, these can also originate from distant sources outside the Baltic Sea catchment area (Kennish, 1997; HELCOM, 2007b).

Once released into the Baltic Sea, heavy metals can remain in the water for very long periods. The concentrations of heavy metals in the Baltic Sea water are up to 20 times higher compared to the North Atlantic (HELCOM, 2007b). In the Baltic Sea, metal concentrations in the sediments increased from the 1950s until the 1970s. Now it seems to be decreasing due to measures taken in the industry. Lead concentrations are today decreasing in fish, due to reduced air emissions from car traffic (Kautsky, Kautsky, 2000). However, even though the concentrations of some heavy metals have decreased in many parts of the Baltic Sea, high concentrations can still be found in certain marine organisms, notably in Baltic herring. Cadmium concentrations in Baltic herring have increased significantly, despite a general declining trend in concentrations in the waters of the Baltic Proper and the Western Baltic Sea. Mercury concentrations in herring have remained at roughly the same level since the 1980s (HELCOM, 2003).

The most important and commonly studied heavy metals are lead, chromium, cadmium, copper, zinc, mercury and arsenic (Gerlach, 1981; Nemerow, 1991; Leivuori, 2000; Swedish EPA, 2000); also nickel and vanadium, which sometimes used as markers for oil contamination (De Mora et al., 2004).

Mercury and cadmium are included in the HELCOM Baltic Sea Action Plan (adopted in 2007) list of hazardous substances or substance groups of specific concern to the Baltic Sea. Mercury and cadmium are also identified as priority hazardous substances; lead and nickel as priority substances by European Commission (Directive 2008/105/EC) and also by Wastewater Treatment Regulation (Order of Minister of Environment No. D1-236 of 17 May 2006; most recent amendments on 18 May 2010, Official gazette, 2010, No. 59-2938) in Lithuanian national legislation. Chromium, copper, tin, zinc, vanadium, aluminum, and arsenic are in the list of other controlled substances in Lithuania.

1.2.2. Oil

Oil has been forming from the organic remains of dead organisms over different periods in the Earth's history. These organics had been preserving till the present in fossil form in places where the absence of oxygen prevented their decomposition. Because different factors influenced the formation and setting of the different sediments, the quality of petroleum is different everywhere it is found (Gerlach, 1981).

Oil contamination is usually caused by an accidental or chronic release of one of three main types of oil: crude oil, heavy fuel and diesel fuel oil (FIMR, 2007). Crude oils, consisting of a complex mixture of hydrocarbon and non-hydrocarbon compounds, vary widely in chemical composition and physical properties. While hydrocarbons comprise more than 75 % by weight of most crude oils, non-hydrocarbons (compounds containing oxygen, nitrogen, sulfur, and metals such as copper, iron, nickel, and vanadium) can predominate in heavy crude oils (Kennish, 1997; Wake, 2005). Among heavy metals, vanadium and nickel are characteristic constituents of crude oil. Vanadium is usually associated with a high sulphur content and nickel with a low sulphur content of crude oil (FIMR, 2007).

Oil spills contaminate the water by creating an oily layer. The oil spreads quickly over the sea surface, often covering extensive areas as slicks varying from micrometers to a centimeter or more in thickness. As the oil spreads and the oil thickness reduces, its appearance changes from the black or dark brown color of thick oil patches to iridescent and silver sheen at the edges of the slick. A common feature of spills of crude oil and some heavy fuel oils is the rapid formation of water-in-oil emulsions which are often characterized by a brown/orange color and a cohesive appearance. Oil slicks travel downwind at 3-4 % of the wind speed, spreading at a rate dependent on water temperature and composition of the oil. Light oils spread faster than heavy oils (Kennish, 1997; Stankevičius, 2008; ITOPF, 2009).

Several physical-chemical processes change the composition of oil in seawater. The main processes are evaporation, photochemical oxidation, emulsification, and dissolution. Low-molecular-weight volatile fractions evaporate, hydrocarbons undergo photo oxidation, water soluble constituents dissolve in seawater, and immiscible components become emulsified. Evaporative loss of volatile hydrocarbons removes the toxic lower-molecular-weight components during the first 24 to 48 hours of an oil spill. The loss of these volatile components substantially lowers the overall toxicity of the oil to water organisms (Kennish, 1997; Stankevičius, 2008). As the density of oil approaches that of seawater, it tends to sink. Sedimentation of oil is facilitated by the sorption of hydrocarbons to particulate matter suspended in the water column (Kennish, 1997).

Microbes play a pivotal role in the degradation of crude oil, often being the dominant factor controlling the fate of toxic hydrocarbons in aquatic environments. All together they can degrade as much as 40 to 80 % of a crude oil spill. Several factors influence the biodegradation rates: oil composition, water temperature, nutrient availability, oxygen levels, and salinity (Kennish, 1997). Anaerobic conditions severely restrict microbial degradation. Investigations show that oil in sediments can persist over 30 years in concentrations of total petroleum hydrocarbons similar to those observed immediately after the spill. Such persistence of oil was attributed to the heavy contamination of the area by the spill, the high organic carbon content, and anoxic conditions in the marsh sediments that hindered microbial degradation (Reddy et al., 2002).

In the cold waters of the Baltic, where the average water temperature is only about 10 degrees, oil decomposes very slowly. Coastal areas contaminated by oil-spills need to be actively cleaned up, which is a very slow and laborious task, especially during the winter. The necessary clean-up operations may themselves unavoidably harm marine life and coastal habitats, e.g., when using oil dispersants (Riepšaitė, Stankevičius, 2005).

Oil pollution is recognised as one of the greatest hazards for the marine environment despite weather it happens in the form of large accidents or long-term small-scale spills and leakage. Oil accidents also cause direct economic losses e.g. by affecting fish stocks and spoiling the recreational use of the sea and coastline. From an ecological point of view, damages occur at all levels of the marine food web including birds and mammals. Oil pollution in the aquatic environment can originate from sea traffic, harbours, drilling activities, ships, and land-based sources. In regard to oil accidents the effects are - at first – acute, causing visible damage on biota and the environment, but at later stage chronic harmful effects might take place. Leakage during continuous activities such as drilling of oil and gas can cause chronic effects on biota (FIMR, 2007). The most visible effects of oil-spills are caused by the oil on the surface: birds and seals are smothered, and their chances of survival are hampered by problems with their mobility or the insulating properties of their feathers or skin (HELCOM, 2003). Other aquatic organisms are also highly impacted by the spilled oil, and massive mortality of marine life including fish, worms, crustaceans, and mollusks occurring in a few days (Burger, 1994; Reddy et al., 2002).

Nevertheless, it appears that long-term chronic contamination by lower levels of oil-derived substances is more harmful to the environment than acute large spills because they deteriorate the overall conditions in the environment and lead to a permanent stress to organisms within the local ecosystem. It should also be pointed out, that chronic contamination can provoke genetic effects in different organisms and initiate damage in the genetic structure of populations of organisms by selecting out the oil-tolerant genotypes, and, likewise, can have an effect on community structure by removing oil-sensitive species and favouring the tolerant ones (FIMR, 2007).

Oil is a serious threat to the Baltic Sea ecosystem. About 10 % of all oil hydrocarbons in the Baltic Sea originate from deliberate, illegal discharges from machinery spaces or cargo tanks of vessels sailing in the Baltic. Surveillance aircraft detect about 400 illegal oil discharges a year happening in

the Baltic Sea (HELCOM, 2003). Long term investigations show that there is an increase in total hydrocarbon concentrations from north to south: the Bothnian Bay towards the Bothnian Sea and further via the Gulf of Finland to the Baltic Proper. The increase is compatible with the greater number of oil spills towards the south (Pikkarainen, Lemponen, 2005).

Oil is in the list of controlled substances in Lithuania (Wastewater Treatment Regulation, Official gazette, 2010, No.59-2938).

1.2.3. Polycyclic aromatic hydrocarbons

Polycyclic aromatic hydrocarbons (PAHs) sources can be broadly divided into two main categories: petrogenic (from fossil fuels) and pyrolytic (from the incomplete combustion of organic material). PAHs are mainly produced by pyrolysis, but are also present in crude oils, coal, coal tar and various refinery products. Some PAHs have both natural and anthropogenic origins because they are the product of both wood and fossil fuel combustion. However, the anthropogenic contribution frequently outweighs PAH input from nearly all other sources (Webster et al., 2003; Barra et al., 2006). Molecular indices, which are based on concentration ratios of selected compounds, can be used as source indicators when evaluating pyrolytic and petrogenic origins of PAH compounds (Baumard et al., 1998a; Kaag et al., 1998; Webster et al., 2003; Barra et al., 2006; Pikkarainen, 2004a, 2004b, 2008).

Polycyclic aromatic hydrocarbons (PAHs) are of concern due to their persistence and potential to accumulate in aquatic organisms, particularly invertebrates. The compounds range from naphthalene ($C_{10}H_8$, two rings) to coronene ($C_{24}H_{12}$, seven rings). Common PAH compounds include two-ring compounds (naphthalene); three-ring compounds (fluorene, phenanthrene, anthracene); four-ring compounds (fluoranthene, pyrene, benzo(a)anthracene); and five-ring compounds (benzo(a)pyrene, benzo(b)fluoranthene, perylene). The low-molecular-weight PAH compounds, containing two or three rings, are acutely toxic to a broad spectrum of marine organisms. Examples of low-molecular-weight PAHs that tend to be toxic are anthracene, fluorene,

naphthalene and phenanthrene. The high-molecular-weight PAH compounds, containing four, five, and six rings, are less toxic but have greater carcinogenic potential. High-molecular-weight PAH compounds that are carcinogenic include banzo(a)pyrene, benzo(c)phenanthrene, dibenzo(a,i)pyrene (Kennish, 1997; Webster et al., 2003).

PAHs exhibit a wide range of physical-chemical properties (vapor pressure, aqueous solubility) that demonstrate their semi-volatile and hydrophobic character and influence their environmental fate (Barra et al., 2006). Organic contaminants in aquatic environment may exist in several forms including dissolved forms, those bound to dissolved organic matter, adsorbed to suspended particulate matter, and associated with surface sediments (Zhou et al., 1998). Due to their low water solubility and hydrophobic nature, PAHs tend to associate with particulate material. The deposition of these particles in rivers and coastal waters can lead to an accumulation of PAHs in the sediment. Accumulation of hydrophobic compounds, such as PAHs, is dependent on sediment type; sediments with high organic carbon content and a smaller particle size (larger surface to volume ratio) have a greater potential to accumulate PAHs compared to coarser, sandy sediments. In addition, PAHs are persistent, especially in anaerobic sediments, with the higher molecular weight PAHs being more persistent than the lower molecular weight compounds (Kennish, 1997; Webster et al., 2003).

The solubility of PAH decreases with increasing molecular weight. Thus, bioaccumulation of PAHs from sediments by marine organisms is generally greater for the lower molecular weight and more water soluble PAHs than for the higher molecular weight compounds. Different profiles of contaminants have been observed in organisms of different trophic levels. These differences were attributed to a partial biotransformation of the contaminants in the organisms of higher trophic levels (Baumard et al., 1998b).

Elevated levels of PAHs are commonly found in estuarine and coastal marine waters near heavily populated areas. Oil related activities, ballast water discharges, dredging activities and disposal, oil pollution and sewage are all

potential sources of PAHs. In the marine environment the introduction of PAHs is through effluent discharges, urban run-off, atmospheric transport, and the spillage or disposal of oil and petroleum products. PAHs also have natural origin: some are synthesized by bacteria, plants, and fungi or derive from natural products and processes, such as coal and oil, grass and forest fires emissions (Kennish, 1997; Webster et al., 2003).

Total PAH concentrations in the Baltic Sea indicate considerable pollution in some areas. Pikkarainen (2008) in her study showed that PAHs in the Baltic were pyrolytic as well as petrogenic, and diesel engine sources of PAHs, caused by steadily increasing shipping in the area, were also indicated. Fluoranthene, benzo(b)fluoranthene, benzo(g,h,i)perylene, and indeno(1,2,3-cd)pyrene dominated in the surface sediment (Pikkarainen, 2008).

Anthracene, benzo(a)pyrene, benzo(b)fluoranthene, benzo(g,h,i)perylene, benzo(k)fluoranthene, and indeno(1,2,3-cd)pyrene are identified as priority hazardous substances; fluoranthene and naphthalene as priority substances by European Commission (Directive 2008/105/EC) and also by Lithuanian Wastewater Treatment Regulation (Official gazette, 2010, No. 59-2938).

1.2.4. Chlorinated hydrocarbon compounds

In marine ecotoxicology, more attention has been given to the higher-molecular-weight chlorinated hydrocarbon compounds, also known as organochlorine compounds, which are common contaminants in estuarine and marine environments. These compounds are mainly derived from pesticides and industrial chemicals (Kennish, 1997). The chlorinated hydrocarbons are broad spectrum poisons that affect many different organisms in one area (e.g., plankton, benthic invertebrates, fish, mammals, and birds), as well as humans (Rylander et al., 1996), and thereby threatening entire communities. Their rapid distribution in marine environments is facilitated by atmospheric dispersal and deposition, current transport, and migration of animals contaminated with the organochlorines (Kennish, 1997).

It is the unique properties of chlorinated hydrocarbon contaminants – chemically stable nature, great mobility, hydrophobicity, resistance to degradation, persistence in the environment, affinity for living systems, bioaccumulative capacity, and general toxicity – that have engendered apprehension and fostered numerous monitoring programs (Kennish, 1997). Due to their lipophilicity, organochlorine compounds tend to concentrate in lipid-rich tissues of animals (Sole et al., 2000), and biomagnify through food webs. Hence, some marine fauna, such as mammals, situated at the uppermost trophic levels, carry very high contaminant residues (Kennish, 1997).

The half-lives of chlorinated hydrocarbon compounds typically range from months to years. The residues of the more persistent compounds, however, may be present for decades or possibly centuries, depending on temperature, light, pH, rate of microbial degradation, and other conditions. The half-life of a contaminant within an organism is in part dependent on body composition and biological functions, such as the concentration of lipids and the reproductive activity of a species (Kennish, 1997).

Most of the chlorinated hydrocarbon compounds are classified as persistent organic pollutants (POPs). According to Stockholm Convention (came into force in 2004; Lithuania signed in 2002), persistent organic pollutants possess toxic properties, resist degradation, bioaccumulate and are transported, through air, water and migratory species, across international boundaries and deposited far from their place of release, where they accumulate in terrestrial and aquatic ecosystems. There have been substantial inputs of POPs into the Baltic Sea from numerous sources over the past 50 years. These sources include industrial discharges, such as the organochlorines in effluents from pulp and paper mills, runoff from farmland, special paints used on ships and boats, and dumped wastes (HELCOM, 2003). A large number of halogenated organic pollutants have been identified in the Baltic Sea and levels in some fish, e.g. herring and cod liver, were so high that recommendations were issued to restrict consumption. Joint international measures to reduce inputs to the Baltic, have resulted in a decline in concentrations of chlorinated hydrocarbons in the

environment of the Baltic Sea since the 1970s, however the presence of these compounds is still detected in all matrices of the Baltic environment (Jonsson et al., 1996; Kautsky, Kautsky, 2000; Ciereszko, 2002; HELCOM, 2003; Pikkarainen, Parmanne, 2006; Pikkarainen, 2007; Bignert et al., 2008).

1.2.4.1. Chlorinated pesticides

Chlorinated hydrocarbon pesticides are extremely harmful biocide agents, acting as a nerve poison to control population sizes of target organisms. The chlorinated hydrocarbon insecticides include a number of well known synthetic compounds, such as DDT, aldrin, chlordane, dieldrin, endosulfan, endrin, lindane, heptachlor, chlordecone, mirex, and toxaphene (Kennish, 1997). In the environment the chlorinated pesticides degrade very slowly – 5 to 15 years (Kennish, 1997), the half-life of DDT can even reach ~150 years in aquatic ecosystems (Mulsow et al., 2002). Officially the use of chlorinated pesticides in Lithuania had stopped in 1970s (Četkauskaitė, 1999).

Dichloro-diphenyl-trichloroethane (DDT)

One of the most well known and ubiquitous chlorinated hydrocarbon contaminant is DDT, which belongs to the diphenyl aliphatics class consisting of an aliphatic or straight carbon chain, with two (di)phenyl rings attached (Kennish, 1997). Solar radiation and the metabolic activities of animals decrease the concentration of DDT in the environment (Kennish, 1997; Mulsow et al., 2002) and degrade it to DDE and DDD. Combination of DDT and its degradation products DDE and DDD is referred to as total DDT. DDD is less toxic to marine organisms than DDT or DDE, and it rarely accumulates in them. DDT is one of the most water-insoluble compounds that was ever artificially synthesized. Its solubility amounts to approximately 6 ppb of water. In contrast, DDT is highly fat soluble. Thus, it tends to partition out of the hydrosphere into biotic compartments (Kennish, 1997).

In the Baltic Sea, there is a significant decrease of DDT concentrations in herring muscle and cod liver since 1970s (Bignert et al., 2008); but the concentrations of DDT are still high in some bivalves and herring muscle

samples from the different areas of the sea (Pikkarainen, Parmanne, 2006; Pikkarainen, 2007).

DDT has been completely banned since the 1980s (HELCOM, 2003). DDT is in the list of persistent organic pollutants (POPs) which are under the Stockholm Convention. European Commission Directive 2008/105/EC as well as Lithuanian Wastewater Treatment Regulation (Official gazette, 2010, No. 59-2938) sets Environmental Quality Standard for DDT in water for inland and other surface waters.

Hexachlorocyclohexanes (HCHs)

There are eight isomers of HCH: β-, γ-, δ-, ε-, η-, θ- and two forms of α-HCH. The structures of these isomers differ in the angles at which the Cl-atoms are attached to the cyclohexane. Technical HCH is dominated by α-HCH (55-80 %). Lindane is a well known insecticide consisting of > 99 % γ-HCH, and is still legally used in many countries. Upon ingestion by animals, lindane tends to be rapidly metabolized to water soluble chlorophenols and chlorobenzenes. Both α- and β- HCH are higher persistent in environment than γ-HCH. HCHs easily accumulate in food chains. β-HCH is the isomer with the highest tendency to accumulate in humans and has a bioconcentration factor (BCF) of over 500 in comparison to 20 for α- and γ-HCH (Kennish, 1997; Sundqvist, 2009).

In recent years, in some areas of the Baltic Sea, concentrations of β-HCH are generally decreasing, and are now approaching the detection limit. The concentrations of lindane have decreased significantly in all matrices except for guillemot eggs and herring (HELCOM, 2003; Bignert et al., 2008). The levels of HCHs in bivalves could be classified as high and moderate (Pikkarainen, 2007).

Hexachlorocyclohexane is identified as priority hazardous substance by European Commission (Directive 2008/105/EC) and Lithuanian Wastewater Treatment Regulation (Official gazette, 2010, No. 59-2938).

Endosulfan, aldrin, endrin, dieldrin

Several chlorinated cyclic hydrocarbons belong to a class of compounds termed the cyclodiene pesticides. These compounds include the most toxic organochlorine insecticides, especially in terms of acute toxicity. Cyclodienes consist of chemicals developed after World War II (e.g., aldrin and dieldrin, 1948; endrin, 1951; endosulfan, 1956). All cyclodienes share common characteristics of low water solubility and extreme persistence. They are particularly stable in soil, and thus have been most commonly employed as soil insecticides. Some of the compounds (e.g., aldrin) are rapidly metabolized by organisms, however, their metabolites (dieldrin) are as toxic and persistent as the parent compounds (Kennish, 1997).

Endosulfan is an insecticide and a multicide applied in cultivation, e.g., fruits, vegetables, maize and rice; it is also used as a wood preservative (HELCOM, 2009b). Technical grade endosulfan contains two isomers, α- and β-endosulfan, in the ratio 7:3 making up 94 % of the content. Endosulfan sulphate is an oxidation product found in technical endosulfan and is also the main microbial oxidation product of α- and β-endosulfans. In the aquatic environment, endosulfan mainly adsorbs to suspended solids and deposits to sediments; however, a certain proportion is likely to remain in the water column due to relatively high water solubility (Cousins et al., 2005).

Aldrin, dieldrin and endrin are POPs which are under the Stockholm Convention. European Commission Directive 2008/105/EC and Lithuanian Wastewater Treatment Regulation (Official gazette, 2010, No. 59-2938) set Environmental Quality Standards for aldrin, dieldrin and endrin in water for inland and other surface waters and identify endosulfan as priority hazardous substance. Endosulfan is also included in the HELCOM Baltic Sea Action Plan list of hazardous substances of specific concern to the Baltic Sea.

Hexachlorobenzene (HCB)

One of the most important fungicides is HCB, a chemical once widely used as a fumigant in grain storage against fungal attacks, as soil fumigant, and as a component in wood preservatives. It is a highly persistent contaminant, largely

found in estuarine and marine environments sorbed to sedimentary particles (Kennish, 1997). HCB is a dioxin-like compound resulting in dioxin-like effects in biota (Falandysz, 2000).

HCB has not been used as a pesticide in the Baltic Sea region since early 1990s, therefore, in the Baltic there is a decrease of HCB in all fish species and in guillemot eggs (Falandysz, 2000; Bignert et al., 2008; HELCOM, 2010a). Nevertheless, due to its persistence in the environment HCB can be still found in seawater, sediments (HELCOM, 2010a) and fish (Pikkarainen, Parmanne, 2006).

HCB is in the list of Stockholm Convention POPs. European Commission Directive 2008/105/EC and Lithuanian Wastewater Treatment Regulation (Official gazette, 2010, No. 59-2938) identify HCB as priority hazardous substance.

Pentachlorophenol (PCP)

Pentachlorphenol (PCP) is a pesticide with a broad application, wide effectiveness, and multiple modes of action. A metabolite of HCB, PCP has been used as a fungicide for wood and textile preservation (fungicide), an insecticide for protection against insects, a nonselective herbicide and a pre-harvest defoliant (Kennish, 1997). PCP is highly toxic to aquatic organisms, persistent and liable to bioaccumulate (OSPAR Commission, 2001).

PCP production in Europe has been banned since the early 1980s. However, due to its persistence in the environment, PCP is still found in the Baltic Sea (Sternbeck et al., 2003; Oehme et al., 2005).

PCP is identified as priority substance by European Commission (Directive 2008/105/EC) and Lithuanian Wastewater Treatment Regulation (Official gazette, 2010, No. 59-2938).

1.2.4.2. Polychlorinated biphenyls (PCBs)

Perhaps the most notable organochlorine contaminants in the marine biosphere are polychlorinated biphenyls (PCBs). It is a group of synthetic halogenated aromatic hydrocarbons consisting of a complex mixture of chlorinated biphenyls with a varying number of substituted chlorine atoms on aromatic

rings. There are 209 possible PCB congeners. Twelve of the 209 PCB congeners have a substitution pattern that allows the molecule to assume a planar conformation conferring a structural similarity to polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofuranes (PCDFs). These dioxin-like PCBs exhibit the same mode of toxicological action as PCDD/Fs (discussed in the next section). Unique physical and chemical properties of PCBs include the following: general inertness, thermal and chemical stability, miscibility with organic compounds, high dielectric constant, non-flammability and low cost. These characteristics enabled PCBs to be widely used wherever such properties were desirable, e.g. in dielectric fluids of transformers and capacitors, heat exchange and hydraulic fluids, lubricants, fire retardants, plastics, and other materials (Kennish, 1997; Sundqvist, 2009).

Due to the highly hydrophobic character, in the environment PCBs are primarily adsorbed to particles (e.g., soil, sediment and aerosols). Furthermore, their high persistence leads to bioaccumulation in organisms. PCBs are deleterious to marine life, especially upper-trophic level organisms that tend to accumulate the compounds in their tissues. PCBs have been contaminants of the marine environment for more than 50 years. Over this time, they have become universally distributed in estuarine and marine environments and occur in nearly all marine plant and animal species (Kennish, 1997; Sundqvist, 2009).

In the Baltic Sea concentrations of polychlorinated biphenyls in sediments, bivalves and herring have decreased significantly since 1970s-80s, probably due to the effect on emissions of stricter regulations and bans in the HELCOM countries (HELCOM, 2003; Pikkarainen, Parmanne, 2006; Pikkarainen, 2007; Bignert et al., 2008), but in spite of this fact, there is still a high degree of contamination by PCBs in some sediment and herring muscle samples (Pikkarainen, Parmanne, 2006; Pikkarainen, 2007).

PCBs are among the persistent organic pollutants (POPs) which are under the Stockholm Convention. In addition, dioxin-like PCBs are included in the

HELCOM Baltic Sea Action Plan list of hazardous substances or substance groups of specific concern to the Baltic Sea.

1.2.4.3. Polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofuranes (PCDFs)

Two related classes of aromatic heterocyclic compounds, the polychlorinated dibenzo-p-dioxins (PCDDs or dioxins) and polychlorinated dibenzofuranes (PCDFs or furanes), cause considerable biological and especially toxic impacts on aquatic organisms. Substitution with different numbers of chlorine atoms gives rise to 75 possible PCDD molecules and 135 possible PCDF molecules, which are jointly, termed congeners (Sundqvist, 2009).

The PCDDs and PCDFs are hydrophobic and lipophilic compounds that resist degradation in environment. They tend to sorb to sediments and ultimately accumulate on the sea floor, which acts as a sink for these compounds. However, in estuarine and marine environments, sediments are not the primary direct source of PCDDs and PCDFs for biota at higher trophic levels, and it appears that the food chain is the most important pathway for bioaccumulation of these compounds (Kennish, 1997).

Despite the global distribution of PCDD/Fs, oddly, they have never been intentionally synthesized. Hundreds of congeners are formed during synthetic processes such as combustion and certain industrial activities. High temperatures in combination with carbon and chlorine sources often lead to the formation of PCDD/Fs. Chemical processes producing significant amounts of PCDD/Fs include the bleaching of pulp using chlorine gas or the chemical manufacture of chlorophenols and other organochlorine chemicals (Sundqvist, 2009). PCDD/Fs may also be formed by natural biological and abiotic processes in the environment. Dioxins can be formed in peat and forest soil, presumably via the enzymatic oxidative dimerization of natural chlorophenols; even a human biosynthesis of dioxins is possible (Gribble, 2003).

In environmental samples dioxins are always found as a mixture of various congeners. The toxicity of the dioxins is very congener-specific, ranging from the most toxic 2,3,7,8-TCDD (tetrachloro-dibenzo-dioxin) or the Seveso-

dioxin to congeners more than 10,000 times less toxic. In order to aggregate the results for the various congeners in a sample and get a "total" dioxin content, some international systems for calculating dioxin toxicity equivalence (TEQ) have been developed. These have been based on the assumption of similar toxic action mechanisms together with the assumption that the interactions are additive. The systems are based on a relative ranking system which gives the congeners toxicity equivalence factors (TEF) with TCDD (the most toxic) assigned a factor of 1. The quantity of each dioxin is multiplied by its TEF to normalize the amount to TCDD equivalent amount (TEQ). The results can then be simply added to give a total TCDD equivalent amount for dioxins (HELCOM, 2004b). The most recent reevaluation of TEF scale was reported by van den Berg et al. (2006).

Elevated levels of PCDD/Fs are present in both coastal and offshore areas of the Baltic Sea. The major hotspots of PCDD/Fs are located close to the shore, and there are large variations in profiles, indicating that local emissions are (or have been) the major cause of pollution (Sundqvist et al., 2009). For example, the dioxin content in herring from the Baltic often exceeds the prescribed maximum limit for dioxin residues (4 WHO-PCDD/F pg/g wet weight) (Bignert et al., 2007, 2008). However, concentrations of dioxins in herring and salmon vary regionally, and the most contaminated fish are found in the Gulf of Bothnia, including herring in the Bothnian Sea, and salmon in the Bothnian Bay. Transfers of dioxins up marine food chains can be observed in fish-eating birds and their eggs. The concentrations of dioxins in guillemot's eggs have now decreased to one third of their 1970-levels. Dioxin concentrations in sediments peaked in the 1970s, but have more recently started to decrease (HELCOM, 2003).

Dioxins and furanes are in the POPs list of the Stockholm Convention. In addition, PCDD/Fs are included in the HELCOM Baltic Sea Action Plan list of hazardous substances or substance groups of specific concern to the Baltic Sea.

1.2.5. Other hazardous substances

Substances are defined as hazardous if they are toxic, persistent and bioaccumulative. Certain contaminants may be hazardous because of their effects on hormone and immune systems (HELCOM, 2007a). A list of hazardous substances or substance groups of specific concern to the Baltic Sea was adopted in Krakow, Poland, on 15 November 2007 with accordance to HELCOM Baltic Sea Action Plan. This list contains eleven hazardous substances. Some of them – cadmium, mercury, endosulfan, dioxins, furanes and dioxin-like polychlorinated biphenyls – have been already described in the previous sections (see above). The rest of important substances from the HELCOM list, such as organotins and substances, which have recently emerged as new contaminants of concern – brominated flame retardants, perfluorinated alkyl substances, alkylphenols and chlorinated paraffins, will be presented in this section.

Moreover, tributyltin, nonylphenol and short-chain chlorinated paraffins has been identified as priority hazardous substances; pentabromodiphenylether (congener numbers 28, 47, 99, 100, 153, 154) and octylphenol as priority substances under European environmental quality standards (EQS) Directive (2008/105/EC) and Lithuanian Wastewater Treatment Regulation (Official gazette, 2010, No. 59-2938).

There is also a large variety of other contaminants, such as organophosphorous compounds, triazine pesticides, volatile organic compounds and other, which have an impact on the aquatic environment, but which are not widely discussed here.

1.2.5.1. Organotin compounds

Organotin compounds are substances composed of tin, directly bound to a number of organic groups. Tributyltin (TBT) is the most hazardous of all tin compounds with considerable biological effects (SOCOPSE, 2009).

In natural waters, TBT has a short residence time, with a half-life ranging from several days to weeks. Organotins are moderately hydrophobic. Adsorbtion of

TBT onto suspended particulate matter is thought to be an important removal process. On the other hand, it also means that organotins, adsorbed to suspended matter, end up in sediment (Ceulemans et al., 1998). As TBT has a strong tendency to adsorb to sediments (Unger et al., 1996; Watanabe et al., 1997; St-Jean et al., 1999), consequently, the bottom fauna can be exposed to significantly high organotin sediment concentrations. Degradation of TBT in sediment is significantly slower – half-life from one to a few years, depending on Redox conditions, water temperature, light and presence of microorganisms (Ceulemans et al., 1998; Michel, Averty, 1999; St-Jean et al., 1999; HELCOM, 2009b). The degradation of TBT in the aquatic environment follows a stepwise debutilation process to the less toxic dibutyltin (DBT) and monobutyltin (MBT) and ultimately to inorganic tin compounds. Triphenyltin (TPhT) is transformed into diphenyltin (DPhT) and further into monophenyltin (MPhT) and inorganic tin. Both TBT and TPhT as well as their degradation products are found in the marine environment (St-Jean et al., 1999; HELCOM, 2009b).

TBT is highly toxic to a wide range of aquatic species (Austen, McEvoy, 1997; Stronkhorst et al., 1999). Trisubstituted organotin compounds such as TBT and TPhT are more strongly bioaccumulated than the less lipophilic mono- and disubstituted organotins such as DBT, MBT, DPhT and MPhT. In fish and marine mammals TBT and TPhT bioaccumulate more strongly in liver than in muscle. The majority of studies suggest that TBT is not biomagnified in aquatic food-chain. On the other hand, TPhT appears to be biomagnified fairly strongly (HELCOM, 2009b).

Historically, since the early 1960s, organotins have been employed as a biocide, due to its effectiveness against algae, gram-positive bacteria, fungi and certain marine organisms. Their main use was as a part of anti-fouling paints applied to ship hulls. Consequently this led to a high contamination of sediments by organotins in harbours and shipyards as shown by different studies from all over the world (e.g., Quevauviller et al., 1989; Ko et al., 1995;

Kan-atireklap et al., 1997; Ceulemans et al., 1998, Shim et al., 1999; De Mora et al., 2003; HELCOM, 2009b).

The antifouling use of organic tin compounds in all vessels was banned in 2003 in the EU (2002/62/EC). According to International Convention on the Control of Harmful Anti-Fouling Systems (AFS Convention), as of 1 Jan 2008, old paint should be removed or permanently covered. Lithuania ratified this Convention on 10 Oct 2006. Thus, currently in the EU the use of TBT and TPhT is assumed to be negligible.

Although the use of trisubstituted organotins in most former applications is now prohibited, small amounts of TBT are still used as a biocide (SOCOPSE, 2009). Nowadays, the main emission source of organic tin compounds is their leaching from ship hulls. Related activities that may cause emissions are sea ship traffic, leaching from contaminated harbour sediments and removal of old antifouling paints (Lilja et al., 2009). In addition, TBT occurs as an impurity in stabilizing agents containing MBT and DBT used in the manufacture of plastics (PVC, polyurethane and polyester) and the emission pathway is via the use of the products (HELCOM, 2009b).

The occurrence of organotin compounds is widespread in the Baltic marine environment. Despite the legislative measures taken, the current levels of the most toxic triorganotin compounds like TBT and TPhT pose a risk to the marine environment. The threat caused by organotin compounds to the Baltic Sea is highest near harbours and shipyards. The elevated levels also occur near sea routes and at the disposal sites for dredged material. The TBT levels are high in water, sediment and biota (e.g. in mussels and fish), whereas high levels of TPhT are mainly found in fish (HELCOM, 2009b).

1.2.5.2. Other contaminants

Polybrominated diphenyl ether (PBDE)

Three polybrominated diphenyl ether (PBDE) flame retardants are available commercially; referred as penta-, octa- and decabromodiphenyl ether, but these products are actually mixtures of diphenyl ethers with varying degrees of bromination (HELCOM, 2009b). Out of a total of 209 PBDE congeners Law

et al. (2006) recommended eight (BDE28, BDE47, BDE99, BDE100, BDE153, BDE154, BDE183, BDE209) to be used as a minimum common congener set in determining BDEs from different matrixes. PBDEs are structurally similar to other environmental pollutants, such as dioxins and PCBs. They are lipophilic and persistent compounds and are widespread in the environment. For certain congeners, bioaccumulation has been observed (Paepke et al., 2004).

Since 2004, the production and use of decaBDE only is permitted in Europe where as pentaBDE and octaBDE have been banned. However, stocks of PBDEs are still present in products in service and waste. PBDEs are used as flame retardants in plastics and to some extent in textiles. Main applications of PBDEs were as flame retardants in (in descending order of importance): high-impact polystyrene, acrylonitrile butadiene styrene, flexible polyurethane foam, textile coatings (not clothing), wire and cable insulation, electrical/electronic connectors and other interior parts (Lilja et al., 2009).

The occurrence of BDEs is widespread in the Baltic marine environment. Penta- and octaBDE levels in the Baltic Sea have probably already decreased due to the current legislative measures. While pentaBDE and octaBDE do not seem to pose a risk to the marine environment in the western Baltic Sea, the situation may be different in the eastern part of the Baltic Sea. Overall of the information on the occurrence of penta-, octa- and decaBDE in the Baltic Sea environment is lacking (HELCOM, 2009b).

Hexabromocyclododecane (HBCDD)

HBCDD is an aliphatic brominated flame retardant. The technical mixture exists mainly as a combination of three different isomers, α-, β- and γ-HBCDD, which are all of environmental interest (Lilja et al., 2009; HELCOM, 2009b). HBCDD has been on the world market since the 1960s. It has been used (and perhaps is still used) as a flame retardant in building insulation materials, as well as electrical and electronic equipment. The main use (90 %) of HBCDD is in polystyrene. The predominant use of polystyrene is in rigid insulation panels/boards for the construction industry. The main pathways of HBCDD to

the marine environment are via rivers and the atmosphere. Living organisms, especially predators, such as mammals and predatory birds are at risk via secondary HBCDD poisoning. Assessment showed that HBCDD is a persistent, very bioaccumulative and toxic substance (PBT substance). At present, information on the occurrence of HBCDD in the environment is very scarce (HELCOM, 2009b).

Perfluorinated alkyl substances (PFAS)

Perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA) and other related compounds belong to chemical family called fluorinated surfactants (i.e. surface-active agent). A surfactant is a substance which, even at low concentrations, effectively lowers the surface tension of its medium by selective adsorption on the interface. In fluorinated surfactants, the hydrophobic part of the surfactant molecule contains fluorine. Perfluorinated surfactants, such as PFOS and PFOA, are fully fluorinated surfactants, where all hydrogens in the hydrophobic part of the molecule have been replaced by fluorine. Perfluorinated surfactants have the unique ability to dramatically lower aqueous surface tension, improve wetting and leveling, while remaining chemically stable under harsh use conditions. Fluorinated surfactants are stable to heat (fire resistant), acids, bases as well as reducing and oxidizing agents. Due to these unique properties, they are often irreplaceable in many applications (Poulsen et al., 2005).

The major uses for the PFOS-related substances are in providing grease, oil and water resistance to materials such as textiles, carpets, paper and other coatings. PFOS has also been used in cleaning products, fire-fighting foams and electrical and electroplating industries. PFOA has been used in the PTFE fluoroplastics industry (Brooke et al., 2004; HELCOM, 2009b).

PFOS is persistent, bioaccumulative and toxic (Brooke et al., 2004). However, when compared to typical persistent organic pollutants, some PFOS- and PFOA-related substances are much more water-soluble and a little more volatile. Unlike most POP compounds, PFOS and PFOA do not accumulate in fatty tissues – but is more likely bind to proteins in liver, kidney and blood

plasma (Kannan et al., 2002; Poulsen et al., 2005; Law et al., 2008). Detectable concentrations of PFOS can be observed in species of higher trophic level like seals, whales, dolphins, porpoises from all over the world, including animals from locations in the Canadian Arctic, demonstrating the widespread distribution of this chemical in the environment (Law et al., 2008). Nevertheless, water is considered to be the target matrix for PFOA (HELCOM, 2009b).

The situation regarding PFAS compounds is complicated because of the large number of substances, and it is not exactly known which substances have harmful properties. The risks and threats of PFOA to the Baltic marine environment are currently difficult to estimate due to the lack of ecotoxicological information (HELCOM, 2009b).

Nonylphenols (NP), octylphenols (OP) and their ethoxylates (NPEs & OPEs)

Nonylphenol and octylphenol are organic compounds of the wider family of alkylphenols, more specifically, they are members of a group called “long-chain alkylphenols” (Lilja et al., 2009).

The name "nonylphenol" is used for a number of isomer substances having a phenol ring structure and alkyl chain of C_9H_{19}. Branched 4-nonylphenol is the most descriptive and commercially available NP. The surfactant products produced from nonylphenol are called "nonylphenol ethoxylates" (NPEs). NPEs are used as emulsifiers, dispersive agents, surfactants and/or wetting agents and are the primary source of inputs to the sea of NP and NPEs. The main users of NP and NPEs are the industrial, institutional and domestic cleaning sectors. The primary source of NP in the environment is considered to be NPEs, which can break down into NP after being released into the environment during their production, their formulation into various other products, and the use of these products. Releases to surface water (rivers, lakes, seas and their sediments) occur via industrial and municipal waste water and waste water treatment plants, as well as via sewage sludge containing NP/NPEs (Lilja et al., 2009).

4-*tert*-octylphenol is a high production-volume substance. It is mainly used to make phenolic resins (Brooke et al., 2005). The remainder is converted into ethoxylates to produce surfactants. It can also be present as an impurity in nonylphenol. Phenolic resins are used in rubber processing to make tires. Minor uses include being a component in printing inks and electrical insulation varnishes, and in production of ethoxylated resins for offshore oil recovery. Octylphenol ethoxylates are mainly used in emulsion polymerisation, textile processing, water-based paints, pesticide and veterinary medicine formulations, and to produce octylphenol ether sulphates (Lilja et al., 2009). Octylphenol has a moderate bioaccumulation potential in aquatic biota. When the substance is released to the environment it mainly partitions to soil and sediment (Brooke et al., 2005).

NP and OP are very toxic to aquatic organisms, especially to aquatic invertebrates and fish. In general, both substances show similar toxicity for particular taxonomic groups (Brooke et al., 2005). More data on NP/NPE and OP/OPE concentrations is needed both from discharges in the catchment area and from sea water, biota and sediment of the Baltic Sea to examine if these substances cause harmful effects to the marine environment. While some available data indicate that levels of NP and OP in sea water and biota are not high, levels in the sediment may have adverse effects on the Baltic marine environment (HELCOM, 2009b).

Short-chain chlorinated paraffins (SCCP or chloroalkanes, C_{10-13}) & medium-chain chlorinated paraffins (MCCP or chloroalkanes, C_{14-17})

Chlorinated paraffins (CPs) are mixtures of polychlorinated *n*-alkanes. The technical mixtures are named based on chain length, C_{10-13} are short chained chlorinated paraffins (SCCPs), C_{14-17} are medium chained chlorinated paraffins (MCCPs), and $C_{>18}$ are long chained paraffins (LCCPs) (Oehme et al., 2005).

SCCP as well as MCCP has been used, for example, in rubber and PVC plastics, paints and metal cutting fluids (HELCOM, 2009b).

SCCPs and MCCPs are persistent, bioaccumulate and are toxic to aquatic organisms (Oehme et al., 2005). The physico-chemical properties of CPs,

(affecting their distribution in the environment) depend on carbon chain length and degree of chlorination. CPs are lipophilic with the potential to bioaccumulate. CPs have been found in aquatic biota such as plankton, mussels, crustaceans, fish, seals, whales, in fish eating birds, and in terrestrial biota such as earthworms, rabbit, moose and reindeer. CPs have the potential to biomagnify and increase in concentration with trophic level (Lilja et al., 2009). CPs have also been detected in air, water and sediments (Muir et al., 2000; Oehme et al., 2005). Existing data indicate that MCCP levels in fish may have adverse effects on the Baltic marine environment. Due to a wider current use of MCCP compared to SCCP, MCCP levels in fish and the sediment are higher than levels of SCCP (HELCOM, 2009b).

More research on SCCP and MCCP concentrations in biota and sediment is needed to conclude if SCCP/MCCP causes harmful effects on the Baltic marine environment (HELCOM, 2009b).

Pharmaceuticals

Pharmaceuticals are compounds that are designed to have biological effects on humans or animals. Over 3000 chemical substances are used in human and veterinary medicines, which include, medicines used as painkillers, antibiotics, contraceptives, beta-blockers, lipid regulators, tranquilizers, and impotence drugs (HELCOM, 2010a). Pharmaceuticals and their metabolites have been subjected to many years of uncontrolled emission into the environment as complex mixtures via a number of pathways; primarily from wastewater treatment plants, effluents or the land application of sewage sludge (Roberts, Bersuder, 2006). Pharmaceuticals are now recognized as relevant environmental contaminants. The examples of pharmaceuticals that have been found in the environment of the Baltic Sea are: clofibric acid, ibuprofen, carbamazepine, gemfibrozil, diclofenac, bezafibrate, naproxen and propyphenazone (HELCOM, 2010a).

Surface waters receive continuous inputs of pharmaceuticals. In addition, the substances undergo various chemical, physical and biological processes that degrade and alter them. Aquatic environment can be seen as the final sink of

the most persistent compounds. Pharmaceuticals are a new threat to marine environment and little is known about the environmental fate, possible accumulation and their effects on biota (HELCOM, 2010a).

1.2.6. Chemical munitions dumped in the Baltic

Large quantities of chemical munitions were dumped in European waters after World War II. More than 40 vessels, loaded with such ordnance, were sunk in Skagerrak and Little Belt, while in the Baltic Sea the warfare agents were mainly discarded overboard in the form of munitions or containers primarily into two basins, with ~11 000 t in the Bornholm Basin at depths of 70-105 m and ~1000 t in the Gotland Basin at depths of 70-120 m (Duursma, 1999; Glasby, 1997; HELCOM CHEMU, 1994; Tørnes et al., 2002). Part of the chemical munitions dumpsite in the Gotland Basin lies within the Lithuanian economic zone.

Chemical warfare agents can be classified according to their effects: tear gases or lachrymators (chloroacetophenon), nose and throat irritants (Clark I, Clark II, adamsite), lung irritants (phosgene, diphosgene), blister gases or vesicants (sulfur mustard, nitrogen mustard, lewisite) and nerve gases (tabun) (HELCOM CHEMU, 1994). Arsenic is a major constituent of chemical munitions such as Clark I, Clark II, adamsite, lewisite and arsine oil. The quantity of these chemical compounds is approximately 1/3 of the chemical warfare agents dumped in the east of Bornholm and southeast of Gotland (HELCOM CHEMU, 1994).

Lewisite ($C_2H_2AsCl_3$) reacts with water to form chlorvinyl arsine oxide, which in alkaline solution can react further to produce arsenic acid and acetylene (HELCOM CHEMU, 1994). Arsine oil is a technical mixture of arsenic (III) chloride, phenylarsine dichloride, diphenylarsine chloride and triphenylarsine (Haas et al., 1998). Clark I ($(C_6H_5)_2AsCl$) and Clark II ($(C_6H_5)_2AsCN$) are expected to adsorb onto sediments and react only very slowly with water. Eventually both degrade to form tetra-phenyldiarsine oxide, which is itself toxic and is hydrolysed very slowly. Similarly, adamsite ($NH(C_6H_4)_2AsCl$) is

practically insoluble in water, adsorbs onto sediments, and hydrolyses very slowly forming phenarsazinic oxide. Thus, the chemical munitions Clark I, Clark II and adamsite, together with toxic reaction products, can be preserved on the sea bed for a long time. However, they might also bioaccumulate in organisms (Glasby, 1997; HELCOM CHEMU, 1994; Tørnes et al., 2002; Sanderson et al., 2010). Clark I, Clark II and adamsite are expected to spread from the chemical munitions source very slowly and contaminate only local sediments. Therefore, elevated arsenic concentrations in the sediments can indicate a leakage of chemicals from the containers (Duursma, 1999; HELCOM CHEMU, 1994; Tørnes et al., 2002; Emelyanov, 2007; Emelyanov, 2007a; Emelyanov, Kravtsov, 2007).

The chemical warfare agents dumped in the sea pose three main threats (Glasby, 1997). The first one is the threat to the general public from agents washed ashore. This could take place only as a result of material in wooden crates being thrown overboard from moving vessels during the original dumping operation. Such occurrences were reported on Polish beaches, mainly between 1952 and 1955 (Glasby, 1997). The possibility that chemical munitions can now be washed ashore from the dumping areas is extremely unlikely. The Bornholm and Gotland basins are characterized by stable stratification with anoxic conditions developing below the halocline, with only slight bottom currents except during exceptional periods of flushing to the basins (Voipio, 1981). In addition, in order to be washed ashore, the dumped material would need to be moved upwards from a depth below 100 m (Glasby, 1997; HELCOM CHEMU, 1994). The second threat is the threat to fisherman who can possibly trawl lumps of viscous mustard gas from the sea floor with their nets. Over the period 2000–2007 from 1 to 25 (in 2003) incidents had been reported where chemical munitions were netted by fishermen. This shows that these chemicals are still a risk for the crews of fishing vessels operating in this part of the Baltic (HELCOM, 2010b). The third is the threat to the marine environment. There is a possibility of bioaccumulation of arsenic compounds in marine organisms (HELCOM CHEMU, 1994; Tørnes et al., 2002).

Investigations in the southern Adriatic (Mediterranean Sea) showed that tissues of fish from the chemical munitions dumping site had higher levels of arsenic than those from a reference site (Amato et al., 2006). Katkova (2010) investigated a scenario in the Baltic Sea, according to which arsenic appears in the sea water as a result of corrosion, hydrolysis and degradation of warfare agents and later accumulates in sea fish and poses a risk for human health.

1.3. Effects of pollutants on aquatic organisms

Marine organisms in their tissues can accumulate contaminants from seawater, suspended particulate matter, sediments, or by uptake through re-suspension of sediment material (UNEP, 1992; Sternbeck et al., 2003). The concentrations of certain contaminants in tissues are related to the concentrations in the organism's surrounding environment. This process is termed bioaccumulation, and it has been used by scientists to assess the marine contamination caused by human activities (UNEP, 1992). Many pollutants accumulate in organisms to concentrations that may become deleterious for a single species, ecosystems or even human (Dybern, Fonselius, 1981).

The widely varying conditions in different marine areas may affect the biological accessibility of pollutants. Among those conditions are salinity, temperature, changes in pH, turbidity (amount of suspended particles), physic-chemical properties and substitution patterns of the individual compounds, as well as different sensitivity of various species. Different species do not bio-accumulate to the same level when they are exposed to the same concentrations of contaminant in sea water, and often have different rates of contaminant elimination. Even individuals of the same species, exposed to the same concentration of contaminants for the same period of time, might not accumulate the substances at the same rate. This is related to factors such as age, sex, size and physiological state of the individual (UNEP, 1992; Karbe et al., 1994; Kersten et al., 1994; Swedish EPA, 2000).

Within the organism tissues, contaminants are bound to various compartments which are different with respect to their sorption capacity. Binding may be of low or of high affinity, unspecific, or specific in the case of contaminants which are bound to specific biochemical structures (receptors). The sorption of contaminants may be short-term or long-term and could be characterized by half-lives of several hours, days, weeks or months (Karbe et al., 1994).

Different contaminants show significant differences with respect to their tendency to be bioaccumulated. Investigations by Karbe et al. (1994) show that in the sediment samples accumulation factors for metals are ranked - Mn > Pb > Hg > Cu > Cd > Fe, in the blue mussel - Hg > Cd > Cu > Pb, in hermit crab - Fe > Cu > Mn > Cd > Hg > Pb. This can be interpreted as a difference exists among metals with respect to their tendency of sorption to inorganic and organic surfaces and uptake by resorptive processes into organic matrices. For example, the tendency of sorption to surfaces is high in case of lead and rather low for cadmium; however, the tendency for bioaccumulation as a consequence of high affinity binding to specific biochemical structures is much stronger for cadmium than for lead.

Investigations by Solé et al. (2000) indicated that bioaccumulation of organic pollutants in aquatic organisms tissue, followed the order PAHs > DDTs > PCBs > OPs (organophosphorous pesticides). Organochlorines and other organic contaminants are enriched from water to sediment and to biota as a consequence of hydrophobic/lipophilic interactions. Octanol-water partition coefficient may be used to quantify the hydrophobic properties of chemicals. The tendency to be accumulated is the highest in case of the highly hydrophobic and lipophilic toxic environmental chemicals (xenobiotics) like DDT and its metabolites, as well as in case of PCBs (Karbe et al., 1994). Due to the lower stability and lower partition octanol-water coefficient, OPs are more environmentally labile. In general, it has been accepted that OPs pose less risk to the ecosystem, due to their low bioaccumulation factor and high biotransformation rate, which prevents their biomagnifications along the food chains. On the contrary, organochlorine pesticides and PAHs may cause

chronic toxicity problems. Organochlorines, in particular, are responsible for negative ecological consequences to wildlife because of their biomagnifications in the top of the food webs (Solé et al., 2000). Generally, organic chemicals with octanol-water coefficient of $K_{ow} > 10^2$ have the potential to biomagnify in marine food chain and they comprise almost two-thirds of all organic chemicals used in commerce. About 40 % of chemicals with these properties have a $K_{ow} > 10^5$ and are potentially bioaccumulative because of their high degree of lipid-water portioning (Kelly et al., 2007).

If concentrations and bioavailability are sufficiently high, negative effects on individual species can occur (Sternbeck et al., 2003). The responses of estuarine and marine organisms to contaminants are manifold, but could be generally classified into four levels of biological organization, i.e. cellular, organismal, population and community levels. The earliest detectable changes inside the cell in response to xenobiotics involve subcellular organelles such as lysosomes, endoplasmic reticulum and mitochondria (Verlecar et al., 2006).

According to Adams (2005), responses of organisms to chemical stressors are the integrated result of both direct and indirect pathways (Fig. 1.3.1). Direct pathways operate primarily through metabolic processes, such as toxic action of chemicals at receptor sites. The effects of stressors acting through direct mechanisms occur initially at the molecular or subcellular level and can be expressed, for example, as changes in biomolecular, biochemical, and physiological components or processes such as protein and gene expression, enzyme activity, metabolism and respiration. Indirect pathways, however, operate mainly through effects on the food chain on habitat availability or through changes in the organism behavior. Indirect effects of stressors can affect interspecific interactions through competition and predation, which may cause a cascade of trophic effects in aquatic systems. Stressors can also indirectly influence the health of marine biota by affecting the quality and quantity of the habitat and result in altered behavior related to reproduction, feeding or selection of habitat (Adams, 2005).

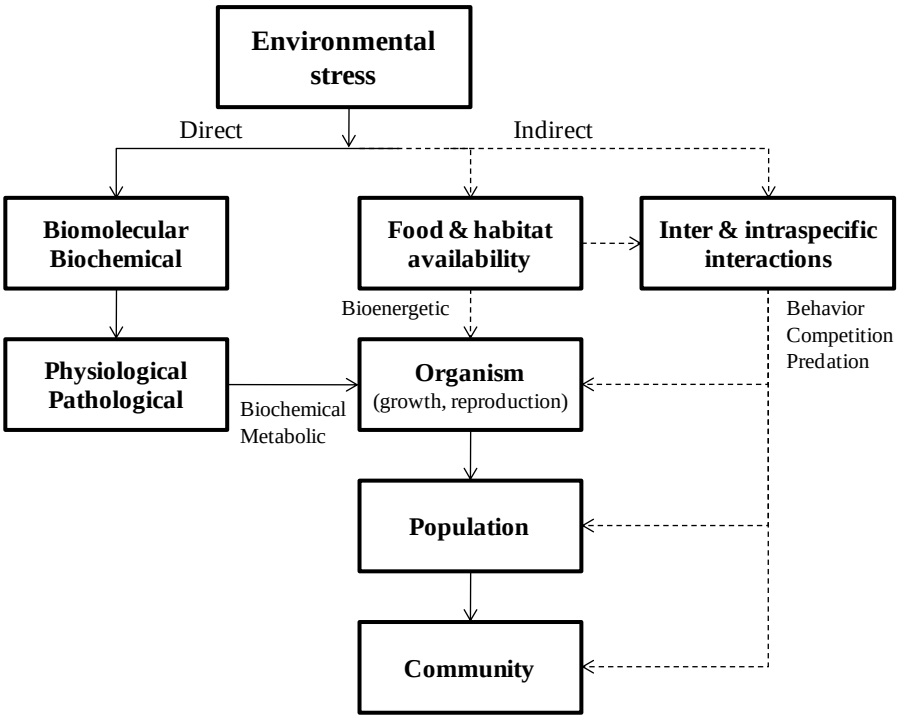


Fig. 1.3.1. Responses of organisms to chemical stressors trough direct and indirect pathways (from Adams, 2005)

Direct and indirect genetic effects have been also described by Medina et al (2007). According to authors direct effects are also related to the damage that genotoxic substances (genotoxins) exert on the molecular structure of the genetic code (i.e. DNA). These include point mutations, chromosomal aberrations like inversions, deletions or additions, DNA adducts, DNA strand breaks, formation of micronuclei and aneugenic effects. Indirect effects are population-mediated processes when pollution decreases the genetic variability of the population (Medina et al., 2007).

It has been shown that certain chemical contaminants, taken up from the environment by living organisms, can bind to DNA. Formation of so-called DNA adducts may result in teratogenesis, mutagenesis and carcinogenesis in many marine vertebrate species. It has been proved that environmental pollutants provoke increased levels of genetic damage in marine invertebrates (Depledge, 1998).

A classic example of the effect of pollutants on marine organisms is that of tributyltin, which has been shown to induce genotoxic, cytotoxic, immunotoxic

and teratogenic effects in some marine invertebrates (Jha, 2004). In British waters, the chemical has had a dramatic effect on the dog whelk *Nucella lapillus* which is hypersensitive to TBT. An extinction of the dog whelks now has been documented in many sites in of UK, where they used to be common until the TBT paints were introduced. TBT causes so called "imposex" effects that triggers growth of a vas deferens and a penis in female dog whelks. These block the opening of the female genital duct so that eggs cannot be released. TBT seems to affect the hormone system that determines the sex of prosobranch mollusks (Walker et al., 1996). Hagger et al. (2006) has provided evidence of DNA damage associated with the development of imposex in the *N. lapillus*; the masculinization of female gastropods considered to be the result of alterations to endocrine-mediated pathways. Thus, the imposex index provides an indication of the exposure of dog whelks to TBT at a site. Similar findings have been made on other gastropode species elsewhere in the world. Since 1998 imposex has been included as a biomarker in the national monitoring program in Denmark, covering both coastal and open waters in the Belt Sea, the Sound, the Kattegat and the Skagerrak. Four key species of gastropods have been selected, *Buccinum undatum*, *Neptunea antiqua*, *Hinia reticulata* and *Littorina littorea*, however, imposex development has also been found in other Danish gastropod species. Studies performed within Danish national monitoring program (NOVA) have shown that imposex is a widespread phenomenon in all regions of Danish waters (HELCOM, 2009b). In addition, other considerable biological effects of TBT also exist including shell malformations of oysters, reduced resistance to infection (e.g. flounder) and effects on the human immune system (SOCOPSE, 2009). By exposing *Mytilus edulis* to environmentally realistic concentrations of tributyltin oxide, Hagger et al. (2005) showed that TBT is cytotoxic and genotoxic to adult mussels. Studies of the same researches also revealed that TBT may adversely affect marine organisms at many stages of the lifecycle and through multiple mechanisms, thus confirming that TBT has a deleterious effect to the marine environment.

At elevated levels metals act as enzyme inhibitors (Kennish, 1997). Heavy metal cations react in part specifically with cellular components to cause toxic effects. Mechanisms of detoxification are accomplished either via the high affinity of metal cations with sulphyhydryl (–SH) groups of metallothionein or via their accumulation in membrane-limited granules, representing a general strategy for metal cation homeostasis (Domouhtsidou, 2004). For example, investigations show that in contaminated areas mercury may be expected to have an impact on bivalve immune functions (Gagnaire et al., 2004). Zinc has been reported to suppress gametogenesis in *Mytilus* (Kalojanni et al., 2005).

Offshore oil industry can contaminate environment with produced water, which in addition to the remaining oil, contains other chemicals, i.e. additives used in drilling and pumping operations, as well as in the oil/water separation process (e.g. such as heavy metals, alkylphenols, PAHs and etc). Investigations of Lavado et al. (2006) showed that exposure of mussels to chemicals present in produced water can lead to alterations on key biochemical pathways that could have physiological consequences for these organisms. He showed that oil and a mixture of oil and alkylphenols can affect endogenous levels of steroids in mussels. Furthermore, studies of Stephens et al. (2000) showed that produced water chemicals can affect and induce the detoxification metabolism in fish. Investigations done after the Prestige oil spill showed that activities of some biomarkers (GST, GR and CAT) were significantly elevated in fish *Lepidorhombu boscii* which came from the most oil impacted area (Martínez-Gómez et al, 2006). In addition, lysosomal membrane stability was reduced in mussels from all locations in the oil spill area, indicating disturbed health (Orbea et al., 2006).

Polycyclic aromatic hydrocarbons (PAHs) are well known genotoxins and carcinogens. PAHs accumulate in tissues to high concentrations in invertebrates at the bottom of the food chains, where uptake rates greatly exceed rates of metabolism and elimination, compared with vertebrates, where metabolism and elimination can cope with uptake (Jha, 2004). Boehm et al. (2005) have recommended using mussels (*Mytilus trossulus*) in monitoring of

PAHs in marine environment, especially following an oil spill. Authors suggest to use mussels as a monitoring tool when the assessments involve food-chain effects. PAHs in the aquatic environment induce acute toxicity in organisms and the presence of PAHs in the sediments has been linked to liver neoplasms and other abnormalities in benthic fish species (Jha, 2004).

During the experiments with *M. edulis* mussels from the Baltic Sea exposed to PCB, the physiological measures (clearance rate and scope for growth) and changes in protein expression indicate the stressed state of the mussels (Olsson et al., 2004). Patterns of PCB congeners in marine organisms, capable of degrading and metabolizing some congeners, are affected by the degree and capacity of biodegradability and the storage capacity of organisms. Organisms at higher trophic levels possess mixed function oxygenase enzyme systems (MFO systems) composed of NADPH and cytochromes, which eliminate lipophilic xenobiotics by converting them to hydrophilic metabolites. In many species PCB congeners are known being MFO system inducers (Karbe et al., 1994).

Aquatic animals can enzymatically biotranform many xenobiotics. Metabolism of most xenobiotics by aquatic organisms results in their detoxification through conversion to more water-soluble and readily excretable products. Detoxification is not the only result of induction of enzymatic biodegradation pathways in living organisms; the increased reactivity of oxygenated metabolites of aromatic compounds can lead to genetic damage. Some xenobiotics, such as PAHs, organophosphorous pesticides, PCBs are enzymatically transformed to metabolites that are more toxic than the original/parent compound (Solé et al., 2000; Pikkarainen, 2008).

However, more investigations should be done to examine harmful effects of contaminants on the aquatic organisms, especially for “new emerging substances” like pentaBDE, NP, NPE, OP and endosulfan which are possible endocrine-disrupting substances (HELCOM, 2009b).

1.3.1. Biomarkers

The need to detect and assess the effects of contamination at ever lower concentrations and in ever more complex mixtures have led to the development of a wide range of molecular, biochemical, sub-cellullar and other indicators of exposure and effects of contaminants and other environmental stressors (CEFAS, 2000). Thus, early warning indicators – biomarkers – that respond before measurable effects on individual performance and population/community dynamics occur were established. Biomarkers indicate that organisms have been or are being exposed to certain chemicals or that organisms are suffering or likely will suffer future impairments of environmental conditions (Forbes et al., 2006).

Several definitions have been given for the term 'biomarker'. Benford et al. (2000) presents 16 definitions of the term 'biomarker' appearing in the literature. In general, the definition includes almost any measurement reflecting an interaction between a biological system and a potential hazard, which may be chemical, physical or biological (WHO, 1993). Three separate terms 'biomarker', 'bioindicator' and 'ecological indicator' of different levels of biological organization can be defined. Biomarker is considered as any biological response to an environmental chemical at the subindividual level, measured inside an organism or in its products (urine, faeces, hair, feathers, etc.), indicating a deviation from the normal status. The term bioindicator is defined as an organism giving information on the environmental conditions of its habitat by its presence or absence or by its behavior, and an ecological indicator is the parameter that describes the structure and functioning of ecosystems (Van Gastel, Van Brummelen, 1994).

Biomarkers can be subdivided into three classes (WHO, 1993; Van der Oost et al., 2003):

- biomarkers of exposure: cover the detection and measurement of an exogenous substance or its metabolite or the product of an interaction between a xenobiotic agent and some target molecule or cell that is measured in a compartment within an organism (e.g.,

acetylcholinesterase inhibition, metallothionein induction, PAH metabolites in bile (Hagger et al., 2008);

- biomarkers of effect: include measurable biochemical, physiological or other alterations within tissues or body fluids of an organism, which can be recognized as associated with an established or possible health impairment or disease. There can be physiological (feeding/clearance rate or heart rate of organisms) or cellular (micronuclei and neutral red retention time) biomarkers (Hagger et al., 2008);
- biomarkers of susceptibility: indicate the inherent or acquired ability of an organism to respond to the challenge of exposure to a specific xenobiotic substance, including genetic factors and changes in receptors which alter the susceptibility of an organism to that exposure.

Biomarkers are also classified as specific or non-specific. For example, the specific biomarker metallothionein has been widely used to indicate the presence of heavy metals (Geffard et al., 2001, 2002; Geret, Cosson, 2002; Mourgaud et al., 2002; Tangui et al., 2002; Domouhtsidou et al., 2004). Acetylcholinesterase activity is considered a specific biomarker of organophosphorus and carbamate pesticides (Bocquené et al., 1995; Bocquené, Galgani, 1998; Monserrat et al., 2001; Binelli et al., 2006). However, it should be pointed out that there is evidence to the confounding effects that some abiotic factors, like salinity or temperature, can have influence on metallothionein (Monserrat et al., 2007) and acetylcholinesterase activity (Radenac et al., 1998; Pfeifer et al., 2005). The determination of oxidative stress (DNA damage, protein oxidation, lipid peroxidation) and antioxidant responses in aquatic species are non-specific biomarkers, since several pollutants can modify directly or indirectly the balance between the concentration of pro-oxidants and antioxidants (Monserrat et al, 2007). Histopathological gill changes in fishes are also non-specific to pollutant exposure (Au, 2004). The responses of general “stress biomarkers” are related to various contaminants exposure: neurotoxicity (acetylcholinesterase inhibition), lysosomal stability, immunotoxicity (macrophage activity),

genotoxicity (micronuclei), oxidative stress (catalase), liver detoxification enzymes (e.g. GST) (Lehtonen, 2005).

Several biomarkers that have been used in the biological effects of pollution studies not only worldwide but also in the Baltic Sea are presented below.

Lysosomal membrane stability (LMS)

Lysosomes are organelles that make up a waste disposal and macromolecular recycling system in cells. Lysosomes are membrane-bound compartment for intracellular digestion of food ingested by the cells. These organelles accumulate chemical contaminants, which results in lysosomal damage leading to cell injury, tissue dysfunction, and reduction in animal "health status" (Moore et al., 2004). Lysosomal membrane stability is a general stress/toxicity biomarker. LMS in flounder, eelpout and blue mussel sensitively reflect effects of general toxicity of various classes of contaminants in different areas of the Baltic Sea. Accidental spill of contaminants, like the oil spill at the Būtingė oil terminal by the Lithuanian coast, caused a significant decrease of the lysosomal membrane integrity in flounder liver and digestive gland of mussels, with an afterward recovery processes and an increase in membrane stability three months after the oil spill. Furthermore, a clear gradient of LMS responses to exposure of pollutants has been detected in flounder and mussels inhabiting different zones in the Baltic Sea (Wismar Bay, Gulf of Gdansk, Lithuanian and Swedish coast) (Lehtonen et al., 2006). The analysis of LMS is a fast, cost-effective and easy-to-learn tool, which provides comprehensive information for the studies of liver/hepatopancreas pathology and related dysfunction. In addition to LMS, studies of immunomodulation (macrophage activity), as well as contamination induced accumulation of unsaturated neutral lipids or phospholipids can be analyzed using histochemical approach (Broeg, Lehtonen, 2006; Lehtonen et al., 2006). LMS is a sensitive biomarker which should be permanently included in regular monitoring activities (Schiedek et al., 2006).

Acetylcholinesterase inhibition (AChE)

Acetylcholinesterase is commonly found in nervous tissues, brain, red blood cells and muscle tissues of most animals. It is responsible for the rapid degradation of the neurotransmitter acetylcholine into the inactive products choline and acetic acid. When AChE is inactivated by a contaminant, the enzyme is no longer able to hydrolyse acetylcholine, keeping its concentration high. As a result, a continuous stimulation of the muscle or nerve fiber occurs, evoking paralysis and even death (Bocquené, Galgani, 1998). AChE inhibition is a neurotoxicity or general stress reflecting biomarker. Monserrat et al. (2006) described it as „a specific biomarker with an old history and new uses". AChE activity has traditionally been used as an indicator of direct neurotoxic effects, especially as an indicator of exposure to organophosphate or carbamate pesticides (Bocquené et al., 1995; Bocquené, Galgani, 1998; Monserrat et al., 2001; Binelli et al., 2006). Large-scale biomarker assessment in fish and mussels from the Baltic Sea revealed seasonal differences in the AChE activity in flounder, eelpout and mussels, which occured due to variations in the presence of deleterious substances, e.g. pesticides from river input or run-off from agricultural sources (Lehtonen et al., 2006). Although the impact of environmental factors also has to be considered, additional studies have showed that AChE in mussels is influenced by temperature and salinity (Pfeifer et al., 2005; Leiniö, Lehtonen, 2005). Hence, abiotic parameters and seasonal differences within the Baltic Sea have to be taken into account when applying AChE activity as a biomarker to monitor contaminant effects in marine organisms. Especially in mussels, the AChE activity often coincide with the environmental pollution gradients. Therefore, AChE has proved to be an indicator of general physiological stress, in many cases having a similar pattern to LMS (Lehtonen et al., 2006). Moreover, investigations show that AChE can be a sensitive indicator of exposure to oil pollution (Moreira et al., 2004).

Ethoxyresorufin O-deethylase (EROD) activity

Ethoxyresorufin O-deethylase activity reflects the active amount of the P450 enzyme CYP1A, which is induced via the cytosolic aryl hydrocarbon receptor. Many planar aromatic molecules, which include some toxic environmental pollutants, such as polycyclic aromatic hydrocarbons, polychlorinated biphenyls and dioxins, are strong agonists to the aryl hydrocarbon receptor. Therefore the induction of EROD activity has been widely used as a biomarker of exposure to PAHs, PCBs and dioxins (Hansson et al., 2006). EROD activity may vary considerably in responce to, for example temperature, sex, reproductive stage and nutrition. Thus it is very important to use well standardised and optimised catching, handling and sampling timing and strategies (Kopecka et al., 2006; Kopecka, Pempkowiak et al., 2008). It is recommended that only one sex should be analyzed, or if both sexes are chosen, the data must be treated separately. In addition, it is also recommended to pay attention to physiological status of studied organisms and not to mix fish groups with developed and undeveloped gonads (Lehtonen et al., 2006). Seasonal differences in EROD activities were found in flounder in the Gulf of Gdansk, attributed to spawning in spring; thus, EROD should not be measured during the reproduction stage. No geographical differences in EROD activity could be found in regard to the pollution gradient in the Gulf of Gdansk (Kopecka et al., 2006). Napierska and Podolska (2005) had also difficulties in interpreting EROD activity levels in flounder at coastal and offshore sites of the southern Baltic Sea. However, there was one clear hotspot – the area of the Vistula River mouth, where EROD activity was regularly elevated in comparison with other areas (Napierska, Podolska, 2005). Interregional comparisons of EROD activities can be difficult, but possible if carefully standardised procedures are used, the condition of individuals is controlled and environmental factors taken into account (Lehtonen et al., 2006).

Metallothionein (MT) induction

Metallothionein induction – a metal exposure or general stress reflecting biomarker. Metallothioneins constitute a family of low molecular weight,

cysteine-rich proteins, which are capable of binding metals. First reported in mammals in the late 1950s, MT have been studied in many aquatic invertebrates (Monserrat et al., 2007). MT induction has widely been used as a specific indicator of exposure to heavy metals (especially Cd, Zn, Cu, Hg) (Geffard et al., 2001, 2002; Geret, Cosson, 2002; Mourgaud et al., 2002; Tangui et al., 2002; Domouhtsidou et al., 2004). Binding of metals into MT during an excess of harmful metals protects an organism against toxicity by limiting availability of the metal cations. Generally, MT expression increases with the elevation of tissue concentrations of MT-inducing metals, reflecting metal bioavailability in the environment (Monserrat et al., 2007). There is seasonal variability in the levels of MT (especially in flounder), which is often greater than between-site differences. A salinity-related change in MT levels can be seen in *Mytilus* sp., with higher levels in the less-saline northern areas. The greatly elevated concentrations in flounder were observed in some areas of the Baltic Sea which was likely related to the reproductive stage of the species but might also be indicative of differential exposure situations during seasonal migrations. Therefore, the interpretation of MT results for the monitoring purposes should be done with care, taking into account different environmental factors (Monserrat et al., 2007; Lehtonen, 2005; Lehtonen et al., 2006).

PAH metabolites in bile

After PAHs penetrate into the fish organism (via gills, body surface, with food or through contaminated sediment), they are rapidly transformed into more hydrophilic metabolites that are excreted. PAH metabolites are determined in fish bile, where they are stored before excretion (Vourinen et al., 2006). Hence, metabolites of PAH compounds in fish bile are indicative tool of exposure to these compounds. Pyrene-type metabolites are the most common ones, e.g. 1-hydroxypyrene is the major metabolite in Baltic Sea perch (Pikkarainen, 2006). Two methods can be used for the analysis of PAH metabolites: the Fixed Wavelength Fluorescence (FF) method and HPLC with fluorescence detection. The FF method, measuring bile fluorescence for

pyrene-type metabolites, does not give as detailed information about PAH exposure as the HPLC-F method, which measures individual metabolites quantitatively. Despite that, the FF method is fast and practical and usually indicates the extent of exposure sufficiently well for biomonitoring purposes (Vourinen et al., 2006). Data from individual fishes of five species demonstrated that there were clear differences in bile PAH metabolite contents between species and between sexes of the same species and this should be considered in monitoring programs. Consequently, fish bile PAH metabolite results indicated differences between reference and polluted areas and also demonstrated gradients in pollution and thus could be useful as a biomarker for PAH exposure (Vourinen et al., 2006; Lehtonen et al., 2006).

Micronuclei (MN)frequency

Micronuclei are small chromatin-containing bodies found in cells. Their formation frequency is used for the indication of gentoxic agent action. The investigations of fish and mussels from the Baltic Sea showed the relevance of the approach in assessment of genotoxic effects, with clear correlations with contaminant gradients. MN is a cost-effective, simple and rapid method which is convenient to employ in field samplings following standard procedures and protocols. The output is well defined, and is easily recognizable. It allows the evaluation of the influence of genotoxic compounds at low concentrations and the assessment of dose-response relationships of genotoxins. Therefore, the MN test is a useful tool in marine monitoring programs (Baršienė et al., 2004). Previous studies on biomarker responses in fish and mussels from the Lithuanian coastal area showed a significant increase of genotoxicity and cytotoxicity, after an accidental oil spill in the Būtingė oil terminal (Baršienė et al., 2004, 2005, 2006a, 2006b). Increased genotoxicity and cytotoxicity levels were found at sites in the Lithuanian economic zone located close to the oil platform D-6 (FIMR, 2007). A higher frequency of micronuclei were detected in mussels from the Būtingė oil terminal and Klaipėda marine port zones in the Baltic Sea (Baršienė, Baršytė Lovejoy, 2000; Baršienė, 2002), in Mediterranean commercial port zone (Magni et al., 2006) and in the Venice

Lagoon polluted by aromatic hydrocarbons (Venier, Zampieron, 2005). In different studies, a significant elevation of micronuclei levels has been described for mussels 30 days after an oil spill, while the cytogenetic damage persisted up to 100 days (Parry et al., 1997) or even 8 months (Baršienė et al., 2004, 2006a). A statistically significant increase of the micronuclei level has also been observed in oysters and fish caged in Haven oil spill zones 10 years after an oil spill (Bolognesi et al., 2006).

Nuclear buds (NB) fragmented-apoptotic (FA) and bi-nucleated (BN) cells

In recent years, growing attention has been paid to morphological nuclear abnormalities like nuclear buds, fragmented-apoptotic or bi-nucleated cells. These nuclear abnormalities can serve as an indicator of genotoxic and cytotoxic damage. The nuclear alterations are related to cell division disturbances, like damaged DNA extrusion from the nucleus, cell death, injures in cytokinesis, or other alterations in cell morphology. Together with micronuclei, NB are used in the assessment of genotoxicity endpoints of pollution, while induction of FA and BN cells indicate cytotoxicity potential of contaminants. Data on other nuclear abnormalities present a wider view on cellular processes following the exposure and permit the measurement of important complementary genotoxicity and cytotoxicity events (Baršienė, Rybakovas, 2006; Baršienė et al., 2006a, 2006c; Baršienė, Andreikėnaitė, 2007; Koukouzika, Dimitriadis, 2008; Rybakovas et al., 2009).

Neutral lipid accumulation

The accumulation of unsaturated neutral lipids in liver of fish and digestive gland cells of invertebrates can be used as a marker of toxically induced disturbance of fat metabolism (Lehtonen et al., 2006). The research of flounder in the Baltic Sea showed that lipid accumulation was significantly higher in autumn, compared to spring with one exception in the Gulf of Gdansk (Kopecka et al., 2006). Analysis of neutral lipid accumulation in the digestive gland of mussels revealed low lipid accumulation only the exception in Wismar Bay and Lithuanian coast (Schiedek et al., 2006; Baršienė et al., 2006b). In addition to LMS test, the biomarker presents information on

pathological status of organisms and on alterations in liver cell metabolism (Lehtonen et al., 2006).

Catalase (CAT) and glutathione S-transferase (GST) activity

Catalase is a haematin-containing enzyme that is responsible for the breakdown of hydrogen peroxide (H_2O_2). Glutathione S-transferase plays important roles in both detoxification and bioactivation reactions; this Phase II conjugation enzyme, is also commonly used as a biomarker of exposure to organic xenobiotics. The activity of both enzymes is related to cellular oxidative stress defense (Lehtonen, 2005).

Studies, carried out on mussels from the Baltic Sea, showed a statistically significant inter-location differences both in CAT and GST activities, which in some cases were connected to the contaminant gradient. In flounder, seasonal differences were observed in CAT activity; a possible connection to tissue contaminant concentrations was also observed (Kopecka et al., 2006). In addition, gender differences were also found in CAT activity – the CAT activities were always higher in male than in female fish (Kopecka, Pempkowiak, 2008). Leiniö and Lehtonen (2005) noticed a high seasonal CAT activity in *Mytilus edulis* in late spring which can be explained by the availability of food and the ensuing start of the growth period after a long period of winter. The similar seasonal differences in CAT activity were described by Bocchetti and Regoli (2006) for the Mediterranean mussel *Mytilus galloprovincialis.* Oxidative changes have been reported as responses in bivalve molluscs during the periods of more intense feeding activities as a consequence of higher oxidative metabolism.

A study with neogastropod *Murex trunculus* showed a seasonality of GST which was probably due to anaerobic metabolism during winter period (Gharbi-Bouraoui et al., 2008). The influence of salinity on GST activity was also described. However, after the evaluation of environmental factors it was determined that the high GST activity was due to copper taken up by the mussel rather than salinity or temperature. Investigations show that GST can also be a sensitive indicator of exposure to oil pollution (Moreira et al., 2004).

Superoxide dismutase (SOD)

Superoxide dismutase processes free oxygen radical intermediates (reactive oxygen species (ROS)), formed as „side products“ (e.g. during biotransformation of organic xenobiotics) to hydrogen peroxide (H_2O_2). Thereafter CAT is responsible for transformation of hydrogen peroxide to water and oxygen (Kankaanpaa et al., 2007). Thus the enzymes SOD and CAT are an efficient pair of cellular antioxidants that neutralize ROS before they initiate radical chain reactions (Moreira et al., 2006).

SOD is a non-specific widely used biomarker. Together with other oxidative stress enzymes, SOD has been used to determine the acute toxic effects of pharmaceuticals (diazepam, clofibrate and clofibric acid) and a detergent (sodium dodecylsulphate) to the crustacean *Artemia parthenogenetica* (Nunes et al., 2006). Moreira et al. (2006) used SOD activity in polychaete *Hediste diversicolor* as an indicator for sediment contamination. Together with other biomarkers SOD was also used in assessments of petrochemical environmental contaminations (Porte et al., 2000; Lima et al., 2007; Gorbi et al., 2008). However, the influence of abiotic parameters such as temperature (Moreira et al., 2006) and nutrients (nitrates, nitrite, ammonia) (Lima et al., 2007) on the activity of SOD was also indicated.

Glutathione reductase (GR) and glutathione peroxidase (GPx)

Oxidative stress enzymes such as glutathione peroxidase and glutathione reductase are of special importance. GPx acts as a scavenger for high concentrations of hydrogen peroxide. During this process, glutathione is oxidized losing its protective capability. GR is an enzymatic species responsible for the reversion of the oxidized form of glutathione, thus leading to the formation of two molecules of glutathione, which can again perform the detoxification role (Nunes et al., 2006).

GR and GPx are used in different environmental contamination studies. For example, GR and GPx showed higher activities in mussels *Mytilus galloprovincialis* from the offshore platform in the Adriatic Sea compared to those caged at the reference site (Gorbi et al., 2008). De Luca-Abbott et al.

(2005) used these biomarkers in the assessments of environmental contamination by transplanting mussels and clams from „clean“ to chemically polluted sites, while Moreira et al. (2006) used them in the assessment of sediments contamination. In another study, GPx and GR activities in crabs and clams correlated with the concentration of Zn and Pb metals in sediment, which means that these metals caused some stress in the exposed organisms, as was reflected in the antioxidant responses (Morales-Caselles et al., 2008).

Lipid peroxidation (LPO)

Lipid peroxidation is another oxidative stress biomarker which indicates oxidative damage to tissues. Many contaminants are known to produce this type of damage, e.g. heavy metals, organic contaminants like pesticides and aromatic compounds, etc. (Chévre et al., 2003). The extent of lipid peroxidation can be measured through the evaluation of the levels of thiobarbituric acid reactive substances (TBARS) (Nunes et al., 2006).

LPO is widely used together with other oxidative stress enzymes. For example, high level of LPO was an indicator of hyperoxic/anoxic cycles generated by the photosynthetic/respiratory process of cyanobacteria (Da Rosa et al., 2005). LPO was also used to study antioxidant responses to polycyclic aromatic hydrocarbons and organochlorine pesticides in green-lipped mussels (*Perna viridis*), where correlations were found for LPO and individual organochlorine compounds (Richardson et al., 2008). Nunes et al. (2006) used LPO to determine the acute toxic effects of pharmaceuticals and sodium dodecylsulphate. When evaluating LPO results, environmental factors should be considered as LPO levels in mussels' digestive glands also seem to be related to pH (Lima et al., 2006).

Reproductive disorders

Common environmental pollutants are capable of disrupting reproductive and developmental processes by interfering with the actions of endogenous hormones. For example, an imposex in the gastropod *Nucella lapillus* is considered to be the result of alterations to endocrine-mediated pathways (Hagger et al., 2006). Studies performed in the Baltic Sea with gastropod

species *Buccinum undatum*, *Neptunea antique* and *Hinia reticulata* have shown that imposex is a widespread phenomenon in all regions of the Danish waters (HELCOM, 2009b).

Intersex – the condition whereby otherwise normal gonochoristic (unisexual) species possess both male and female characteristics (Ford, Fernandes, 2005) – is considered to be a reliable biomarker of contaminant effects. Eelpout (*Zoarces viviparus*) is used as a bioindicator to analyze impairments provoking reproductive failure. Because of its viviparous mode of reproduction, this species presents a unique opportunity to evaluate the reproductive success and disturbances in larval developmental on early ontogenetical stages. In eelpout there is also a possibility to investigate reproductive disorders in both sexes in the process of gonadal development. Presence of intersex and ovotestis could be detected in male eelpout histological analysis. Intersexuality status of fish is considered to be an indicator of endocrine disruption. For example, in polluted coastal waters of Germany the prevalence of intersex and malformation of larvae was high, whereas in reference sites (Kvädöfjärden, Swedish coast) these injures rarely observed (Gercken et al., 2006). Compared to other marine fish (e.g. flounder) eelpout seems to be more sensitive to develop an intersex condition; that is why the histological examination of testis can be restricted to a rather low number of specimens. Female eelpout living in polluted areas frequently show a degeneration of the oocytes in the developing gonad. When the oocytes are in a vitellogenic stage this condition (follicular atresia) can be assessed macroscopically. The obtained results clearly showed that female eelpout from the German Baltic coastal areas exhibit reduced reproductive success compared to females from less contaminated Swedish coastal sites. In conclusion, eelpout proved to be an ideal species to monitor contaminant induced reproductive disorders at different stages of a reproductive cycle (Gercken et al., 2006).

Intersex has been studied in other Baltic Sea fish species – three-spined stickleback (*Gasterosteus aculeatus L.*) in Swedish coastal waters. Intersex was observed in only one individual from the whole sampling set of

sticklebacks. The contradiction in the field observations for eelpout and sticklebacks may be attributable to the two species having different sensitivity and/or having been exposed to different extent of contamination during the time of their gonadal development (Petterson et al., 2007).

Liver histopathology and externally visible fish diseases

Different histopathological alterations in fish and other aquatic organisms have been developed and used as biomarkers in pollution monitoring. Liver is the primary organ for biotransformation of organic xenobiotics, for the excretion of harmful trace metals, food digestion and storage, and for metabolism of sex hormones. In general, liver histopathological lesions are not specific to pollutants. Moreover, species-specific differences in sensitivity to chemicals have also been reported (Au, 2004). Liver histopathology studies in the Baltic Sea have been carried out in flounder from coastal as well as from offshore sampling sites. Lesions identified have been categorised into major classes: nonspecific lesions, early non-neoplastic toxicopathic lesions, pre-neoplastic lesions, benign liver tumours and malignant liver tumours. The results revealed marked and consistent spatial variation in the prevalence of the lesion categories, indicating contaminant effects (Lang et al., 2006).

Externally visible fish diseases can serve as a primary indicator of exposure to environmental pollution, and certain diseases have proven to be reliable biological indicators of toxic and carcinogenic effects of pollutants (Au, 2004). In the Baltic externally visible fish diseases have been studied in flounder and cod from offshore sampling sites. The results clearly showed spatial differences in the health status and an impact of host-specific and site-specific factors on the prevalence. Analysis of the results obtained by applying other biological effect techniques showed some relationships between the occurrence of externally visible diseases and other biomarkers. Thus, it is important to take into account the health status of the fish when assessing biological effects of contaminants (Lang et al., 2006).

1.4. Integrated assessment of the state of the aquatic environment

The complexity of marine systems, their high variability, and the influence of multiple environmental factors on these systems suggest that no single measure is adequate for assessing the effects of multiple stressors on biota. An appropriate number of measures is required for determining the biological significance of stress and for understanding the underlying cause of the observed effects. Some studies have used chemical criteria exclusively for assessing effects of stressors and in helping to identify the possible causes of observed effects on biota. There is no doubt that, results of chemical analyses is an important indication of whether or not organisms are exposed to pollutants at unacceptable high levels (Den Besten, 1998). But relying on chemical criteria alone for assessing the effects of stressors and understanding the mechanistic basis of these effects on surface water resources may provide an inadequate assessment of the biological and ecological condition of aquatic systems. Use of chemical criteria alone in the assessment of water quality can be incomplete for legislation related issues as they do not include broader ecological measures. Chemical criteria alone fail to describe the influence of other environmental factors that can impair aquatic ecosystems, such as sedimentation, changes in habitat and natural flow regimes, varying temperature and oxygen regimes, and changes in ecological factors such as food availability and predator-prey interactions (Adams, 2005). In addition, contaminants in the environment can occur as a complex mixture, and the risk associated with such mixtures cannot be adequately evaluated on the basis of the effect and behaviour of individual components. Since biological systems are the main target of the toxicant action, they could provide important information which is not readily available from chemical analyses (Jha, 2004). Biomarkers may be useful tools, indicating bioavailability of contaminants and adverse effects at the same time. Biomarkers could also be used to detect subtle effects on the population or community level (Den Besten, 1998).

Studies of biological effects have many advantages in comparison to chemical analyses. Molecular, cellular, biochemical and physiological effects of

environmental contaminants are rapidly manifested, and the obtained data may be used to focus subsequent detailed chemical analyses on problem areas. Numerous chemicals and their metabolites are potent genotoxins, causing pathological lesions and reproductive disorders, which may strongly affect populations and communities. Therefore, biomarkers indicating effects of chemicals serve as early warning signals, a pre-requirement when following the precautionary principle (Lehtonen et al., 2006).

Biological-effect methods are important elements in environmental monitoring programs. They can indicate links between contaminants and ecological responses. Biological-effect methods can be used to indicate the presence of substances, or combinations of substances of concern, but also to identify regions of decreased environmental quality or reduced ecosystem health (Thain et al., 2008).

In order to improve the assessment of the effects of chemical contaminants and their mixtures in the Baltic Sea marine ecosystem, an integrated approach is needed (Lehtonen, Schiedek, 2006b). Van der Oost et al. (2003) suggest five environmental monitoring methods which may be performed in order to assess risks of contaminants on organisms and to classify the environmental quality of ecosystems:

- chemical monitoring: measuring levels of contaminants in abiotic environmental compartments;
- bioaccumulation monitoring: measuring contaminant levels or bioaccumulation in biota;
- biological effect monitoring: the assessment of exposure and effect of contaminants using a set of biomarkers;
- health monitoring: the assessment of contaminants effect by examining the occurrence of irreversible diseases or tissue damage in organisms;
- ecosystem monitoring: the assessment of ecosystem quality by evaluation of e.g. species composition, density or biodiversity.

Thus, an integrated monitoring program is a study consisting of coordinated monitoring activities comprising both chemical and biological measurements

in a variety of environmental media or compartments (Van der Oost et al., 2003). An integrated monitoring will provide a reliable assessment of ecosystem health of the aquatic environment. It will also support political measures to be taken to improve the state of the environment (Lehtonen, Schiedek, 2006b).

The HELCOM Baltic Sea Action Plan (BSAP) has been adopted in 2007. The vision to have a healthy Baltic Sea environment is built on four pillars in the HELCOM BSAP: the sea unaffected by eutrophication, life undisturbed by hazardous substances, favourably conserved biodiversity, and environmentally friendly maritime activities. Among the ecological objectives concerning life undisturbed by hazardous substances are: to reach concentrations of hazardous substance close to natural levels, to ensure that all Baltic fish are safe to eat, to safeguard the health of wildlife. The HELCOM countries have therefore decided to work together to build up more information about the sources of the selected hazardous substances, the extent of their occurrence in the Baltic marine environment, as well as about their biological effects. By sighning the BSAP, HELCOM countries also agreed to develop biological effects monitoring which would facilitate a reliable ecosystem health assessment (HELCOM, 2007a). Nevertheless this measure still remains to be undertaken. Biological effects monitoring is not established not only in Lithuania but also in some other countries around the Baltic Sea. When developing biological effects monitoring, a harmonized implementation of biomarkers as an early warning system in the whole Baltic Sea should be the final aim. Although the specific sub-regional conditions of the Baltic Sea should also be considered (HELCOM, 2010a).

The EU Marine Strategy Framework Directive (2008/56/EC) (MSFD) aims for the good status of the marine environment by 2020. MSFD addresses hazardous substances by the Annexes I and III, setting one of the qualitative descriptors for determining good environmental status as „Concentrations of contaminants are at levels not giving rise to pollution effects“. According to MSFD, progress towards good environmental status will depend on whether

pollution is progressively being phased out, i.e. wether the presence of contaminants in the marine environment and their biological effects are kept within acceptable limits, so as to ensure that there are no significant impacts on or risk to the marine environment. Thereafter, the assessment of the present environmental condition, setting the indicators of good environmental status, the establishment of environmental targets and monitoring programs are needed for the successful implementation of the MSFD. And that is the case where integrated view of the situation is needed.

Thus, integrated monitoring of hazardous substances and their effects is as an important step forward to reach one of our future goals – the Baltic Sea life undisturbed by hazardous substances.

2. MATERIAL AND METHODS

2.1 Study area

Nearly 83 % of Lithuanian territory belongs to the catchment area of the Baltic Proper, including the river catchment areas of the Nemunas, the Bartuva, the Venta and the Akmena-Dane. The population density of this territory is 57 inhabitants per km². The Lithuanian sub-basin catchment area is dominated by agricultural land (54 %) and forests (31 %), with 5 % urban areas, 4 % inland waters, 2 % wetlands and 4 % devoted to various other land uses. The area's main river, the Nemunas, discharges into the semi-enclosed Curonian Lagoon (HELCOM, 2004a). Only 90.6 km of the shore belongs to Lithuania, with its main part (51 km) being on the Curonian sand spit which is declared the National Park and entered into the UNESCO list of protected areas (Žaromskis, 2007). The study area includes the Lithuanian waters of the Baltic Sea, which are situated in the south-eastern part of the Baltic Proper. Lithuanian waters are divided into 4 different types: transitional waters (central part, northern part and the plume of the Curonian Lagoon waters), heavily modified waterbody (Klaipėda Strait), coastal waters (northern stony coast and southern sandy coast) and open sea waters (Fig. 2.1.1).

The offshore waters show the typical stratification pattern for the Baltic Proper with the upper layer (mean salinity 7-8 PSU) separated by a permanent halocline at 70-80 m depth from the more saline subhalocline water layer which is oxygen deficient. The composition of the sediment is quite diverse: from coarse clastic material, to sands of various grade, to silt. There are three main lithological facies: boulders with shingle and gravel, coarse and medium sand, and fine sand (Bitinas et al., 2005; Olenin, 2005).

In the area north of Klaipėda, major hydrological features are determined by the interaction between the southeastern Baltic offshore waters and the runoff of the mostly freshwater Curonian Lagoon. Due to prevailing northern direction of currents this area is much more influenced by the freshwater outflow than the rest part of the coastal areas. The mainland sub-marine

coastal slope, extending from the shore down to 25-30 m, is characterized by diverse bottom types. Its uppermost part, at 0-6 m, is covered by mobile quartz sand, while at greater depths the sand alternates with pebble-gravel deposits and large boulders. Benthic communities on the hard bottom are dominated by the blue mussel *Mytilus edulis* and invasive barnacle *Balanus improvisus* (Olenin, Daunys, 2004; Olenin, 2005).

Southward of the Klaipėda Strait, there are typical Baltic Proper waters. Along the Curonian Spit the bottom sediments are much more homogenous, with sand prevailing throughout the area. In general, the character of sediments changes from the mixture of sand and gravel in the wave affected coastal area to aleurites and pelitic muds in deeper areas. Sandy bottoms at the depths of 20 m and downward are dominated by the bivalve *Macoma balthica* (Olenin, Daunys, 2004; Olenin, 2005).

The narrow (width 400–600 m) Klaipėda Strait area connects the Curonian Lagoon and the south-eastern part of the Baltic Sea. This area is artificially deepened and its maximum depth is about 14 m. It is oligohaline with irregular salinity fluctuations from 0.5 to 8 PSU (Olenin, Daunys, 2004; Zaiko et al., 2007).

The Curonian Lagoon is the largest coastal lagoon in the Baltic Sea. It is an enclosed shallow lagoon (mean depth 3.7 m). The southern and central parts of the lagoon are freshwater due to discharge from Nemunas (98 % of total) and other rivers, while the northern part is oligohaline with irregular salinity fluctuations from 0 to 8 PSU. The Lagoon is a highly eutrophied water body and blue-green algae blooms are a regular annual phenomenon. The main water current in the Curonian Lagoon is the outflow of the Nemunas river, which empties into the Baltic Sea near the port of Klaipėda. Almost the whole bottom of the Curonian Lagoon is covered by recent sediments. The relict glacigenic sediments occur only locally and are exposed as small fields of boulders with pebbles and gravel accumulations overgrown by mollusc *Dreissena polymorpha* colonies (Trimonis et al., 2003; Olenin, 2005).

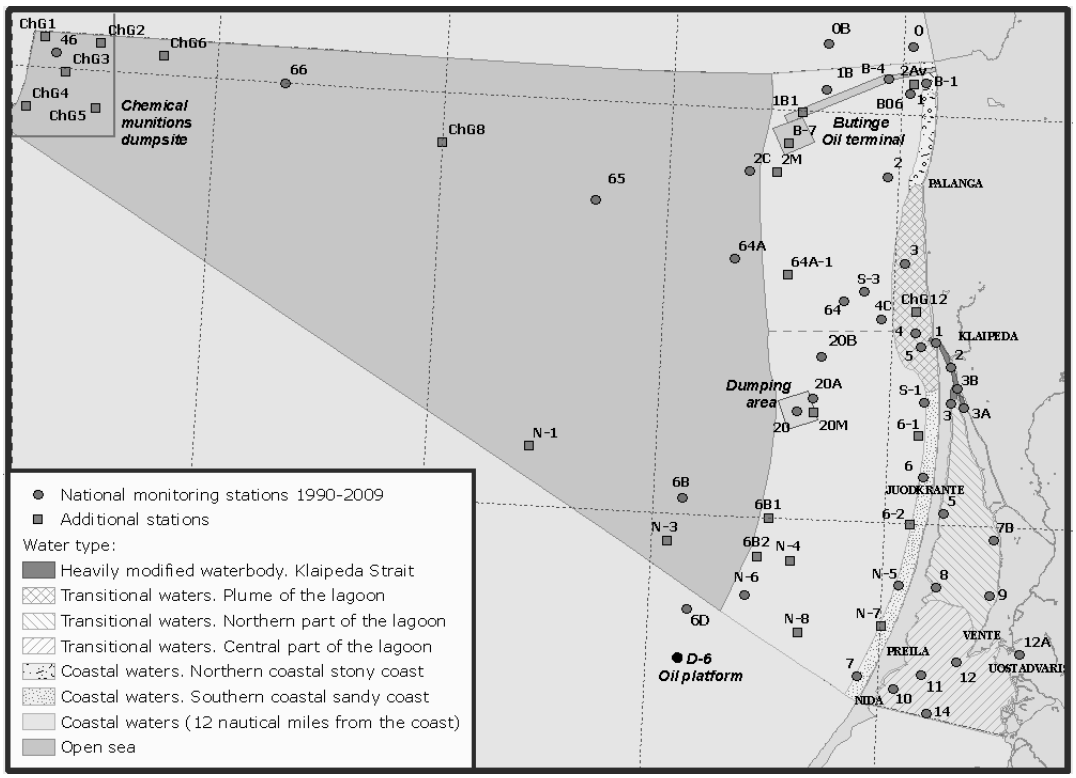


Fig. 2.1.1. Lithuanian monitoring and study stations in the Baltic Sea and Curonian Lagoon

2.2 Sampling campaigns

Several environmental parameter sets were compiled during the period of the study. All analysed data sets and case studies are presented in Table 2.2.1. Coordinates of the stations are presented in Annex I, Table A1.

Table 2.2.1. Environmental parameters analyzed in different projects and case studies and used in the study

Data sets and case studines (stations)	Date	Matrix[4]	Parametres[5]	Analysing laboratory[6]	Author's contribution
Lithuanian national monitoring data (Baltic Sea: 46, 66, 65, 64A, 2C, 6B, 0B, 0, 6D, 1B, B-4, B-1, 1, 2, 3, S-3, 64, 4C, 4, 5, 20B, 20A, 20, S-1, 6, N-5, 7, N-6; Curonian Lagoon: 10, 14, 12, 12A, 8, 9, 7B, 5, 3A, 3, 3B, 2, 1)	1990-2009	Water, sediments	Hg, Cd, Cu, Pb, Cr, Zn, Ni, total oil hydrocarbons, organochlorine pesticides	CMR	Planning annual monitoring activities, sampling of water and sediments, sampling and preparation of biota samples, supervision of the chemical analyses of metals, oil and pesticides
			PAHs, PCBs	EPA ERD	
		M. edulis *M. balthica* *D.polymorpha* *P. flesus* *C. harengus* *G. morhua*	Hg, Cd, Cu, Pb, Zn, organochlorine pesticides	CMR	

Data sets and case studines (stations)	Date	Matrix[4]	Parametres[5]	Analysing laboratory[6]	Author's contribution
Arsenic in sediments from the southeastern Baltic Sea (ChG1, ChG2, ChG3, ChG4, ChG5, ChG6, ChG8, ChG11, ChG12, 66 (ChG7), 65 (ChG9), 6B, B-1, S-3, 64 (ChG10), 4 (ChG13), 5 (ChG14), 20A, 20, S-1, 6, N-5, 7, N-6)	2003-2004	Sediments	As, Fe	IAEA MEL	Chemical analysis, data evaluation
D-6 project[1] (65, N-1, 6B (N-2), N-3, N-4, N-5, N-6, N-7, N-8, 7 (N-9), 4, 6)	2005-2006	Water, sediments	Oil hydrocalrbons	CMR	Coordinator of the Lithuanian party of the project, sampling, supervision of the oil analysis, hydrochemistry data evaluation
LIFE project[2] (1B, B-4, 1B-1, B-7, 2M, 2, 3, 64A-1, 20A, 20, 20M, S-1, 6-1, 6, 6-2, N-5, 7, N-6, N-8, N-4, 6B2, 6B1)	2006	Sediments	Hg, Cd, Pb, Zn, Cu, oil hydrocarbons	CMR	Project partner, planning of sampling sites, sampling, supervision of the metals and oil analyses, data evaluation
			PAHs	JSC VT	
		M. edulis *M. balthica*	Hg, Cd, Pb, Zn, Cu	CMR	
Genotoxic and cytotoxic effects in bivalves from the Baltic Sea (65, 1B, B06, 20M, N-4, N-8)	2006	*M. edulis* *M. balthica*	Genotoxicity: MN, NB; cytotoxicity: FA, BN	VU IE	Planning of sampling sites, sampling, participation in geno- and cytotoxicity analyses
Būtingė oil spill 31 January 2008 (1B, B-7, 2AV, 1, 2, Palanga)	2008	Water, sediments	Oil hydrocarbons	CMR	Planning of sampling sites, sampling, supervision of oil analysis, taking part in geno- and cytotoxicity analyses
		M. edulis	Genotoxicity: MN, NB; cytotoxicity: FA, BN	VU IE	
Biomarkers in some invertebrates of the coastal area (Baltic Sea: 65, 1B, 7; Curonian Lagoon: 12, 2)	2008	*M. eduis* *M. balthica* *D.polymorpha* *N.diversicolor*	Biomarkers: AChE, CAT, GST, LPO	FIMR, SYKE	Planning of sampling sites, sampling, analysis of biomarkers
Dioxins and TBT in sediments from the coastal area (Baltic Sea: 65, 4, 20, 7; Curonian Lagoon: 3B, 2)	2008	Sediments	Dioxins, furanes, dioxin-like PCBs, TBT	GALAB	Planning of sampling sites, sampling
Screening of the selected hazardous substances in the eastern Baltic marine environment[3] (65 and 4)	2008-2009	Water	PFAS, NP/NPE, OP/OPE	IVL	Coordinator of the Lithuanian party of the project, development of sampling strategy, sampling of water samples, chairing the final project meeting
		P.flesus *C.harengus*	TBT, TPhT, PBDE, HBCDD, NP/NPE, OP/OPE, endosulfan	IVL	
			PFAS, SCCP and MCCP	NILU	

[1] Joint Finnish-Lithuanian Project „Evaluation of the environmental state of the sea area in the Lithuanian territorial waters and economic zone adjacent to the Russian oil platform D-6".
[2] LIFE Nature project "Marine Protected Areas in the Eastern Baltic Sea" (LIFE 05 NAT/LV/000100).
[3] HELCOM project "Screening study on occurrence of hazardous substances in the eastern Baltic Sea" (Nordic Council of Ministers).
[4] *Mytilus edulis, Macoma balthica, Platichthys flesus, Clupea harengus, Gadus morhua, Dreissena polymorpha, Nereis diversicolor.*

[5]PAHs – polycyclic aromatic hydrocarbons; PCBs – polychlorinated byphenyls; PFAS – perfluorinated substances; NP – nonylphenol; NPE – nonylphenol ethoxylate; OP – octylphenol; OPE – octylphenol ethoxylate; TBT – tributyltin; TPhT – triphenyltin; PBDE – polybrominated diphenyl ethers; HBCDD – hexabromocyclododecane; SCCP – short chained chlorinated paraffins; MCCP – medium chained chlorinated paraffins; MN – micronuclei; NB – nuclear buds; FA – fragmented apoptotic cells; BN – bi-nucleated cells; AChE – acetylcholinesterase; CAT – catalase; GST – glutation S-transferase; LPO – lipid peroxidation.

[6] CMR – Center of Marine Research, Ministry of Environment, Lithuania; EPA ERD – Environmental Protection Agency, Environmental Research Department, Lithuania; IAEA MEL – International Atomic Energy Agency, Marine Environment Laboratory, Monaco; JST VT – Joint Stock Company „Vandens tyrimai“, Lithuania; VU IE – Vinius University, Institute of Ecology, Lithuania; FIMR – Finnish Institute of Marine Research, Finland; SYKE – Finnish Environment Institute, Finland; GALAB – GALAB Laboratories GmbH, Germany; IVL – Swedish Environmental Research Institute, Sweden; NILU – Norwegian Institute for Air Research, Norway.

2.2.1 National monitoring cruises

Water, sediment and biota samples were collected during the national monitoring cruises of research vessel „Vėjas“ (in the Baltic Sea) or expedition boat „Gintaras“(in the Curonian Lagoon) (Fig. 2.2.1.1). Locations of sampling stations are presented in Fig. 2.1.1. There are 4 seasonal cruises (February, May, August, October-November) to the Baltic Sea and 12 sampling cruises (every month) to the Curonian Lagoon. According to the annual monitoring plans of 2008 there were 16 sampling stations for contaminants (heavy metals, oil hydrocarbons, chlorinated pesticides) in the Baltic Sea monitoring plan, which included coastal and open sea waters. The monitoring plan of Transitional waters (Curonian Lagoon, Klaipėda Strait and plume of the lagoon) included 13 stations. Monitored contaminants, matrix and sampling frequency are presented in table 2.2.1.1.

Water sampling

Water samples for the analysis of contaminants were taken by plastic 5 liter water sampler (PWS). Samples for the analysis of metals (except Hg) were taken into the plastic or borosilicate glass colorless bottles (250 ml); samples for the Hg analysis were taken into the borosilicate glass (500 ml) bottles. Bottles were rinsed with the samples and filled with the sample till the top.

Samples for the analysis of oil hydrocarbons, PAHs, organochlorine pesticides and PCBs were taken into the clean glass bottles (1000 ml). Bottles shouldn’t be rinsed with the sample and shouldn’t be filled till the top.

A **B**

Fig. 2.2.1.1. Research vessels of the Center of Marine Research: A – research vessel "Vėjas" for the national monitoring of the Baltic Sea (1980–2009; length: 55.6 m; width: 9.3 m; drought: 4.3 m; 7 laboratories; 12 crew members; 20 members of scientific group); B – expedition boat of for the national monitoring of the Curonian Lagoon (1983; length: 21 m; width: 4 m; drought: 1.6 m; 1 laboratory; 2 crew members; 5 members of scientific group)

Table 2.2.1.1. Contaminants, matrix and sampling frequency from the national monitoring annual plans

Parameter	Matrix	Sampling campaign frequency in the Baltic Sea
Hg, Cd, Cu, Pb, Cr, Zn	Water	4/year
Hg, Cd, Cu, Pb, Cr, Zn, Ni	Sediment	3/year
Hg, Cd, Cu, Pb, Zn	Biota	1/year
Petroleum hydrocarbons	Water	4-12/year
Petroleum hydrocarbons	Sediment	3/year
Organochlorines: Drins (3), DDTs (3), HCHs (3), Endosulfan (2), HCB	Water/sediment/biota	1/year
PCB (7)[1]	Water/sediment	1/year
PAH (8)[2]	Water/sediment	1/year

[1] Concentrations of PCBs were determined in the laboratory of Environmental Research Department (Lithuanian EPA) by gas chromatography with ECD detector.

[2] Concentrations of PAHs were determined in the laboratory of Environmental Research Department (Lithuanian EPA) by high-performance liquid chromatography with fluorescence detection. PAHs in water were determined by LST EN ISO 17993:2004 method, in sediments – by ISO 13877:1998 method.

Sediment sampling

Sediment samples were collected using a large Van Veen grab sampler (75 kg, with a sampling area of 0.1 m^2). Sediment from the top ~1-3 cm was sub-sampled to plastic (for metal analysis, except Hg) and glass (for other contaminants) containers. Samples were frozen immediately onboard. After transportation to the laboratory, samples were stored in a deep-freezer at a temperature of ≤ - 20 ^oC until analysis.

Sampling of biota

Mytilus edulis mussels in the Baltic Sea and *Dreissena polymorpha* mussels from the Curonian Lagoon were collected from the sea bottom using dredge haul and *Macoma balthica* mussels in the Baltic Sea – using Van Veen grab sampler. Mussels were separated from the substratum and kept in the clean seawater from the area of collection.

Fish is sampled by the Lithuanian State Pisciculture and Fisheries Research Centre. Frozen fish is delivered to the Center of Marine Research together with the netting cards.

2.2.2 Arsenic in sediments from the southeastern Baltic Sea

The sediment samples were collected during two expeditions of the scientific research vessel "Vėjas" (Fig. 2.2.1.1, A) organized by the Ministry of Environment (Centre of Marine Research) and the Ministry of National Defense of Lithuania. The first mission in June 2003 went to the chemical weapons dumpsite. Sampling locations are illustrated in Fig. 2.1.1, distinguishing samples collected on the first mission by the prefix ChG: stations ChG1, ChG2, ChG3, ChG4, ChG5, ChG6, ChG8, ChG11, ChG12 and also monitoring stations 66 (ChG7), 65 (ChG9), 64 (ChG10), 4 (ChG13), 5 (ChG14). Sampling stations at the dumpsite (ChG1, ChG2, ChG3, ChG4, ChG5) were chosen near chemical weapon units, according to sonar data obtained from scanning the seafloor by a Lithuanian naval vessel. The second mission in August 2004 visited Lithuanian national monitoring stations: 66, 65, 6B, N-6, 20, 20A, 64, S-3, B-4, 4, 5, S-1, N-5, 7, B-1, 6. Five stations (66, 65, 64, 4, 5), which were sampled during the first mission, were revisited to check sampling variability. Fourteen sediment samples were collected during the first expedition in June 2003, of which 5 samples were from the chemical munitions dumpsite (ChG1-ChG5 stations), and sixteen samples during the expedition in August 2004. Sediment samples were collected according to procedure described in 2.2.1.

2.2.3 Evaluation of the environmental state of the sea area in the Lithuanian territorial waters and economic zone adjacent to the Russian oil platform D-6

According to the research plan of the project, the sampling cruise has been organized in November 2005 to evaluate the environmental state of the Baltic sea area adjacent to the Russian oil platform D-6. Experts from Finnish Institute of Marine Research (FIMR, Finland) have participated in the project to make this evaluation independent. Among the measured parameters were: total hydrocarbons and PAHs in biota and sediments; heavy metals in biota and sediments; organotins in biota and sediments; alkylated phenols in sediments; biological effects in biota. Center of Marine Research was responsible for the sampling campaign and the analysis of oil hydrocarbons in water and sediments. The station network was based on the standard monitoring stations in the region (4, 6, N-5, 7, N-6, 6B, 65); new stations near the Russian border were also included (N-1, N-3, N-4, N-7, N-8) (Fig. 2.1.1). Water, sediment and biota samples were sampled according to the procedures described in 2.2.1.

2.2.4 LIFE Nature project "Marine Protected Areas in the Eastern Baltic Sea"

Sampling stations were planned in the two LIFE project areas: 12 LIT and 13 LIT. Sediment and biota samples were collected in 2006 during three sampling cruises in May, August and October on the scientific research vessel "Vėjas" (Fig. 2.2.4.1). Sediment and biota samples were sampled according to the procedures described in 2.2.1.

Two length classes of mussels were taken for the analysis of heavy metals: for *Macoma* 15-18 mm and 19-21 mm; for *Mytilus* – 15-24 mm and larger than 25 mm At one site quantity of *Mytilus* mussels for such arrangement was insufficient, there was only one size class.

The analysis of 15 PAHs in sediments was done by the JSC "Vandens tyrimai" laboratory. Samples were extracted with petroleum ether. The analysis was done by high performance liquid chromatography with fluorescence detection.

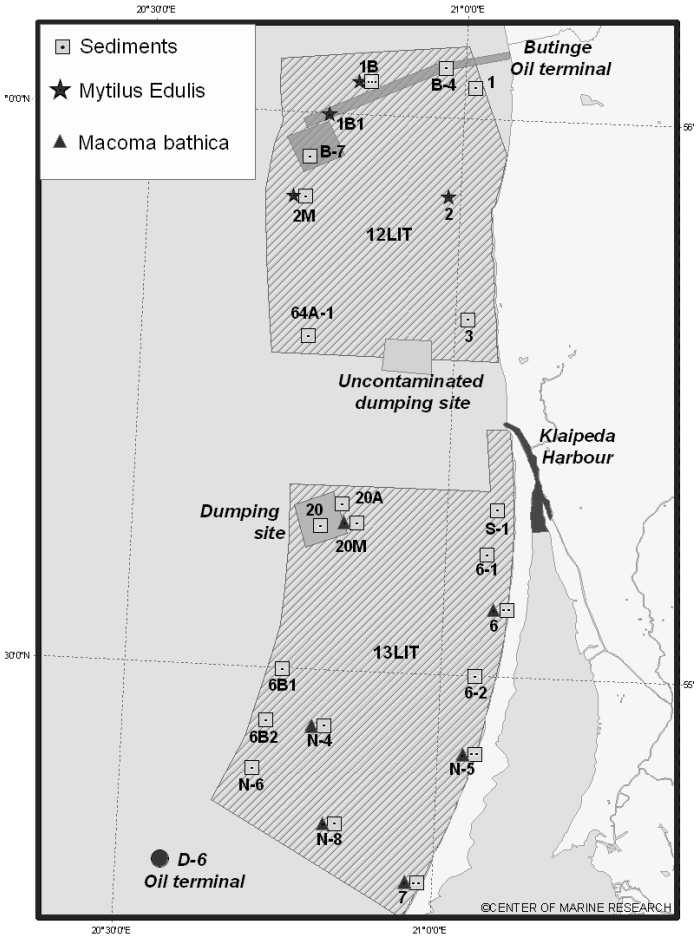


Fig. 2.2.4.1. LIFE project areas and sampling sites. Dots show number of sampling campaigns (2006-05; 2006-08 or 2006-10)

2.2.5 Environmental genotoxicity and cytotoxicity effects in bivalve mollusks from the Baltic Sea

Environmental genotoxicity and cytotoxicity at stations 65, 1B, B06, 20M, N-4, N-8 were measured in gill cells of bivalve mussels *Macoma balthica* and *Mytilus edulis*. Mussels were sampled in May, June and August 2006, according to the procedure described in 2.2.1. Sampling stations are shown in Fig. 2.1.1.

2.2.6 Būtingė oil spill

In 31 January 2008, the accidental spill of oil products from the tanker "Stena Antarctica" occurred during the pumping of oil from the Būtingė oil terminal. Oil spilled due to the weather conditions, the spill has been distributed on Lithuanian coast near Šventoji. On 11-12 of February 2008 blue mussels (*Mytilus edulis*) were sampled from three study sites (1st, 1B and 2AV) closely located to "Stena Antarctica" oil spill site in the Būtingė oil terminal area. In addition to annual monitoring sites, station 2AV was sampled because it was the place where the oil spill was firstly noticed (Fig. 2.2.6.1). Three months later, in May 2008, 16 mussels were collected from two contaminated by accidental oil spill sites and 16 specimens were sampled in June 2007 from the reference site Palanga. In August 2008, 30 mussels additionally were sampled from three contaminated by oil spill sites. The main objective of this study was to evaluate the level of environmental genotoxicity and cytotoxicity in different sites of the Lithuanian economic zone of the Baltic Sea, which were affected by the oil spill from the tanker "Stena Antarctica".

2.2.7 Biomarkers in some invertebrates of the coastal area

For the biomarker studies, in August 2008 *Macoma balthica* mussels were sampled from the national monitoring stations 7 and 65 in the Baltic Sea; *Mytilus edulis* – from the station 1B (Fig. 2.1.1). In the Curonian Lagoon bivalves *Dreissena polymorpha* were sampled closely to the station 12 (Fig. 2.1.1). Mussels were sampled according to the procedure described in 2.2.1. *Nereis diversicolor* specimen were sampled in the Curonian Lagoon station 2 (Fig. 2.1.1) using a Van Veen grab sampler.

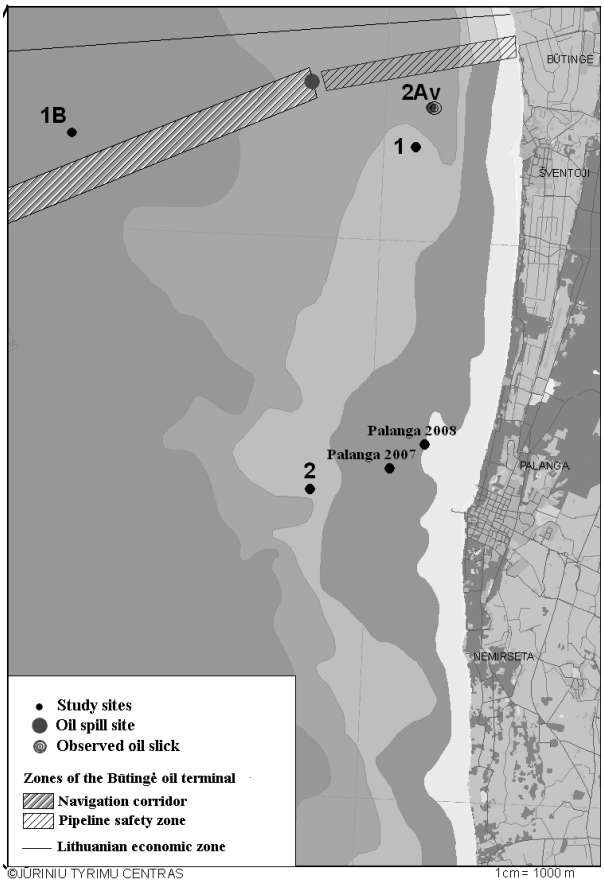


Fig. 2.2.6.1. Sampling stations for the monitor of environmental geno-cytotoxicity after the oil spill in 2008

2.2.8 Dioxins and TBT in sediments from the coastal area

Sediment samples were taken in August 2008 by the procedure described in 2.2.1. Four samples (stations 7, 4, 20 and 65) were taken during the National monitoring cruise to the Baltic Sea from the research vessel „Vėjas“. Stations were selected consider the distance to the main anthropogenic activity areas: station 7 is situated near the coast rather close to the border with Russia and has sandy sediments, thus it can be used as a reference station. Station 4 is situated near the entrance to the Klaipėda harbour and the Curonian Lagoon. Station 20 is a station from the dumping site were dredged sediments from the harbour are dumped. And station 65 is an open sea station. Other two samples were taken during the National monitoring cruise from the Curonian Lagoon using the research vessel “Gintaras”. Both stations (2 and 3B) are situated in the Klaipėda harbour (Fig. 2.1.1).

Sediments were sent to GALAB Laboratories GmbH in Germany for the analysis of dioxins and furanes, dioxin-like PCBs (gas chromatography/mass spectrometry method) and TBT (DIN 19744 method).

2.2.9 Screening of the selected hazardous substances in the eastern Baltic marine environment

During 2008/2009 under the leadership of HELCOM, a screening study in the eastern Baltic Sea environment on the occurrence of eight of the substances/substance groups identified as hazardous under the Baltic Sea Action Plan has been performed. Two matrices (water and fish) were chosen for the analysis of hazardous substances. Two sampling stations 4 and 65 were chosen for water sampling. Water samples were sampled according to the procedure described in 2.2.1. Two fish species herring (*Clupea harengus*) and flounder (*Platichtys flesus*) were chosen for the research as a widely distributed species. Fish samples were taken by the Vilnius University, Institute of Ecology using gill nets (14-90 mm).

Water and frozen fish samples were sent to the Swedish Environmental Research Institute for the analysis of hazardous substances.

2.3 Analysis methods

2.3.1 Chemical analysis

As and Fe were measured in the Marine Environment Laboratory, IAEA (Monaco), other contaminants in the laboratory of the Center of Marine Research (Lithuania).

Preparation of biota samples for the analysis

After sampling mollusks were kept for 24 hours in clean water from the original area. After that, the length of each mussel was measured and all mussel specimens were arranged in length classes. Whole soft body of the mollusks were carefully removed from the shell and pooled with others samples of same size class in one sample in plastic zip-lock bags and were stored at $\leq$ - 20 ˚C below zero in freezer for further analytical procedures.

Heavy metals

Heavy metal (HM) concentrations in mollusk tissues were detected in wet samples, HM concentrations in sediments – in dried samples. Mussel tissues were thawed and homogenized with Ultra Turrax homogenizer and digested with nitric acid in microwave oven Anton Paar Multiwave 3000. Content of Cd, Pb and Cu in analytical solutions were detected using Perkin Elmer AAnalyst 800 electro thermal atomizer with Zeeman background correction according to LST EN ISO 15587-2:2004. Content of Zn was detected on Perkin Elmer AAnalyst 800 with flame atomisation according to LST ISO 8288:2002. Ammonium dihydrogen phosphate and magnesium nitrate with nitric acid were used as matrix modifiers when analyzing Pb and Cd. Mercury content in sediments and mollusks (GOST 26927-86) was measured by cold vapour technique by Perkin Elmer MAS 50A. Quality control was ensured by the use of blanks and reference materials IAEA-433 (Trace elements and methylmercury in marine sediments) and NIST Mussel Tissue 2976, which were analyzed simultaneously according to the same procedures as the samples.

Arsenic and iron

Sediment samples were freeze-dried and digested using a CEM MARS5 high-pressure microwave digestion system in acid-cleaned Teflon microwave vessels using nitric and concentrated hydrofluoric acids. After cooling, the sample digestates were transferred to 50-ml plastic test tubes containing 0.8 g boric acid. Following dilution to 50 ml with Milli-Q water, tubes were capped and placed in an ultrasonic bath for ~1 h to ensure the complete dissolution of residual solid material. Arsenic was determined by electrothermal atomic absorption spectrometry using a Varian SpectrAA Zeeman 220 instrument equipped with Zeeman background correction. $Pd(NO_3)_2$ and $Mg(NO_3)_2$ matrix modifiers were used. Calibration was carried out by the standard addition method. Replicate blanks, replicate samples and reference material (IAEA 433) were analysed for quality control purposes. Iron was determined by flame atomic absorption spectrometry on a Varian instrument SpectrAA 220FS.

Organochlorine pesticides

Chlorinated pesticides (α-HCH, β-HCH, γ-HCH, DDT, DDD, DDE, α-endosulfan, β-endosulfan, aldrin, diendrin, endrin, HCB) in water were determined by LST EN ISO 6468:2000 method, using extraction with hexane and purification with the aluminum-oxide column. Chlorinated pesticides in sediments were determined by ISO 10382:2002 method, using extraction with acetone and petrol ether and purification by aluminum-oxide/Ag(NO_3) column. Chlorinated pesticides in biota samples were determined by LST EN 1528-1:2001, LST EN 1528-2:2000, LST EN 1528-3:2000, LST EN 1528-4:2000 methods, using acetone/hexane mixture for extraction and aluminum oxide column eluting by petrol ether.

Oil products

In the Baltic Sea cruises, the extraction of water samples for total oil hydrocarbons was done on board with carbon tetrachloride. Samples from the Curonian Lagoon were transported to the laboratory of Center of Marine Research for analysis. In laboratory, after the purification with Al-oxide column, samples were analyzed by infrared spectrometry. As a calibration standard a mixture of benzene, hexadecane and iso-octane in carbon tetrachloride was used. A mixture of diesel fuel and lubricating oil was used for quality control (LAND 49-2002). For total oil hydrocarbon determination, sediment samples were extracted with carbone tetrachloride and cleaned through aluminum oxide column. Concentrations of total oil hydrocarbons were determined by infrared spectrometry (Methodical recommendations…, 1979).

2.3.2 Biochemical biomarkers

After the sampling according to the procedure described in 2.2.1, the collected bivalve specimens were kept in the fridge in buckets containing water from the sampling site and mussels were dissected within 8 hours after the sampling. Foot tissues of 6 individuals of *M. balthica* or gill tissues of 6 blue mussels or 7 zebra mussels were pooled in one Eppendorf tube (5 tubes for each station)

for AChE analysis. For oxidative stress biomarkers the digestive glands of 6 specimens were pooled in one tube, with 5 tubes per station. *N. diversicolor* sample contained the whole organism. Each sample was coded and deposited in liquid nitrogen immediately after dissection and stored at –80 °C until analysis. Measurements were done according to the procedures, validated in the Marine Research Laboratory of Finnish Environment Institute.

Acetylcholinesterase (AChE)

Sample tissues were homogenized with 0.02 M phosphate buffer (pH 7.0) containing 0.1 % Triton X 100 in a 1/2 ratio (w/v) using ultra-turrax (3 times × 20 sec). The homogenates were then centrifuged at 10 000 × g for 20 min at 2-4 ^{o}C. Aliquots of the supernatant were taken for further analysis of AChE or were stored below –20 ^{o}C.

Total proteins were determined according to Bradford (1976). Bovine serum albumin (BSA) has been used as the protein standard. This method was adapted to be used with a microplate reader. Absorbance was read at 600 nm and the sample concentration was calculated from the standard curve.

AChE activity was determined using the method of Ellman et al. (1961) adapted to microplate reader by Bocquené and Galgani (1998). To each well of the microplate, 300 µl of 0.02 M phosphate buffer (pH 7), 20 µl of 0.01 M DTNB (dithio*bis*nitrobenzoate) and 20 µl of supernatant were added in succession. After 5 min incubation to allow the DTNB to react with the sulfhydryl groups of the amino acids in the sample, 10 µl of 0.1 M ACTC (acetylthiocholine iodide) was added to begin the enzymatic reaction. Samples were measured in quadruplicates on a microplate reader at 412 nm (kinetic) for 8 min, every 15 sec. AChE activity is expressed as nmoles of product developed per minute per mg of proteins.

Homogenization of samples for LPO, GST, GR, CAT and SOD analyses

Sample tissues were homogenized with 100 mM K-phosphate buffer (pH 7.4) in a 1/3 ratio (w/v) using ultra-turrax (3 times × 20 sec). Subsamples for LPO analysis were taken: 55 µl of subsample was mixed with 55 µl of K-phosphate

buffer and 2.2 μl of 4 % BHT (2,6-Di-tert-butyl-4-methylphenol) solution in methanol. The homogenates were then centrifuged at 10 000 × g for 20 min at 2-4 ^{o}C to isolate the post-mithocondrial supernatant (PMS). The aliquots of PMS were taken: 30 μl for GST analysis, 30 μl for CAT, 50 μl for GR, 20 μl for SOD and 50 μl the analysis of proteins. All subsamples were stored in – 80 ºC until the analyses. Total proteins were determined using the same procedure as for AChE according to Bradford (1976).

Lipid peroxidation (LPO)

Lipid peroxidation was quantified as thiobarbituric acid reactive substances. 100 μl of subsample for LPO was added to 15 ml tubes containing 1 ml of 12 % TCA (Trichloroacetic acid). 900 μl of mixture of 60 mM Tris-HCl (Trizma hydrochloride) with 0,1 mM DTPA (Diethylenetriaminepentaacetic acid) was added followed by 1 ml of 0.73 % TBA (2-Thiobarbituric acid). Tubes were placed in water and were heated at 100 ºC for 60 min. Then samples were transferred to 1.5 ml microtubes and centrifuged for 5 min at 11500 rpm. The aldehyde formed was estimated spectrophotometrically at 535 nm. Results are expressed as TBARS (thiobarbituric reactive substances) – nmol TBARS g^{-1} wet weight.

Catalase (CAT)

Before analysis the subsamples for CAT were diluted 1:10 with K-phosphate buffer 100 mM (pH 7.0). 1350 μl of K-phosphate buffer, 75 μl of the 600 nM H_2O_2 and 75 μl of the subsample were added to the cuvette of the spectrophotometer. The absorbance was measured at 240 nm every 5 sec during 3 min. Every measurement was done in 2 replicates. The absorbance of the blank was checked and didn't exceed 1.2. CAT activity was calculated as μmol H_2O_2 consumed min^{-1} mg^{-1} of proteins.

Glutathione S-transferase (GST)

Before analysis the subsamples for GST were diluted 1:10 with K-phosphate buffer 100 mM (pH 7,4). 765 μl of 100 mM K-phosphate reaction buffer (pH 7.0), 45 μl of 20 mM CDNB (1-Chloro-2,4-dinitro-benzene), 45 μl of 20 mM

GSH (L-Glutathione reduced) and 45 μl of the subsample were added to the cuvette of the spectrophotometer. The absorbance was measured at 340 nm every 5 sec during 1 min. Every measurement was done in 2 replicates. The results were expressed as nmol of substrate hydrolised per min per mg of protein.

Glutathione Reductase (GR)

To each well of the microplate 180 μl of reaction buffer (100 mM K-phosphate, pH 7.5 + 2 mM EDTA), 25 μl of 5 mM GSSG (oxidized glutathione) and 20 μl of subsample were added. 25 μl of 1 mM of NADPH was added after 10 min of incubation. Samples were measured on a microplate reader at 340 nm for 5 min, every 15 sec. The results were expressed as nmol of substrate hydrolised per min per mg of protein.

Superoxide dismutase (SOD)

Before analysis the subsamples for GST were diluted 1:30 with K-phosphate buffer 100 mM (pH 7.4). The reaction solution was prepared before the analysis: 50 mM sodium phosphate with 1 mM EDTA (pH 7.8) was mixed with Na(OH) (0.1 M) and Xanthine; afterwards Cytochrome C was dilute in the prepared mixture. To each well of the microplate 50 μl of reaction solution, 12.5 μl of subsample and 12.5 μl of Xanthine oxidase (0,3 U ml^{-1}) were added. SOD standards (30 U/ml) were used for calibration. Samples were measured in quadruplicates on a microplate reader at 550 nm for 2 min, every 20 sec. SOD activity of samples was calculated and expressed in units per mg of protein.

2.3.3 Environmental genotoxicity and cytotoxicity

Blue mussels were dissected, gills removed and two gill arches placed in a drop of 3:1 ethanol acetic acid solution on clean microscopic slide and gently nipped with tweezers for 2-3 minutes (until cells spread within a drop). Then the produced cell suspension was softly smeared on a surface of the slide and air-dried. Dried smears were subsequently fixed in methanol for 10 min and stained with 4 % Giemsa solution in phosphate buffer pH 6.8. The stained slides were analyzed under a light microscope Olympus BX51 at final

magnification of 1.000×. Blind scoring of micronuclei and other nuclear abnormalities was performed on coded slides, the origin of samples being unknown. Micronuclei (MN) were identified according to the following criteria: (1) round and ovoid-shaped non-refractory particles in the cytoplasm, (2) color and structure similar to chromatin, (3) diameter of 1/3-1/20 of the main nucleus, (4) particles completely separated from the main nucleus. Nuclear buds, bi-nucleated and fragmented-apoptotic cells were identified using criteria described by M. Fenech with co-authors (2003). For each studied specimen of mussels, 2000 cells with intact cytoplasm were scored. Final results were expressed as the mean value (‰) of sums of the analyzed individual lesions scored in 1.000 cells per mussel collected from every study location (Baršienė et al., 2004). The analysis was made in the Genotoxicology laboratory of the Institute of Ecology.

2.4 Experimental exposure of mussels to different PAHs and their mixtures

An experiment has been carried out in the laboratory of the Finnish Institute of Marine Research. Mussels were collected by diving from Hanko (Gulf of Finland, Finland) in October 2008 and acclimatized in aquariums during 4 days until the beginning of the experimental exposure. After the acclimation 30 *Mytilus edulis* mussel specimens were placed to each of 16 5-liters aquariums filled with filtered water. The exposure aquariums were: control (water without chemicals), solvent control, three aquariums with fluoranthene (F) (4, 12, 36 μg l^{-1}), three with pyrene (Py) (4, 12, 36 μg l^{-1}), two aquariums with the mixture of F (4, 12 μg l^{-1}) and benzo(a)pyrene (BaP) (4 μg l^{-1} – constant concentration in all aquariums with BaP), two aquariums with the mixture of Py (4, 12 μg l^{-1}) and BaP (4 μg l^{-1}), two aquariums with the mixture of F (4, 12 μg l^{-1}l) and Py (4, 12 μg l^{-1}), two aquariums with the mixture of F (4, 12 μg l^{-1}), Py (4, 12 μg l^{-1}) and BaP (4 μg l^{-1}) (Fig. 2.4.1). The duration of

the experiment was 48 h. Water with PAHs in the aquariums has been changed once – after the 24 h of exposure.

After 48 hours of exposure antioxidant biomarkers including catalase (CAT), glutathione-S-transferase (GST), glutathione reductase (GR), superoxide dismutase (SOD) and lipid peroxidase (LPO) were measured from the digestive gland and acetylcholinesterase (AChE) for neurotoxic effects was measured from the gills, according to protocols described in 2.3.2 section. Slides for the environmental geno-cytotoxicity studies were prepared from mussel gills following the procedure described in 2.3.3 section. Filtering activity was checked by counting filtering mussels every few hours during the day. In all aquariums, there were permanently filtering more than 50 % of the mussels.

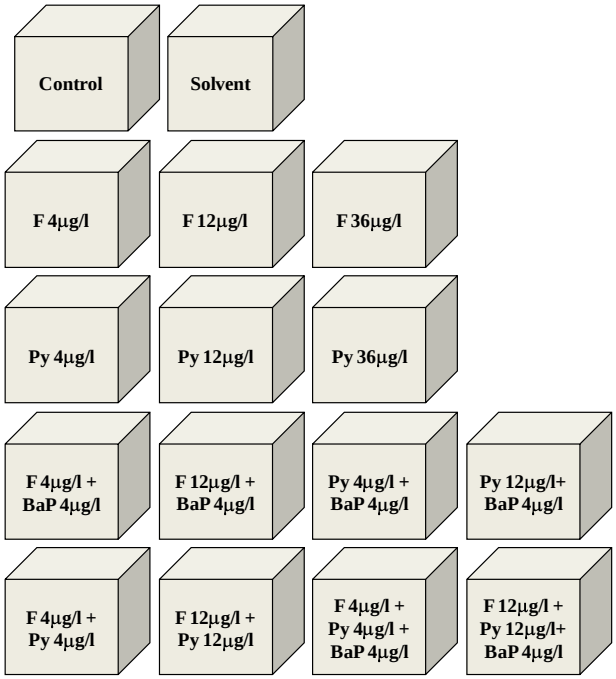


Fig. 2.4.1. Design of the experiment. Summed concentrations of PAHs are shown F – fluoranthene, Py – pyrene, BaP – benzo(a)pyrene

2.5 Mathematical and statistical data analysis

Statistical data analysis

Analysing monitoring data, values less than the limit of quantification were assigned as a one-half of the value of the reported quantification limit.

The dataset of mercury and total oil hydrocarbons in water have been analysed for significant trends. First the data from all the stations and water horizons has been sorted according to areas of interest: open sea, northern coastal water, southern coastal waters, plume of the lagoon, Klaipėda Strait and Curonian Lagoon. Limits of determination appeared in the dataset only starting from 2004 (for oil) and 2005 (for mercury). Before that all nominal concentrations, even "zeros" (no signal), were reported. To make the data more comparable to the data of the latest period all "zeros" were changed to presumptive limit of quantification 0.01 mg l^{-1} (the same for oil and mercury data). It is the lowest concentration that has been determined in the data set. So, "zeros" will not lower down the average of the year. Starting from 2004 or 2005 half of the limit of quantification has been used for further calculations. After that transformation, the datasets of every year (every area separately) were tested for the outliers using the Grubbs'outlier test. Significant outliers (significance level: 0.05 (two-sided)) were identified in each data set. However, an expert judgment has been applied in order to remove the data point from further data analysis. Criteria for removal were: concentration itself (too high concentrations) concentrations at all horizons; concentrations in adjacent stations; concentration at the same station during the year.

Mercury and total oil hydrocarbons in water plots and statistical analysis was made using Plot and Image Analysis (PIA) statistical software. Plots display the geometric mean concentration of each year (circles) and the 95 % confidence intervals of the geometric means. The overall geometric mean value for the time-series is depicted as a horizontal, thin line. The trend is presented by a regression line (plotted if $p < 0.05$, two-sided regression analysis). The log-linear regression lines fitted through the geometric mean concentrations follow smooth exponential functions. A smoother is applied to test for non-linear trend components. The smoothed line is plotted if $p < 0.05$. Below the header of each plot the results from several statistical calculations are reported:

n(tot) = Total number of analyses included together with the number of years (**n(yrs)=**).

m = The overall geometric mean value together with its 95 % confidence interval (the number of degrees of freedom = n of years - 1).

slope = The slope, expressed as the yearly change in percent together with its 95 % confidence interval.

sd(lr) = The square root of the residual variance around the regression line, as a measure of between-year variation, together with the *lowest detectable change* in the current time-series with a power of 80 %, one-sided test, *alpha* = 0.05. The last figure is the estimated *number of years* required to detect an annual change of 5 % with a power of 80 %, one-sided test, *alpha* = 0.05.

power = The power to detect a log-linear trend in the time-series. The first figure represents the power to detect an annual change of 5 % with the number of years in the current time-series. The second figure is the power estimated as if the slope were 5 % a year and the number of years were ten. The third figure is the *lowest detectable change* (given in percent per year) for a ten year period with the current between year variation at a power of 80 %.

y(09) = The concentration estimated from the regression line for the last year together with a 95 % confidence interval, e.g. y(09)=0.022 (0.015, 0.032) is the estimated concentration of year 2009 where the residual variance around the regression line is used to calculate the confidence interval. Provided that the regression line is relevant to describe the trend, the residual variance might be more appropriate than the within-year variance in this respect.

r^2 = The coefficient of determination (r2) together with a P-value for a two-sided test (H_0: slope = 0), i.e. a significant value is interpreted as a true change, provided that the assumptions of the regression analysis is fulfilled.

Tau = The Kendall's ' **tau** ' as a result from the non-parametric Mann-Kendal trend test, and the corresponding P-value.

sd(sm)= The square root of the residual variance around the smoothed line. The significance of this line could be tested by means of an Analysis of

Variance. The P-value is reported for this test. A significant result will indicate a non-linear trend component.

Box-and-whisker plots (software STATGRAPHICS Centurion XV) were used for the comparison of the concentrations of metals in water in different areas of the Lithuanian Baltic Sea. The plots are designed to illustrate important features of a numeric data when grouped according to the value of a second variable. Box-and-whisker plots summarize data samples through 5 statistics: minimum, lower quartile, median, upper quartile and maximum. They also indicate the presence of outliers.

All data was checked for normality using standardized skewness and/or kurtosis. If standardized skewness and/or kurtosis is outside the range of -2 to +2, this indicated some significant nonnormality in the data, which violates the assumption that the data come from normal distributions. That was the case for the data on metals in water in different areas. Non-parametric Kruskal-Wallis test was usded to compare the medians of metal concentrations in different areas instead of the means. The Kruskal-Wallis test tests the null hypothesis that the medians of metals within each of the 6 areas are the same. The P-value showed if there is a statistically significant difference amongst the medians at the 95 % confidence level.

The normalization of data was used to present arsenic concentrations in sediments, which is based on calculating the residuals about the regression line. The residual is the difference between measured concentration and that calculated from the regression equation. Large positive residuals may be regarded as representing samples with higher than expected contaminant concentrations (Whalley et al., 1999).

The maps of spatial distribution of total oil concentrations in water were produced using Thematic Images and Spatial Statistics (TISS) software. The annual geometric mean is presented on the maps.

In the genotoxic and cytotoxic effects in bivalve mollusks *Macoma balthica* and *Mytilus edulis* study and in Būtingė oil spill study the final results were expressed as the mean value (‰) of the sums for the individual lesions scored

in 1000 cells per mussel collected from every study location. The statistical analysis was carried out using PRISM statistical package. The mean and the standard error were calculated for each group of bivalves. The non-parametric Mann–Whitney U-test was used to compare MN frequencies in mollusks from the reference and contaminated sites, from the site before the oil spill with those from contaminated by oil or between time-related groups of mussels collected from the same study location. One Way ANOVA was used to compare results between the studied mussels groups in the Būtingė oil spill study.

For the laboratory exposure data, non-parametric Spearman correlation coefficients (r-values) were calculated for significant correlations between biochemical biomarker responses in mussels and fluoranthene, pyrene or total PAHs concentration. Non-parametric Mann-Whitney U-test was used to compare cellular alteration frequencies between control and treatment groups. The same statistical test was used to evaluate the influence of benzo(a)pyrene on biochemical biomarker responses and also on genotoxicity and cytotoxicity effects; the comparison of the responses in mussels exposed solely to fluoranthene or pyrene with those responses after exposure to their mixtures with benzo(a)pyrene was made using statistical software STATGRAPHICS Centurion XV.

Responses of CAT, SOD, GST, GR, LPO, AChE and MN biomarkers were related to the concentration of fluoranthene (F), pyrene (Py) and the presence of benzo(a)pyrene (BaP) using redundancy analysis (RDA). Missing data values of biomarker responses were changed by the average value of the exposure group. Average values of each exposure group were used for MN, as MN samples were taken from the different mussels than for biochemical biomarkers. RDA biplot for square root transformed data on F, Py and BaP as explanatory variables and CAT, SOD, GST, GR, LPO, AChE and MN as response variables is presented. The data of biomarker responses and the concentration of F and Py were used as numerical variables, the presence of

BaP was included as an explanatory nominal variable. The RDA was performed using Brodgar (Highland Statistics Ltd.) software.

Pollution Index

For the evaluation of contamination of the waterbody, concentrations of pollutants are often compared with the Environmental Quality Standards (EQS). Pollution Index (*PI*) takes into account effects of all pollutants for the evaluation of the total damage of pollution (Nemerow, 1991).

For the calculation of the PI, the ratio of the concentration (*C*) of the pollutant (*i*) and the EQS of that pollutant should be calculated: C_i/EQS_i. If the concentration of pollutant is equal to the EQS value, than this ratio is 1.0.

When $(C_i/EQS_i) \leq 1.0$,

$$\frac{C_i}{EQS_i} \leq 1.0, \qquad \frac{C_i}{EQS_i} = \text{the actual value of } \frac{C_i}{EQS_i} \quad (1)$$

When $(C_i/EQS_i) > 1.0$,

$$\frac{C_i}{EQS_i} > 1.0, \qquad \frac{C_i}{EQS_i} = 1.0 + p \cdot \log_{10} \frac{C_i}{EQS_i} \quad (2)$$

Here, p = a constant value (as a standard value for a relative comparison, Nemerow (1991) arbitrarily suggests to employ 5.0).

Ratios C_i/EQS_i should be calculated for all pollutants at the same station. The maximum and mean values of the C_i/EQS_i ratio are taken for the further calculation of PI. The Pollution Index (PI) is calculated according to equation (3):

$$PI = \sqrt{\frac{max(C_i / EQS_i)^2 + mean(C_i / EQS_i)^2}{2}} \quad (3).$$

Integrated Biomarker Response Index (IBR)

The IBR index for the biomarker responses in experimental laboratory studies was calculated according to procedure described in Broeg, Lehtonen (2006).

The IBR is calculated by summing up triangular Star Plot areas calculated for each two neighboring biomarkers in a given data set. The procedure described below was used:

For each biomarker: (1) Calculation of mean and SD for each station. (2) Standardisation of data for each station: $x'_i = (x_i$ - mean $x) / s$, where x'_i = standardised value of the biomarker, x_i = mean value of a biomarker from each station, mean x = mean of the biomarker calculated for all the stations, and s = standard deviation calculated for the station-specific values of each biomarker. Result: variance = 1, mean = 0. (3) Using standardised data, addition of the value obtained for each station to the absolute (= non-negative) value of the minimum value in the data set: $B = x'_i + | x_{min} |$. Result: adjusts the lowest value in the set to zero. For all the biomarkers treated this way: (4) calculation of Star Plot areas by multiplication of the obtained value of each biomarker (B_i) with the value of the next biomarker, arranged as a set, dividing each calculation by 2 and (5) summing-up of all values: $\{[(B_1 \times B_2)/2] +[(B_2 \times B_3)/2] + \ldots [(B_{n-1} \times B_n)/2]\}$. Result: IBR (average of different arrangements of biomarkers in the set).

The IBR is directly dependent on the number of biomarkers in the set. Thus, the values of IBR are given divided by the number of biomarkers used in each case and termed as IBR/n. It should also be noted that because the calculation method of IBR is based on relative differences between the biomarker responses in each given data set it is necessary to re-calculate all the index values each time when making new comparisons (Broeg, Lehtonen, 2006).

2.6 Hazardous Substances Status Assessment Tool (CHASE)

The hazardous substances status of the Lithuanian Baltic Sea and Curonian Lagoon waters has been assessed using HELCOM Hazardous Substances Status Assessment Tool (CHASE), which is a multimetric indicator-based tool developed for the HELCOM integrated thematic assessment for hazardous substances in the Baltic Sea. The integrated CHASE assessment is based on monitoring and other projects data (1999–2007) on various chemicals, the radionuclide cesium-137 and certain indicators of biological effects. These data are considered in relation to the four ecological objectives in the hazardous substances segment of the HELCOM Baltic Sea Action Plan

reflecting the HELCOM strategic goal for hazardous substance. The quantification of the "hazardous substances status" is based on a Contamination Ratio (CR), which is the ratio of the current status (measurement of the concentration of a substance or biological effect) and a threshold level or quality criterion, which is used as an approximation for an environmental target for that particular substance or biological effect. The CRs of all substances or indicators within an ecological objective are integrated to yield a status classification ("high", "good", "moderate", "poor" or "bad") of that particular ecological objective. The ecological objective receiving the lowest status classification serves as the overall classification of the assessed site or area, giving the classification of the "hazardous substances status" of that site or area according to one of five classes. "High" and "good" classes indicate that areas are not disturbed by hazardous substances, while "moderate", "poor" and "bad" indicate different degrees of disturbance by hazardous substances. The threshold levels used in CHASE were obtained from national legislation, international agreements or EU directives (e.g., EC Environmental Quality Standards and OSPAR Environmental Assessment Criteria because at the present time there are no thresholds specific to the Baltic Sea (HELCOM, 2010a). An example of the CHASE tool Excel sheet is shown in the Figure 2.6.1.

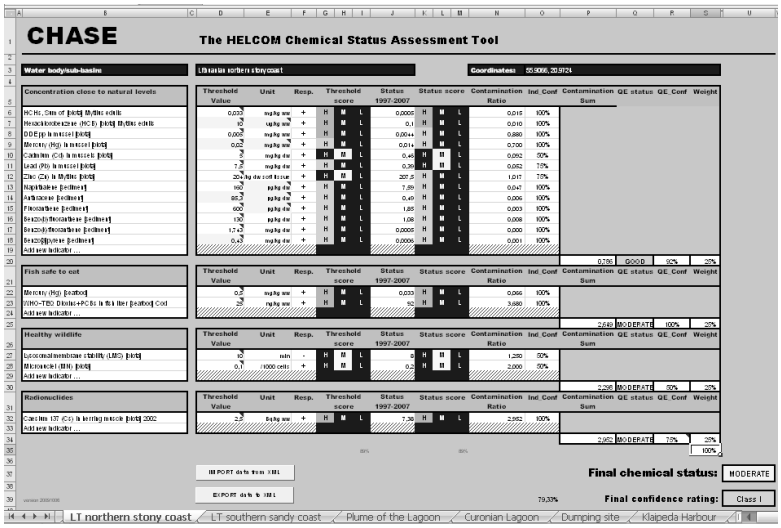


Fig. 2.6.1. An example of the CHASE tool Excel sheet for Lithuanian northern stony coast

3. RESULTS

3.1 Contaminants in the Baltic Sea

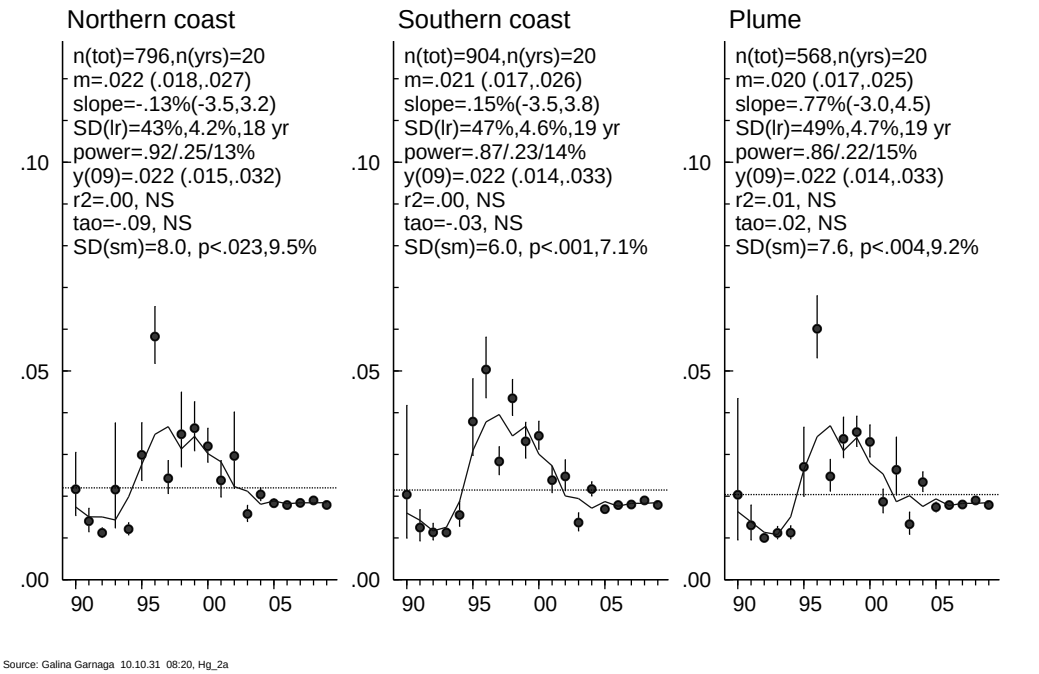
3.1.1 Heavy metals

Long-term concentrations of **mercury** in water for the period of 1990–2009 are shown in Fig. 3.1.1.1. Data analysis showed the decrease of mercury concentrations in water and sediments starting from 1995–1996.

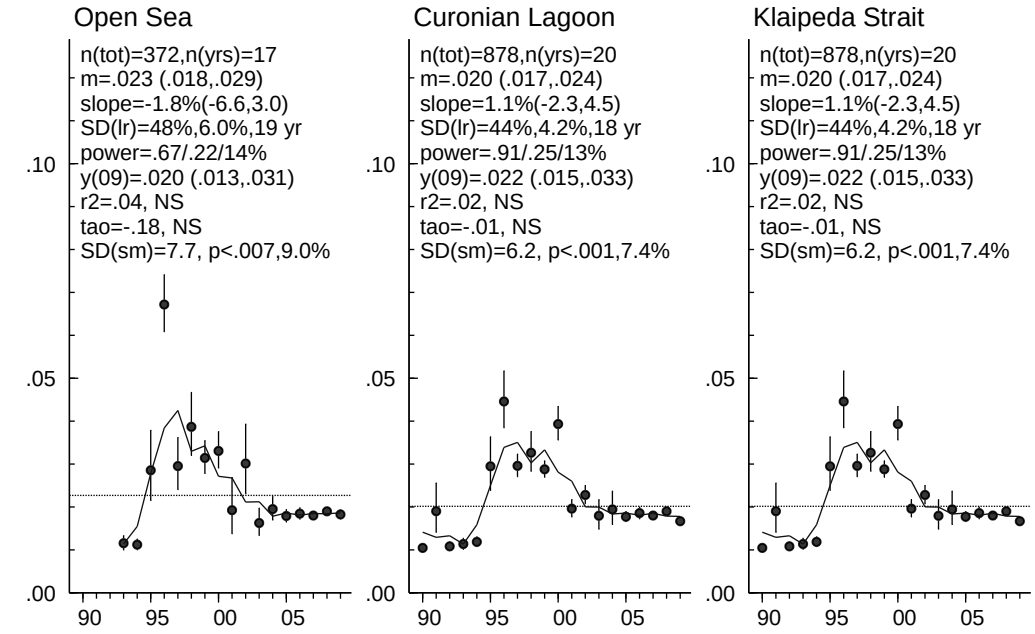
Determination of heavy metals (Cd, Cr, Cu, Ni, Pb and Zn) have been added to the National monitoring programme in 2003–2004.

For the comparison of metal concentrations in **water** between the different areas of the Lithuanian Baltic Sea the year 2009 has been chosen as the recent situation. The concentrations of metals in water in 2009 are shown in the Box-and-whisker plots (Fig. 3.1.1.2) The numeric data is shown in the Table 3.1.1.1. The Kruskal-Wallis test has identified a statistically significant difference in concentrations of Cu and Cd between the areas. Median concentrations of Cu were higher in the Curonian Lagoon. Elevated concentrations of Cu in the Curonian Lagoon were detected in August and November 2009. Concentrations of Cu in other areas of the Baltic Sea were mostly near or below the limit of quantification (2 μg/l) with only several exceptions.

Higher median concentrations of Cd were found for Northern coastal waters, plume area and open sea waters. However, all median concentrations were under the limit of quantification for Cd (0.07 μg/l). Higher Cd concentrations were detected in northern coastal waters and plume mostly in August, 2009. Open sea station 65 had elevated concentrations of Cd in February, May and August. Although, the annual average Environmental Quality Standard (EQS) for Cd (0.2 μg/l) has not been exceeded for the year 2009.



Source: Galina Garnaga 10.10.31 08:20, Hg_2a



Source: Galina Garnaga 10.10.31 08:27, Hg_2b

Fig. 3.1.1.1. Long-term 1990–2009 mercury concentrations in different areas of the Lithuanian zone of the Baltic Sea (the legend for the plots is presented in Material and Methods / Statistical data analysis chapter)

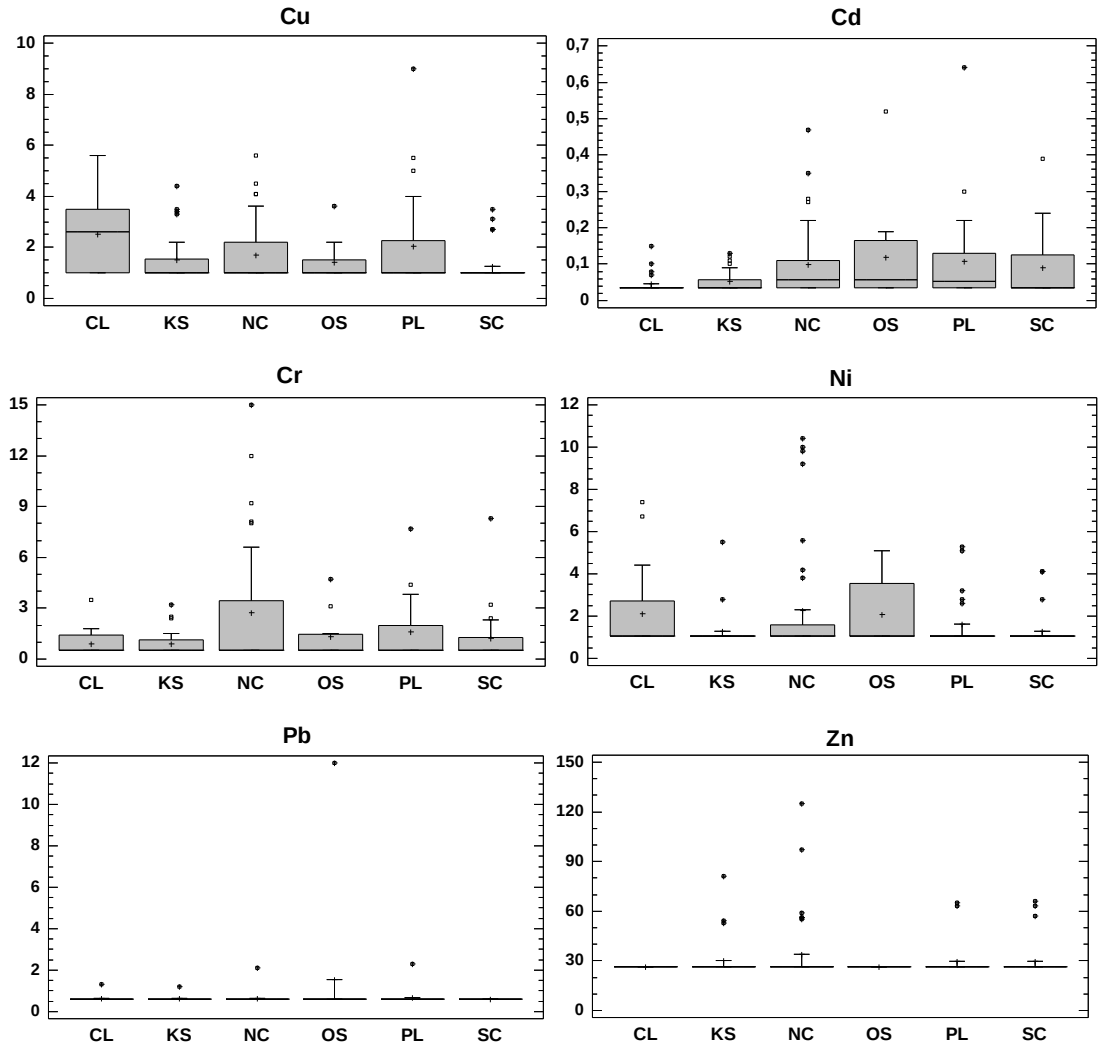


Fig. 3.1.1.2. Box-and-whisker plots for metals in water of the Curonian Lagoon (CL), Klaipėda Strait (KS), northern coastal waters of the Baltic Sea (NC), open sea (OS), plume of the lagoon in the sea (PL) and southern coastal waters of the Baltic Sea (SC). Units are in μg l^{-1}. Data is from the year 2009

Table 3.1.1.1. Median and average (± standard deviation) concentrations of the metals in the different areas of the Lithuanian Baltic Sea in 2009. Units are in μg l^{-1}. Kruskal-Wallis test compares the medians of metal concentrations in different areas. Statistically significant differences are marked with *. n – the number of measurements

		Curonian Lagoon (CL)	**Klaipėda Strait (KS)**	**Northern coastal waters (NC)**	**Open Sea (OS)**	**Plume (PL)**	**Southern coastal waters (SC)**
n		22	28	40	12	24	32
Cu	Median	2.6	1.0	1.0	1.0	1.0	1.0
	Average±St dev	2.5±1.4	1.5±1.0	1.7±1.2	1.4±0.8	2.0±2.0	1.3±0.7
	Kruskal-Wallis	Test statistic = 17.8681 p-value = 0.00311637* (p<0.05)					
Cd	Median	0.035	0.035	0.058	0.058	0.053	0.035
	Average±St dev	0.05±0.03	0.05±0.03	0.10±0.10	0.12±0.14	0.11±0.13	0.09±0.09
	Kruskal-Wallis	Test statistic = 11.9854 p-value = 0.0349889* (p<0.05)					

		Curonian Lagoon (CL)	Klaipėda Strait (KS)	Northern coastal waters (NC)	Open Sea (OS)	Plume (PL)	Southern coastal waters (SC)
Cr	Median	0.5	0.5	0.5	0.5	0.5	0.5
	Average±St dev	0.91±0.74	0.91±0.71	2.72±3.51	1.30±1.33	1.61±1.78	1.23±1.47
	Kruskal-Wallis	Test statistic = 6.37041 p-value = 0.271826					
Ni	Median	1.05	1.05	1.05	1.05	1.05	1.05
	Average±St dev	2.12±1.91	1.27±0.89	2.28±2.74	2.08±1.59	1.62±1.26	1.30±0.80
	Kruskal-Wallis	Test statistic = 9.31465 p-value = 0.0971529					
Pb	Median	0.6	0.6	0.6	0.6	0.6	0.6
	Average±St dev	0.63±0.15	0.62±0.11	0.64±0.24	1.55±3.29	0.67±0.35	0.60±0.00
	Kruskal-Wallis	Test statistic = 2.44239 p-value = 0.785146					
Zn	Median	26.5	26.5	26.5	26.5	26.5	26.5
	Average±St dev	26.5±0.0	30.4±12.2	33.7±20.4	26.5±0.0	29.6±10.6	29.8±10.6
	Kruskal-Wallis	Test statistic = 5.24221 p-value = 0.387041					

The concentration of Cd in water during the period of 2005–2009 (Table 3.1.1.2) often exceeded the environmental quality standard (EQS) (0.2 μg l^{-1}); in 2009 the 13 % of measured concentrations were above the EQS in northern coastal waters, 8 % in open sea, 13 % in the plume of the lagoon and 13 % of the southern coastal waters. During the same period, the concentration of Cu also exceeded the maximum allowable concentration (MAC) (10 μg l^{-1}). The highest number of measurements (15) above the MAC was observed in 2006 in Klaipėda Strait, that number comprises 54 % of all measurements. Nevertheless, Cu concentration in 2009 was low and didn't exceed MAC in all areas. Concentrations of other metals exceeded MAC or EQS only episodically (from 1 to 5 measurements per year).

Table 3.1.1.2. The percentage of measurements above the maximum allowable concentration (MAC) or environmental quality standard (EQS) (Official gazette, 2010, No. 59-2938) for different areas of the Baltic Sea and Curonian Lagoon (the number of measurements exceeding the limits is shown in brackets)

	Curonian Lagoon (CL)	Klaipėda Strait (KS)	Northern coast (NC)	Open Sea (OS)	Plume (PL)	Southern coast (SC)
Cu (MAC=10 μg l^{-1})						
2005	45% (9)	38% (9)	6% (4)	6% (1)	8% (3)	3% (2)
2006	55% (12)	54% (15)	1% (1)	4% (1)	0%	0%
2007	15% (7)	24% (12)	10% (7)	4% (1)	11% (4)	17% (10)
2008	0%	16% (5)	0%	0%	0%	2% (1)
2009	0%	0%	0%	0%	0%	0%
Cr (MAC=10 μg l^{-1})						
2005	0%	0%	0%	0%	3% (1)	0%
2006	0%	0%	0%	0%	0%	0%
2007	0%	0%	0%	0%	0%	0%
2008	0%	0%	0%	0%	0%	0%
2009	0%	0%	5% (2)	0%	0%	0%

	Curonian Lagoon (CL)	Klaipėda Strait (KS)	Northern coast (NC)	Open Sea (OS)	Plume (PL)	Southern coast (SC)
Cd (EQS=0.2 μg l^{-1})						
2005	30% (6)	25% (6)	34% (24)	61% (11)	61% (22)	52% (34)
2006	27% (6)	14% (4)	30% (21)	21% (5)	44% (16)	33% (19)
2007	30% (14)	29% (14)	53% (36)	46% (11)	44% (16)	42% (23)
2008	13% (3)	28% (9)	8% (4)	17% (3)	23% (8)	26% (12)
2009	0%	0%	13% (5)	8% (1)	13% (3)	13% (4)
Pb (EQS=7.2 μg l^{-1})						
2005	0%	8% (2)	4% (3)	0%	0%	8% (5)
2006	0%	4% (1)	0%	0%	0%	0%
2007	0%	2% (1)	0%	0%	3% (1)	2% (1)
2008	4% (1)	0%	0%	0%	3% (1)	0%
2009	0%	0%	0%	8% (1)	0%	0%
Ni (EQS=20 μg l^{-1})						
2009	0%	0%	0%	0%	0%	0%
Zn (MAC=100 μg l^{-1})						
2005	15% (3)	0%	1% (1)	0%	0%	0%
2006	14% (3)	7% (2)	1% (1)	0%	3% (1)	2% (1)
2007	2% (1)	0%	0%	0%	3% (1)	4% (2)
2008	13% (3)	6% (2)	0%	0%	0%	0%
2009	0%	0%	3% (1)	0%	0%	0%
Hg (MAC-EQS=0.07 μg l^{-1})						
2005	0%	0%	0%	0%	0%	0%
2006	0%	0%	0%	0%	0%	0%
2007	0%	0%	0%	0%	0%	0%
2008	0%	0%	0%	0%	0%	0%
2009	0%	0%	0%	0%	0%	0%

Determination of heavy metals in **sediments** (Cd, Cr, Cu, Ni, Pb and Zn) have been added to the environmental monitoring programme in 2003. Figs. 3.1.1.3 – 3.1.1.8 present metal concentrations in sediments in 2003–2009 in open sea (station 65), northern coast (station 1B), dumping site (stations 20, 20A and 20B), plume of the lagoon (stations 4 and 5), Klaipėda harbour (station 3B) and Curonian Lagoon (stations 10 and 14). Stations with similar sediment type were chosen to represent the area. According to the Lithuanian legislation document (Order of Minister of Environment No. 77 of 26 February 2002; most recent amendments on 26 November 2008, Official gazette, 2008, No. 139-5521) on "Sediment dredging in sea and sea-port areas and dredged sediment treatment rules", there are 4 categories of sediments according to pollution by heavy metals, oil hydrocarbons, PCB and PAHs. I pollution

category is shown in every figure for comparison (II pollution category is also shown for Ni).

Concentrations of Cu are shown in Fig. 3.1.1.3. Concentration of Cu are decreasing starting from 2005 in the dumping site sediments, starting from 2006 in the open sea, harbour and Curonian Lagoon. The highest mean concentration – 30.6 mg kg^{-1} dry weight (d. w.) – was detected in Klaipėda harbour (station 3B) in 2006. This concentration together with concentrations in 2003 (25.6 mg kg^{-1} d. w.) and 2007 (16.8 mg kg^{-1} d. w.) and also in the Curonian Lagoon in 2006 (13.3 mg kg^{-1} d. w.) and 2007 (10.3 mg kg^{-1} d. w.) exceeded I pollution category (10 mg kg^{-1} d. w.) of sediments.

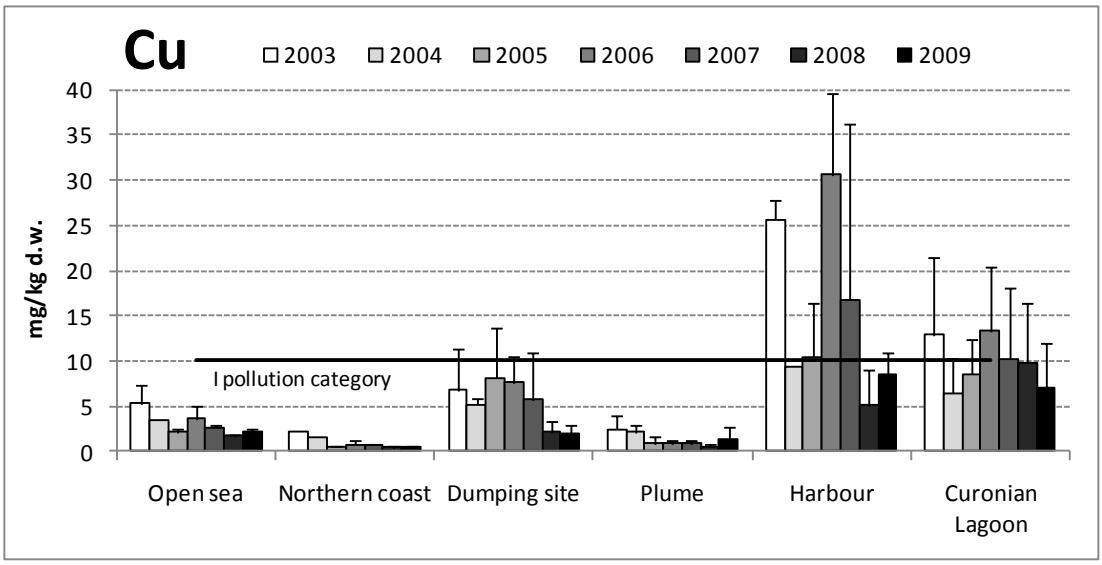


Fig. 3.1.1.3. Concentrations of copper (mg kg^{-1} dry weight) in sediments in 2003–2009 in open sea (station 65), northern coast (station 1B), dumping site (stations 20, 20A and 20B), plume of the lagoon (stations 4 and 5), Klaipėda harbour (station 3B) and Curonian Lagoon (stations 10 and 14). Mean values with standard deviations are presented

The highest mean concentration of Cd – 0.66 mg kg^{-1} dry weight (d. w.) – was detected in the sediments of the Curonian Lagoon (stations 10 and 14) in 2005. This concentration together with concentration in 2007 (0.61 mg kg^{-1} d. w.) exceeded I pollution category (0.5 mg kg^{-1} d. w.) of sediments (Fig. 3.1.1.4).

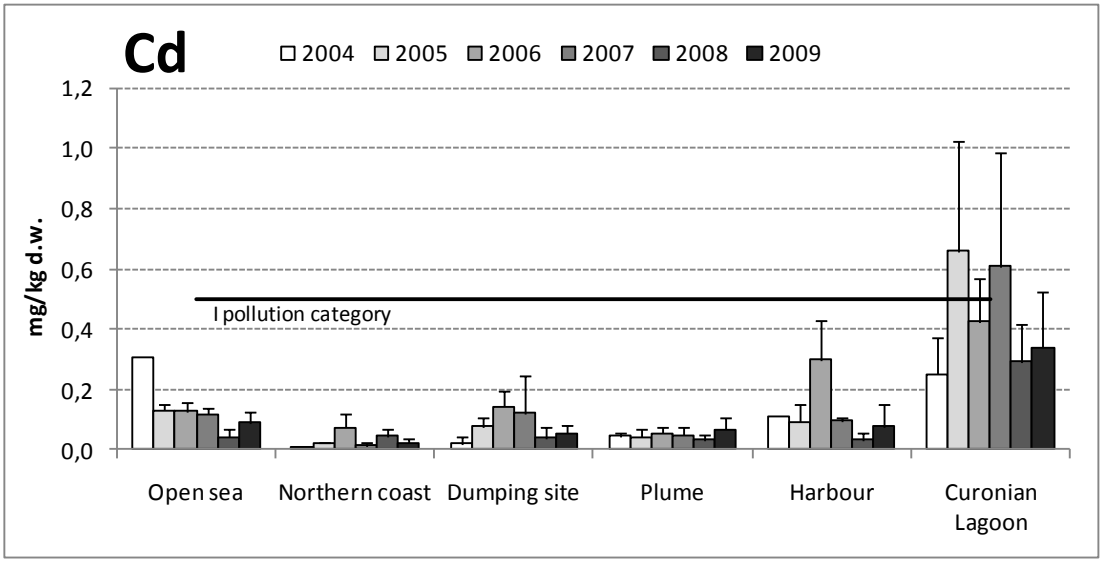


Fig. 3.1.1.4. Concentrations of cadmium (mg kg^{-1} dry weight) in sediments in 2003–2009 in open sea (station 65), northern coast (station 1B), dumping site (stations 20, 20A and 20B), plume of the lagoon (stations 4 and 5), Klaipėda harbour (station 3B) and Curonian Lagoon (stations 10 and 14). Mean values with standard deviations are presented

The decrease of Cr concentrations can be seen in the dumping site, harbour and Curonian Lagoon stations starting from 2006. The highest mean concentration – 54.1 mg kg^{-1} dry weight (d. w.) – was found in the Curonian Lagoon in 2006. This concentration exceeded I pollution category for Cr (30 mg kg^{-1} d. w.). Mean concentrations of Cd in the Curonian Lagoon during the period 2003–2008 exceeded I pollution category. There were separate measurements that exceeded 30 mg kg^{-1} d. w. in the open sea, dumping site and harbour in different years, but mean concentrations were below this limit (Fig. 3.1.1.5).

Concentrations of Ni are decreasing starting from 2005 almost in all areas except the open sea station 65. High Ni concentrations were measured in 2003–2005 at the dumping site, in 2003–2006 in the Klaipėda harbour sediments and in 2003–2008 in the Curonian Lagoon. These mean concentrations have exceeded I pollution category (10 mg kg^{-1} d. w.). The highest mean concentration – 47.2 mg kg^{-1} d. w. – was detected in the Curonian Lagoon in 2005, but it didn't exceed the II pollution category for silty sediments (50 mg kg^{-1} d. w.).

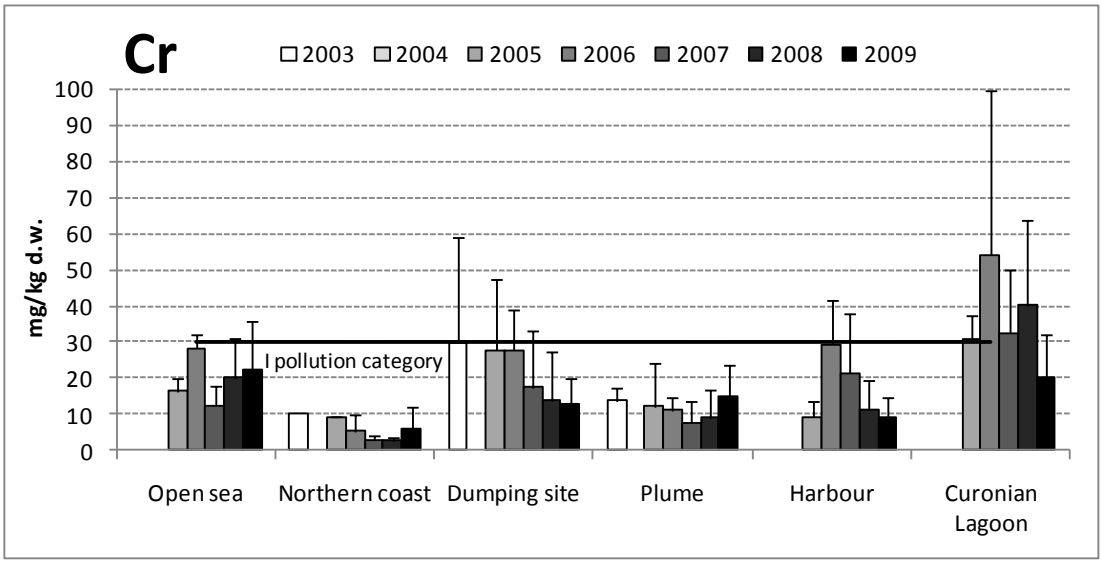


Fig. 3.1.1.5. Concentrations of chromium (mg kg^{-1} dry weight) in sediments in 2003–2009 in open sea (station 65), northern coast (station 1B), dumping site (stations 20, 20A and 20B), plume of the lagoon (stations 4 and 5), Klaipėda harbour (station 3B) and Curonian Lagoon (stations 10 and 14). Mean values with standard deviations are presented

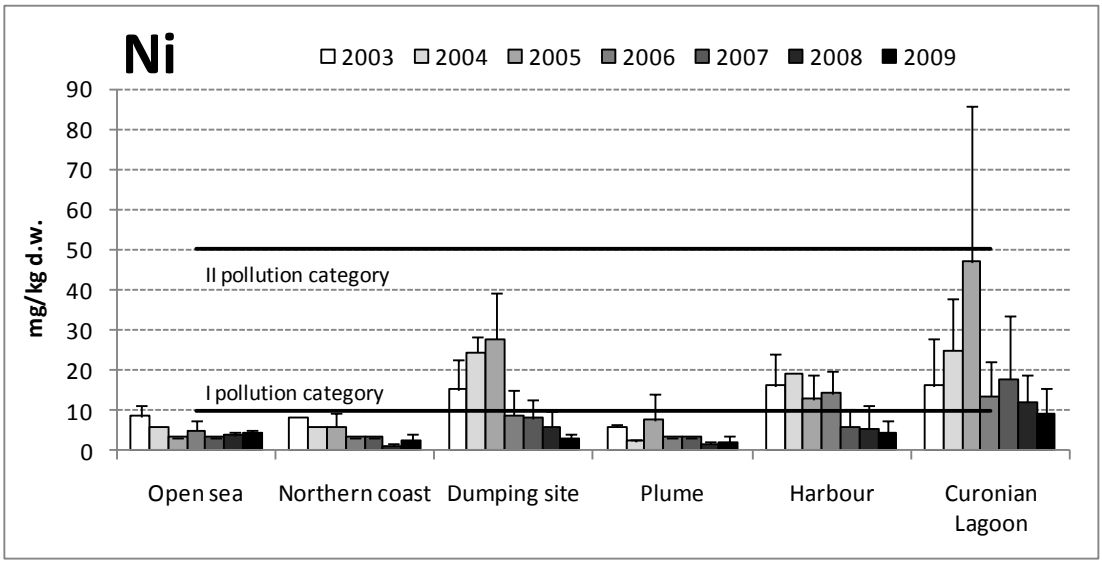


Fig. 3.1.1.6. Concentrations of nickel (mg kg^{-1} dry weight) in sediments in 2003–2009 in open sea (station 65), northern coast (station 1B), dumping site (stations 20, 20A and 20B), plume of the lagoon (stations 4 and 5), Klaipėda harbour (station 3B) and Curonian Lagoon (stations 10 and 14). Mean values with standard deviations are presented

A decrease in Pb concentrations can be noticed in dumping site starting from 2006 and in the Curonian Lagoon starting from 2007. Highest Pb

concentrations were found in Klaipėda harbour. In 2008 mean concentration of Pb in 3B station reached 18.8 mg kg^{-1} d. w. Although, annual mean Pb concentrations didn‘t exceed I pollution category for Pb (20 mg kg^{-1} d. w.) (Fig. 3.1.1.7).

Highest Zn concentrations were also found in Klaipėda harbour. In 2003 mean concentration of Zn in 3B station reached 136 mg kg^{-1} d. w. and exceeded I pollution category for Zn (60 mg kg^{-1} d. w.). In harbour I pollution category was also exceeded in 2005, 2006, 2007 and 2009. In the Curonian Lagoon the I category exceeded in 2005 and 2007 and reached 71.8 and 60.9 mg kg^{-1} d. w. respectively (Fig. 3.1.1.8).

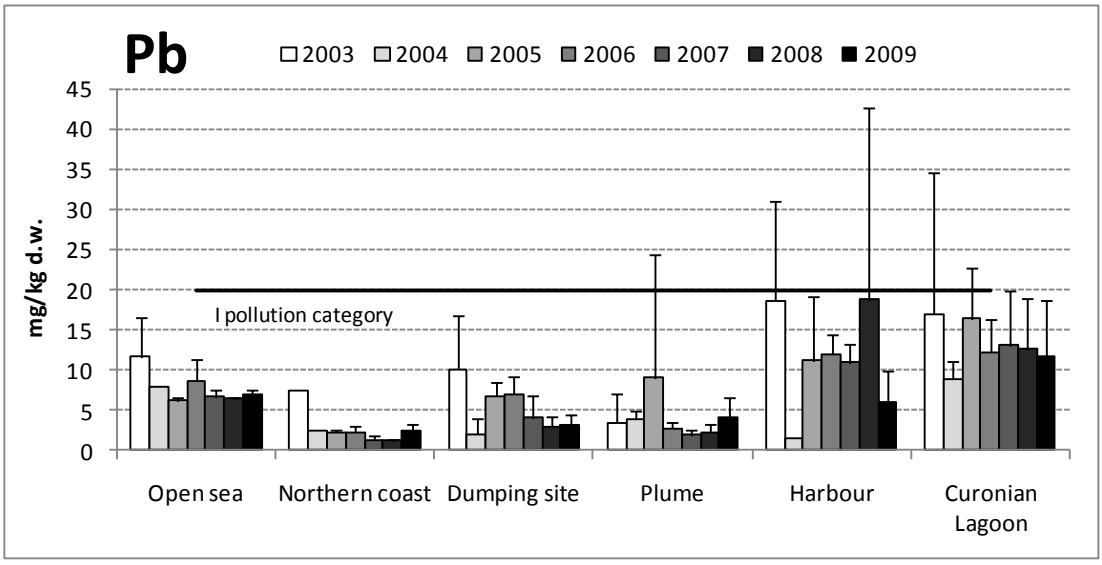


Fig. 3.1.1.7. Concentrations of lead (mg kg^{-1} dry weight) in sediments in 2003–2009 in open sea (station 65), northern coast (station 1B), dumping site (stations 20, 20A and 20B), plume of the lagoon (stations 4 and 5), Klaipėda harbour (station 3B) and Curonian Lagoon (stations 10 and 14). Mean values with standard deviations are presented

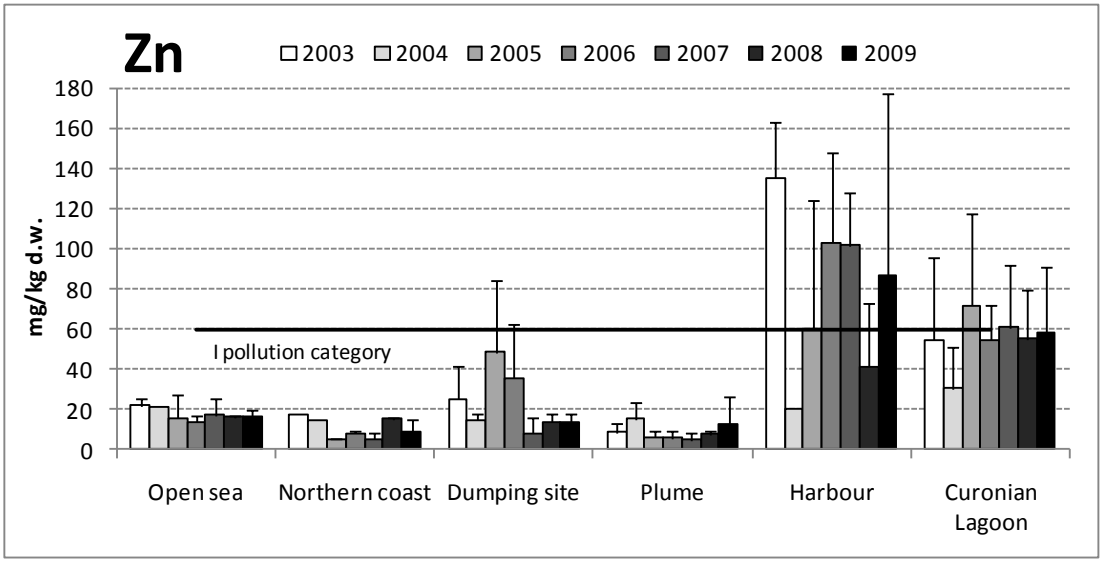


Fig. 3.1.1.8. Concentrations of zinc (mg kg^{-1} dry weight) in sediments in 2003–2009 in open sea (station 65), northern coast (station 1B), dumping site (stations 20, 20A and 20B), plume of the lagoon (stations 4 and 5), Klaipėda harbour (station 3B) and Curonian Lagoon (stations 10 and 14). Mean values with standard deviations are presented

During the LIFE project study a spatial distribution of trace metals in 2006 was investigated. Results show that there are elevated concentrations of all metals at the stations 20, 20A and 20M which are situated at the dumping site of the dredged sediments from the Klaipėda harbour. According to the Lithuanian legislation document on "Sediment dredging in sea and sea-port areas and dredged sediment treatment rules" (Official gazette, 2008, No. 139-5521), almost all concentrations of Zn, Cu, Cd, Pb and Hg in the studied regions fall within the cleanest I category. However, Cu concentrations at the dumping site of dredged sediments from the harbour (stations 20, 20A and 20 M) and Zn concentration at the station 20A have exceeded the I category values (about 11 mg kg^{-1} d. w. of Cu and about 88 mg kg^{-1} d. w. of Zn) (Fig. 3.1.1.9).

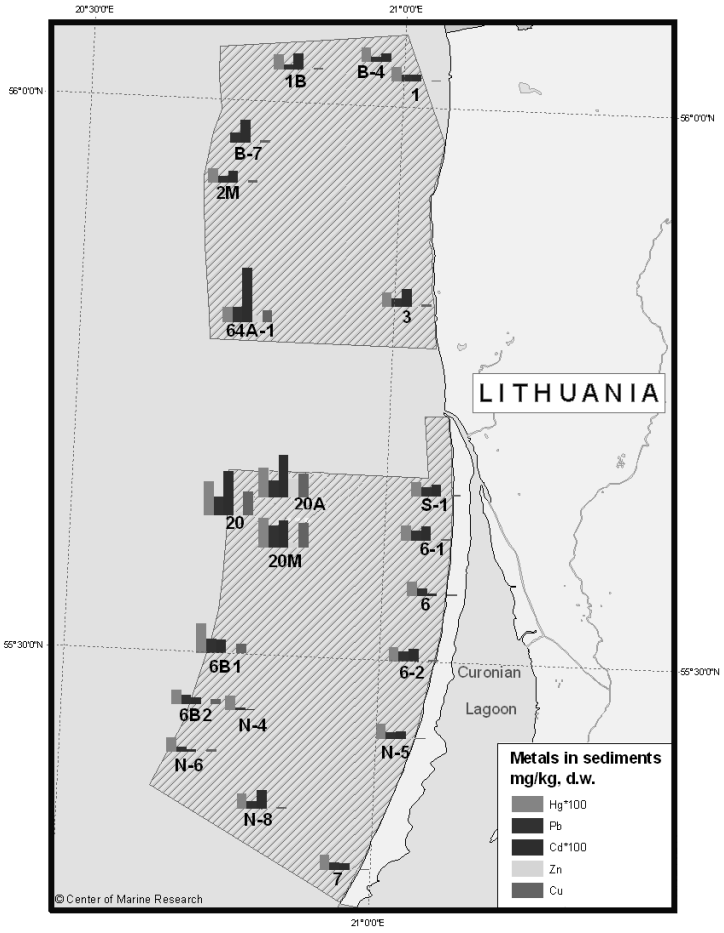


Fig. 3.1.1.9. Spatial distribution of heavy metals in sediments in 2006

The concentrations of mercury in **biota** during the period of 1999–2006 is shown in Fig. 3.2.1.1.10. Starting from 1999–2000 mercury concentration in fish and mussels is decreasing.

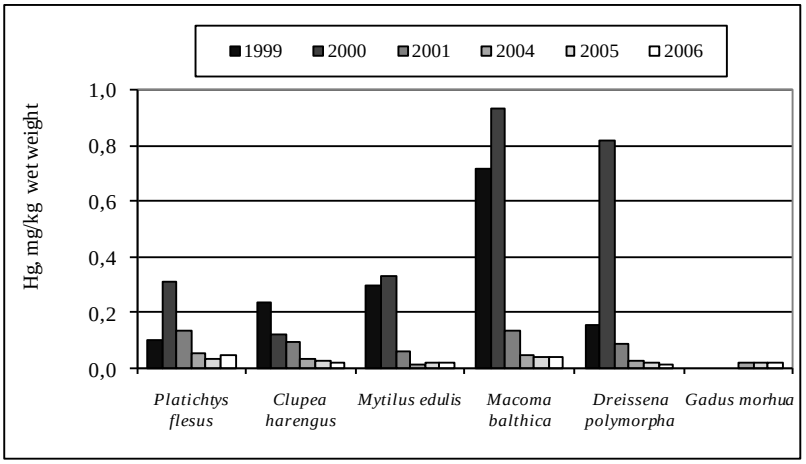


Fig. 3.2.1.1.10. Concentration of mercury in fish and bivalves in 1999–2006

In the Table 3.2.1.1.3 concentrations of metals in biota in 2006 are compared to the concentrations measured in 1998–2000. In most cases concentrations of metals have decreased or left at the same level. In 2006 highest concentrations were found in *Macoma balthica* and in liver of flounder (*Platichthys flesus*). Rather high concentrations of cadmium (0.411 mg kg^{-1} w. w.) were found in *Mytilus edulis*.

Table 3.2.1.1.3. Concentrations of heavy metals in biota in Lithuanian coastal area in 1997–2000 and 2006 (minimum and maximum values are shown; number of samples is shown in brackets)

	Zn	**Pb**	**Cd**	**Cu**	**Cr**
	mg kg^{-1} wet weight				
Dreissena polymorpha					
1998–2000 (6)	5.6–50	0.04–0.89	0.02–0.18	0.10–3.28	0.03–4.84
2006 (2)	<5.15–5.44	<0.13–0.23	0.09–0.17	1.80–1.89	<0.27–0.39
Mytilus edulis					
1997–2000 (4)	6.12–113	0.16–1.32	0.08–0.51	1.69–2.21	0.12–12.3
2006 (4)	10.8–42.8	<0.13–0.21	0.29–0.54	0.77–1.01	<0.27–0.40
Macoma balthica					
1999–2000 (2)	7.30–24.2	0.97–1.04	0.04–0.10	16.2–35.4	0.03–1.96
2006 (2)	119–154	0.21–0.22	0.11–0.18	25.7–35.3	0.28–0.54
Platichtys flesus					
1998–2000 (9)	5.12–23.7	0.03–0.58	0.01–0.23	0.94–22.4	0.05–7.36
2006 (3)	24.0–32.7	<0.13	0.34–0.44	21.8–28.4	<0.27
Gadus morhua					
1998 (1)	–	0.08	0.02	1.04	–
2006 (5)	11.3–21.5	<0.13	<0.008–0.04	6.25–40.5	<0.27

3.1.1.1 Arsenic in sediments of the Baltic Sea

Arsenic concentrations in sediments of the Lithuanian economic zone are shown in Fig. 3.1.1.1.1 The concentration of arsenic in the sediments ranged from 1.1 to 19.0 mg kg^{-1}. The concentration of iron ranged from 2.2 to 47.8 g kg^{-1}, with an average of 10.7 g kg^{-1}. Environmental heterogeneity for arsenic and iron can be estimated based on the five stations (ChG7(66), ChG9(65), ChG10(64), ChG13(4), ChG14(5)) sampled during both expeditions and ranged from 2 to 30 %. The relatively large variability can be explained in part due to the very low concentrations, which ranged from 1.1 to 3.7 mg kg^{-1} for arsenic and from 2.2 to 10.9 g kg^{-1} for iron. Four of the five

sites in the vicinity of the chemical munitions dumpsite exhibited slightly higher arsenic content than observed at the other locations.

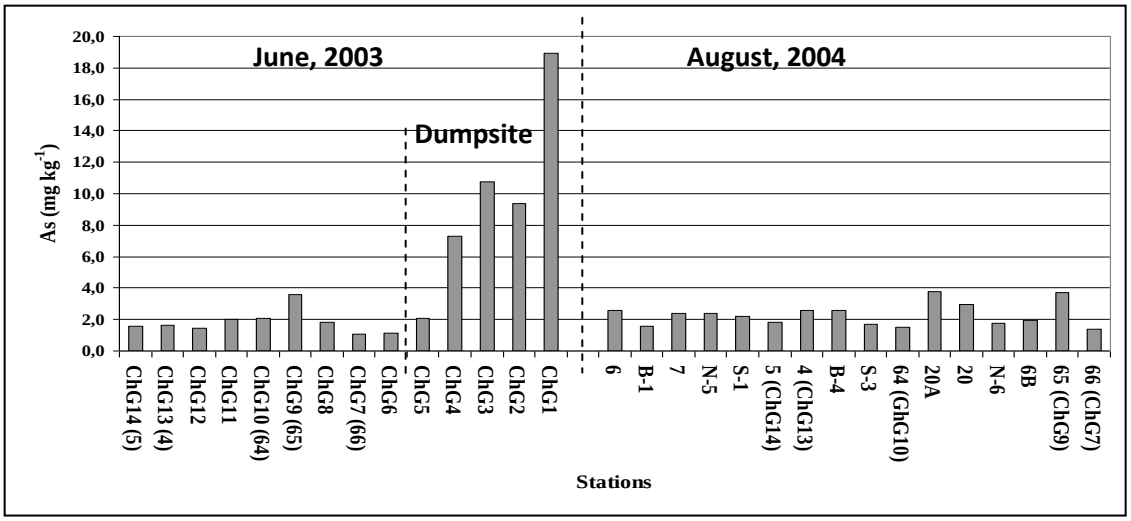


Fig. 3.1.1.1.1. Arsenic concentrations in the surface sediments in the southeastern Baltic

The interpretation of metal distributions in sediments has often relied upon some method of normalisation in order to account for variations based on grain size rather than source strength. In this vein, iron has commonly been used for the normalization of heavy metal distributions in the marine sediments (Whalley et al., 1999). In the Baltic Sea, iron plays an important role in diagenetic geochemical reactions in sediments. Although, it has been suggested that iron may not be appropriate for normalizing heavy metal concentrations (Leivuori, 1998), arsenic does display positive correlations with iron in both the Baltic (Borg, Jonsson, 1996) and the North Sea (Whalley et al., 1999). Therefore, iron has proved to be a useful reference element specifically in the case of arsenic.

An approach to present normalised data is based on calculating the residuals about the regression line. The residual is the difference between measured concentration and that calculated from the regression equation. Large positive residuals may be regarded as representing samples with higher than expected contaminant concentrations (Whalley et al., 1999).

Examining inter-element ratios provides the procedure to interpret sediment metal data. Specifically As:Fe relationships have been used to assess the extent of As pollution in the North Sea (Whalley et al., 1999). Arsenic as a function of iron in the sediments from Lithuania is shown in Fig. 3.1.1.1.2. Two distinct trends are evident for samples from inside and outside the chemical munitions dumpsite; both sets of data have good correlation coefficients. Clearly, one site (ChG5) located in the southeast corner of the dumpsite area comprises a background sample. Arsenic residuals are plotted for the various stations (Fig. 3.1.1.1.3). There are slightly higher concentrations in four samples from the chemical munitions dumpsite, notably at station ChG1. This representation of the data has the benefit of not being influenced by possible confounding grain size effects, as might the total As concentrations depicted in Fig. 3.1.1.1.1.

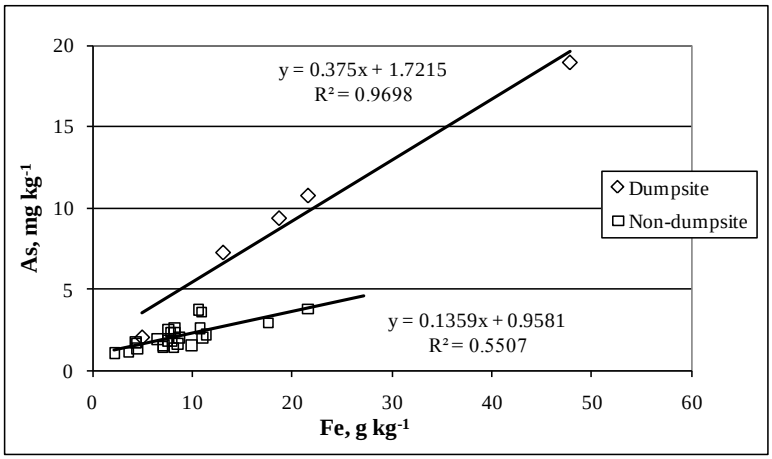


Fig. 3.1.1.1.2. Correlation between Fe and As in the Baltic Sea sediments

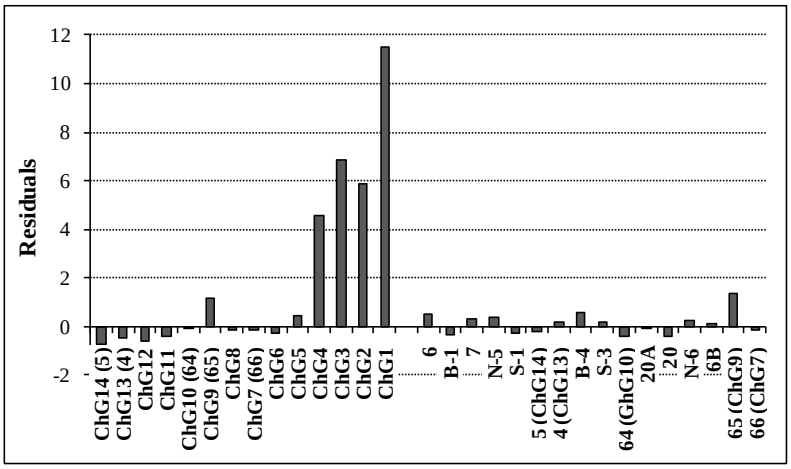


Fig. 3.1.1.1.3. Residual arsenic normalized to iron

3.1.2 Total oil hydrocarbons

Long-term monitoring data is available for total oil hydrocarbons in **water** for the period of 1990–2007. Starting from 2008 a different method – gas chromatography – has been started to use. Two methods can't be directly compared, the sensitivity of two methods are different – the limit of determination of the infrared spectrometry method was 0.03 mg l^{-1} and for gas chromatography method – 0.1 mg l^{-1}. That is why oil hydrocarbons data obtained by the infrared sprectrometry method is treated separately. The data of total oil hydrocarbons was grouped for different types of waters. Sampling stations and a number of measurements for each type of waters are shown in the Table 3.1.2.1.

In Lithuanian legislation the maximum allowable concentration (MAC) for total oil hydrocarbons in water was 0.05 mg l^{-1}, the sensitivity of the infrared total oil determination method was taken into account. From 2010 MAC for total oil in water was changed to 0.2 mg l^{-1}, the limit of quantification of gas chromatography method (0.1 mg l^{-1}) was taken into account. Nevertheless 1990–2007 oil data was compared to more strict 0.05 mg l^{-1} MAC. For the period of 1990–2007 14 % of values were above the MAC in the open sea, 13 % – in the transitional waters. Klaipėda Strait and Coastal waters had 9 % of exceeding the 0.05 mg l^{-1} limit values. Fig. 3.1.2.1 shows, how the percent of values above the MAC had changed during 18-years period. During the period from 1998 till 2004 the percent of values above the MAC didn't exceed 15 %, although starting from 2005 the number of high THC concentrations increased. During the last three years (2005–2007) the percent of high values have increased in every type of waters up to 25 % in Klaipėda Strait and 28 % in the open sea (Table 3.1.2.1).

Table 3.1.2.1. Sampling stations, number of measurements and percentage of values above the MAC (0.05 mg l^{-1}) for 1990–2007 and 2005–2007 periods

Type of waters	Stations	Number of measurements	Values above the MPL 1990-2007 (%)	Values above the MPL 2005-2007 (%)
Transitional waters	Central part of the lagoon: 10, 12, 12A, 14 Northern part of the lagoon: 5, 7B, 8 Plume of the lagoon: 3, 4, 5	1676	13	22
Heavily modified waterbody	Klaipėda Straight: 1, 2, 3, 3A, 3B	1490	9	25
Coastal waters	Northern stony coast: 1, 1B, B-1, B-4, 2, S-3, 64 Southern sandy coast: 20, 20A, S-1, 6, N-5, N-6, 7	1788	9	12
Open sea	2C, 6B, 65, 66, 46	553	14	28

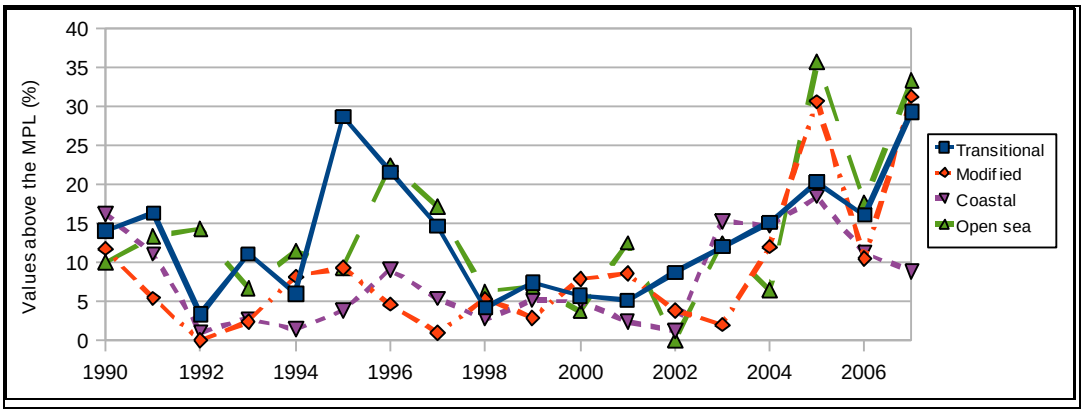


Fig. 3.1.2.1. The percentage of values of total hydrocarbon concentration in water above the maximum allowable concentration (MAC) (0.05 mg l^{-1})

The dataset of total oil hydrocarbons in water for the period of 1990–2007 has been analysed for significant trends. The data from all the stations and water horizons has been sorted according to areas of interest: open sea, northern coastal water, southern coastal waters, plume of the lagoon, Klaipėda Strait and Curonian Lagoon (Fig.3.1.2.2). Data was tested for significant outliers. The outliers that were removed: open sea (2 outliers in 1995 and 1996), northern coastal water (2 outliers were removed in 1996), Klaipėda Strait (1 outlier in 2006) and Curonian Lagoon (4 outliers: 1 in 1991, 1 in 1995; 2 in 2006). The outliers had a high concentration of total oil hydrocarbons with low concentrations in other horizons of the station or in the area in the vicinity to the sampling point.

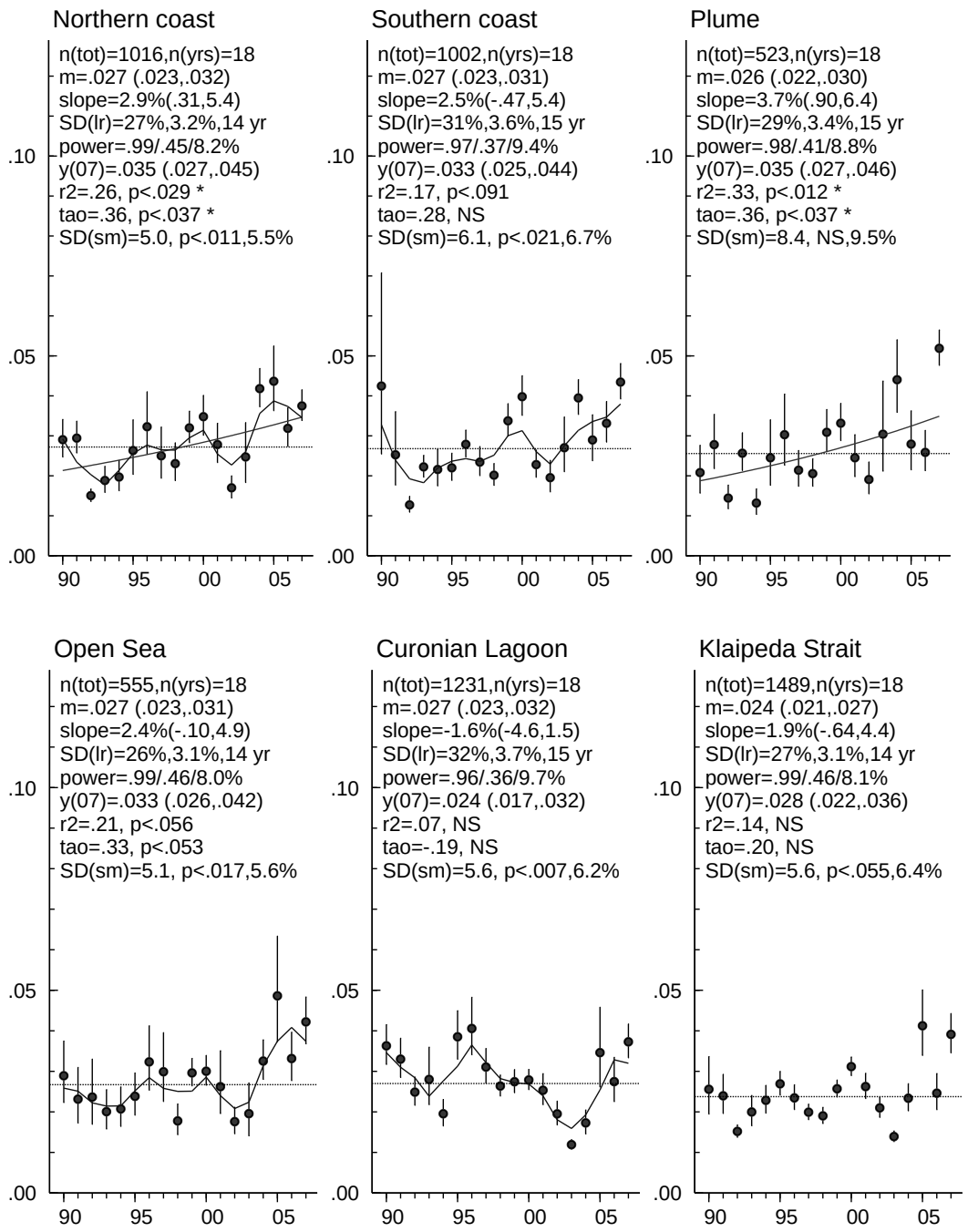


Fig. 3.1.2.2. Concentration of total hydrocarbons (THC) in different types of waters of the south-eastern Baltic Sea (the legend for the plots is presented in Material and Methods / Statistical data analysis chapter)

Spatial distribution of total oil concentrations in water in 1992, 1997, 2000 and 2007 are shown in Fig. 3.1.2.3–3.1.2.6. The annual geometric mean is presented on the maps. It can be noticed that the concentration of total oil in water in selected years is increasing.

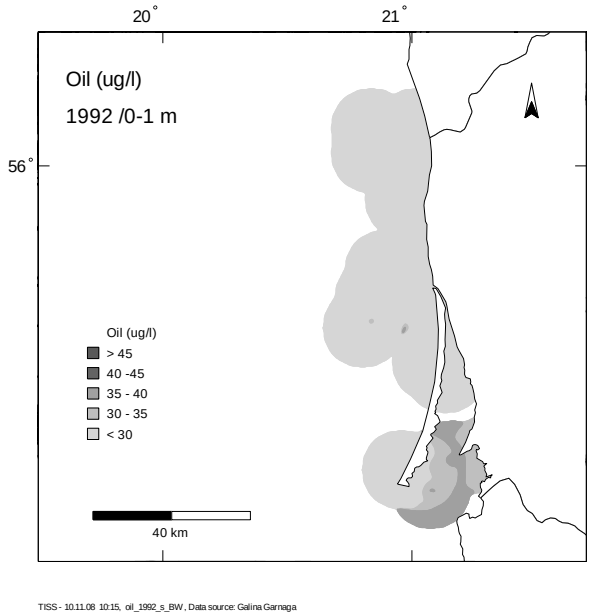


Fig. 3.1.2.3. Distribution of total oil in water in the south-eastern Baltic Sea in 1992

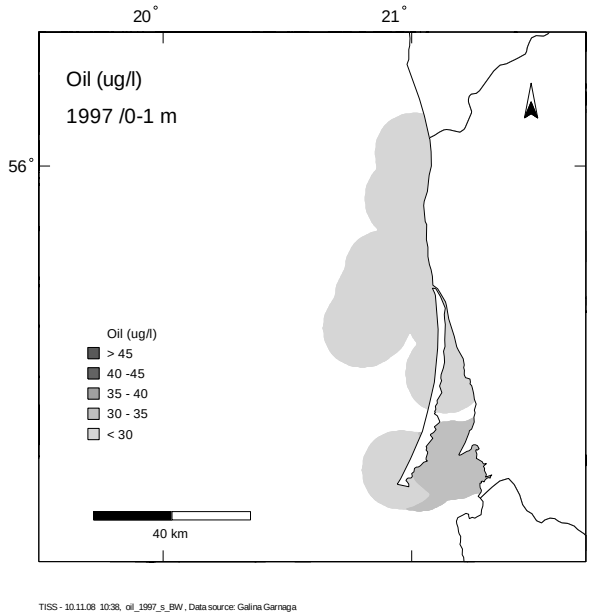


Fig. 3.1.2.4. Distribution of total oil in water in the south-eastern Baltic Sea in 1997

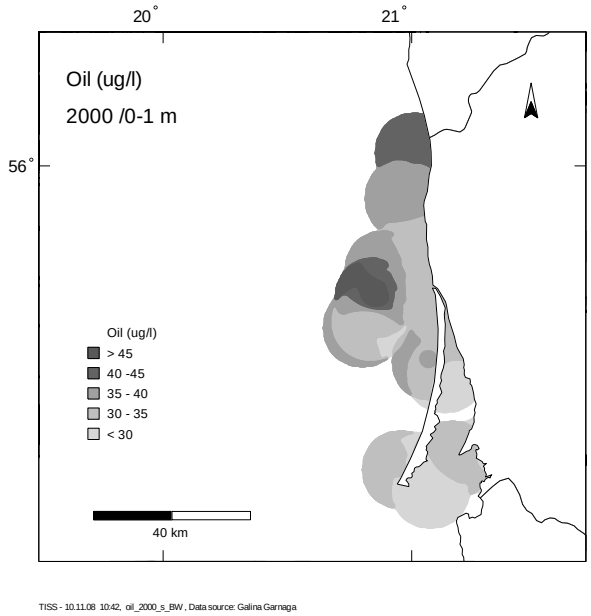


Fig. 3.1.2.5. Distribution of total oil in water in the south-eastern Baltic Sea in 2000

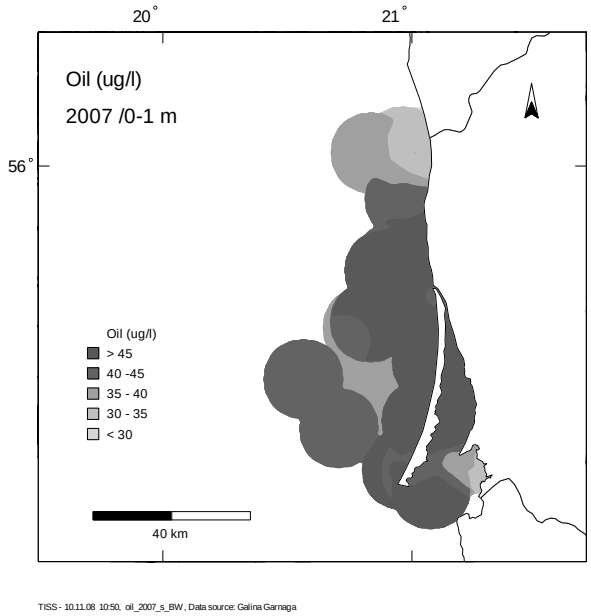


Fig. 3.1.2.6. Distribution of total oil in water in the south-eastern Baltic Sea in 2007

Spatial distribution of total oil hydrocarbons in **sediments** (Fig. 3.1.2.7) shows that there was elevated concentration of total oil at the station B-4 (26 mg kg^{-1} d. w.) which is situated near the Būtingė oil terminal. Higher concentrations were also at the dumping site stations 20, 20A and 20M and at some stations along the Curonian spit (S-1, 6-1, 6-2), comparing to other stations. Although

according to the Lithuanian legislation document on "Sediment dredging in sea and sea-port areas and dredged sediment treatment rules" (Official gazette, 2008, No. 139-5521) almost all values (except station B-4) fall within the cleanest *I* category (< 20 mg kg^{-1} d. w. of total oil hydrocarbons).

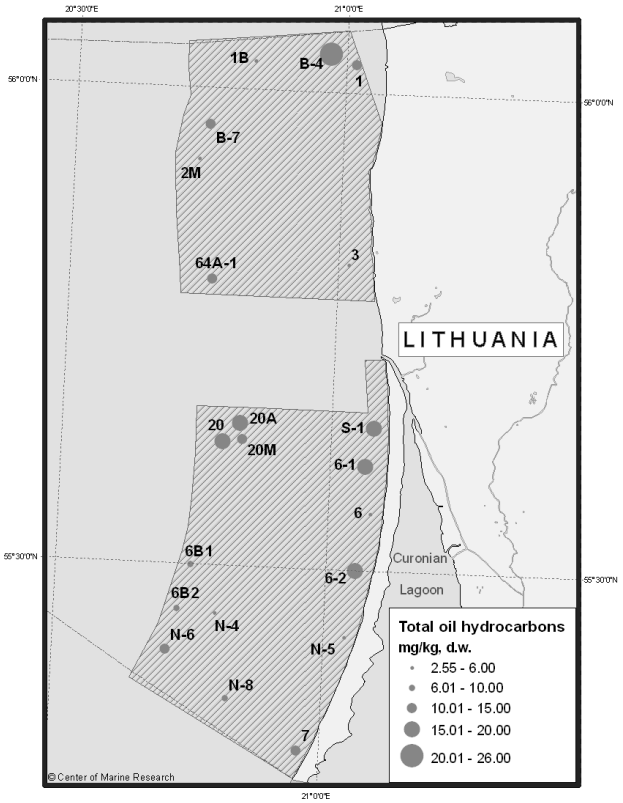


Fig. 3.1.2.7. Spatial distribution of total oil hydrocarbons in sediments in 2006

3.1.3 PAHs

Concentrations of PAHs in 2006–2008 in **sediments** of the Baltic Sea and Curonian Lagoon are shown in Fig. 3.1.3.1. Almost in all samples the highest amount of fluoranthene was detected (from 22 to 72 %), at the stations 12A and 20 dominated benzo(b)fluoranthene (69 and 24 %).

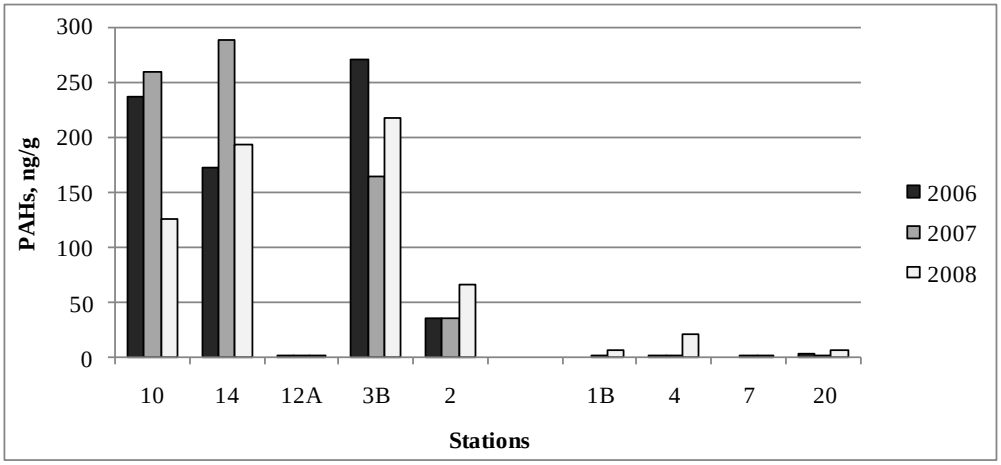


Fig. 3.1.3.1. Concentrations of PAHs in sediments in 2006–2008

The investigation of concentrations of PAHs in sediments was also done in 2006 (during the LIFE project). The highest concentrations of summed PAHs were found at the station N-4, 20M (near the dumping site) and 1B (near the Būtingė oil terminal) (Fig. 3.1.3.2).

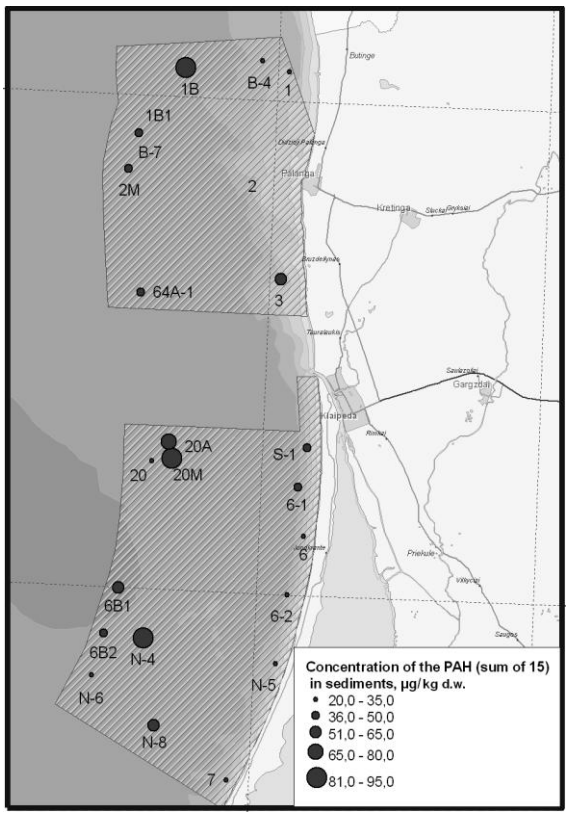


Fig. 3.1.3.2. Average concentrations of summed polycyclic aromatic hydrocarbons in sediments in 2006

Molecular indices, which are based on concentration ratios of selected compounds, can be used to distinguish pyrolytic and petrogenic origins of PAH compounds (Webster et al., 2003; Pikkarainen, 2004). PAHs of pyrolytic origin could be attributed to urban and industrial activities while petrogenic contamination is coming from either vessels or oil installations (Webster et al., 2003).

Five indices were calculated: phenanthrene/anthracene, fluoranthene/pyrene, chrysene/benz(a)anthracene, fluoranthene/(pyrene + fluoranthene), indeno(1,2,3-cd)pyrene/(indeno(1,2,3-cd)pyrene + benzo(ghi)perylene) (Table 3.1.3.1).

Table 3.1.3.1. Molecular Indices of selected PAHs (indices marked in **bold** indicate petrogenic origin of PAHs; in ***bold and italic*** – indicate a contribution from diesel engines; PAHs under the detection limit marked as grey cells)

Station	Sampling date	*Phen/Ant*	*Fluoranth/Pyr*	*Chr/BaA*	*Fluor/(Pyr+Fluor)*	*Ind/(Ind+BghiP)*
1B	2006-05	**17,8**	1,2	**2,0**	0,55	
	2006-08	**21,8**	1,2	**1,7**	0,55	
	2006-10	9,6	1,7	0,4	***0,63***	0,76
2M	2006-05	14,6	1,4	0,9	0,59	0,83
N-8	2006-05	**21,1**	1,0	**1,1**	0,51	0,75
6	2006-05	13,7	1,4	0,7	0,58	
	2006-10	**22,8**	**0,8**	**2,0**	0,45	
7	2006-05	**21,3**	**0,9**	**1,7**	0,47	
	2006-10	**25,1**		**2,2**		
N-4	2006-08	14,5	1,9	**1,2**	***0,66***	
N-5	2006-08	**20,9**	1,2	**2,4**	0,54	
	2006-10	**20,1**	**0,7**	**2,0**	0,42	
1	2006-10	**22,1**	**0,9**		0,48	
3	2006-10	12,7	1,5	0,7	***0,60***	0,72
20	2006-10	13,7	1,3	0,7	0,57	
20A	2006-10	14,0	1,1	0,9	0,52	0,73
20M	2006-10	6,8	1,8	0,2	***0,64***	0,73
S-1	2006-10	**27,3**	1,4	**1,1**	0,58	
B-4	2006-10	13,4	1,0	**2,3**	0,50	
B-7	2006-10	**24,2**	**0,7**	**2,4**	0,39	
N-6	2006-10	**22,6**	**0,8**	**2,9**	0,44	
64A-1	2006-10	14,4	1,3	0,6	0,57	***0,63***
6-1	2006-10	**17,5**	1,1	**1,6**	0,52	
6-2	2006-10	**19,4**	1,2	**1,5**	0,55	
6B1	2006-10	14,0	1,1	0,8	0,53	0,79
6B2	2006-10	**18,9**	**0,9**	**1,3**	0,49	0,78

A phenanthrene/anthracene ratio <10 and fluoranthene/pyrene ratio >1 indicate a pyrolytic origin, whereas a phenanthrene/anthracene ratio >15 and fluoranthene/pyrene ratio of <1 indicate a petrogenic origin. Concentration ratio of chrysene/benz(a)anthracene below 1 indicate pyrolytic origin and values above 1 – petrogenic origin (Pikkarainen, 2004). Samples from the Lithuanian part of the Baltic are both of pyrolytic and petrogenic origins. All three indices indicated that the source of PAHs at the 6, 7, N-5, B-7, N-6 and 6B2 stations is petrogenic (coming from either vessels or oil installations).

Two concentration ratios are considered to be indicative of diesel engines: fluoranthene divided by the sum of pyrene and fluoanthene with a range of 0.60 to 0.70 and indeno(1,2,3-cd)pyrene divided by the sum of indeno(1,2,3-cd)pyrene and benzo(ghi)perylene with the range of 0.35 to 0.70 (Pikkarainen, 2004). In this study a diesel engine source was indicated at the 1B, N-4, 3, 20M, 64A-1 stations which had comparatively high concentrations of summed PAHs compared to other stations.

3.1.4 Organochlorine compounds

3.1.4.1 Organochlorine pesticides

Concentrations of organochlorine pesticides in **water** in 1997–2006 are presented in Fig. 3.1.4.1.1. The concentration of organochlorine pesticides (α-HCH, β-HCH, γ-HCH, α-endosulfan, β-endosulfan, 4,4‘-DDE, 4,4‘-DDD, 4,4‘-DDT, aldrin, dieldrin, endrin, heksachlorbenzene) in water in 2008 mostly didn‘t exceed the limit of determination of the method. The concentrations higher than the limit of determination were obtained at the station 1 in the Baltic Sea – here the concentrations of the α-HCH was 11 ng l^{-1} (could be an influence of the Šventoji river) – and at dredged sediments dumping site (station 20A): the concentration of 4,4‘-DDE was 5.7 ng l^{-1}, 4,4‘-DDD – 10 ng l^{-1}, 4,4‘-DDT – 13 ng l^{-1}.

The measurements of DDT in **biota** (mussels) from the Lithuanian part of the Baltic Sea (*M. balthic* and *M. edulis*) and Curonian Lagoon (*D. ploymorpha*) were made starting from 1999. Long-term studines show that the DDT

concentration in *M. balthica* and *M. edulis* is at the same level about 3 µg kg^{-1} w. w. In the Curonian Lagoon mussel *D. polymorpha* the concentration of DDT is about 1 µg kg^{-1} w. w. (Fig. 3.2.1.2.1).

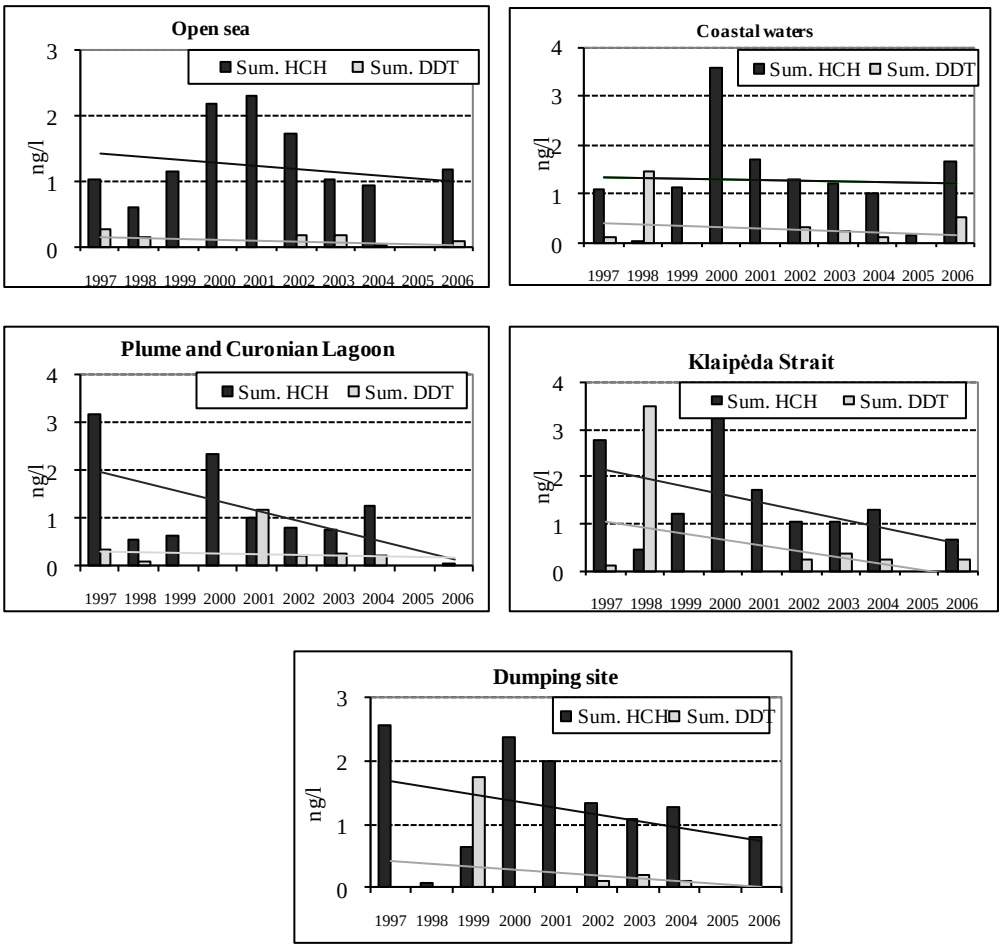


Fig. 3.1.4.1.1. Summed concentrations of DDT and HCH in the Baltic Sea and Curonian Lagoon water in 1997–2006 m

Concentrations of organochlorine compounds in **sediments** are presented in Fig. 3.1.4.1.2.

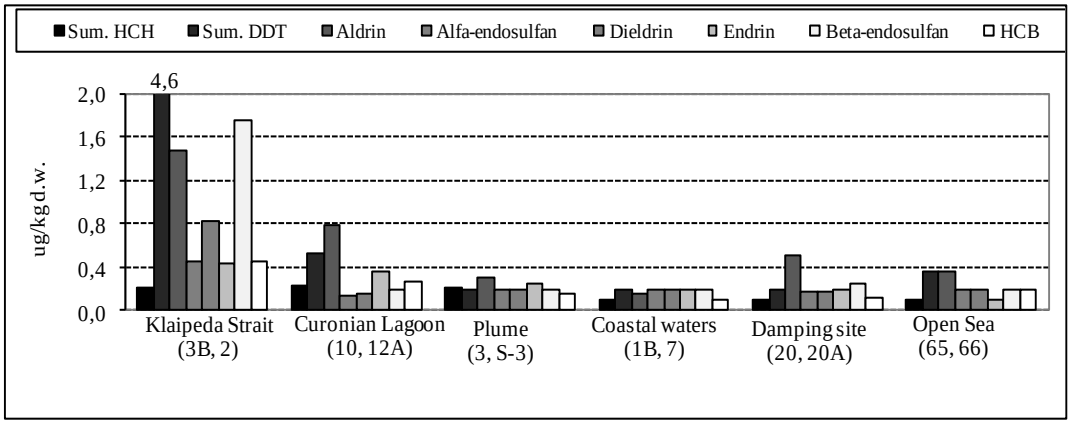


Fig. 3.1.4.1.2. Concentrations of organochlorine compounds in sediments in 2006

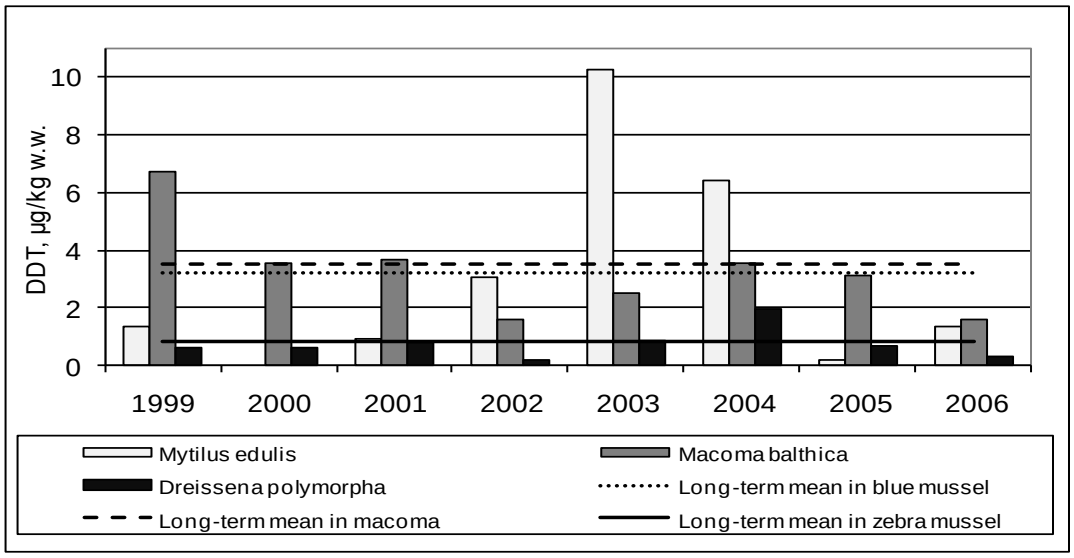


Fig. 3.2.1.2.1. Summed concentration of DDT in *Mytilus edulis*, *Macoma balthica* and *Dreissena polymorpha* mussels in 1999–2006

High concentrations of organochlorine pesticides were found in *Clupea harengus* muscles. Especially high concentrations of DDT were found: 4,4‘-DDT – 1.8 µg kg^{-1} w. w., 4,4‘-DDD – 4.3 µg kg^{-1} w. w., 4,4‘-DDE – 12.1 µg kg^{-1} w. w. That could be explained by the higher amount of fat found in Baltic herring comparing to other biota species (Fig. 3.2.1.2.3).

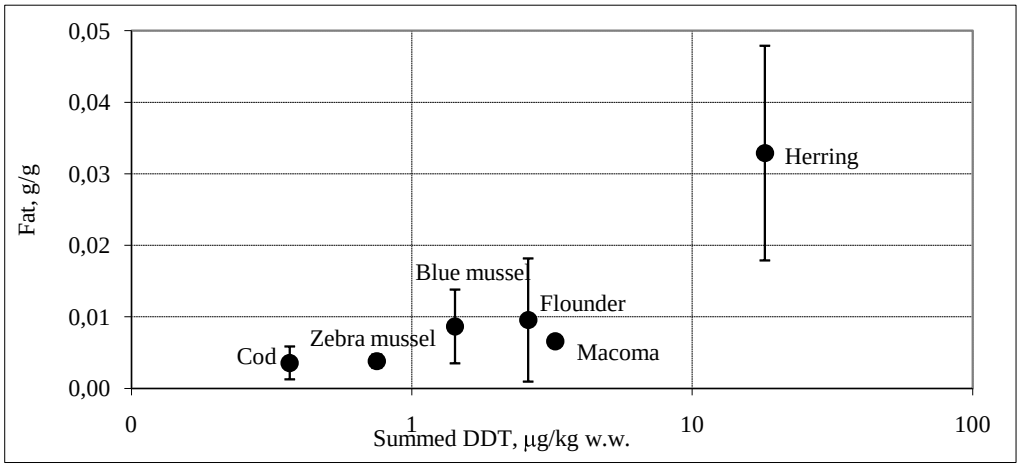


Fig. 3.2.1.2.3. Summed concentration of DDT versus fat amount (± standard deviation) in biota from the Lithuanian part of the Baltic Sea in 2006 (x – logarithmic scale)

3.1.4.2 PCBs

Polychlorinated biphenyls (PCBs) (28, 52, 101, 118, 138, 153, 180) were added to Lithuanian national monitoring program only in 2008. The concentrations of PCBs in 2008 and 2009 in **water** and **sediments** of the Baltic Sea (1B, 4, 7, 20 stations) and Curonian Lagoon (10, 14, 12A, 3B, 2 stations) didn‘t exceed limits of determination of the methods (in water – 0.01 µg l^{-1}; in sediments – 1.0 µg kg^{-1}).

Dioxin-like PCBs (105, 114, 118, 123, 126, 156, 157, 167, 169, 189, 77, 81) were analyzed in sediments of the Baltic Sea (7, 4, 65, 20 stations) and Curonian Lagoon (stations 3B and 2) in August 2008. Summed concentrations of dioxin-like PCBs and concentrations according to toxic equivalency factors (TEF) are shown in Figs. 3.1.4.2.1 and 3.1.4.2.2. In all stations of the Baltic Sea concentrations of dioxin-like PCBs were under the limit of determination. The highest concentration of dioxin-like PCBs (WHO-PCB-TEF 0.076 ng kg^{-1} d. w.) was found in the Klaipėda harbour (station 3B).

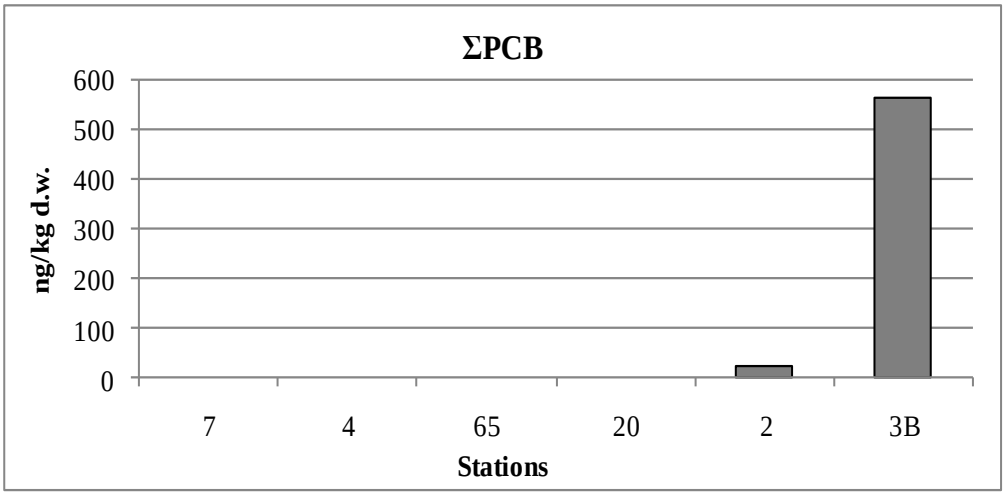


Fig. 3.1.4.2.1. Summed concentrations of the dioxin-like PCBs in sediments of the Baltic Sea (7, 4, 65, 20 stations) and Curonian Lagoon (2, 3B stations) in 2008 (values which are under the limit of quantification are not included in calculations)

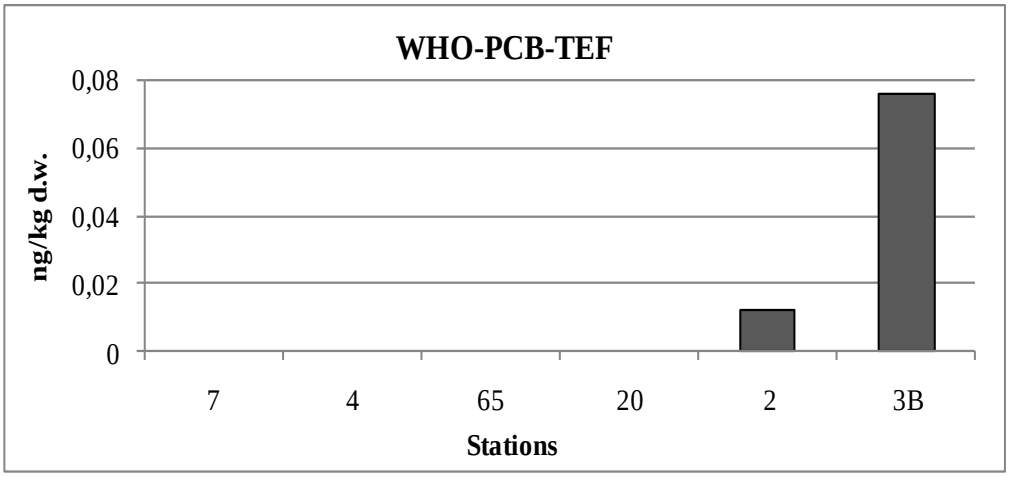


Fig. 3.1.4.2.2. Concentrations of dioxin-like PCBs in sediments of the Baltic Sea (7, 4, 65, 20 stations) and Curonian Lagoon (2, 3B stations) according to toxic equivalency factors (TEF) in 2008 (values which are under the limit of quantification are not included in calculations)

3.1.4.3 Dioxins and furans

Summed concentrations of dioxins and furans in **sediments** and concentrations according to toxic equivalency factors (TEF) are shown in Figs. 3.1.4.3.1 and 3.1.4.3.2. In the Baltic Sea stations 7 and 4 concentrations of dioxins and furans were under the limit of quantification. The highest summed concentration of dioxins and furans (29 ng kg^{-1} d. w.) was found in dredged sediments dumping site (station 20), however at this station the dioxin and

furans compounds were less toxic (PCDD/F TEF 0.199 ng kg^{-1} d. w.) than at the open sea station 65 (PCDD/F TEF 0.438 ng kg^{-1} d. w.).

3.1.5 TBT

Organotin compounds: monobutyltin, dibutyltin, tributyltin, tetrabutyltin, monooctyltin, dioctyltin, tricyclohexyltin, triphenyltin were analyzed in **sediments** of the Baltic Sea (7, 4, 65, 20 stations) and Curonian Lagoon (stations 3B and 2) in August 2008. Organotin compounds were detected at three stations: 3B station near the Malkų bay in the Klaipėda harbour – the highest concentration of tributyltin (57 µg kg^{-1} d. w.) and dibutyltin (3.1 µg kg^{-1} d. w.) were detected at that station; station 2 in the Klaipėda Strait (the concentration of tributyltin – 8.8 µg kg^{-1} d. w.); station 20 at the dredged sediments dumping site (the concentration of tributyltin – 2.3 µg kg^{-1} d. w.). The concentrations of organotin compounds at other stations were under the limit of quantification (1 µg kg^{-1} d. w.) (Fig. 3.1.5.1).

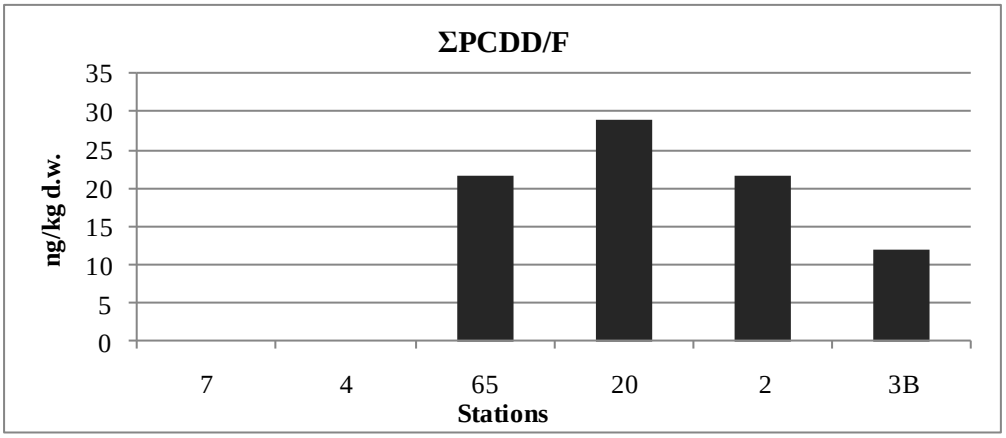


Fig. 3.1.4.3.1. Summed concentrations of dioxins and furans in sediments of the Baltic Sea (7, 4, 65, 20 stations) and Curonian Lagoon (2, 3B stations) in 2008 (values which are under the limit of quantification are not included in calculations)

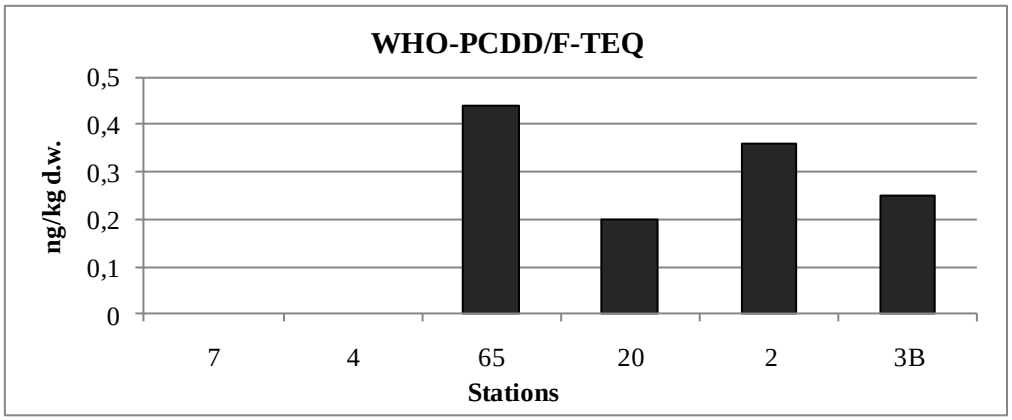


Fig. 3.1.4.3.2. Concentrations of dioxins and furans in sediments of the Baltic Sea (7, 4, 65, 20 stations) and Curonian Lagoon (2, 3B stations) according to toxic equivalency factors (TEF) in 2008 (values which are under the limit of quantification are not included in calculations)

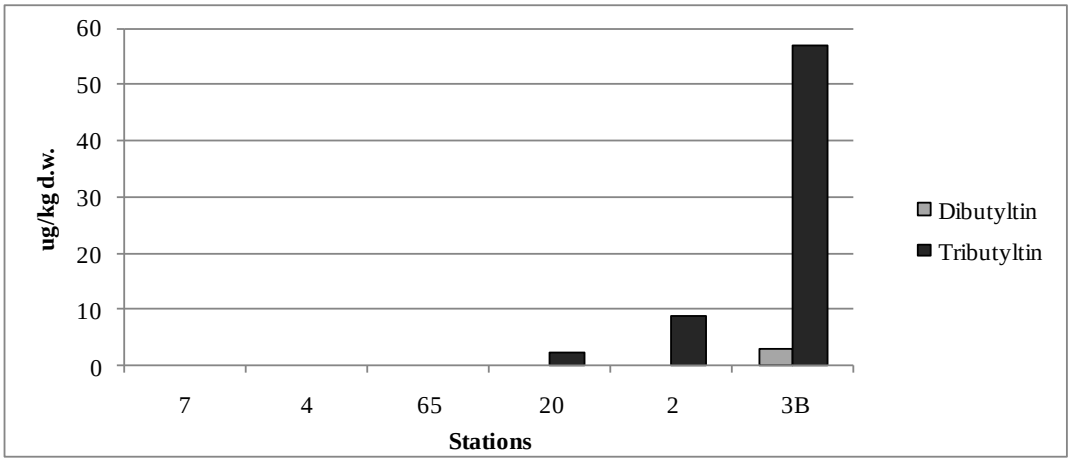


Fig. 3.1.5.1. Tri- and dibityltin concentrations in sediments of the Baltic Sea (stations 7, 4, 65, 20) and Curonian Lagoon (stations 2, 3B) in 2008

3.1.6. Screening of the selected hazardous substances in the eastern Baltic marine environment

During the HELCOM "Screening of the selected hazardous substances in the eastern Baltic marine environment" project hazardous substances which are in the HELCOM Baltic Sea Action Plan list were detected in water and fish (flounder and herring). Results of the study are presented in Tables 3.1.6.1 and 3.1.6.2.

Table 3.1.6.1. Concentrations of perfluorinated substances and phenolic substances in water in coastal area north from Klaipėda (station 4) and in the open sea area north-east from Klaipėda (station 65)

	Coastal area north from Klaipėda	**Open sea area north-east from Klaipėda**
Perfluorinated substances in water, ng l^{-1}		
6:2 FTS	<3	<3
PFOSA	<0.2	<0.2
PFBS	<0.4	<0.4
PFHxS	<0.3	<0.3
PFOS	<1.0	<1.0
PFDcS	<0.2	<0.2
PFBA	<2.0	<2.0
PFHxA	<2.0	<2.0
PFHpA	<4.0	<4.0
PFOA	<5	<5
PFNA	<2.0	<2.0
PFDcA	<4.0	<4.0
PFUnA	<2.0	<2.0
Phenolic substances in water, ng l^{-1}		
4NP	29	50
4NP-EO1	<20	<20
4NP-EO2	<10	<10
4-t-OP	1.2	<0.5
4-t-OP-EO1	<0.5	<0.5
4-t-OP-EO2	<0.5	<0.5
Bisfenol A	<14	<14
Triclosan	<1	<1

Table 3.1.6.1. Concentrations of hazardous sustances in flounder and herring from the coastal area north from Klaipėda and in the open sea area north-east from Klaipėda

	Coastal area north from Klaipėda		**Open sea area north-east from Klaipėda**	
	Flounder	Herring	Flounder	Herring
Organic tin compounds, ng g^{-1} fresh weight (f. w)				
MBT	<1; <30	<1; <15	<10	<2
DBT	<1.3 - 2.1	<1.3	<1.3	<1.3
TBT	<2	<2 - 6.4	<2	3.1
MPhT	<0.8	<0.8	<0.8	<0.8
DPhT	2.3 - 3.4	<0.9 - 4.4	3.3	4.6
TPhT	<0.1	<0.1	<0.1	<0.1
MOT	<0.4	<0.4	<0.4	<0.4
DOT	<0.4	<0.4	<0.4	<0.4

	Coastal area north from Klaipėda		Open sea area north-east from Klaipėda	
	Flounder	Herring	Flounder	Herring
Brominated flame retardants in fish muscle, ng g^{-1} f. w.				
BDE-47	0.014 - 0.021	0.092 - 0.18	<0.01	0.26
BDE-85	<0.01	<0.01	<0.01	0.011
BDE-99	<0.01	0.024 - 0.036	<0.01	0.054
BDE-100	<0.01 - 0.010	0.046 - 0.11	<0.01	0.15
BDE-138	<0.01	<0.01	<0.01	<0.01
BDE-153	<0.01	<0.01	<0.01	0.013
BDE-154	<0.01	0.021 - 0.038	<0.01	0.058
BDE-183	<0.01	<0.01	<0.01	<0.01
BDE-197	<0.1	<0.1	<0.1	<0.1
BDE-201	<0.1	<0.1	<0.1	<0.1
BDE-202	<0.1	<0.1	<0.1	<0.1
BDE-209	<0.1	<0.1	<0.1	<0.1
HBCDD	<0.1	<0.1	<0.1	<0.1
Perfluorinated substances in fish liver, ng g^{-1} f. w.				
6:2 FTS	<0.7; <1.0	<0.5; <0.6; <1.1	<0.8	<1.3
PFOSA	<0.6; <0.8; <0.5	<0.2; <0.3; <0.1	<0.6	1.8
PFBS	<0.1; <0.2	<0.2; <0.1	<0.1	<0.2
PFHxS	0.40 - 1.1	0.23 - 0.39	0.26	0.30
PFOS	11 - 20	6.1 - 10	6.9	11
PFDcS	<0.07; <0.1	<0.1; <0.07; <0.05	0.06	<0.1
PFBA	<0.5; <0.7; <1.0	<0.8; <0.5	0.45	<1.1
PFHxA	<1.5; <2.0; <2.3	<2.6; <1.3	0.78	<2.0
PFHpA	<1.6; <1.7; <2.9	<1.7; <0.9; <1.2	2.0	<2.0
PFOA	<1.8; <2.1; <3.1	<3.3; <1.5; <1.3	<1.9	<2.7
PFNA	<2.7 - 1.8	<1.8; <1.4	3.5	<2.4
PFDcA	<1.3; <2.1; <2.3	<1.8; <1.5; <2.6	<2.0	<2.5
PFUnA	<2.4 - 1.5	<1.5; <0.9; <0.8	<1.2	<2
Phenolic substances in fish muscle, ng g^{-1} f. w.				
4NP	<10 - 12	<10	<10	<10
4NP-EO1	<20	<20	<20	<20
4NP-EO2	<10	<10	<10	<10
4-t-OP	<1	<1; <2	<1	<1
4-t-OP-EO1	<1	<1	<1	<1
4-t-OP-EO2	<1	<1	<1	<1
Bisfenol A	0.98 - 3.9	<0.6 - 3.1	0.95	1.6
Triclosan	<1	<1	<1	<1
Chlorinated paraffins in fish liver, ng g^{-1} f. w.				
SCCP	17 - 62	6,5 - 19	15	34
MCCP	<0,46; <0,57; <0,67	<1,9; <0,74; <0,30	<0,26	<0,25
Endosulfan in fish muscle, ng g^{-1} f. w.				
α-endosulfan	<0.2	<0.2	<0.2	<0.2
β-endosulfan	<0.2	<0.2	<0.2	<0.2
Endosulfan sulphate	0.011 - 0.014	0.057 - 0.088	0.016	0.12

3.1.7 Pollution index

For the calculation of Pollution Index (PI) the Baltic Sea (20, 7, 1B, 4) and Curonian Lagoon (10, 14, 12A, 3B, 2) stations with the complex monitoring of contaminants were chosen. All samples were taken in August of 2006. The EQS values for the calculation of PI were taken from the Order of Minister of Environment on "Wastewater treatment regulation" (17.05.2006, No. D1-236). Substances with the concentrations under the limit of quantification at all stations were not taken into calculation. If the concentration of other substances were under the limit of quantification, the concentration was considered as "zero" and the ratio C_i/EQS_i was also "zero" (Table 3.1.7.1).

Results show that more contaminated are the water of the Curonian Lagoon. The highest Pollution Index was calculated in the Klaipėda Strait (station 2) *PI* = 4.4 and near the Malkų bay (station 3B), *PI* = 3.0. Oil hydrocarbon concentration was high at these stations.

Table 3.1.7.1. Ratios C_i/EQS_i and Pollution Indexes (*PI*) at some stations of the Baltic Sea and Curonian Lagoon in August 2006

	Stations	Oil hydrocarbons	Pb	Cu	Cd	Zn	Cr	Anthracene	Fluoranthene	Benzo(b)fluoranthene	Benzo(k)fluoranthene	Benzo(a)pyrene	Benzo(ghi)perylene	Indeno(1,2,3-c,d)pyrene	Mean C_i/EQS_i	Max. C_i/EQS_i	Pollution Index (PI)
Baltic Sea	**1B**	1.0	0	0	0.04	0	0.3	0.1	0.02	0	0	0	0	0	0.1	1.0	**0.7**
	4	0	0	0	0	0	0	0.1	0	0	0	0	0	0	0.01	0.1	**0.1**
	7	1.0	0	0	0	0	0	0.2	0.1	1.0	0.4	0.4	0.7	0.5	0.3	1.0	**0.7**
	20	0	0	0	0.02	0	0	0	0.02	0	0	0	0	0	0.0	0.02	**0.0**
Curonian Lagoon	**10**	2.71	0.4	0	0	0	0	0.1	0	0	0	0	0	0	0.2	2.7	**1.9**
	14	0	0	0	0	0	0	0.2	0.1	0.6	0.3	0.2	0	0	0.1	0.6	**0.4**
	12A	0	0.3	0	0	0	0	0.1	0	0	0	0	0	0	0.03	0.3	**0.2**
	3B	0.8	0	4.2	0.03	0	0	0	0	0	0	0	0	0	0.4	4.2	**3.0**
	2	6.21	0	1.6	0.03	0.7	0	0.1	0	0	0	0	0	0	0.7	6.2	**4.4**

3.2 Effects of contaminants on biota

3.2.1 Biochemical biomarkers in Lithuanian coastal area

In August 2008 samples for biomarker measurements were collected in the Baltic Sea and Curonian Lagoon.

Acetylcholinesterase activity was measured in gills of *Mytilus edulis* and *Dreissena polymorpha*, in foot of *Macoma balthica* and in the whole organism of *Nereis diversicolor* (Fig. 3.2.1.1). There was a difference in acetylcholinesterase activity in *M. balthica* mussels between 2 stations: 13.1 ± 6.4 nmol ACTC min^{-1} mg^{-1} protein at the 65 stations and 69.6 ± 30.1 nmol ACTC min^{-1} mg^{-1} protein at the station 7.

There was a high variability in acetylcholinesterase activity among studied organisms: the lowest activity was obtained in *D. polymorpha* mussels – 0.58 nmol ACTC min^{-1} mg^{-1} protein. In *N. diversicolor* an acetylcholinesterase activity was 32.7 ± 11.0 nmol ACTC min^{-1} mg^{-1} protein. There was a large variability among replicates in all samples, especially in *M. balthica* collected from the station 7.

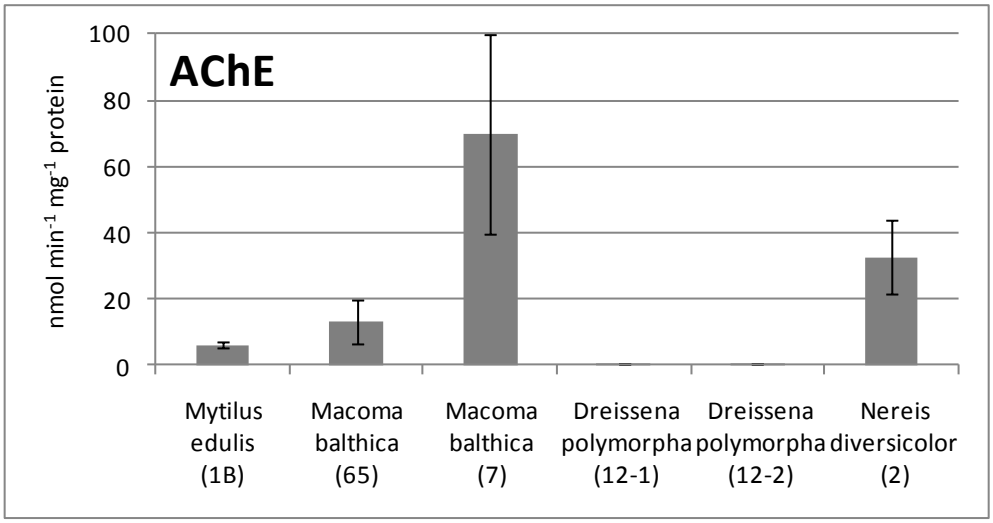


Fig. 3.2.1.1. Acetylcholinesterase activity (mean ± standard deviation) in aquatic organisms from the Baltic Sea (*M. edulis* and *M. balthica*) and Curonian Lagoon (*D. polymorpha* and *N. diversicolor*) in August 2008

GST, CAT, SOD activities and LPO are shown in the Fig. 3.2.1.2.

GST varied from 132.2 ± 37.8 nmol min^{-1} mg^{-1} protein in *N. diversicolor*, to 339.6 ± 52.0 nmol min^{-1} mg^{-1} protein in *M. edulis*, and 717.5 ± 192.6 and 1034

± 82 nmol min^{-1} mg^{-1} protein in *M. balthica* at the stations 65 and 7 respectively.

CAT varied from 74.2 ± 41.0 μmol min^{-1} mg^{-1} protein in *N. diversicolor*, to 104 ± 20 μmol min^{-1} mg^{-1} protein in *M. edulis*, and 89.4 ± 15.1 and 114 ± 15 μmol min^{-1} mg^{-1} protein in *M. balthica* at the stations 65 and 7 respectively.

The activity of SOD in *M. balthica* varied from 4.2 ± 2.0 to 11.1 ± 1.8 units min^{-1} mg^{-1} protein at the stations 65 and 7. In *M. edulis* 11.0 ± 8.7 units min^{-1} mg^{-1} protein of SOD were found, in *N. diversicolor* – 10.7 ± 3.8 units min^{-1} mg^{-1} protein.

LPO varied from 67.5 ± 7.3 nmol TBARS g^{-1} w. w. in *N. diversicolor*, to 158 ± 71 nmol TBARS g^{-1} w. w. in *M. edulis*, and 179 ± 38 and 190 ± 54 nmol TBARS g^{-1} w. w. in *M. balthica* at the stations 7 and 65.

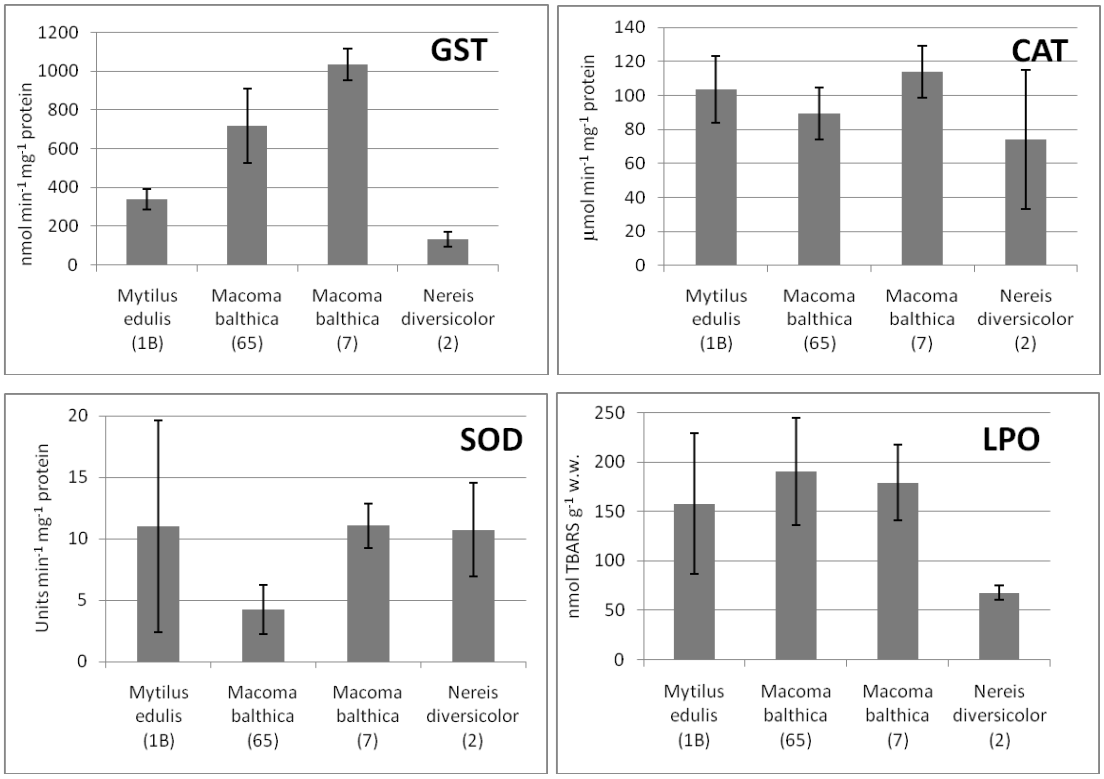


Fig. 3.2.1.2. Biomarker responses (mean ± standard deviation) in aquatic organisms from the Baltic Sea (*M. edulis* and *M. balthica*) and Curonian Lagoon (*N. diversicolor*) in August 2008. Abbreviations: GST – glutathione S-transferase activity; CAT – catalase activity, SOD – superoxide dismutase activity; LPO – lipid peroxidation

3.2.2 Genotoxic and cytotoxic effects in bivalve mollusks *Macoma balthica* and *Mytilus edulis*

In May 2006, bivalves *Macoma balthica* and *Mytilus edulis* were sampled from six study locations. The frequency of micronuclei (MN/1000 cells) in *M. balthica* varied from 1.28 to 3.63 ‰, in mussels – from 1.74 to 3.38 ‰ (Fig. 3.2.2.1). Low level of micronuclei was observed in *M. balthica* inhabiting station N-8, which was considered as a reference site. Significant increase of genotoxicity was found in clams from the location N-4 (P = 0.0155) and in clams from the location 65 (P = 0.0003) (Fig. 3.2.2.1).

Similar levels of nuclear abnormalities were occurred in mussels collected in the Būtingė oil terminal area. MN frequency in mussels from 1B station was analyzed in May and August 2006. In August, the mean of MN (3.38 ‰) was two-fold higher than in May (1.74 ‰). The value of the parameter in *M. balthica* from 6B station in June was equal to 2.86 ‰ (Fig. 3.2.2.1).

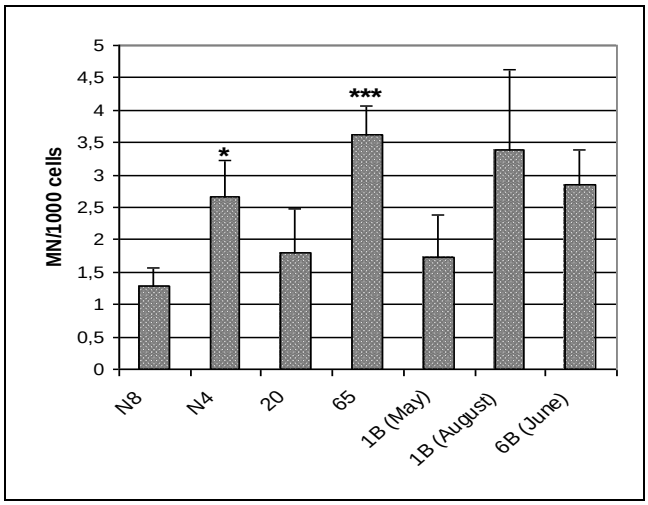


Fig. 3.2.2.1. Frequency of micronuclei in gill cells of clams and blue mussels from different study locations in the Lithuanian economic zone of the Baltic Sea. Differences between *M. balthica* clams from the reference station N-8 and other locations: one asterisk at level P < 0.05, three asterisks – P < 0.0001

The occurrence of other nuclear abnormalities was analyzed in *M. balthica* and *M. edulis* bivalves. In *M. balthica* the frequency of nuclear buds (BD/1000 cells) varied in a range from 0.50 to 1.49 ‰, fragmented-apoptotic cells (FA/1000 cells) – from 0.53 to 1.72 ‰, bi-nucleated cells (BN/1000 cells) –

from 1.51 to 2.23 ‰. The highest levels of nuclear buds was observed in mussels collected in May from the station 1B, fragmented-apoptotic cells – in mussels collected from the same station in August and bi-nucleated cells – in bivalves inhabiting station 65 (Fig. 3.2.2.2).

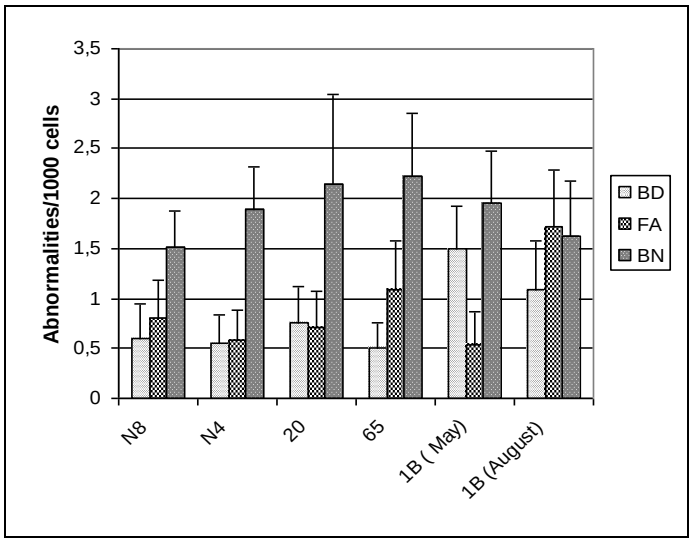


Fig. 3.2.2.2. Frequency of nuclear buds (BD), fragmented-apoptotic (FA) and bi-nucleated (BN) cells in bivalve gills from different locations in the Lithuanian economic zone of the Baltic Sea

3.2.3 Būtingė oil spill

In *Mytilus edulis* specimens collected on 11-12 February 2008 (12 days after the oil spill in the Būtingė oil terminal) the frequency of micronuclei (MN/1000 cells) varied from 1.99 to 2.38 ‰, nuclear buds (NB/1000 cells) – from 1.28 to 2.45 ‰, fragmented-apoptotic cells (FA/1000 cells) – from 0.14 to 0.49 ‰, bi-nucleated cells (BN/1000 cells) – from 0.94 to 1.20 ‰. The lowest values of micronuclei, nuclear buds and fragmented-apoptotic were found in mussels from the 1st location (Fig. 3.2.3.1). The concentration of total oil hydrocarbons in water at the same station was not elevated and did not exceed maximum allowable concentration (MAC) (0.05 mg l^{-1}). The highest level of genotoxicity (4.83 ‰ MN and NB incidences) was registered in mussels from the 2AV station, where elevated concentration of total oil hydrocarbons (up to 0.11 mg l^{-1}) in water was determined.

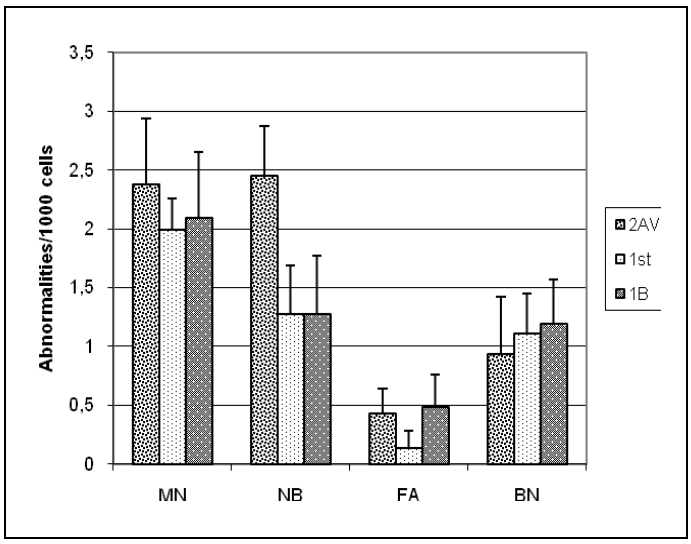


Fig. 3.2.3.1. Frequency of micronuclei (MN), nuclear buds (NB), fragmented-apoptotic (FA) and bi-nucleated (BN) cells in gills of mussel collected in February 2008 after the oil spill

The analysis of nuclear abnormalities in gills of mussels was performed in May 2008, three months after the oil spill to determine the persistency of the damage. Similar genotoxicity and increased cytotoxicity levels in 1st and 2nd stations compared to the responses in February 2008 were detected. Since we have performed annual analysis (2001–2008) of genotoxicity and cytotoxicity in mussels from the Lithuanian coast, it was possible to compare the levels of responses in mussels before and after the oil spill. In the long-term studies, location close to Palanga has served as a reference site. Comparison of genotoxicity and cytotoxicity levels in the Palanga location in June 2007 and after the accidental spill in May 2008 showed statistically significant increase of micronuclei ($P = 0.0036$) and fragmented-apoptotic cells ($P = 0.0286$) in mussels inhabited 2nd (Palanga) station, and nuclear buds ($P = 0.0264$) in mussels from the 1st station (Fig. 3.2.3.2). One way ANOVA analysis showed significant differences in MN ($P = 0.0258$, $F = 4.161$), in NB ($P = 0.0125$, $F = 5.121$), and FA ($P = 0.0164$, $F = 4.750$) between the studied mussels groups.

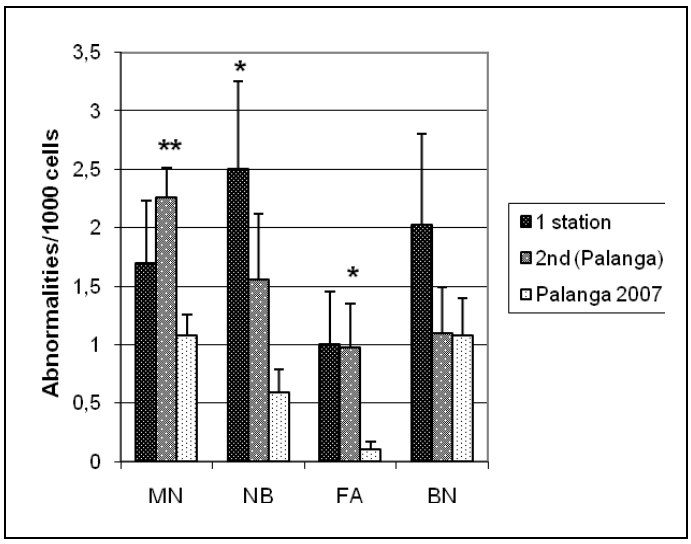


Fig. 3.2.3.2. Frequency of MN, NB, FA and BN cells in gills of mussel collected in May 2008 from the polluted by oil locations (1st and 2nd stations) and from the reference (before the oil spill) Palanga site in June 2007. Differences between mussels from the reference and contaminated stations shown: one asterisk at level $P < 0.05$, two asterisks – $P < 0.001$

The environmental genotoxicity and cytotoxicity levels were significantly increased in mussels collected 6 months after the oil spill, in August 2008, compared to the background levels detected in mussels before the oil spill. In *M. edulis* from contaminated by oil stations (1st, 1B and Palanga 2008), the frequency of micronuclei varied from 3.74 to 6.06 ‰, nuclear buds – from 1.69 to 2.65 ‰, fragmented-apoptotic cells – from 0.97 to 1.96 ‰, bi-nucleated cells – from 1.38 to 2.36 ‰ (Fig. 3.2.3.3). In August 2008, significantly higher genotoxicity was observed in mussels after the oil spill compared to genotoxicity before the accident. Compared to the reference data from Palanga (June 2007), statistically significant elevation of MN ($P = 0.0001$, $P < 0.0001$), NB (P values varied from 0.0020 to 0.0120) and FA (P values varied from 0.0013 to 0.0086) was found in mussels from all three contaminated stations.

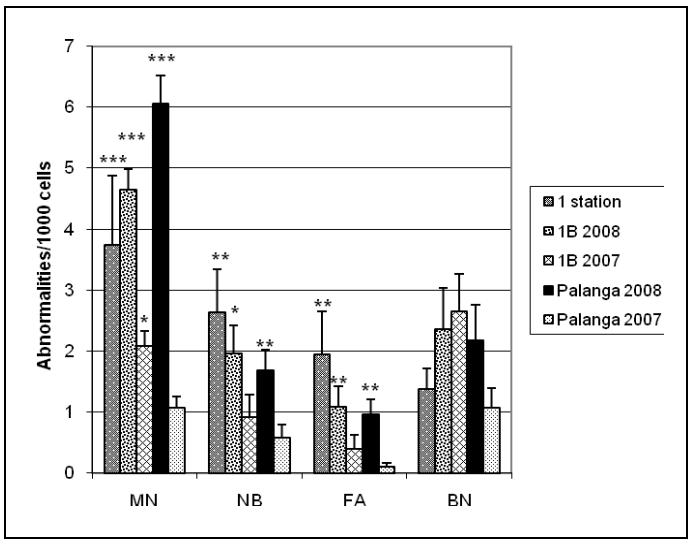


Fig. 3.2.3.3. Frequency of micronuclei (MN), nuclear buds (NB), fragmented-apoptotic (FA) and bi-nucleated (BN) cells in gills of mussel collected in August 2008 from the polluted by oil locations (stations 1st, 1B and Palanga 2008) and from the reference (before the oil spill) Palanga 2007 site. Differences between mussels from the reference and contaminated stations shown: one asterisk at level $P < 0.05$, two asterisks – $P < 0.001$, three asterisks – $P < 0.0001$

Investigation of environmental genotoxicity and cytotoxicity in mussels inhabiting 1B station was performed earlier in May and August 2006, in August 2007, and after the oil spill in February and August 2008. The lowest responses were in August 2007, the highest – after the oil spill in August 2008 (Fig. 3.2.3.4). Increase of MN in August 2008 was significant ($P < 0.0001$) compared to the parameter value in August 2007. One way ANOVA revealed significant time-related differences only in frequencies of micronuclei ($P = 0.0036$, $F = 4.647$).

After the oil spill environmental genotoxicity and cytotoxicity endpoints were repeatedly measured three times in mussels from the 1st station. Time-related elevation of all responses was detected (Fig. 3.2.3.5). Compared to the values of February 2008, there were extremely increased levels of fragmented-apoptotic cells in August (14-fold) and in May (7-fold). The frequency of micronuclei has increased only in August (1.9-fold), 2-fold increase of nuclear buds was observed in May and in August, and 1.8-fold increase of bi-nucleated cells was found in May. Nevertheless, due to the high variation in individual

responses, time-related statistically significant elevation was detected only for fragmented-apoptotic cells.

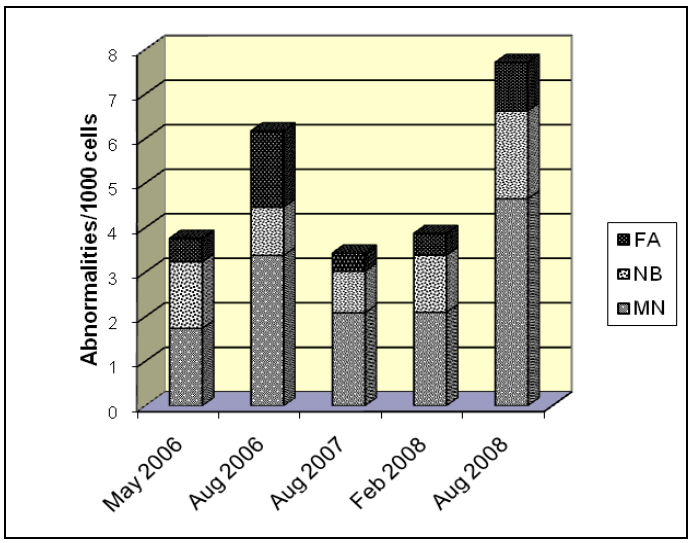


Fig. 3.2.3.4. Incidences of micronuclei (MN), nuclear buds (NB), and fragmented-apoptotic (FA) cells in gills of mussel collected in 2006–2008 from the location 1B near the Būtingė oil terminal area

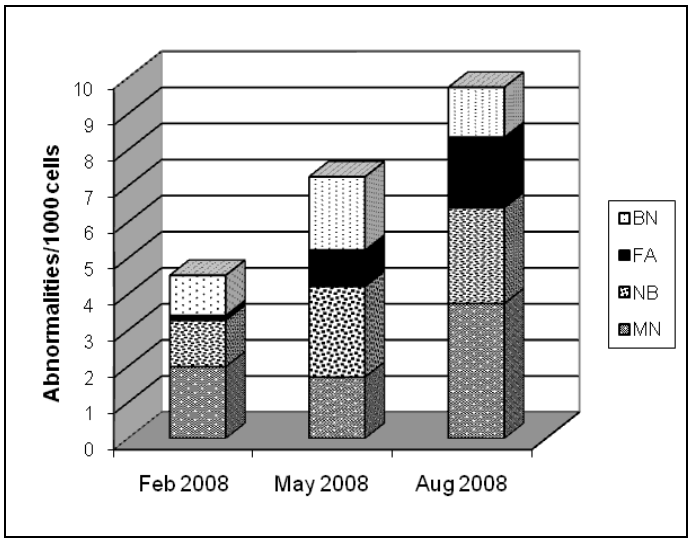


Fig. 3.2.3.5. Incidences of micronuclei (MN), nuclear buds (NB), fragmented-apoptotic (FA) and bi-nucleated (BN) cells in gills of mussel collected from the 1st station in 12 days (February 2008), in 3 months (May 2008) and in 6 months (August 2008) after the oil spill in the Būtingė oil terminal

3.2.4 Biomarker responses to different PAHs – laboratory experiment

The results of biochemical biomarkers are shown in Fig. 3.2.4.1, 3.2.4.2, 3.2.4.3, 3.2.4.4, 3.2.4.5, 3.2.4.6. Non-parametric Spearman correlation

coefficients (r-values) for significant correlations between biomarker responses in mussels and fluoranthene, pyrene or total PAHs concentration are shown in Table 3.2.4.1.

Mean activity of CAT in digestive glands of experimental mussels ranged between 20.9 (after the treatment with 12 μg l^{-1} of pyrene) and 39.2 μmol min^{-1} mg $protein^{-1}$ (after the treatment with 4 μg l^{-1} of pyrene) (Fig. 3.2.4.1). Rather high values were found in mussels from the control and control with solvent treatments (31.2 and 35.6 μmol min^{-1} mg $protein^{-1}$ respectively). Higher response was also found in the treatment with the mixture of all three PAHs (fluoranthene 12 μg l^{-1}, pyrene 12 μg l^{-1} and benzo(a)pyrene 4 μg l^{-1}). CAT response was increasing from fluoranthene 4 μg l^{-1} to fluoranthene 36 μg l^{-1}, but showed the highest values for pyrene 4 μg l^{-1} decreasing to pyrene 12 μg l^{-1} and pyrene 36 μg l^{-1}. Statistically significant positive correlation was observed between CAT activity and fluoranthene concentration in the single contaminant exposure. However, no correlations were detected between CAT activity and fluoranthene concentration together with control treatments or between CAT and fluoranthene in all experimental exposures. Negative correlations were detected between biomarker and pyrene in single and all experimental exposures (Table 3.2.4.1).

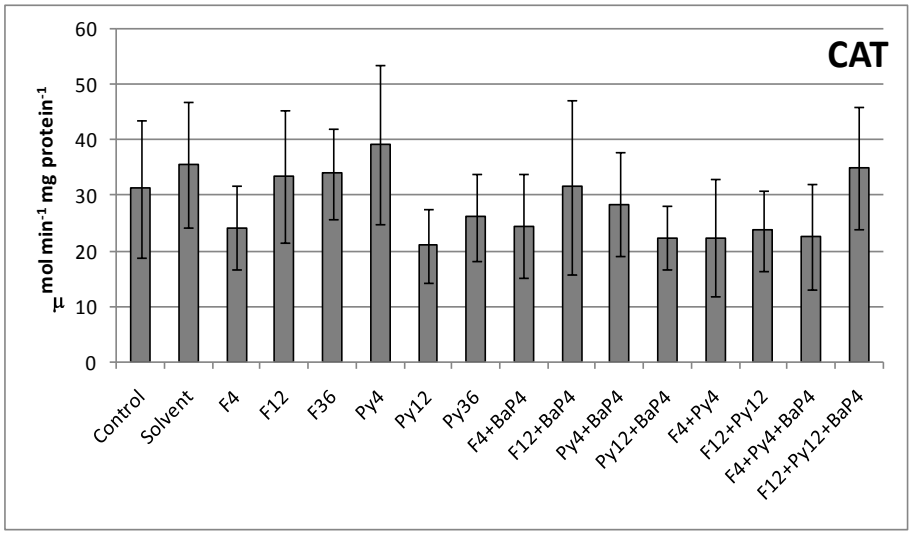


Fig. 3.2.4.1. Catalase (CAT) activity in mussel digestive gland after exposure to PAHs. Values are presented as mean ± SD, n = 20

The highest mean activity of SOD 25.3 units min^{-1} mg $protein^{-1}$ in digestive glands of mussels was found in control treatment, rather high activity of SOD 22.9 units min^{-1} mg $protein^{-1}$ was also found in control treatment with solvent (Fig. 3.2.4.2). Due to high SOD response in controls, negative correlation was detected between SOD activity and fluoranthene concentration in all treatments and in single fluoranthene exposure (0, 4, 12 and 36 mg l^{-1}) (Table 3.2.4.1). SOD showed high values of activity for pyrene 4 μg l^{-1} (24.5 units min^{-1} mg $protein^{-1}$), which decreased with the increase of pyrene concentration and showed similar activities for pyrene 12 μg l^{-1} and pyrene 36 μg l^{-1} (14.0 and 14.5 units min^{-1} mg $protein^{-1}$). Negative correlations were detected between biomarker and pyrene in single exposures of contaminant, nevertheless no consistent relationship was found with pyrene in all experimental treatments (Table 3.2.4.1).

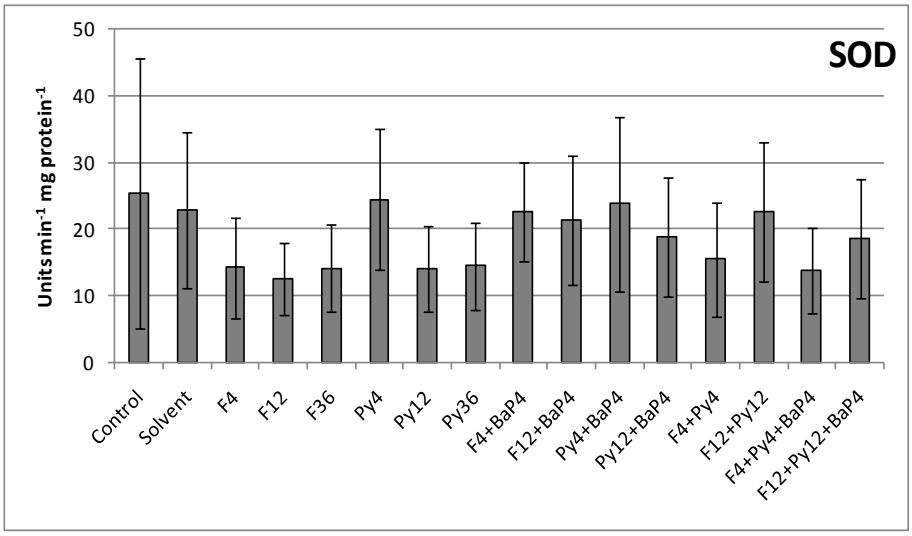


Fig. 3.2.4.2. Superoxide dismutase (SOD) activity in mussel digestive gland after exposure to PAHs. Values are presented as mean ± SD, n = 20

GST activities in control treatments were also high – 93.2 nmol min^{-1} mg $protein^{-1}$ in mussels from control and 90.7 nmol min^{-1} mg $protein^{-1}$ in control with acetone (Fig. 3.2.4.3). High GST activities (92.4 and 92.5 nmol min^{-1} mg $protein^{-1}$) were found in mussels treated with mixtures of pyrene (4 μg l^{-1} and 12 μg l^{-1}) and benzo(a)pyrene (4 μg l^{-1}). Although, GST response was slightly increasing from fluoranthene 4 μg l^{-1} to fluoranthene 36 μg l^{-1}, negative correlations were detected with fluoranthene in single contaminant and all experimental exposures due to high control values. Pyrene 12 μg l^{-1} had the highest GST activity (86.5 nmol min^{-1} mg $protein^{-1}$) from all single contaminant treatments with pyrene. Negative correlation was observed between GST activity and pyrene in single exposure with control (Table 3.2.4.1).

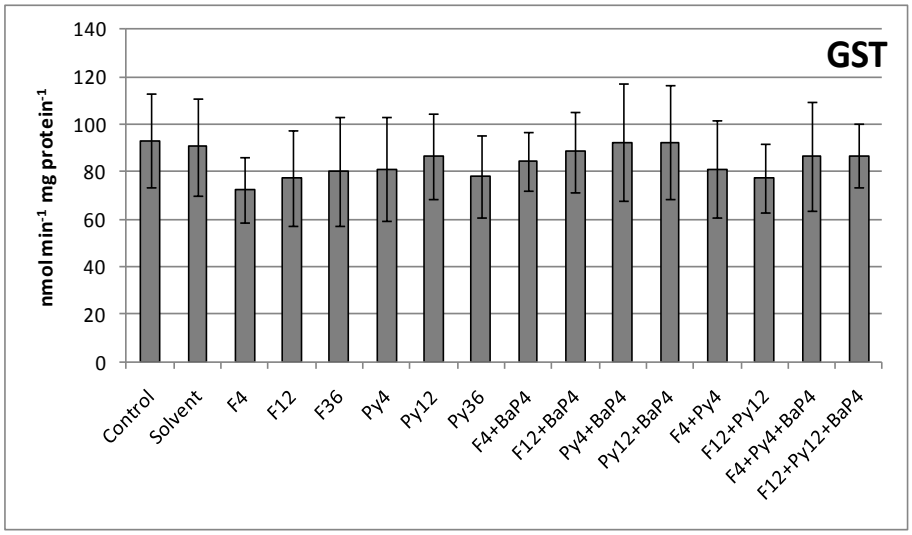


Fig. 3.2.4.3. Glutathione S-transferase (GST) activity in mussel digestive gland after exposure to PAHs. Values are presented as mean ± SD, n = 20

Mean activity of GR in digestive glands of mussels ranged between 14.1 (after the treatment with the mixture of fluoranthene 4 μg l^{-1}, pyrene 4 μg l^{-1} and benzo(a)pyrene 4 μg l^{-1}) and 19.6 μmol min^{-1} mg $protein^{-1}$ (after the treatment with the mixture of fluoranthene 12 μg l^{-1}, pyrene 12 μg l^{-1} and benzo(a)pyrene 4 μg l^{-1}) (Fig. 3.2.4.4). GR response was increasing from fluoranthene 4 μg l^{-1} to fluoranthene 36 μg l^{-1}, and rather high GR activity was found after the treatment with fluoranthene 36 μg l^{-1} (19.4 μmol min^{-1} mg $protein^{-1}$). Pyrene 4 μg l^{-1} had the highest GR activity (18.7 μmol min^{-1} mg $protein^{-1}$) from all single contaminant treatments with pyrene. However, no statistically significant correlations were detected between GR activity and fluoranthene or pyrene concentrations (Table 3.2.4.1).

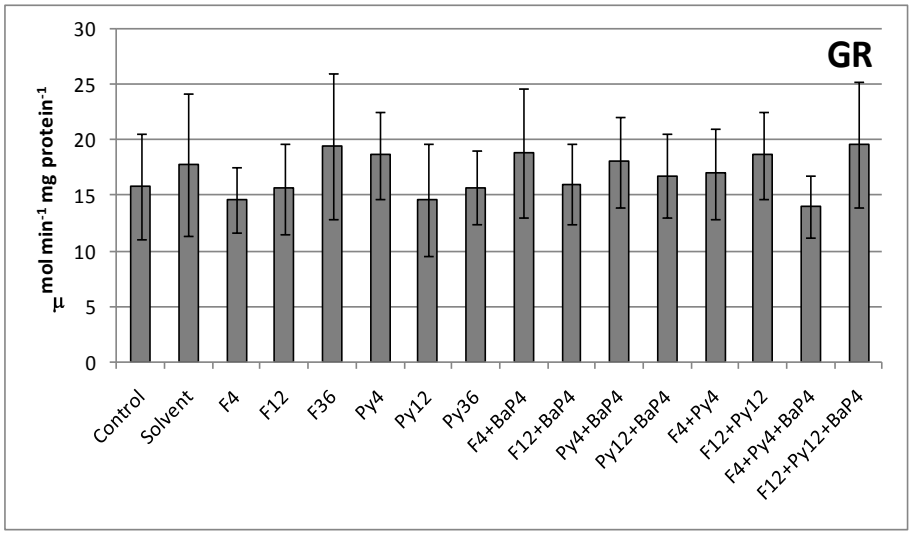


Fig. 3.2.4.4. Glutathione reductase (GR) activity in mussel digestive gland after exposure to PAHs. Values are presented as mean ± SD, n = 20

LPO in digestive gland of mussels had a high variability. There were individual mussels having extremely high LPO in all treatments. Mean values of LPO in digestive glands ranged between 123 (after the treatment with the mixture of pyrene 12 μg l^{-1} and benzo(a)pyrene 4 μg l^{-1}) and 206 nmol TBARS g wet $weight^{-1}$ (after the treatment with the mixture of fluoranthene 12 μg l^{-1} and pyrene 12 μg l^{-1}) (Fig. 3.2.4.5). Rather high mean LPO value was found after the exposure to fluoranthene 12 μg l^{-1} (204 nmol TBARS g wet $weight^{-1}$). Positive correlation was observed between LPO and fluoranthene in all experimental exposures. LPO showed the high value for pyrene 4 μg l^{-1} decreasing to pyrene 12 μg l^{-1} and pyrene 36 μg l^{-1}. However, no consistent relationship was observed between LPO and pyrene concentration (Table 3.2.4.1).

AChE activities measured from gills were highly variable showing both increased and decreased levels between different treatments compared to controls. Also individual variation within the treatments was high. Mean activity of AChE varied from 16.2 (after the treatment with 4 μg l^{-1} of pyrene) to 8.0 μmol min^{-1} mg $protein^{-1}$ (after the treatment with 36 μg l^{-1} of fluoranthene) (Fig. 3.2.4.6). Rather low AChE activities were found in mussels after the treatment with pyrene 12 μg l^{-1} and the mixture of fluoranthene

12 μg l^{-1} with benzo(a)pyrene (9.1 μmol min^{-1} mg $protein^{-1}$ in both treatments). Negative correlations were observed between AChE activity and fluoranthene concentration in the single contaminant and in all experimental exposures. Negative correlations were detected between biomarker and pyrene in single and all experimental exposures. AChE activity was not correlated with pyrene (Table 3.2.4.1).

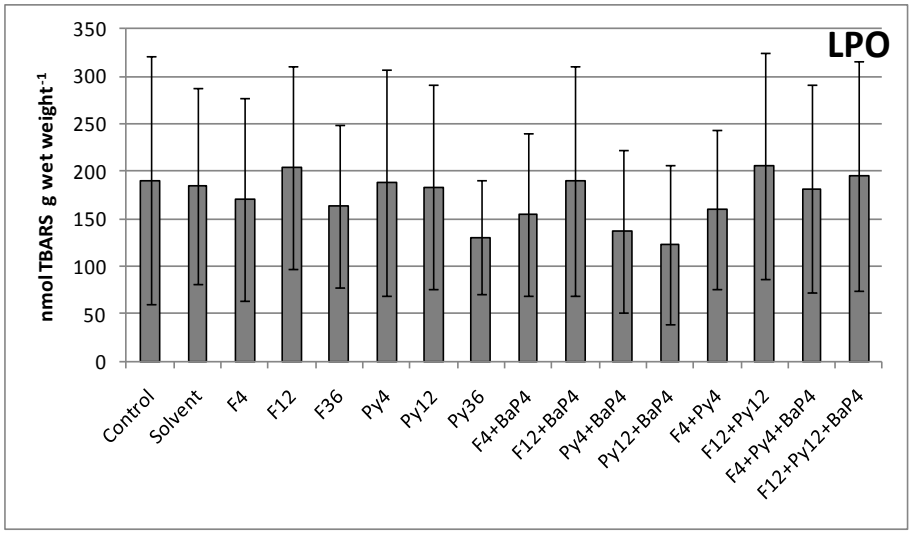


Fig. 3.2.4.5. Lipid peroxidation (LPO) in mussel digestive gland after exposure to PAHs. Values are presented as mean ± SD, n = 20

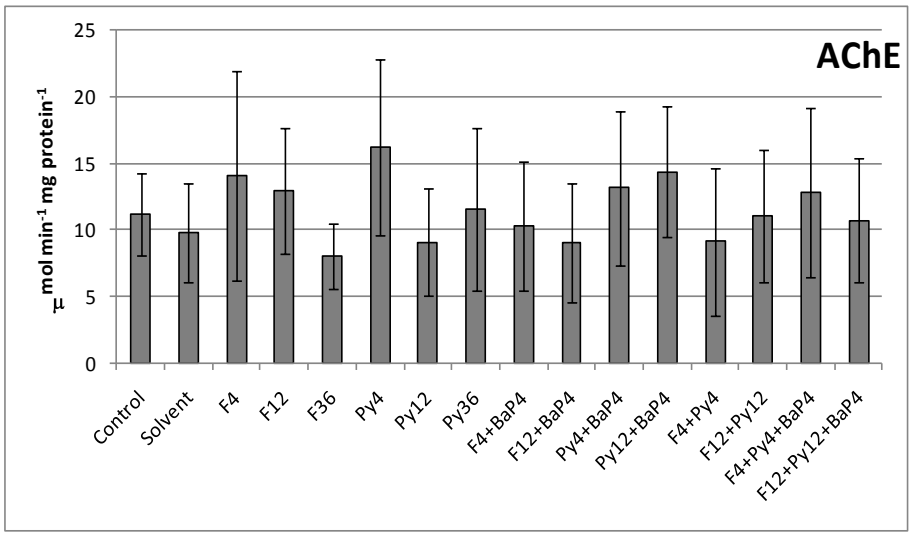


Fig. 3.2.4.6. Acetylcholinesterase (AChE) activity in mussel gills after exposure to PAHs. Values are presented as mean ± SD, n = 10

No consistent relationships were observed between activities of biomarkers (except SOD) and total concentration of PAHs (Table 3.2.4.1).

Table 3.2.4.1. Non-parametric Spearman correlation coefficients (r-values) for significant correlations between biomarker responses in mussels and fluoranthene, pyrene or total PAHs concentration ($^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, ns $P > 0.05$)

	CAT	SOD	GST	GR	LPO	AChE
Fluoranthene						
0 , 4, 12, 36 μg l^{-1}	ns	-0.358**	-0.317**	ns	ns	ns
4, 12, 36 μg l^{-1}	0.442***	ns	ns	ns	ns	-0.449*
in all treatments	ns	-0.151*	-0.153**	ns	0.124*	-0.032*
Pyrene						
0, 4, 12, 36 μg l^{-1}	-0.371***	-0.236*	-0.240*	ns	ns	ns
4, 12, 36 μg l^{-1}	-0.345*	-0.399**	ns	ns	ns	ns
in all treatments	-0.213***	ns	ns	ns	ns	ns
Total concentration of PAHs						
0, 4, 8, 12, 16, 24, 28, 36 μg l^{-1}	ns	-0.130*	ns	ns	ns	ns

The results of genotoxicity and cytotoxicity levels in the gill cells of experimental mussels are shown in Figs. 3.2.4.7; 3.2.4.8; 3.2.4.9; 3.2.4.10.

Analysis of micronuclei in gill cells of mussels exposed to PAHs and their mixtures showed the lowest frequency of MN in mussel groups treated with pyrene 4 μg l^{-1} (Py4) and 12 μg l^{-1} (Py12) and fluoranthene 4 μg l^{-1} (F4) concentrations (Fig. 3.2.4.7). Exceptionally high levels of MN were determined in mussels treated with model mixture of fluoranthene 12 μg l^{-1} and benzo(a)pyrene 4 μg l^{-1} and after exposure to fluoranthene 12 μg l^{-1} concentration. MN frequency in mussel gill cells reached 11.43 ‰ (MN/1000 cells; 4.7-fold increase via control) in F12 + BaP4 and 10.57 ‰ (MN/1000 cells) in F12 group of mussels. High MN induction (over 6 MN/1000 cells) was registered in mussels after exposure to mixtures of pyrene 4 and 12 μg l^{-1} with benzo(a)pyrene and the mixture of pyrene 4 μg l^{-1} and fluoranthene 4 μg l^{-1} (Fig. 3.2.4.7). Non-parametric Mann-Whitney U-test showed statistically significant increase of MN frequency compared to control group in mussels from five exposure groups – F12 + BaP4 ($P = 0.0021$), F12

(P = 0.0032), Py12 + BaP4 (P = 0.0290), F4 + Py4 (P = 0.0315), Py4 + BaP4 (P = 0.0392). Since all responses in control and solvent groups statistically were not different, the significance of induction of studied nuclear abnormalities were detected compared to control group of mussels.

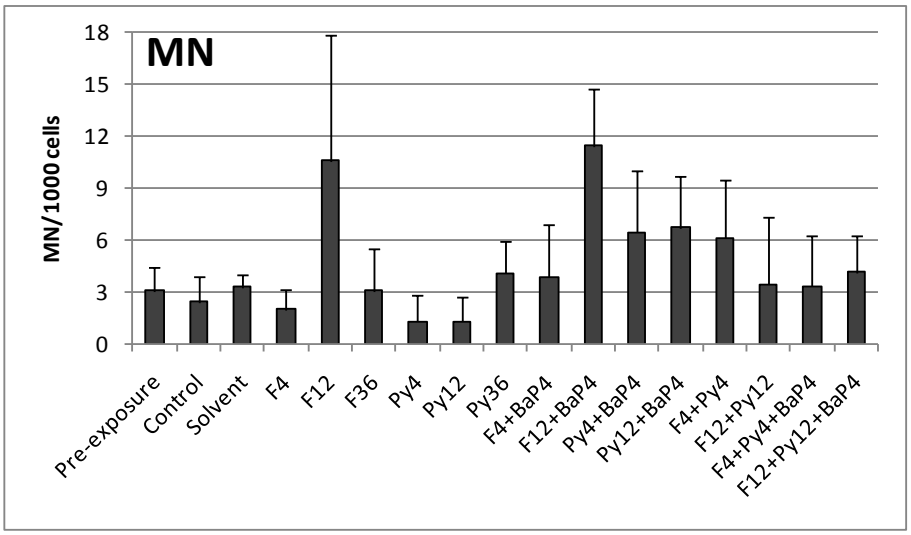


Fig. 3.2.4.7. Frequency of micronuclei (MN) in mussel gills after exposure to PAHs. Mean values with standard deviations are presented

In most exposure groups of mussels, the induction of nuclear buds was higher compared to the induction of micronuclei. The highest level of nuclear buds (14.14 NB/1000 cells; 6.6-fold increase compared to control) was observed in the same exposure group (F12 + BaP4), which showed the highest frequency of MN. In F4 + Py4 and Py12 + BaP4 groups NB levels exceeded 10 NB/1000 cells. High NB induction was determined in Py4 + BaP4 (9.24 ‰ – NB/1000 cells), F12 (8.71 ‰), F12 + Py12 + BaP4 (6.89 ‰). Comparatively high response (5.86 ‰) was observed in mussels after treatment with mixture of fluoranthene 12 μg l^{-1} and pyrene 12 μg l^{-1} (Fig. 3.2.4.8). The non-parametric Mann-Whitney U-test showed significant differences between control group of mussels and those from F12 + BaP4 (P = 0.0006), Py12 + BaP4 (P = 0.0006), F12 (P = 0.0126) and F12 + Py12 + BaP4 (P = 0.0260) exposure groups.

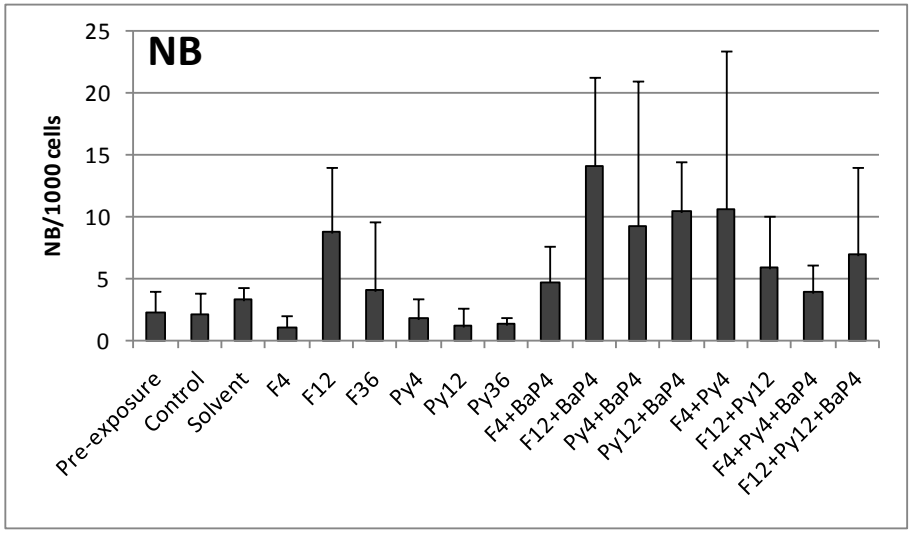


Fig. 3.2.4.8. Frequency of nuclear buds (NB) in mussels after exposure to PAHs. Mean values with standard deviations are presented

The highest frequency of fragmented-apoptotic (9.50 ‰; 7.5-fold increase via control) cells was observed in mussels from F4 + Py4 exposure group. High levels of FA cells were detected also in two other experimental groups treated with mixtures of 8 μg l^{-1} total concentration – F4 + BaP4 (6.14 ‰) and Py4 + BaP4 (5.14 ‰). In four groups (F4, Py12, Py36 and in pre-exposure), the response did not exceed the background level – one alteration per 1000 cell (Fig. 3.2.4.9). Statistically significant differences between control and F4 + Py4, F4 + BaP4, F12 + BaP4, Py12 + BaP4 exposure groups were calculated.

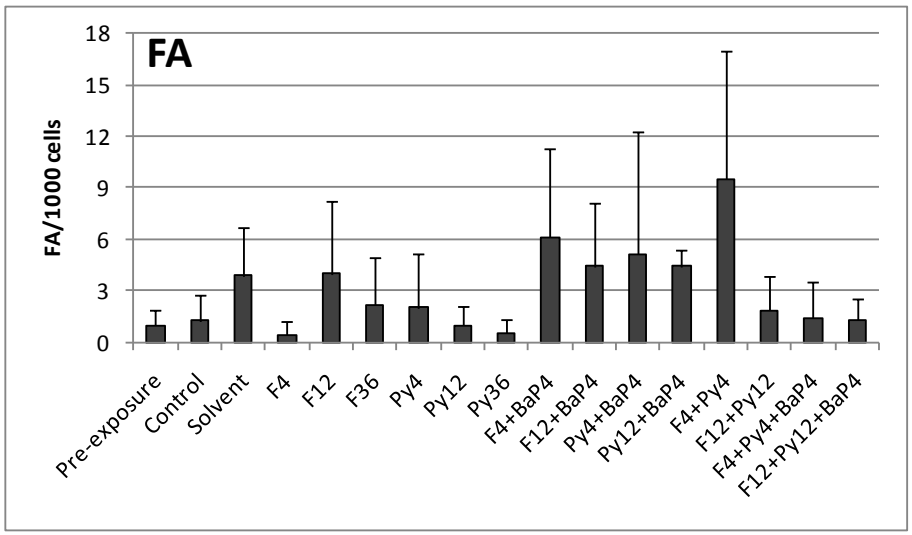


Fig. 3.2.4.9. Frequency of fragmented-apoptotic (FA) cells in mussel gills after exposure to PAHs. Mean values with standard deviations are presented

The results of the experimental treatment showed an extremely high induction of bi-nucleated cells in mussel gills. Total concentrations of 8 and 16 μg l^{-1} of PAHs in mixtures provoked the highest formation of bi-nucleated cells, which reached an extreme level of 19.91 BN/1000 cells (7.5-fold increase via control) in Py4 + BaP4 group of mussels. Extremely high responses were detected in mussels after exposure to F12 + Py12 μg l^{-1} (5.3-fold increase via control) and to fluoranthene 12 μg l^{-1} (Fig. 3.2.4.10). Statistically significant induction was found in nine exposure groups, which were treated solely with pyrene, or fluoranthene at 12 μg l^{-1}, or with model mixtures with total PAHs concentrations of 8, 12, 16 and 24 μg l^{-1}. There were no significant differences between control and Py4, F4, Py36, F36 and F12 + Py12 + BaP4 groups.

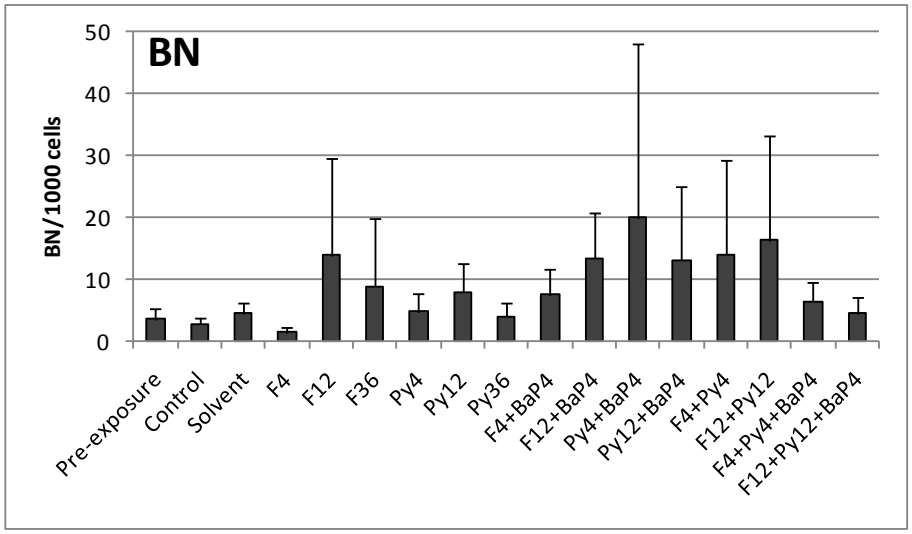


Fig. 3.2.4.10. Frequency of bi-nucleated (BN) cells in mussel gills after exposure to PAHs. Mean values with standard deviations are presented

Analysis of the both genotoxicity endpoints (MN + NB) together revealed low responses in mussel groups treated with pyrene 12 μg l^{-1} (Py12) and 4 μg l^{-1} (Py4) and fluoranthene 4 μg l^{-1} (F4) concentrations. Exceptionally high levels of genotoxicity responses were determined in mussels treated with mixture of flouranthene 12 μg l^{-1} and benzo(a)pyrene and after exposure to fluoranthene 12 μg l^{-1} concentration (Fig. 3.2.4.11).

Considering total cytotoxicity (FA + BN), the lowest responses were appeared in mussels after exposure to fluoranthene 4 μg l^{-1} (F4) and pyrene 36 μg l^{-1}, the highest values of the parameter were found in Py4 + BaP4 and F4 + Py4 groups (Fig. 3.2.4.11).

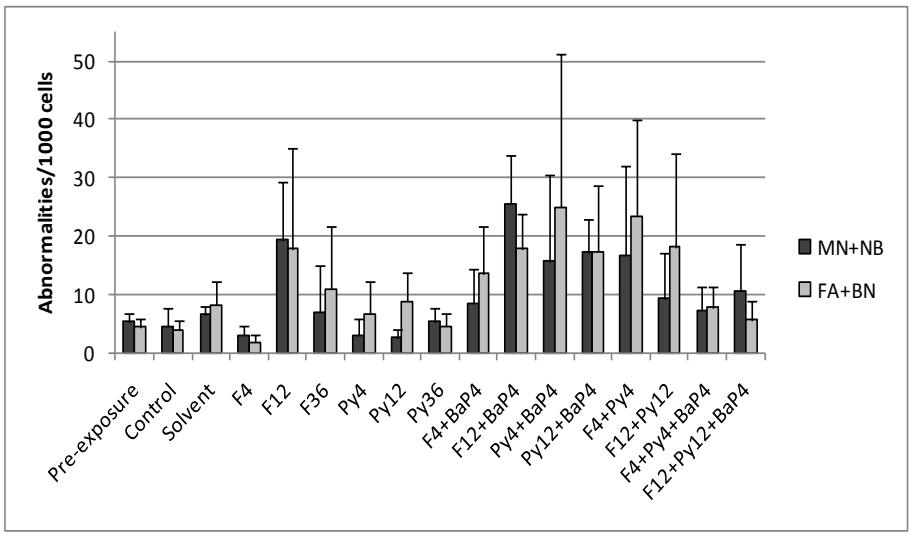


Fig. 3.2.4.11. Induction of genotoxicity (MN + NB) and cytotoxicity (FA + BN) in mussel gills after treatment with different PAHs and their mixtures

The influence of benzo(a)pyrene on biochemical biomarker responses and also on genotoxicity and cytotoxicity effects was evaluated by comparison of the responses in mussels exposed solely to fluoranthene or pyrene with those responses after exposure to their mixtures with benzo(a)pyrene.

Statistically significant increase of activities of SOD, GST and GR in mixtures of BaP with fluoranthene at 4 μg l^{-1} was revealed. However, at higher concentration of fluoranthene (12 μg l^{-1}) the addition of BaP caused the significant elevation of SOD activity only. The activity of CAT after the treatment with the mixture of pyrene 4 μg l^{-1} and BaP and LPO values after the treatment with the mixture of pyrene 12 μg l^{-1} and BaP showed a statistically significant decrease. Whereas activity of AChE have significantly increased after the treatment with pyrene 12 μg l^{-1} and BaP.

Statistically significant increase of genotoxicity in mixtures of BaP with fluoranthene at 4 μg l^{-1} was revealed. However after treatment with BaP and

pyrene mixtures, significantly elevated levels of genotoxicity were detected at 4 μg l^{-1} and 12 μg l^{-1}. Elevation of cytotoxicity caused by BaP was detected only after treatment of mussels with mixture of fluoranthene 4 μg l^{-1} and benzo(a)pyrene 4 μg l^{-1}.

Table 3.2.4.2. Significance levels (P values) in induction of biochemical biomarkers and nuclear abnormalities between mussels exposed to pyrene or fluoranthene and to their mixtures with benzo(a)pyrene (Mann-Whitney (Wilcoxon) U-test, P<0.05).

Parameters	**F4 + BaP4 via F4**	**Py4 + BaP4 via Py4**	**F12 + BaP4 via F12**	**Py12 + BaP4 via Py12**
CAT	0.0859	***0.0142***	0.9860	0.2689
SOD	***0.0010***	0.8149	***0.0048***	0.2406
GST	***0.0033***	0.0859	0.0962	0.4407
GR	***0.0003***	0.7251	0.0720	0.2616
LPO	0.8817	0.5162	0.4094	***0.0239***
AChE	0.3075	0.2413	0.1212	***0.0200***
MN	0,4004	***0,0120***	0.4042	***0.0055***
NB	***0,0031***	0,1548	0.0960	***0.0008***
FA	***0,0094***	0,8913	0.7425	***0.0024***
BN	***0,0020***	0,1198	0.3695	0.6511
MN+NB	***0,0102***	***0,0176***	0.1788	***0.0021***
FA+BN	***0,0026***	0,1244	0.3374	0.0949

To combine all the obtained data, responses of CAT, SOD, GST, GR, LPO, AChE and MN biomarkers were related to the concentration of fluoranthene, pyrene and the presence of benzo(a)pyrene using redundancy analysis (RDA). RDA biplot for square root transformed data on F, Py and BaP as explanatory variables and CAT, SOD, GST, GR, LPO, AChE and MN as response variables is presented (Fig. 3.2.4.13). The RDA showed that fluoranthene positively correlated with CAT, LPO and MN and showed a negative relation with AChE. The concentration of pyrene positively related with AChE but

showed a negative correlation with CAT, LPO and MN. The presence of BaP was positevily related with MN, GR, SOD and GST responses.

Three variables, the concentration of fluoranthene, pyrene and the presence of BaP, explained 27 % of the variation in biomarker responses. The two-dimensional approximation explained 91 % of this (64.8 % on axis 1 and 26.2 % on axis 2).

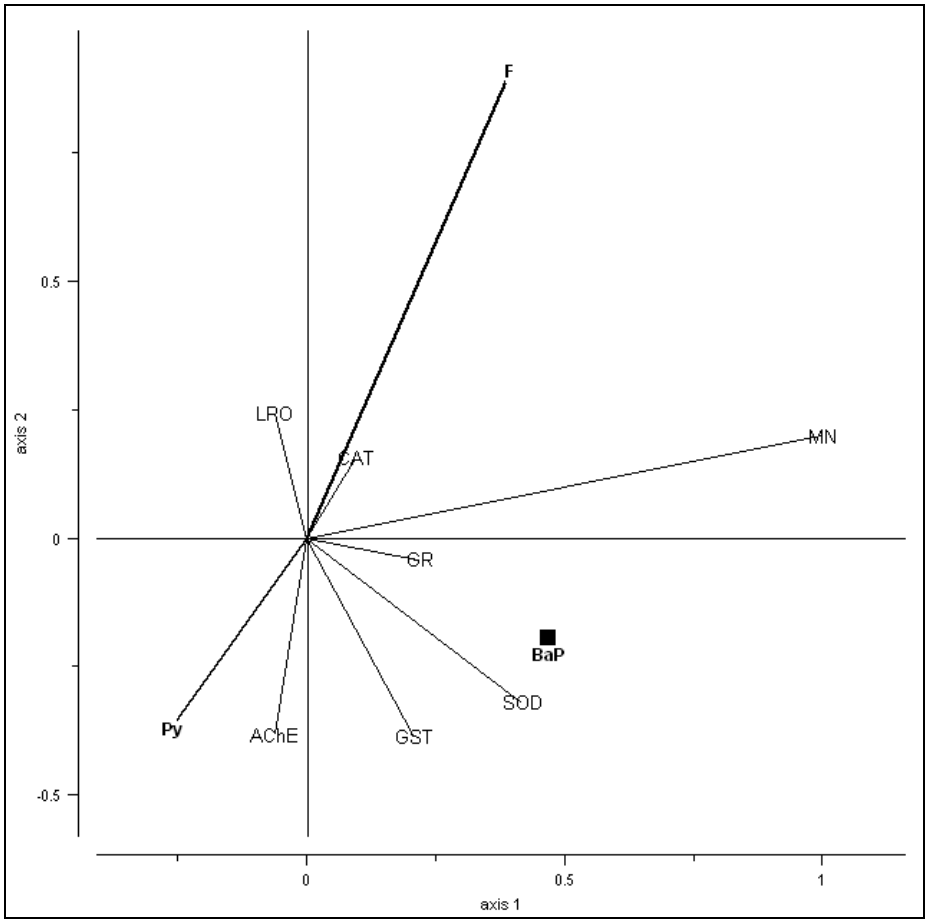


Fig. 3.2.4.13. RDA biplot for square root transformed data on fluoranthene (F), pyrene (Py) and benzo(a)pyrene (BaP) as explanatory variables and CAT, SOD, GST, GR, LPO, AChE and MN as response variables.

F statistic and P-values of conditional effects are shown in Table 3.2.4.3. All three explanatory variables have a statistically significant ($P < 0.05$) effect on biomarker responses.

Table 3.2.4.2. F statistic and P-values of conditional effects in RDA analysis

Variable	F-statistic	P-value
Benzo(a)pyrene	13.197	0.001
Fluoranthene	7.250	0.001
Pyrene	2.499	0.011

Integrated Biomarker Indexes (IBR) were calculated for the experimental mussels using biomarkers CAT, SOD, GST, GR, LPO, AChE and MN (Fig. 3.2.4.13). The value of IBR increased from 0.03 to 0.35 with the increase of fluoranthene concentration from 4 to 36 μg l^{-1}. High IBR values were calculated for treatments with mixtures of fluoranthene 4 and 12 μg l^{-1} with BaP (0.31 and 0.64 respectively). In general, the IBR was not high for pyrene and its' mixtures with BaP; it varied from 0.10 for pyrene 12 μg l^{-1} to 0.30 for Py4 + BaP4. High IBR was obtained for the treatment with the high concentration mixture of all three PAHs (F12 + Py12 + BaP4) – 0.44. The IBR for mussels in control treatments varied from 0.37 in control to 0.47 in the control with solvent.

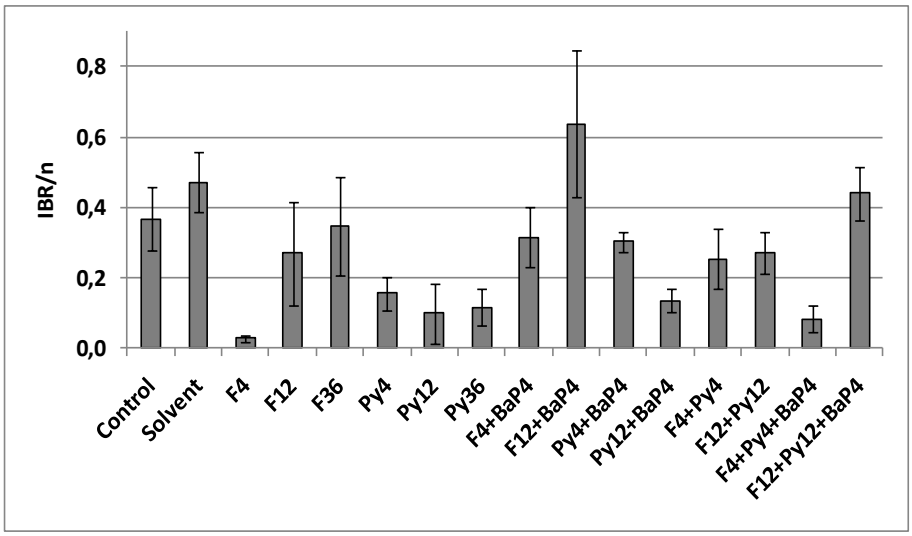


Fig. 3.2.4.13. Integrated Biomarker Index (IBR), calculated using biomarkers CAT, SOD, GST, GR, LPO, AChE and MN. IBR is given here as IBR/n, n being the number of biomarker parameters used in the calculation of the index.

3.3 Results of CHASE

The results of chemical status of Lithuanian waters from the CHASE tool is shown in Table 3.3.1.

Table 3.3.1. The hazardous substances status and final confidence rating of Lithuanian waters from the integrated assessment tool CHASE

	Final chemical status	**Final confidence rating**
Northern coastal waters	Moderate	Class I
Southern coastal waters (except dumping area)	Moderate	Class II
Dumping site	Moderate	Class I
Plume of the Lagoon	Moderate	Class I
Klaipėda Strait	Poor	Class III
Curonian Lagoon	Good	Class I

4. DISCUSSION

4.1. Contaminants in water, sediments and biota in the Lithuanian part of the Baltic Sea

National environmental monitoring data have shown that there were apparent trends in concentrations of some contaminants within the Lithuanian part of the Baltic Sea. For example, the decreasing trend of mercury was detected in all compartments of the aquatic environment: in the water, sediments and biota. Although, according to HELCOM (2007b) data, in 2004 waterborne inputs of mercury from Lithuania to the Baltic Sea made 13 % – it was the third place after Estonia (61 %) and Poland (18 %). There is also an increase (about 20 times) in atmospheric emissions of mercury in Lithuania: from 0.018 tones in 1990 to 0.418 tones in 2006 (Bartnicki et al., 2008). However, there are no clear trends in total load of mercury in the whole Baltic Sea. A sudden increase in waterborne load occurred during 1999 and 2000. It is mentioned in the HELCOM report, that the observed increase in waterborne loads in 2000 can be in part due to improved monitoring techniques adopted by the HELCOM monitoring program (HELCOM, 2007b). The same to some extent could be applied to the national monitoring data. The purity of reagents has become higher; a strong attention nowadays is paid to the quality control of the data.

The decreasing trend was observed for DDT pesticide and its metabolites DDD and DDE. During the last few years the values of DDT in water and sediments were mostly under the limit of quantification. But these compounds can be still detected in biota. Although the use of DDT has been banned it still persists in the food web of the Baltic Sea. The example of this was the high concentration of DDT in dead seal found on the Lithuanian beach in 2003. Summed concentrations of DDT and it’s metabolites in different biota species are shown in Fig. 4.1.1.

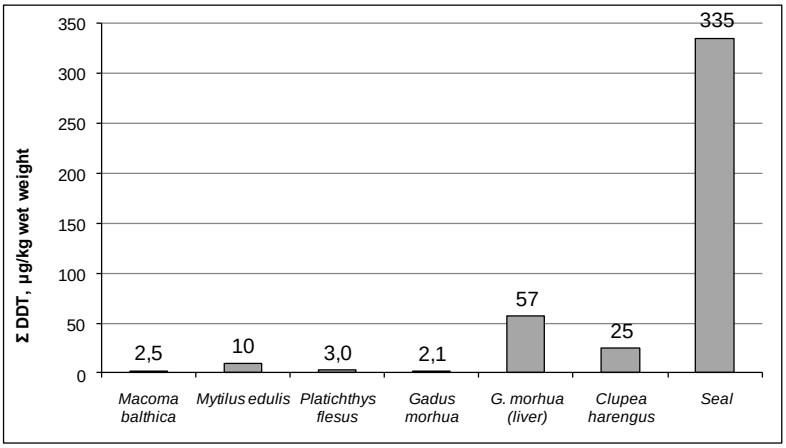


Fig. 4.1.1. Summed concentration of DDT in different Baltic Sea biota species in 2003

Different results were obtained from the analysis of the long-term data on oil hydrocarbons. The concentrations of oil in water have shown increasing trends in some areas of the Lithuanian part of the Baltic Sea.

Polycyclic aromatic hydrocarbons (PAHs) concentration is a suitable monitoring parameter for the determination of oil pollution in aquatic environment. PAHs have been added to the monitoring program already in 2006, but the amount of stations is not sufficient. Indices have shown that PAHs in Lithuanian part of the Baltic Sea are of both pyrolythic and petrogenic origin.

There are no long term data on metal concentrations in aquatic environment, as they were added to the monitoring program only in 2003. However, the existing data already allows to evaluate the pollution of Lithuanian waters by metals. Areas that contaminated by metals were the dredged sediments dumping site, Klaipėda harbuor and Curonian Lagoon area close to Nida. It is known that concentrations of metals in sediments depend on the grain size composition. Normalization procedure can be used to correct both background and contaminant concentrations for the influence of the natural variability in sediment granulometry and mineralogical composition mediated by the ambient energy of the aquatic system (Kersten, Smedes, 2002). Unfortunately, the normalization of sediments is not currently used for the evaluation of metal data in national monitoring programme of Lithuania. According to Kersten and

Smedes (2002), there are two main normalization methods: granulometric and geochemical normalization. Clay fractions of sediments have high contaminant affinity and binding capacity. The clay content in sediments (< 2 μm clay fraction) can be considered as a primary normalizer. The geochemical normalization is based on the assumption that there are components in sediment which may serve as hosts for contaminants with high sorptive capacity, such as Al, Si, Fe, Mn, sulfide minerals, organic matter, total organic carbon (TOC). Normalization to iron has been the best method in case of arsenic (see section 4.3). In HELCOM countries the most popular procedure is normalization to TOC. The granulometric composition of sediments in the abovr mentioned areas was dominated by the clay fraction. That partly explains higher contaminants concentrations observed there.

In 2005–2007 during the project carried out by Lithuanian EPA and other institutions "Screening of Hazardous Substances in the aquatic environment of Lithuania" the selected WFD priority substances and some other pollutants in wastewater, sewage sludge and the receiving environment (surface water and sediments) were investigated. However, only few samples were taken in the Klaipėda Strait. The findings of the project were that the most problematic "new-generation" substances for Lithuanian aquatic environment are phthalates and organotin compounds. These substances were detected in wastewater, sewage sludge as well as in the receiving environment and often exceeded the applied limits. Thus, high concentrations of organotin compounds were detected in Klaipėda harbour during previous studies (Dudutytė et al., 2007). However, samples taken in 2008 from the several stations in the Baltic Sea did not reveal high concentrations of organotins. The monitoring of these compounds in Klaipėda harbour should be performed to evaluate the pollution of the harbour area by these contaminants.

Investigations of dioxins and furans showed that the summed concentrations of these pollutants in the Baltic Sea and Curonian Lagoon ranged from the concentrations under the limit of quantification to 29 ng kg^{-1} d. w.; according

to TEQ – from 0 to 0.44 ng kg^{-1} d. w. For the comparison, the summed concentrations of dioxins and furans in the coastal areas of Denmark, Sweden, Finland and Germany varied from 500 to 1500 ng kg^{-1} d. w.; according to TEQ – from 10 to 30 ng kg^{-1} d. w. (HELCOM, 2004b). This survey showed that sediments at the sampled stations were rather unpolluted by dioxins and furans compared to other sites in the Baltic Sea. But the current aim is to reach concentrations close to zero, as these anthropogenically generated contaminants do not occure naturally in the environment.

There are still no Environmental Quality Standards (EQS) existing for contaminants in sediments. The existing document for dredged sediments "Sediment dredging in sea and sea-port areas and dredged sediment treatment rules" (Official gazette, 2008, No. 139-5521) gives only a rough estimation of sediment pollution and served for another purpose. The EQS for sediments and for biota are highly needed.

Biota is the most important component of the aquatic environment. The concentrations of heavy metals found in mussels from the Lithuanian part of the Baltic Sea have been compared with the investigations from the other studies. Comparing the concentration of heavy metals in *M. edulis* the similar concentrations were found as in Latvian Nida–Bernati area in 2007: zinc – 10.3, lead – 0.098, cadmium – 0.36, copper – 1.04 mg kg^{-1} w. w. (Poikane et al., 2007). The determination of heavy metal concentrations in *M. balthica* has been made by researchrs of the Finnish Institute of Marine Research in November 2005 (FIMR, 2007). Mollusks were taken from the Lithuanian national monitoring station 65. The concentrations of metals were: zinc – 76.5, lead – 0.50, cadmium – 0.101, copper – 27.5 mg kg^{-1} w. w. According to these results, the concentrations of metals at the station 65 are higher compared to the concentrations in mollusks from the sandy sediments of the coastal area near the Curonian spit (FIMR, 2007).

During the HELCOM project “Screening of the selected hazardous substances in the eastern Baltic marine environment” measurements of “new-generation” substances in the Lithuanian area of the Baltic Sea were performed. The

concentrations of six studied substances or substance classes were above LOQ, i.e. organic tin compounds, PBDEs, PFAS, nonylphenol, chlorinated paraffins and endosulfan (endosulfan sulphate). Substances that were detected in fish samples from both sampling sites were: TBT, DPhT, PBDEs (BDE 47, BDE 99, BDE 100, BDE 154), PFHxS, PFOS, PFNA, Bisphenol A, chlorinated paraffins (SCCP), and endosulfan (endosulfan sulphate). It can be noted that herring mostly accumulated TBT and brominated flame retardants, flounder – perfluorinated substances. DPhT, Bisphenol A, SCCP and endosulfan sulphate were found in both fish species. Notably, for PBDEs, the highest concentrations between Estonian, Latvian, Polish and Swedish sampling sites were found in Gulf of Gdansk and outside the Lithuanian coast.

There is a lack of data on the "new-generation" substances in Lithuania. New studies are needed to evaluate the sources of contamination by these substances and find appropriate measures to control the pollution of the environment by hazardous substances. For example, there are no information at all on pharmaceuticals in the marine environment of Lithuania.

4.2. Sources of pollutants in Lithuanian waters of the Baltic Sea

Pollution sources in the Baltic Sea can be divided as point and diffuse sources. Discharges from point sources mostly include municipal and industrial effluents. Lithuania reported 25 point pollution sources in 2000. Total wastewater discharge into the Baltic Sea from Lithuanian catchment area was $47 \cdot 10^6$ m^3 $area^{-1}$ in 2000 (HELCOM, 2004a). Diffuse sources are defined as any sources of pollutants not accounted for as point sources. Small, dispersed point source discharges are considered as diffuse sources. Riverine load consists of discharges and losses from different sources within a river's catchment area, and include discharges from industrial plants, municipal wastewater treatment plants, scattered dwellings, discharges from rainwater, fish farms and losses from agriculture and managed forests, as well as natural background losses and atmospheric deposition on inland surface waters (HELCOM, 2004a). According to modelling results (using MSC-E Eulerian

Heavy Metal transport model MSCE-HM (Bartnicki et al., 2008 according to Travnikov, Ilyin, 2005) atmospheric deposition of pollutants in Lithuanian waters occur not only from Lithuania itself but also from other countries like Latvia, Poland, Russia, Germany, Denmark and Sweden. In 2006, the total anthropogenic emission of lead in Lithuania decreased about 8 times from 47 tones in 1990 to 6 tones in 2006; the 10 fold decrease is observed for cadmium – from 3.8 to 0.367 tones. The 2 fold increase is also observed for atmospheric emissions of PCDD/F from 5.6 g TEQ/year in 90ies to 11 g TEQ/year in 2006 (Bartnicki et al., 2008).

Klaipėda harbour is the most important source of pollution. According to the monitoring data, high concentrations of different contaminants can be detected in both sediments and water. Together with dredged sediments pollutants can enter the environment of the Baltic Sea. An evidence of transferring contaminants from the harbour to the sea is distribution of TBT compounds in sediments of the harbour and dumping site (Fig. 3.1.5.1). TBT compounds were detected only at harbour and dumping site stations, but not at the other stations of the coastal area. One more evidence is the concentration of heavy metals in sediments. The concentration of metals at the dumping site usually was not high and did not exceed the I or II pollution categories. But comparing with the data in the neighboring areas, the concentrations of metals in the dumping site is higher. However, the distribution of dioxins and furans in sediments of the same stations (Fig. 3.1.4.3.1) do not point at the harbour as the source of contamination – harbour stations have even lower concentration of contaminants then dumping site station; similar concentration was also found at the open sea station (65). Such distribution of dioxins and furans could point that the pathways of these contaminants to the marine environment could be through the atmospheric deposition.

The analysis of data on oil hydrocarbons and PAHs has indicated that area near Būtingė oil terminal polluted by oil products. Accidental oil spill events have particularly strong influence on the contamination of environment. More about the Būtingė oil terminal will be discussed below in section 4.6.

D-6 oil platform in Russia is also a potential hazard of oil pollution. Modelling results showed, that if an accident has occured at the platform, the oil under certain meteorological and hydrological conditions would reach Lithuanian coast (FIMR, 2007). The evaluation of the environment state of the area adjacent to the oil platform didn't reveal any higher contaminants concentrations, although environmental genotoxicity was higher in the Lithuanian area adjacent to the D-6 oil platform (Baršienė et al., unpublished data).

4.3 Chemical munitions dumping site

Arsenic concentrations data from sediments, even from the weapons dumpsite region, were low relative to other investigations of sediments in the Baltic and North Seas (Table 4.3.1). For comparison, other studies have reported background values of total arsenic in sediments of the Baltic Sea ranging from 4 mg kg^{-1} in the Gulf of Finland (Vallius, Lehto, 1998) to 320 mg kg^{-1} in the Bothnian Bay (Leivuori, 1998). The concentration of arsenic was about 15 mg kg^{-1} in Baltic Proper sediments (Borg, Jonsson, 1996) and in the surface sediments of Bornholm Basin (Kunzendorf, Vallius, 2004). However, the latter authors found lower values of about 9 mg kg^{-1} in the Gotland and North Central Basins of the Baltic Sea. In the Western North Sea, minimum concentrations of arsenic in the sediments reported by Whalley et al. (1999) were less then 0.15 mg kg^{-1} and reached 135 mg kg^{-1} in the coastal area.

With respect to chemical munitions dumpsites, some much higher concentrations in sediments have been reported whereby total arsenic varied from 9 to 480 mg kg^{-1}. The highest concentration was found in the samples from the dumpsite in Skagerrak; high concentrations of Clark I, triphenylarsine and bis(diphenylarsine)oxide were found in the same samples (Tørnes et al., 2002). In 1992, 10 mg kg^{-1} of Clark I was found in the samples from the Bornholm dumpsite; this site is characterized by high dispersion and sharp anomalies for total arsenic, which range from 18 to 210 mg kg^{-1} (HELCOM CHEMU, 1994; Paka, Spiridonov, 2002). High arsenic levels of up to 200 mg

kg^{-1} were found in the samples from the Skagerrak several centimetres below the bottom/water interface (Paka, Spiridonov, 2002).

Table 4.3.1. Investigations of arsenic in the Baltic and North Sea sediments

Year	Area	As concentration (mg kg^{-1})	Reference
		Non-dumpsites	
1990-1995	Western North Sea	<0.15-135	(Whalley et al., 1999)
1996-1997	North Sea (Dutch coast)	11-29	(De Boer et al., 2001)
2002	Skagerrak	43-49	(Tørnes et al., 2002)
1996	Baltic Proper	7-23	(Borg, Jonsson,1996)
1996-1997	Gotland	26*	(Stepanets et al., 2000)
1998	Gulf of Finland	4-27	(Vallius, Lehto,1998)
1998	Gulf of Finland	8-28	(Leivuori, 1998)
1998	Bothnian Sea	46-85	(Leivuori, 1998)
1998	Bothnian Bay	167-320	(Leivuori, 1998)
Chemical munitions dumpsites			
1997-2001	Skagerrak	9-200 (average 25)	(Paka, Spiridonov, 2002)
2002	Skagerrak	75-480	(Tørnes et al., 2002)
1992	Bornholm	185 and 210	(HELCOM CHEMU, 1994)
1992	Bornholm	<100	(HELCOM CHEMU, 1994)
1997-2001	Bornholm	18-150 (average 25)	(Paka, Spiridonov, 2002)
1996-1997	Gotland (Liepaja dumpsite)	100*	(Stepanets et al., 2000)
1997-2002	Gotland (Liepaja dumpsite)	18-28 (average 24)	(Paka, Spiridonov, 2002)

* 10-11 cm layer of sediments

At the Gotland (Liepaja) dumpsite, deeper sediments horizons (10-11 cm) had elevated arsenic contents, with concentrations up to 100 mg kg^{-1} (Stepanets et al., 2000). Such pollution was not evident in the upper layers of sediments. In contrast, Paka and Spiridonov (2002) concluded that arsenic distribution in the surface sediments of the Gotland dumpsite is characterised by low dispersion and the absence of high levels, reporting arsenic concentrations from 18 to 28 mg kg^{-1}. The maximum arsenic value obtained in the Lithuanian sector as reported here, namely 19 mg kg^{-1}, falls within this interval.

An analysis of sediment samples taken from the Lithuanian economic zone of the Baltic Sea showed that there were higher arsenic concentrations near the chemical munitions dumpsite (average 9.7 mg kg^{-1}) compared to other coastal locations (2.1 mg kg^{-1}). Normalization of results to iron showed slightly elevated residual arsenic concentrations near the dumpsite. However, arsenic concentrations data, even from the dumpsite region, were low relative to other investigations in the Baltic and North Seas. This preliminary study focused only on arsenic. For a definitive assessment of the possible leakage of poisonous contaminants, more research is needed, such as long-term monitoring of the area and determination of various individual toxic substances, including arsenic-containing munitions.

4.4 Biochemical biomarkers in Lithuanian coastal area

There were only few studies of biomarkers in mussels in the Lithuanian coastal area. Data from previous studies together with this study data are shown in Table 4.4.1 for *Mytilus edulis* and in Table 4.4.2 for *Macoma balthica*. AChE activity in *M. edulis* mussels in 2008 was about 6 times lower then it was in 2002 (Table 4.4.1). AChE activity in *M. balthica* at the station 7 (N-9) was about 2 times higher in August 2008. However, at the station 65 in was lower in August 2008, then in November 2005. The areas of these stations differ in important physical properties such as water depth, near-bottom temperature, oxygen conditions and also in sediment type.

Table 4.4.1. Acetylcholinesterase (AChE) activity in *Mytilus edulis* from the Palanga-Būtingė area

Biomarker, units	June 2002*	September 2002*	August 2008**
AchE, nmol min^{-1} mg^{-1} protein	36.2 – 39.3	28.5 – 33.9	4.9 – 7.0

* - data according to Baršienė et al. (2006);
** - this study.

Table 4.4.2. Acetylcholinesterase (AChE), glutathione-S-transferase (GST), catalase (CAT) and superoxide dismutases (SOD) activities in *Macoma balthica* collected from the stations 65 and 7 (N-9)

Biomarker, units	Station	November, 2005*	August, 2008**
AChE, nmol min^{-1} mg^{-1} protein	65	~ 22	13.3 ± 6.4
	7 (N-9)	26.4 ± 2,8	69.6 ± 30.1
GST, nmol min^{-1} mg^{-1} protein	65	900 ± 60	718 ± 193
	7 (N-9)	~ 1250	1034 ± 82
CAT, μmol min^{-1} mg^{-1} protein	65	~ 180	89.4 ± 15.1
	7 (N-9)	216 ± 27	114 ± 15
SOD, units min^{-1} mg^{-1} protein	65	33.7 ± 1,8	4.2 ± 2.0
	7 (N-9)	~ 25	11.1 ± 1.8

* - data according to FIMR (2007) (approx. values are taken from figures);
** - this study.

GST values in both studies at both stations were very similar. CAT activity was about 2 times lower in August 2008. SOD activity was 8 times lower at the station 65 and 2 times lower at the station 7 (N-9). The differences between two studies can be because of the different environmental conditions at the stations. It is known that such factors as temperature, Seccki depth, near bottom oxygen saturation have an effect on biomarkers activities and responses (Leiniö, Lehtonen, 2005). From the other hand there are still no standardised procedures of biochemical biomarker analysis. Most methods are still under development. Thus there is a need for standardized analytical methods to make results more comparable.

This study presents the first attempt in Lithuania to analyze AChE, CAT, GST and SOD in organisms inhabiting the Curonian Lagoon (*D. polymorpha*) and Klaipėda Strait (*N. diversicolor*). *D. polymorpha* is widely spread in the central part of the Curonian Lagoon and could be a useful organism for biomarker monitoring. *N. diversicolor* could be a useful biomarker in the Klaipėda harbour and coastal waters of the Lithuanian part of the Baltic Sea. The use of *N. diversicolor* gives an opportunity to investigate the same species in all Lithuanian coastal areas, as there are different species of bivalves in the

northern and southern parts of the coastal waters which can't be really compared.

There is definitely only a few data on biochemical biomarkers in aquatic organisms from the Lithuanian coastal area of the Baltic Sea (Baršienė et al., 2006). To investigate the effect of pollution the screening study on biomarkers has to be done, using different species, taking samples at different environmental conditions (e.g. in different seasons). Having reliable background data, the interpretation of accidental spills of contaminants would be more efficient and reasonable.

4.5 Genotoxicity and cytotoxicity biomarkers in Lithuanian waters

The micronucleus test (MN) is one of the most popular and promising approaches in environmental genotoxicity studies; it has served as an index of cytogenetic damage for over 30 years. The MN test has been used successfully in marine mollusks as a biological indicator of pollution *in situ* (Brunetti et al., 1992; Burgeot et al., 1995; Bolognesi et al., 1996, 2004, 2006; Izquierdo et al., 2003; Baršienė et al., 2004, 2005, 2006a, 2006c; Magni et al., 2006; Schiedek et al., 2006). Micronuclei and other nuclear abnormalities were analysed in two indigenous bivalve mollusk species *Mytilus edulis* and *Macoma balthica* inhabiting different sites in the Lithuanian zone of the Baltic Sea. The study revealed the highest level of micronuclei in *M. balthica* inhabiting the offshore station 65. A comparatively high frequency of micronuclei was found in *M. edulis* from station 1B in August 2006 and in *M. balthica* from station N-4. The reference level of micronuclei incidences was observed in *M. balthica* inhabiting the comparatively uncontaminated coastal station N-8. Analysis of other nuclear abnormalities revealed no significant differences among the bivalves from the study sites. Marine traffic is the main source of pollution at the offshore station 65. Long-term data on total oil hydrocarbons and heavy metal contamination disclosed elevated oil hydrocarbon concentrations in water in 2003. Especially high total oil hydrocarbon concentrations were observed in autumn 2005. In sediments from station 65, elevated

concentrations of Cu, Pb and Zn were detected in 2006. The concentration of TBT reached 6.0 μg kg^{-1} dry weight, the concentration of total PAHs being 120 μg kg^{-1} dry weight (FIMR, 2007). In 2006, DDT concentrations in *M. balthica* tissues were lower than the average level. The study station 1B is situated near the Būtingė oil terminal area, where increased levels of total oil hydrocarbons have been observed episodically. Elevated concentrations of Cd were detected in mussels in the present environmental genotoxicity study, but DDT concentrations were lower than the average level.

The station N-4 is located close to the Russian D-6 oil platform. A higher frequency of micronuclei has been detected earlier in mussels taken close from the oil terminal and marine port zones in the Baltic Sea (Baršienė, Baršytė Lovejoy, 2000; Baršienė, 2002). A number of studies have reported an increase of environmental genotoxicity in zones affected by accidental oil spills (Parry et al., 1997; Harvey et al., 1999; Pietrapiana et al., 2002; Baršienė et al., 2004; 2006a, 2006b; Laffon et al., 2006; Bolognesi et al., 2006). Higher incidences of micronuclei and nuclear abnormalities were found in fish from areas affected by effluents from a shale processing plant (da Silva Souza, Fontanetti, 2006). Significantly increased levels of micronuclei and other nuclear abnormalities were observed in mussel gills after exposure to 0.5 ppm of Statfjord B (the North Sea) crude oil and to 0.5 ppm of spiked oil (Baršienė, Andreikėnaitė, 2007).

Hazardous effects of PAHs arise as a result of oxidative biotransformation producing highly DNA-reactive metabolites which have been recognized as carcinogenic, mutagenic and cytotoxic compounds (Torres-Bugarin et al., 1998; Woodhead et al., 1999). The genotoxic potency of ten polycyclic aromatic hydrocarbons (anthracene, 7,12-dimethylbenz[*a*]anthracene, benz[*a*]anthracene, dibenz[*a*,*h*]anthracene, dibenz[*a*,*c*]anthracene, 3-methylcholanthrene, benzo[*a*]pyrene, benzo[*e*]pyrene, chrysene and pyrene) has been demonstrated (Nishikawa et al., 2005). Cells with micronuclei were found to increase in the gills or hemolymph of marine mollusks treated with

benzo(a)pyrene (Burgeot et al., 1995; Venier et al., 1997; Siu et al., 2004), and with dimethylbenz(a)antracene (Bolognesi et al., 1996).

Analysis of heavy metals in bivalve tissues showed increased levels of Cd, Cr, Zn, Pb and Cu in bivalves collected from the study sites of the Lithuanian zone of the Baltic Sea. MN elevation has been described in eel (*Anguilla anguilla*) after treatment with cadmium and mercury (Sanchez-Galan et al., 2001; Teles et al., 2005), in *Carassius auratus gibelio* treated with chromium (Al-Sabti, Härdig, 1990). A statistically significant increase in micronuclei was observed in rainbow trout *Oncorhynchus mykiss* after exposure to a model mixture composed of Cu, Zn, Pb, Ni, Cr and Mn (Andreikėnaitė et al., 2007). The genotoxicity of chromium, cadmium and copper has been detected in different tissues of common carp (*Cyprinus carpio*), Prussian carp (*Carassius gibelio*) and Peppered corydoras (*Corydoras paleatus*), and the MN test was proposed as a suitable approach for the screening of genotoxic compounds in aquatic ecosystems *in situ* (Cavas et al., 2005).

There is a growing concern over the presence of genotoxic compounds in the marine environment and a rising need to elaborate appropriate methods for the assessment of genotoxicity in indigenous marine organisms. Nevertheless, there is a shortage in our knowledge on short- and long-term implications of mutagenic complex mixtures in wild marine populations (Moore et al., 2004). The application of cytogenetic assays using ecologically relevant species offers a chance to perform early tests on ecosystem health in relation to exposure to contaminants (Jha, 2004). Chemicals with a genotoxic potential for the aquatic environment are of serious concern since they can bind to DNA molecules and provoke a damaging chain of biological changes such as impaired enzyme function or general metabolism, cytotoxicity, immunotoxicity, disturbances in reproduction, inhibition of growth, or carcinogenesis (Ohe et al., 2004). Nevertheless, a comparatively low response in indigenous organisms could arise as a result of adaptation to chronically contaminated habitats such as dumping sites (station 20M) in Lithuanian economic zone of the Baltic Sea. Therefore, adaptation to chronically

contaminated habitats should be one of the priorities for the future studies of environmental genotoxicity in dumping areas, in marine port zones, in the oil refinery or drilling sites, dredging and other areas with a long history of anthropogenic influence. Deployment of organisms from uncontaminated sites and caging in these ecologically stressful areas, as well as in zones of accidental spills of contaminants should be applied for assessing changes in genotoxicity of environments.

4.6 Būtingė oil spill – assessment of the state of the environment

Petroleum industry cause environmental pollution problems worldwide and biological effects in indigenous biota could be provoked by different oil compounds. The main objective of the study was to estimate genotoxicity and cytotoxicity potential caused by the accidental oil spill (in 31 January 2008) in the Būtingė oil terminal. Būtingė oil terminal, an oil installation on the Lithuanian coast is among contributing activities to the oil pollution of the Baltic Sea. An accidental oil spills in the terminal have occurred in winter 2001 (23 November) and in 2008 (31 January). In both cases, oil slicks were driven to the Lithuanian coastline by the direction of the main Lithuanian recreational sites Palanga and Šventoji.

After an accident, as soon as oil gets into water it begins to spread on its surface making thin film of oil. Part of oil evaporates, the other part dissolves, forms emulsions, degrades, oxidizes, aggregates. The remaining part of oil can sink and settle on the bottom or even penetrate into the sand (Stankevičius, 2008). Although these processes are not fast – after 12 days after the oil spill the concentration of total oil hydrocarbons in water was still 2 times higher, compared to the concentrations observed at the same station before (Fig. 4.6.1). Concentration of oil hydrocarbons in water at tankers anchoring area was about 13 times above the maximum allowable concentration (MAC, 0.05 mg l^{-1}). Further monitoring of oil in water of Būtingė terminal after the oil spill showed that higher concentrations of total oil hydrocarbons were detected mostly in near-bottom water layer in May and August of 2008 (Mažeikių

nafta, 2008). Therefore effects of oil contamination in organisms can be seen even in 6 months after the oil spill as it was determined in our study. Concentration of PAHs (naphthalene, anthracene, fluoranthene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, benzo[*ghi*]perylene, indeno[1,2,3-*cd*]pyrene) in sediments of the 1B station were analysed in August of 2006, 2007 and after the oil spill in August of 2008. Summed concentrations of PAHs after the oil spill were about 5 times higher than before the accident.

Environmental genotoxicity and cytotoxicity data in the resident mussels before (one to two years) and after the accidental spill in 2008 have been analysed. Statistically significant difference in micronuclei and other nuclear abnormalities was observed in mussels inhabiting station 1B, which is located near the Būtingė oil terminal area. Furthermore an extremely elevated environmental genotoxicity and cytotoxicity responses were observed in mussels from the Palanga location in August 2008, 6 months after the oil spill. Since our previous studies on environmental genotoxicity in different sites of the Baltic and North Seas revealed the MN baseline in mussels consisting of 1-1.5 MN/1000 cells (Baršienė et al., 2004, 2006a), the frequency of MN after the oil spill was 6-fold elevated.

Environmental genotoxicity and cytotoxicity measurements in mussels and different fish species from the Lithuanian coastal and offshore areas were started in September 2001 and now there are long-term results regarding changes of the parameters. In the Lithuanian coast, MN frequency in mussels ranged from 1.08 ‰ (MN/1000 cells) in Palanga location in June 2007 to 6.06 ‰ in the same location in August 2008.

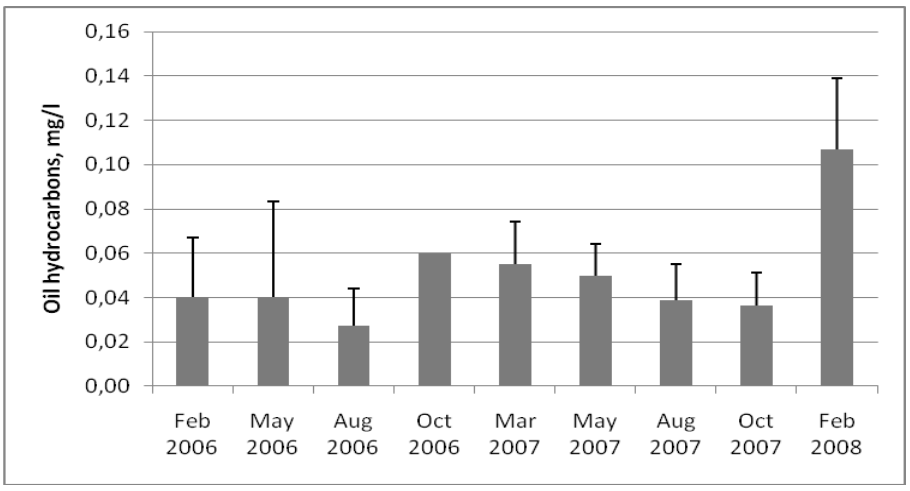


Fig. 4.6.1. Average concentrations of total oil hydrocarbons in water at 1B station in 2006–2008

The reference level (1.2 ‰) in Palanga site was found also in June 2001, but after the accidental oil spill in Būtingė oil terminal (in November 2001), Palanga location was contaminated by oil and the genotoxicity level in 2002–2003 have increased up to 3 MN/1000 cells (Baršienė et al., 2004) and remained significantly elevated until 2005. Full recovery of mussels was found only in June 2007. However, in January 2008, the oil spill accident has recurred and very similar scenarios of spilled oil distribution and genotoxicity elevation appeared again. As a result, the frequency of micronuclei in mussels from the Palanga site increased up to 6.06 ‰, and reached the highest level registered in 2001–2008 at different study locations on the Lithuanian coastal and offshore zones.

It is noteworthy to stress that 2-4-fold elevation of nuclear buds, the other endpoint of the environmental genotoxicity, was found after the oil spill in 2008 compared to the level before the spill. The induction of bi-nucleated cells in gills of mussels was elevated up to two times; induction of fragmented-apoptotic cells was increased up to 9 times. The phenomenon in mussels appears evidently as a result of action of genotoxic and cytotoxic agents constituting the spilled oil. Therefore, in assessment of oil spill damage, a usefulness of other than micronuclei nuclear abnormalities has been confirmed. Nuclear buds, fragmented-apoptotic, bi-nucleated cells and some

other nuclear abnormalities have earlier been successfully applied in mussels with the aid to assess pollutant effects (Izquierdo et al., 2003; Venier, Zampieron, 2005; Baršienė et al., 2006a, 2006c; Baršienė, Rybakovas, 2006; Baršienė, Andreikėnaitė, 2007; Koukouzika, Dimitriadis, 2008).

Increased genotoxicity and cytotoxicity was defined after 3-week treatment with 0.5 ppm crude oil processed from the Statfjord B platform in the North Sea. Moreover, the co-exposure to 0.5 ppm of oil spiked with a mixture of alkylphenols (Σ = 0.1 ppm) and 0.1 ppm of PAHs induced 2.8-fold increase of MN and 4-fold increase of nuclear buds and fragmented-apoptotic cells via control levels (Baršienė, Andreikėnaitė, 2007).

Crude oil consists of different components, like various hydrocarbons, heavy metals, nitrogen-oxygen compounds. The data on genotoxicity of 10 polycyclic aromatic hydrocarbons in mice skin cells was reported and it was pointed to genotoxicity of these compounds and general correlated with their reported carcinogenicity (Nishikawa et al., 2005). Genotoxic effects of benzo[*a*]pyrene (BaP) and dimethylbenz[*a*]antracene have been shown earlier in the gills and hemolymph of marine molluscs (Burgeot et al., 1995; Bolognesi et al., 1996; Venier et al., 1997; Siu et al., 2004). Comet and MN assays have been presented clear dose- and time-dependent responses to BaP exposure in *Mytilidae* bivalve *Perna viridis* (Siu et al., 2004). Increased frequency of MN was observed in mussels after 15-day exposure to 0.1 μg l^{-1} of phenanthrene (Koukouzika, Dmitriadis, 2008), in zebra mussel *D. polymorpha* after 2-, 3- and 4-day treatment with different concentrations (2 μg l^{-1} and 10 μg l^{-1}) of BaP (Binelli et al., 2008). Recent analysis of environmental genotoxicity within the water column monitoring near the Statfjord B oil platform revealed a clear gradient related increase in MN in haemocytes of mussels and liver erythrocytes of Atlantic cod, caged in 2004 for 6 week in the area of the platform. Significantly increased levels of micronuclei were detected in mussels and cod deployed in 500 m from the platform, compared to the reference site (Hylland et al., 2008). Among the biomarkers used to monitor impacts (EROD, DNA adducts, PAH metabolites

etc.), lysosomal destabilization in mussel hepatopancreas and MN induction in haemocytes were only methods indicating, that caged mussels suffer from the contaminants in studied area of the North Sea. Micronulei formation was suggested as a sensitive biomarker which should be used to monitor low levels of petroleum contaminants (Hylland et al., 2008).

The results of the present study pointed to comparatively quick formation of oil spill induced genotoxicity and cytotoxicity in winter at low temperature and the need to highlight harmful effects after the oil spillage in marine environment. The parameters used serve as an early warning sign of pollution-induced genetic damage in wildlife species, MN test as well as morphological alterations of cell nuclei including nuclear buds and fragmented-apoptotic could further be successfully apply in the monitoring of genotoxins in zones of oil industry. Taking into consideration species-specific responses to contaminants and pollution pattern, the ecologically relevant information from oil industry areas could be obtained by assessment of genotoxic effects in indigenous species (fish and mussels) both *in situ* and in caged organisms from wild populations. Consider the oil spill danger in oil installations, further elaboration of laboratory-controlled studies using environmentally realistic doses of genotoxic compounds should help to describe in details harm to resident species inhabiting areas of chronic contamination. Laboratory-controlled experiments, active monitoring approaches, and *in situ* assessment of DNA damage in various tissues of target species will help to archive a substantial progress in assessment of early responses as well as short- or long-term adaptations to chronic pollution originating from petroleum installations.

4.7 Biomarker responses in *Mytilus edulis* to different PAHs – laboratory experiment

Results of the present study showed, that an exceptionally high levels of miconuclei (11.43 ‰) and nuclear buds (14.14 ‰) were determined in the mussels *Mytilus edulis* treated for 48 hours with mixture of 12 μg l^{-1} of

flouranthene and 4 μg $l^{-1}$of benzo(a)pyrene. High frequency of MN (10.57 ‰) was found after exposure solely to fluoranthene 12 μg l^{-1} concentration, high induction of nuclear buds (NB) – in F4 + Py4 and Py12 + BaP4 groups of mussels, in which NB levels exceeded mean of 10 NB/1000 cells. Consequently, this study results showed a high genotoxicity and cytotoxicity potential of polycyclic aromatic hydrocarbons in their mixtures. On the other hand, current data indicated that less than additive effects are presented in exposure system *M. edulis* – model mixture of PAHs. Very similar response to mixture of PAHs has been described in freshwater bivalves, and was pointed on effects of saturation of metabolic pathways in activating process of mutagenic PAHs (White, 2002).

The data contribute to the sparse information on PAH behavior in marine invertebrates after exposure to complex mixtures and point that PAH genotoxicity and cytotoxicity in their mixtures is not simply the sum of effects from each mixture component. In mussel gills, genotoxicity and cytotoxicity of studied PAHs was significantly higher in model mixtures compared to solely treatment with pyrene or fluoranthene. Interestingly, the micronuclei were formed extensively under the fluoranthene action, nuclear buds – under fluoranthene and pyrene influence. It could be presumed, that fluoranthene acts as clastogenic and aneugenic agent in mussels, pyrene – mainly as clastogenic agent provoking DNA damage. In overall, the induction of both genotoxicity endpoints (MN and NB) was exceptionally high. The significant difference of genotoxicity responses with controls after 48 h exposure to the PAHs may be indicative of a weak defense capacity of the *M. edulis* against DNA damage induced by metabolites of the compounds. The threshold might be reached above which repair systems are not capable to eliminate the genetic damage. Moreover, the total concentration of 8 and 16 μg l^{-1} of PAHs in mixtures provoked the highest formation of bi-nucleated cells, which reached an extreme level of 19.91 BN/1000 cells in Py4 + BaP4 group of mussels. At

higher concentration (in F36, Py36, F4 + Py4 + BaP4, F12 + Py12 + BaP4) the responses were as minimum 2 times lower than in Py4 + BaP4 group.

In scientific literature there is deficiency of data on genotoxicity of pyrene and fluoranthene or their mixtures with other PAHs in marine mussels, but there are data on genotoxicity potential of benzo(a)pyrene. In *Mytilus galloprovincialis* after 48 h exposure to benzo(a)pyrene (0.025, 0.075, 0.225, 0.675 mg l^{-1}), MN levels in control and exposed groups were significantly different (Scarpato et al., 1990). In digestive gland cells of *M. galloprovincialis,* 19 μg l^{-1} BaP exposure induced significant MN elevation with a maximum response after 72 h treatment (Banni et al., 2010). In Venier et al. (1997) study, statistically elevated levels of MN in gill and haemolymph cells were shown in mussels *M. galloprovincialis* exposed for 48 h to 50, 100, 500, 1000 ppb of BaP. Higher incidences of micronuclei, bi-nucleated, fragmented or abnormally shaped nuclei were recorded after 2-day BaP exposure in mussel groups compared with control mussels. It was pointed that such rapid increase of the cytogenetic damage could be due to formation of intermediate metabolites (Venier et al., 1997). Significantly increased levels of DNA strand breaks has been observed in *Perna viridis* mussels after one-day treatment with 0.3 and 3 μg l^{-1} of benzo(a)pyrene (Ching et al., 2001). In exposure of this species to 0.3, 3 and 30 μg l^{-1} BaP, dose- and time-related formation of micronuclei (MN) was shown in gill cells and in haemocytes during the entire 12 days. There have been detected significant differences between mussels exposed to 0.3 and 30 μg l^{-1} BaP after 3, 6 and 12 days (Siu et al., 2004). The high potential of BaP to provoke induction of DNA damage and produce irreversible cytogenetic alterations has been described in freshwater zebra mussel *D. polymorpha* after 1-, 2-, 3- and 4-day treatment with 0.4 nM, 7.9 nM and 40 nM concentrations of BaP. Since the results of the study showed clear statistical differences between all exposed and control groups, it was supposed very fast capacity (starting 24 h) of the zebra mussel to transform BaP into active metabolites (Binelli et al., 2008).

Comet assay and micronuclei test in mussel digestive gland cells have demonstrated the BaP potential to induce significant DNA damage with a maximum response after 72 h exposure (Banni et al., 2010). DNA strand breaks formation in green-lipped mussels *Perna viridis* was found after one-day treatment with low concentrations of BaP (0.3 and 3 μg l^{-1}) and was supposed being over the threshold of the repair system. It should be pointed, that high concentration of BaP did not showed differences between exposed to 30 μg l^{-1} and control groups of mussels (Ching et al., 2001).

Our experimental treatment was designed with the aim to describe relationships between genotoxicity and cytotoxicity endpoints in mussels treated with different concentrations of pyrene, or fluoranthene, and after exposure to their mixtures with BaP of 4 μg l^{-1}. The statistical Mann-Whitney (Wilcoxon) U-test have revealed the significant influence of BaP in elevation of total genotoxicity (MN + NB) in F4 + BaP4, Py4 + BaP4 and in Py12 + BaP4 groups and elevation of total cytotoxicity (FA + BN) in F4 + BaP4 mussel group. Therefore only in model mixture with fluoranthene 12 μg l^{-1}, BaP did not increased neither genotoxicity, nor cytotoxicity responses. Exposure solely to fluoranthene 12 μg l^{-1} caused exceptionally high levels of genotoxicity and cytotoxicity in mussel gill cells.

An important fact was identified showing that adding 4 μg l^{-1} of BaP significantly increased genotoxicity and cytotoxicity of model mixtures at low (4 μg l^{-1}) fluoranthene or pyrene concentrations, and did not showed significant increase of genotoxicity in mussels treated with model mixtures consisting of 12 μg l^{-1} of fluoranthene. Analysis of protein expression biomarkers in the Baltic Sea blue mussels after 3-day exposure to low (2.8 μg $animal^{-1}$ day^{-1}), intermediate (28 μg $animal^{-1}$ day^{-1}), or high (280 μg $animal^{-1}$ day^{-1}) nominal doses of benzo(a)pyrene (BaP) showed significantly up-regulated expression in the mussels treated with the low dose (Prevodnik et al., 2007). The finding on significant role of BaP in model mixtures with low level concentrations, also suppression of genotoxicity and

cytotoxicity responses in mussels treated with highest concentrations (36 μg l^{-1}) with fluoranthene, or pyrene, as well as with model mixtures of all three PAHs, indicates that in our experiment used 12 μg l^{-1} of fluoranthene concentrations were at threshold levels for the mussels. However, the same concentration of pyrene did not induced micronuclei, nuclear buds, fragmented-apoptotic cells at all and only caused 2-fold increase of bi-nucleated cells. Thus, pyrene at 12 μg l^{-1} has shown only risk for the cell division alterations, whilst genotoxicity, nor apoptosis was not appeared. A high induction of bi-nucleated cells in mussels exposed to pyrene and benzo(a)pyrene mixtures could be explained by cytostatic action of pyrene metabolites. The data of our study let to suppose, that in mussels after 48 hours exposure to pyrene or fluoranthene metabolic activity is presented and metabolites actively affect the genetic material and influence on cell division apparatus.

Siu with co-authors (2008) has shown a strong correlation between BaP concentrations and MN induction in mussels *P.viridis* after 8, 12, 16 and 30-day deployment into polluted by PAHs sites of Hong Kong coastal area. Significant dose- and time-dependent levels of MN and DNA strand breaks has detected at heavily polluted sites. Micronucleus frequencies in Brazil inter-tidal bivalve *Perna perna* haemocytes were also significantly related to PAH concentrations, and increase of the response appears at concentrations as low as 300 μg kg^{-1} Σ 35 PAH (Francioni et al., 2007). In the field studies, certain unpredictable environmental and physiological factors could affect bioaccumulation of genotoxins in tissues and influence subsequent biological effects in marine organisms. Since presence of mixtures of chemicals and variation of their concentrations in aquatic ecosystems could make difficulties in interpretation of the responses and introduce certain modifications in organism responses to complex of agents, the controlled laboratory studies of the model mixtures of contaminants would be performed. The chemical and toxicological assessment of toxic substances can indicate the hazard, but do

not reflect genotoxicity risk to organisms during exposure. Multi-biomarker approach measuring genotoxicity and cytotoxicity potential presents early warning signals of damage in DNA, chromosomal levels, or indicates alterations in cell division and their morphology.

Markers of genotoxicity effects are at high priority due to reflection of damage caused by hazardous substances to genetic material of organisms (Moore et al., 2004).

Most biochemical biomarkers in single compound exposures were increasing from fluoranthene 4 μg l^{-1} to 36 μg l^{-1}, but showed high values for pyrene 4 μg l^{-1} decreasing to pyrene 12 μg l^{-1} and 36 μg l^{-1}. This indicates that pyrene elicits stronger oxidative stress response in mussels in lower concentrations than fluoranthene. Mixed exposures with F and Py in different concentrations are in line with single exposures, and some additive responses are seen. In mixtures of either fluoranthene with BaP or pyrene with BaP the biomarker responses are induced showing the effect of BaP.

The results of Integrated Biomarker Indices (IBR) revealed the effect of fluoranthene on the neurotoxicity, oxidative stress and genotoxicity biomarkers, and some additive responses were observed. Low concentrations of pyrene (4 μg l-1) elicited stronger oxidative stress response (CAT, SOD, GR and LPO) in mussels. However, the response of micronuclei, the biomarker of genotoxicity, was higher at 36 μg l-1 concentration of pyrene. The results of IBR also showed that the biomarker responses were induced (particularly in mixtures with fluoranthene) showing the effect of BaP.

This study demonstrated a usefulness of multi-biomarker approach in assessment of the influence of PAHs on marine organisms and revealed feasibility to apply the tool for the other PAHs, with the aid to detect more precisely the damage and evaluate the risk to the marine wildlife and human. The extensive development of oil and other industry in marine environment requires development of environmentally meaningful endpoints for integrated approach to estimate ecological risk of PAH contamination in marine

ecosystems. Consider environmentally realistic routes of the contamination and presence of genotoxin's mixtures, the information generated in the present study, should help to fulfill our knowledge gaps in understanding of ecological significance of polyaromatic hydrocarbons in marine ecosystems. Multi-species laboratory-controlled experiments in future, assessment of damage in various tissues of target species will uncover pattern of early responses as well as short- or long-term adaptations to chronically produced pollution at low concentrations of PAHs.

4.8 Integrated assessment of the contamination of the Baltic Sea

There is a great number of different anthropogenically produced substances in the marine environment which have an effect on living organisms. At the particular moment the concentration of one contaminant can be higher than the other and additive effects of the contaminants can occur. That is why the useful tool for the evaluation of the contamination of the environment is the Pollution Index (PI). The PI calculated for the Lithuanian waters in 2006 showed elevated values for the Klaipėda Strait. However, the PI evaluates only the contamination of water at the particular moment. The concentration of water is a subject of change. It would be more practical to use PI for sediment or biota as the concentration of pollutants in these compartments of environment are more stable. But the EQSs ratified by the legislative document are needed. As was already mentioned before, the EQS values in Lithuania exist only for water. Anyway, the PI helps to estimate the state of the environment taking into consideration all contaminants that are present. It helps to compare the overall pollution in space and time.

In the case of Lithuanian national monitoring, the part of oil national monitoring data set ends up in 2007. The different method (gas chromatography) for the determination of oil hydrocarbons in water has been started to use in 2008. Unfortunately, the new gas chromatography method is not so sensitive. The limit of detection of the method is 0.1 mg l^{-1} and that is already 2 times the MAC (0.05 mg l^{-1}). The monitoring survey in 2008 and

2009 already showed that most of the data of oil hydrocarbons in water is under the limit of quantification of the method. That is why the amount of stations and the frequency of sampling can be reduced. The monitoring of oil concentrations in water should be done only in the areas of concern like Būtingė area, the area in adjacent to D-6 oil platform near the border with Russia and in Klaipėda harbour. A new method is good for evaluating of the state of the environment after the accidental oil spill. As the chemical method is not sensitive, micronuclei method can be sensitive enough to detect an effect of chronic oil pollution by rather low concentrations, which are not detectable by the chemical method. Our results show that biomarkers, especially micronuclei, are a useful tool in monitoring of oil pollution.

Sometimes biomarkers are considered as complicated tool because of the interpretation of the data. The response of biomarkers can depend on such environmental factors like salinity, temperature, season, condition index of organisms, microorganisms. But biological effects studies have been already used in monitoring programs all over the world and in the Baltic Sea. More investigations are done the more information is coming on the interpretation of the effects of contaminants. Besides this, there are some other useful tools like Integrated Biomarker Index (IBR) which sums up all the activities of biomarkers and gives the overall view on the situation.

This study was the first attempt of an integrated assessment of environmental state of the marine environment in Lithuania using national monitoring data of specific contaminants together with biological effects data.

The thesis reflects the overall goals of the hazardous substances segment of the HELCOM Baltic Sea Action Plan – to achieve a Baltic Sea with life undisturbed by hazardous substances and of the EU Marine Strategy Framework Directive – concentrations of contaminants are at levels not giving rise to pollution effects. To achieve these goals an integrated holistic assessment of the state of the environment of the Baltic Sea is needed. For that purpose HELCOM developed a multi-metric Chemical Status Assessment Tool CHASE.

For the first time the assessment of the chemical status of Lithuanian marine waters has been made using CHASE. This provided us with the opportunity of the comprehensive and integrated evaluation of pollution. The results have shown that the chemical status of the most analyzed areas is moderate, whilst in the Klaipėda Strait it is poor. Thus, relevant measures have to be taken to improve the status of the Baltic marine environment and to achieve established goals.

CONCLUSIONS

1. Long-term data analysis showed the decrease of mercury concentrations in water starting from 1995–1996; mercury concentrations in fish and mussels were decreasing starting from 1999–2000. From 2005–2006 concentrations of copper, chromium, nickel and lead were decreasing in sediments at the dumping site and in the Curonian Lagoon. The analysis of the long-term (1990–2007) data on the total oil concentrations in water showed that there were statistically significant increasing trends in the northern coastal waters and plume of the lagoon. Statistically significant trends were not detected in other areas of the Lithuanian Baltic Sea. During the period of 1997–2009 there was a decrease in DDT and hexachlorocyclohexane (HCH) pesticides and their metabolites concentrations in water and sediments. DDT concentrations in *Macoma balthica, Mytilus edulis* and *Dreissena polymorpha* stayed at the same level during the analysed period of time.
2. Higher contaminants concentrations were detected in the Klaipėda harbour area and at the dredged sediments dumping site. During the period of 2005–2007 25 % of the measured total oil hydrocarbon concentrations in the Klaipėda Strait were above the maximum allowable concentration (MAC). The concentrations of copper, nickel, and lead in sediments of the Klaipėda harbour often exceeded the I pollution category, the highest concentration of dioxin-like PCBs was also found in the Klaipėda harbour. The highest concentration of tributyltin was detected in sediments of Malkų bay. The highest summed concentration of dioxins and furans was found at dredged sediments dumping site. Tributyltin was also found in sediments of dumping site area.
3. Būtingė oil terminal and Russian D-6 oil platform are potential oil pollution sources. After the oil spill in Būtingė oil terminal in the 31 of January 2008, concentration of oil hydrocarbons in water at tankers anchoring area was about 13 times above the MAC. Calculated molecular

indices of polycyclic aromatic hydrocarbons (PAHs) at tankers anchoring area and adjacent to the D-6 oil platform showed that the source of PAHs in the sediments was petrogenic, related to shipping or oil platforms. A diesel engine source of PAA was indicated at the 1B station.

4. Analysis of sediment samples showed that there were higher arsenic concentrations near the chemical munitions dumpsite (average 9.7 mg kg^{-1}) compared to other coastal locations. Normalization of results to iron showed slightly elevated residual arsenic concentrations near the dumpsite. However arsenic concentrations, even from the dumpsite region, were low compared to other dumping sites in the Baltic and North Seas.
5. Integrated assessment of pollution and biomarker responses showed that *M. edulis* and *M. balthica*, as well as *D. polymorpha* and *Nereis diversicolor* can be used as sentinel species for the biological effects studies and monitoring in the Lithuanian zone of the Baltic Sea.
6. The highest level of environmental genotoxicity and cytotoxicity in *M. balthica* mussels was found in the open sea area of the Lithuanian zone. A comparatively high frequency of micronuclei was found in *M. edulis* from station 1B and in *M. balthica* from station N-4, the reference level of micronuclei incidences was observed in *M. balthica* gill cells from the coastal station N-8. Statistically significant increase of micronuclei and other nuclear abnormalities was observed in mussels after the oil spill in the Būtingė oil terminal. Elevated environmental genotoxicity and cytotoxicity responses were observed in mussels from the Palanga location 6 months after the oil spill event.
7. The results of the laboratory PAHs exposure showed statistically significant effects of fluoranthene, pyrene and benzo(a)pyrene on biomarker responses (AChE, GST, CAT, GR, SOD, LPO, MN) in *M. edulis*. BaP could be suspected as a provocative factor for the formation of genotoxicity and cytotoxicity in *M. edulis* under the influence of fluoranthene or pyrene.

8. According to CHASE evaluation, a chemical status of the Curonian Lagoon is good; northern coastal waters, southern coastal waters, dredged sediments dumping site and plume of the lagoon – moderate, Klaipėda Strait – poor.

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SCIENTIFIC APPROVAL

List of publications

1. Garnaga G., Stankevičius A., 2005. Arsenic and other environmental parameters at the chemical munitions dumpsite in the Lithuanian economic zone of the Baltic Sea. Environmental research, engineering and management, 3 (33): 24-31.
2. Garnaga G., Wyse E., Azemard S., Stankevičius A. and de Mora S., 2006. Arsenic in sediments from the southeastern Baltic Sea. Environmental Pollution, 144 (3): 855-861.
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4. Garnaga G., Štukova Z., 2008. Contamination of the South-eastern Baltic Sea and the Curonian Lagoon with oil products. US/EU-Baltic International Symposium, 2008 IEEE/OES, pp.1-8, ISBN: 978-1-4244-2267-8.

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1. Garnaga G., Wyse E., Azemard S., Stankevičius A. and de Mora S., 2006. Arsenic in sediments from the southeastern Baltic Sea. EU-USA International Symposium „Integrated Ocean Observation Systems for Managing Global and Regional Ecosystems Using Marine research, Monitoring and Technologies“, Klaipėda, Lithuania.
2. Garnaga G., 2007. Chemical munitions in the Lithuanian economic zone of the Baltic Sea. Baltic SeaBreeze International Conference “Baltic Sea for future generations”, Palanga, Lithuania.
3. Garnaga G., Štukova Z., Kondratjeva L. 2007. Pollution of the southeastern Baltic and Curonian Lagoon with oil products. Scientific conference “Biodegradation of oil and other environmental contaminants”, Public Agency “Soil Remediation Technologies”, Vilnius, Lithuania.

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ANNEX I

Table A1. Monitoring and other studies stations coordinates (decimal degrees) in the Baltic Sea and Curonian Lagoon

Station	Latitude	Longitude
Baltic Sea		
0	56.0850	21.0167
0B	56.0850	20.8333
1	56.0283	21.0167
1B	56.0283	20.8333
1B-1	56.0000	20.7830
2	55.9250	20.9750
2C	55.9250	20.6733
2M	55.9250	20.7333
3	55.8167	21.0167
4 (ChG13)	55.7350	21.0500
4C	55.7500	20.9733
5 (ChG14)	55.7183	21.0617
6	55.5583	21.0783
6-1	55.6100	21.0660
6-2	55.5000	21.0550
6B (N-2)	55.5200	20.5633
6B1	55.5000	20.7490
6B2	55.4540	20.7280
6D	55.3833	20.5833
7 (N-9)	55.3117	20.9567
20	55.6333	20.8000
20A	55.6500	20.8333
20B	55.7000	20.8500
20M	55.6340	20.8360
46	56.0200	19.1467
64 (ChG10)	55.7650	20.8917
64A	55.8167	20.6500
64A-1	55.8000	20.7660
65 (ChG9)	55.8817	20.3417
66 (ChG7)	56.0000	19.6500
S-1	55.6500	21.0750
S-3	55.7833	20.9333
B-1	56.0417	21.0500
B-4	56.0450	20.9667
B-7	55.9620	20.7500
N-1	55.5750	20.2250
N-3	55.4667	20.5333
N-4	55.4500	20.8000
N-5	55.4250	21.0350
N-6	55.4050	20.7067
N-7	55.3750	21.0017
N-8	55.3617	20.8250
ChG1	56.0383	19.1200
ChG2	56.0350	19.2433
ChG3	55.9967	19.1717
ChG4	55.9517	19.0900
ChG5	55.9550	19.2417
ChG6	56.0250	19.3833
ChG8	55.9400	20.0000
ChG11	55.7500	20.9733
ChG12	55.7617	21.0500
Curonian Lagoon		
1	55.7117	21.1117
2	55.6851	21.1200
3	55.6667	21.1333
3A	55.6464	21.1617
3B	55.6467	21.1633
5	55.5300	21.1367
7B	55.4900	21.1700
8	55.4150	21.1233
9	55.4167	21.2333
10	55.3050	21.0267
12	55.3333	21.1667
12A	55.3467	21.3017
14	55.2633	21.0783