Available online at www.sciencedirect.com
ScienceDirect
Ecotoxicology and Environmental Safety 69 (2008) 39–48
Ecotoxicology and Environmental Safety
www.elsevier.com/locate/ecoenv

# A multispecies approach for monitoring persistent toxic substances in the Gulf of Gdańsk (Baltic sea)

S. Galassi[a], R. Bettinetti[b], M.C. Neri[a], R. Jeannot[c], T. Dagnac[c], S. Bristeau[c], V. Sakkas[d], T. Albanis[d], V. Boti[d], T. Valsamaki[d], J. Falandysz[e], U. Schulte-Oehlmann[f]

[a]*Department of Biology, University of Milan, Via Celoria 26, 20133 Milano, Italy*
[b]*Department of Chemistry and Environmental Sciences, University of Insubria, Via Valleggio, 11, 22100 Como, Italy*
[c]*Bureau de Recherches Géologiques et Minières, Av. C. Guillemin, 45060 Orléans, France*
[d]*Department of Chemistry, University of Ioannina, Panepistimioupolis, 1 45110 Ioannina, Greece*
[e]*Department of Environmental Chemistry, University of Gdańsk, 18 Sobieskiego Str. 80-952, Gdańsk, Poland*
[f]*Department of Ecology and Evolution, J.W. Göethe Universität, Siesmayerstraße 70, 60054 Frankfurt, Germany*

Received 16 February 2006; received in revised form 25 October 2006; accepted 18 November 2006
Available online 8 February 2007

**Abstract**

Bivalve mussels are usually used for biomonitoring persistent toxic substances (PTS) in coastal ecosystems. Nevertheless, these organisms, which live attached on hard substrates, can be found along the sandy coasts only on human manufactured products. In this work different species collected in the Gulf of Gdańsk were compared to evaluate their suitability for monitoring PTS pollution at a local scale. The clam *Mya arenaria* seems to represent an excellent indicator of sediment pollution, mainly for organotin compounds which are selectively bioaccumulated. Organochlorine compounds are bioaccumulated in the different species mainly in function of their lipid body burden. Habitat conditions (salinity, substrate, pollution), however, strongly limited the occurrence of different species in the sampling sites; the most ubiquitous species, the common shrimp *Crangon crangon,* resulted therefore the most suitable to be used for the comparison of PTS pollution in this aquatic environment.

Although the blue mussel (*Mytilus trossulus*) was confirmed to be a very useful sentinel species to compare pollution level inside and outside the Gulf of Gdańsk, we recommend the use of other species to give a more detailed picture of the pollution situation in coastal areas.


*Keywords:* Biomonitoring; Endocrine disruptors; Gdańsk gulf; Persistent toxic substances

## 1. Introduction

The Baltic Sea, an enclosed and shallow sea, is particularly vulnerable to toxic organic pollutants (Falandysz et al., 2000) because it is a cold-water body with complete water renewal time of about 10 years, receiving land based pollution transported by rivers and trapping persistent toxic substances (PTS) deposited from the atmosphere. Chemicals are also discharged into this sea as wastes of the industrial towns located on the coast. One of the most polluted zones along the Baltic coast is the Gulf of Gdańsk, receiving the discharge of the wastewaters from the two big Polish cities of Gdańsk (956,000 p.e.) and Gdynia (441,000 p.e.), and also from the Vistula River (Falandysz et al., 1999). The Gulf of Gdańsk, surrounded by a heavily populated region and agricultural land, covers an area of 3800 $km^2$ with a maximum depth of 53 m. The drainage basin of the gulf is 194,000 $km^2$.

The Vistula river flows from the mountains in southern Poland and 26 million people live in its catchment area. It has the second largest drainage basin among the Baltic rivers, equalling 12% of the total catchment area of the Baltic Sea. About half the agricultural area of the Baltic Sea region is located in the area of the Vistula River basin.

The Gulf of Gdańsk is affected by chemical pollution and by eutrophication. Concentrations of phosphates,

*E-mail address:* silvana.galassi@unimi.it (S. Galassi).

0147-6513/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.ecoenv.2006.11.015

ammonia compounds and nitrites as well as biochemical oxygen demand ($BOD_5$) in the coastal water of the Gulf of Gdańsk are on the average 5–10-fold higher than in the water of the open Baltic Sea (Szumilas and Sobol, 1990). High concentrations of the following substances have been found in the gulf: nutrients (N, P), detergents, pesticides, polycyclic aromatic hydrocarbons, aromatic amines, phenols, heavy metals (Cd, Cr, Se, As, Ni, Pb, Mn, Cu, Fe). There are several industrial plants located on the Polish coast of the Gdańsk Basin: seven metal works, one oil refinery, three chemical plants, 11 fish and food-processing industries, and five energy and harbour installations.

Falandysz et al. (2002a, b, 2006) found that sediments, invertebrates and vertebrates in the Gulf of Gdańsk are contaminated with PCBs, organotins (OT), insecticides like DDT and cyclodienes. Potrykus et al. (2003) performed further biomonitoring studies in 1997 using the blue mussel *Mytilus trossulus* and showed much higher PCB and *p,p′*-DDE levels inside the Gulf of Gdańsk, near the ports of Gdynia and Gdańsk than in the Pomeranian Bay and in open sea. OT pollution was also assessed in mussels, fishes (Senthilkumar et al., 1999; Albalat et al., 2002) and sediments (Szpunar et al., 1997) along the Polish coast. Fish analyses highlighted that the highest OT concentrations occurred in a sampling site inside the Gulf of Gdańsk (Albalat et al., 2002).

Recent data suggest that loads of some hazardous compounds have been reduced over the past 20 years in the Baltic Sea (HELCOM, 2003). DDT is no longer used in Eastern European Countries and it was legally banned in 1996 in Poland (Münch and Axenfeld, 1999).

However, some other agrochemicals recently introduced into the market might become of concern for this aquatic environment. It was recognised that a comprehensive knowledge of the impact of micropollutants on wildlife and human health in the Baltic Sea is still lacking. Therefore, several studies are ongoing to evaluate biological effects of environmental pollution in this area by a battery of biomarkers of pollutant exposure.

In the EU-project COMPRENDO (EVK1-CT2002-00129) aimed to investigate the effects of some PTS with androgenic/anti-androgenic activity in different animal models, the Gulf of Gdańsk has been selected as representative of polluted areas to compare effect levels determined in laboratory with the levels found in a real ecosystem. With this aim all marine and brackish benthic species available along this coast were collected and analysed for the determination of the androgenic and anti-androgenic chemicals selected in the COMPRENDO project. This was a good occasion to test which of them could be used as an indicator species for monitoring PTS pollution at a local scale.

Fat content, trophic position and habitat of the different organisms were taken into account to appraise whether they could be used as sentinel species for the monitoring of the spatial distribution and of the PTS pollution trend. Besides the androgenic/antiandrogenic chemicals studied in the project (*p,p′*-DDE, OT compounds, vinclozolin, fenarimol and phenylurea herbicides), PCB, *p,p′*-DDT and *p,p′*-DDD that could be quantitatively recovered and analysed with the methodology used for *p,p′*-DDE determination were also measured in all the samples for which enough material was available.

## 2. Materials and methods

### 2.1. Sampling

Table 1 and Fig. 1 give a short description of the sampling stations along the coastal zone and in the inner channels. Sampling took place in March 2003. Pools of at least six individuals of each species were prepared for analytical determinations for each sampling stations and stored frozen until freeze-drying. Relevant biological and ecological characteristics of the species analysed were considered important for the understanding of pollutant bioaccumulation potential:

*Mytilus trossulus* (blue mussel): is a filter feeder bivalve mussel, 6–10 cm length that lives attached to rocks, docks, pilings, floats and gravel.

*Mya arenaria* (soft-shelled clam or sand gaper): is a large long-lived bivalve: large specimens may reach 12–15 cm length. It lives in burrows down to 50 cm, deep in sand, mud, sandy mud, and sandy gravels. Its habitat is from the mid shore to the shallow sub-littoral tanks. This bivalve has siphons that can reach the surface (about 20–40 cm or up to 40 cm in large specimens).

*Crangon crangon* (common shrimp): is a crustacean that resides from the middle shore down to submerged depths of around 150 m; it also lives into estuaries and typically buries into the sand. Although most specimens measure between 30 and 50 mm length, some may grow to 90 mm. The common shrimp feeds on a range of worms, molluscs and crustaceans.

Table 1
Description of the sampling stations

| Site | Description | Remarks | Animals found |
|---|---|---|---|
| 1 | Gdańska Stocznia Remontowa (*major source*) | Antifouling paints main use area: near the dock no. 4 | No alive bottom fauna could be collected |
| 2 | Dead Vistula River Canal, Ostrów (*possible source area*) | Near of the fuel base of the Orlen Co. | No alive bottom fauna could be collected |
| 3 | Dead Vistula River Canal, Nowy Port site | | No alive bottom fauna could be collected |
| 4 | Dead Vistula River Canal, Siennicki site | | No alive bottom fauna could be collected |
| 5 | Dead Vistula River Canal, the Motława River site in the down town Gdańsk | | No alive bottom fauna could be collected |
| 6 | Dead Vistula River Canal, sewage treatment plant outlet pipe (*major source*) | Municipal sewage treatment plant for the city of Gdańsk | No alive bottom fauna could be collected |

Table 1 (*continued*)

| Site | Description | Remarks | Animals found |
|---|---|---|---|
| 7 | Dead Vistula River Canal, sewage treatment plant outlet | Site distant 500 m | No alive bottom fauna could be collected |
| 8 | Dead Vistula River Canal/Sea interface region | Westerplatte site | *Mytilus trossulus* |
| 9a | Dead Vistula River Canal/Sea interface region | The entrance area | *Mytilus trossulus, Crangon crangon, Platychthis flesus, Gobius microps, Hyperoplus lanceolatus, Gasterosteus aculeatus* |
| 9b | Dead Vistula River Canal/Sea interface region | Brzeźno site | *Mytilus trossulus, Crangon crangon* |
| 11 | Puck Bay, Puck (*source*) | Near the outlet of small river highly contaminated with municipal sewage | No alive bottom fauna could be collected |
| 12 | Puck Bay, the Mechanical Works area | | *Cardium glaucum, Mya arenaria, Macoma baltica, Crangon crangon, Palaemon adspersus, Psetta maxima* |
| 13 | Puck Bay, the port area (*possible source*) | | *Cardium glaucum, Macoma baltica, Crangon crangon, Pungitius pungitius* |
| 14 | Puck Bay, Marina (recently established) (*possible source*) | Relatively small marina | No live bottom fauna could be collected. |
| 15 | Puck Bay, 500 m east of marina | | *Cardium glaucum, Macoma baltica, Crangon crangon, Platichthys flesus, Psetta maxima, Gobius microps, Gasterosteus aculeatus* |
| 20 | Reda River outlet (*point source*) | Birds refuge | *Cardium glaucum,Macoma baltica*, *Mya arenaria, Crangon crangon, Gasterosteus aculeatus, Platichthys flesus, Gobius microps* |
| 21 | Reda River outlet region-200 m to outlet | | *Mya arenaria* |
| 22 | Reda River outlet region-500 m to outlet | | *Mya arenaria*, *Platychthis flesus* |
| 30 | Mechelinki site, former sewage pipe outlet area (*point source*) | Raw sewage outfall site for the city of Gdynia till the 1990s. | *Mytilus trossulus, Mya arenaria* |
| 32 | Mechelinki site, former sewage pipe outlet area | distance of 300 m | *Mytilus trossulus, Macoma baltica, Cardium glaucum, Crangon crangon, Platichthys flesus, Psetta maxima* |
| 33 | Mechelinki site, former sewage pipe outlet area | distance of 600 m | *Crangon crangon* |
| 40 | Vistula River outlet-point zero (*point source*) | Agricultural soil runoff, municipal and industrial effluents—main drainage for Poland | *Crangon crangon, Platychthis flesus, Gasterosteus aculleatus, Gobius microps, Psetta maxima, Hyperoplus lanceolatus* |
| 41 | Vistula River outlet region-500 m north-east (*transect*) | | *Crangon crangon, Platichthys flesus, Psetta maxima* |
| 42 | Vistula River outlet region-1000 m east (*transect*) | | *Crangon crangon, Platichthys flesus* |
| 43 | Vistula River outlet region (*transect*) | Opposite to Sobieszewo site | *Mya arenaria, Macoma baltica*, *Cardium glaucum, Crangon crangon, Platichthys flesus, Gobius microps* |
| 44 | Vistula River outlet region (*transect*) | Brave Vistule outlet | *Mytilus trossulus, Crangon crangon, Eriocheir sinensis, Platychthys flesus, Gobius microps, Psetta maxima* |
| 60 | Kacza River outlet-point zero (*point source*) | | *Mytilus trossulus*, *Crangon crangon, Platychthis flesus* |
| 61 | Kacza River outlet region-100 m form point zero (*transect*) | | *Macoma baltica*, *Cardium glaucum, Mya arenaria*, *Mytilus trossulus, Eriocheir sinensis, Crangon crangon, Platychthis flesus,Gasterosteus aculeatus* |
| 70 | Jelitkowski Creak outlet (*point source*) | | *Macoma baltica, Cardium glaucum, Mytilus trossulus, Mya arenaria, Eriocheir sinensis, Crangon crangon, Platychthis flesus, Hyperoplus lanceolatus, Psetta maxima, Gobius microps* |
| 80 | Skwer Kościuszki (*transect*) | | *Mya arenaria, Macoma baltica,Cardium glaucum*, *Crangon crangon, Eriocheir sinensis, Platichthys flesus, Psetta maxima* |
| 81 | Gdynia Shipyard (*major source*) | | No live bottom fauna could be collected |
| 82 | Ships terminal (*source/transect*) | | No live bottom fauna could be collected |
| 83 | Nauta Shipyard (*major source*) | | No live bottom fauna could be collected |

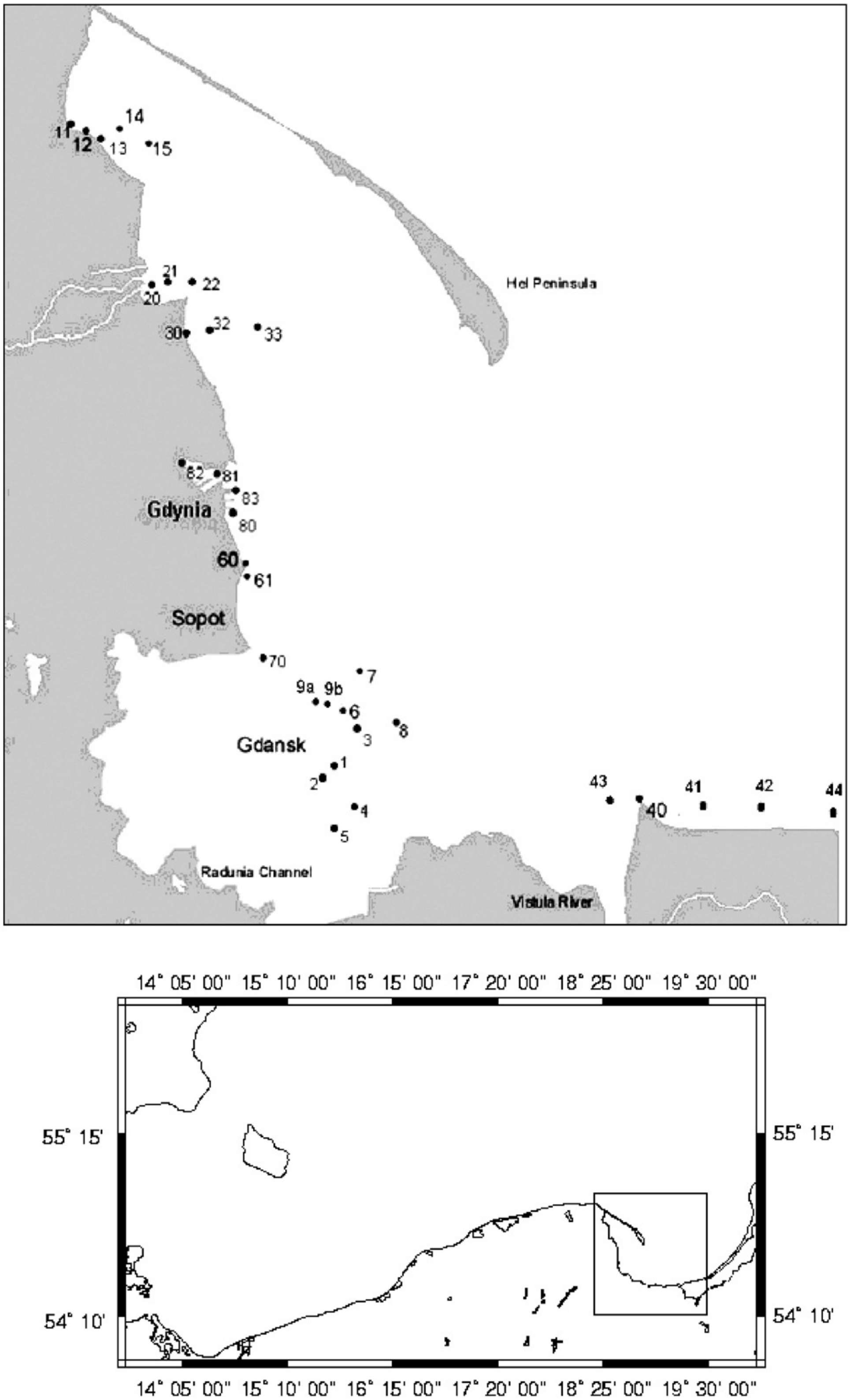


Fig. 1. Location of the sampling stations in the Gulf of Gdańsk.

*Cerastoderma glaucum* (lagoon cockle): is a sedentary bivalve mussel living in sandy and sandy gravels of littoral zones. It is a filter feeder organism reaching a maximum length of 5 cm.

*Eriocheir sinensis* (chinese mitten crab): is a crustacean, which inhabits the bottoms and banks of freshwater rivers and estuaries at the adult stage, before migrating to the brackish and salt waters of estuaries for reproduction. This crab, whose carapace can reach over 80 mm of width, consumes a wide variety of plant and animal materials, including algae, macrophytes, terrestrially derived detritus, invertebrates (both hard and soft-shelled) and will scavenge fish carcasses.

*Platichthys flesus* (flounder): is a bottom dwelling fish living in inshore waters up to depths of 50 m, growing up to 50 cm length. It feeds on a variety of bottom-living animals, e.g., crustaceans, worms and molluscs. Juveniles live in shallow water close to the shore. This fish is very tolerant of reduced salinities and is frequently found in estuaries. Only juvenile ($<10$ cm) specimens were selected in this study.

*Psetta maxima* (turbot): is a highly esteemed food fish living on sandy, rocky or mixed bottoms; it is rather common in brackish waters. It mainly feeds on other bottom-living fishes (sand-eels, gobies, etc.), and also, to a lesser extent, on larger crustaceans and bivalves. Young fish may be found inshore in the breaker zone or in shore pools. Only juvenile ($<12$ cm) specimens were selected in this study.

*Gobius microps* (*Pomatoschistus microps*) (common goby): is a small fish growing up to 6 cm of length migrating downstream, or into shallow waters, mainly at the onset of the breeding season, in spring. It feeds on small crustaceans like caprellids and worms.

*Gasterosteus aculeatus* (stickleback): lives in shallow waters amongst seaweeds, seagrass and pondweeds in freshwater, estuaries, rock pools, and saline lagoons or coastal waters. It feeds on worms, crustaceans, aquatic insects, small fish and even eggs and fry of its own species.

### 2.2. Analytical determinations

#### 2.2.1. Organochlorine compounds

Soxhlet extraction was performed on 2–3 g of lyophilised sample with 100 mL of *n*-hexane for 8 h. After solvent evaporation under reduced pressure, extractable organic matter (EOM) was determined by gravimetric measurement. Organic matter was then destroyed with $H_2SO_4$ (98%) and chlorinated hydrocarbons were recovered by several *n*-hexane washing. Hexane extract was concentrated to about 2 mL and cleaned-up on Florisil column ($4 \times 0.7$ cm).

The purified extracts (1 μL) were introduced by on-column injection into a gas chromatograph Carlo Erba TOP 8000 (ThermoQuest Italia) equipped with a capillary column (WCOT fused silica CP-Sil 8 CB, Varian USA, $50 \times 0.25$ mm I.D., film thickness 0.25 μm). A Carlo Erba ECD 80 (ThermoQuest Italia) was used as electron capture detector heated at 320 °C.

Reference standard mixtures of *p,p′*-DDE, *p,p′*-DDD (Dr. Ehrenstorfer, Germany) and *p,p′*-DDT Pestanal® (Riedel-de Haen, Germany) were prepared dissolving 10 mg of pure compounds in iso-octane and diluting this solution to the final concentration of 10 μg $L^{-1}$. A commercial mixture of Aroclor 1260 (10 mg $L^{-1}$ in iso-octane, Dr. Ehrenstorfer, Germany) was used to prepare the reference standard for PCB determination. Single PCB congeners were identified and quantified both by reference-pure PCBs (BCR, Brussels, Belgium) and by data from literature (Newman et al., 1998). Good laboratory practices were tested on the standard reference material® 2977 (lyophilized mussel) of the National Institute of Standard & Technology (NIST), Gaithersburg, MD, USA, kindly provided by IAEA (Monte Carlo) (Table 2) extracting and analysing the sample in triplicate. According to recovery efficiency and to the results of a previous intercalibration exercise (CIPAIS Commissione Internazionale per la Protezione delle Acque Italo-Svizzere, 1999) the total analytical variability is assumed to be about 25%.

#### 2.2.2. Organotin (OT) compounds

OT standards were purchased from STREM Chemicals (Bischheim, France). 2,2,4-trimethylpentane (VWR, France) was used in GC/MS/MS. Methanol for HPLC (JT Baker, France) and hydrochloric acid 99% (JT Baker, France) were used as constituents of the extraction mixtures. Sodium tetraethylborate, min 98% (STREM Chemicals, Bischheim, France), acetic acid glacial, 99% (Lancaster, Bischheim, France) and ammonium acetate 98% (Lancaster, Bischheim, France) were used for the derivatization procedure.

GC/MS/MS analyses were performed using a ThermoQuest (Les Ulis, France) system consisting of a Trace GC 2000 gas chromatograph equipped with a PTV split–splitless temperature injector, an AS 2000 autosampler and a POLARIS Q ion-trap mass spectrometer (Thermofinnigan, Les Ulis, France). For data processing, Excalibur software from Thermofinnigan was used. The injector was equipped with a $12 \times 2$ mm I.D. Silcoseeve liner (Thermofinnigan). Of the sample, 2 μl were injected onto the PTV injector in constant flow mode set at 1 mL $min^{-1}$ and with an injection rate of 1 μl $s^{-1}$. The split flow was set at 50 mL $min^{-1}$. The temperature of the injector was initially set at 85 °C then increased to 300 °C at a rate of 10 °C $s^{-1}$ where it was maintained during 12 min. The PTV split/splitless valve was operating in splitless mode until the temperature of 300 °C was achieved. Once the temperature stabilized, it was maintained for a period of 1.5 min and then changed to split mode.

Table 2
Average organochlorine compound recoveries (%) and LOQs on a reference material

| Pesticides | Recoveries ± RSD (%) | $LOQ_s$ (μg/kg) |
|---|---|---|
| *Pp′*DDT | 98.33 (7.37) | 0.01 |
| *Pp′*DDD | 63.33 (15.27) | 0.01 |
| *Pp′*DDE | 76.23 (5.05) | 0.05 |
| PCB 151 | 101.00 (1.00) | 0.05 |
| PCB 149 | 76.33 (1.53) | 0.05 |
| PCB 153 | 90.00 (6.00) | 0.05 |
| PCB 187 | 76.50 (0.75) | 0.05 |
| PCB 180 | 64.50 (5.50) | 0.05 |
| PCB 170 | 73.00 (4.00) | 0.05 |
| PCB 194 | 76.00 (3.46) | 0.05 |

Compounds were separated on a $30 \times 0.25$ mm I.D. column, coated with 0.25 μm of 65% dimethyl-35% phenyl polysiloxane phase (BPX-35, SGE, Courtaboeuf, France). The temperature of the column was initially set at 85 °C for 1 min, then increased from 85 to 130 °C at a rate of 10 °C $min^{-1}$, from 130 to 225 °C at 4 °C and finally from 225 to 280 °C at 15 °C $min^{-1}$. Helium was used as the carrier gas at a constant flow of 1 mL $min^{-1}$. The transfer line was set at 300 °C with the external ion source at 280 °C. The ions in electronic impact (EI) for the target species were selected and fragmented with helium gas by collision induced dissociation (CID) in the ion trap. The second order mass spectra resulting from the most intense fragment were scanned from $m/z$ ion 50 to the mass of the selected ions.

The concentrations were calculated using the calibration curves established for each compound in internal standardisation mode with tripropyltin and diheptyltin as internal standards.

Biota samples were freeze-dried and 500 mg of each freeze-dried sample was extracted with 30 mL of HCl (0.2 M) in methanol, during 30 min. After extraction, 5 mL of extract were mixed with 2 mL of Sodium tetraethlylborate (2%) at pH 4.8 (100 mL of acetate buffer 0.6–1 M). OT species were recovered in 2–5 mL of 2,2,4-trimethylpentane and evaporated to 1 mL under gentle nitrogen flow, before analysis by GC-MS/MS.

Butyltin compound average recoveries for certified reference material (mussel, CRM 477, Brussels, Belgium) were $84 \pm 12$ and $94 \pm 25$. Recovery tests were done in triplicate.

For all biota for which there is no CRM available, successive recovery experiments were carried out after spiking the tissues with OT standards. Table 3 shows the recoveries for OT compounds in biota samples. *Mytilus trossulus*, *Platychtis flesus* and *Mya arenaria* were extracted in triplicate to assess the standard deviation of the procedure and the standard deviation is reported in bracket. The other results are single determinations of pool of individuals of the same species. Recoveries are quantitative for all species for TBT and below 70% in some species only for disubstituted and monosubstituted OT (Table 3). Limit of quantification (LOQ) in biota samples was 20 ng $g^{-1}$ on wet weight as Sn for TBT and 10 ng $g^{-1}$ for MBT and DBT.

#### 2.2.3. Fungicides

Spiked and non-spiked samples, 1 g, were gently blended and dispersed on 1 g of Florisil into a glass mortar using a pestle in order to obtain a homogeneous mixture (5 min). The mixture was then transferred into a column constructed from a syringe barrel, which already contained 1 g of alumina and a frit that retains the entire sample. To the column, 10 mL (2 × 5 mL) of dichloromethane were added and the sample was allowed to elute drop-wise by applying a slight vacuum. The effluent was collected and concentrated, under a gentle stream of nitrogen to 0.05–0.02 mL for LC and GC analysis, respectively. Extracts were stored at 4 °C until being analysed.

The selected phenylurea herbicides as well as their metabolites were analyzed by means of a reversed-phase high performance liquid chromatography Shimadzu, Kyoto, Japan (Model LC-10ADVp) coupled to a UV diode array detector (Shimadzu, Model SPD-10AVp). The analytical column was a 25 × 4.6 mm $C_{18}$ packed with 5 μm particles (Supelco, Bellefonte, PA, USA). The detector was set at 252 and 250 nm. The mobile phase consisted of acetonitrile (HPLC-grade): water 10:90% (v:v). The flow rate was 1 mL $min^{-1}$ and the volume injected 20 μL. The oven temperature was set to 30 °C.

The analyses of vinclozolin and fenarimol were performed using a Shimadzu 14A gas chromatograph equipped with $^{63}Ni$ electron capture detector (ECD) at 300 °C and with a dimethylpolysiloxane (DB-1) column, 30 m × 0.32 mm × 0.25 μm. The temperature programme used for the analysis was from 55 °C (2 min) to 210 °C (15 min) at 5 °C $min^{-1}$ and to 270 °C at 10 °C $min^{-1}$. The injector was set to 220 °C in the splitless mode. Helium and nitrogen were used as carrier and make-up gas respectively for the GC-ECD system.

Confirmation of compounds was performed by GC-MS, using a QP 5000 Shimadzu instrument equipped with a DB-5-MS capillary column, 30 × 0.25 mm × 0.25 μm, containing (5% Phenyl)-methylpolysiloxane (J&W Scientific, Folsom, CA, USA) under the following chromatographic conditions: Injector temperature 220 °C, oven temperature program 55 °C (2 min) to 210 °C (20 min) at 5 °C $min^{-1}$ and to 270 °C at 10 °C $min^{-1}$. Helium was used as the carrier gas. The interface was kept at 290 °C and the spectra were obtained at 70 eV. The splitless mode was used for injection. The analyses of the compounds were performed in the selected ion-monitoring (SIM) mode. In this way, it was possible to establish the best conditions with respect to sensitivity and selectivity. The low pesticide levels made the use of the SIM mode necessary, which provided response factors up to 10 times higher than the full scan mode.

Recoveries (%) of these pesticides and metabolites spiked at 50.0 μg $kg^{-1}$ and $LOQ_s$ are reported in Table 4 as means and standard deviations of determinations performed in triplicate.

Table 3
Average organotin compound recoveries (spiked at 6 μg/g as cation) in biota samples from Gdańsk Gulf (SD in brackets fro extractions performed in triplicate)

| Biota | Recoveries (%) | | |
|---|---|---|---|
| | MBT | DBT | TBT |
| *Plathichtys flesus* | 135 (12) | 95 (3) | 111 (4) |
| *Psetta maxima* | 99 | 89 | 106 |
| *Gasterosteus aculeatus* | 95 | 79 | 126 |
| *Gobius microps* | 94 | 87 | 111 |
| *Mya arenaria* | 102 (6) | 106 (8) | 76 (8)[a] |
| *Mytilus trossulus* | 107 (16) | 109 (15) | 107 (2) |
| *Cerastonema glaucum* | 56 | 76 | 104 |
| *Eriocheir sinensis* | 116 | 66 | 86 |
| *Crangon crangon* | 70 | 57 | 101 |

[a]Not spiked with TBT.

Table 4
Average recoveries (%) of pesticides and metabolites spiked at 50.0 μg $kg^{-1}$ and LOQs

| Pesticides | Recoveries ± RSD (%) | $LOQ_s$ (μg/kg) |
|---|---|---|
| Diuron | 89 ± 9 | 1.5 |
| Linuron | 92 ± 8 | 1.20 |
| DCPU = 1-(3,4-dichlorophenyl)urea | 68 ± 11 | 2.3 |
| DCPMU = *N*-(3,4-dichlorophenyl)-*N*-Methylurea | 87 ± 9 | 2.0 |
| 3,4-DCA = 3,4-dichloroanaline | 60 ± 13 | 3.0 |
| Fenarimol | 79 ± 7 | 1.8 |
| Vinclozolin | 72 ± 8 | 2.0 |

## 3. Results

No living bottom fauna was found in the Dead Vistula River canal (stations from 1 to 7) as well as in Gdynia shipyard (stations 81–83), and at sites 11 and 14 (Table 1).

OT and OC compound concentrations in biota samples are shown in Table 5. For some species, only OT compounds could be determined because the amount of sample was not enough for OC compound determination. The species occasionally found (*Hyperoplus lanceolatus*, *Macoma baltica*, *Pungitius pungitius, Palaemon adspersus*) were not taken into consideration for analytical determinations.

PCBs and *p,p′*-DDE are the major pollutants among the organochlorine compounds. The parent *p,p′*-DDT compound could also be detected in the organisms living in these coastal waters. TBT is in higher concentrations than OCs while DBT was detected in a lower number of samples and MBT was always below the LOQ of 10 ng $g^{-1}$ w.w. The highest concentrations are reached in *M. arenaria* for OT compounds (Table 5) while *Eriocheir sinensis*, which has a very high level of lipid content in its tissues (Table 6), shows the highest OC contamination levels.

The TBT concentrations in *Crangon crangon* are rather the same in all the sampling sites (Fig. 2a) but station 20 where these compounds are significantly lower. The opposite situation is observed for organochlorine compounds, which reach the highest levels tissues at station 20 (Fig. 2b) in correspondence of the Reda river outlet (Table 1).

The phenylurea herbicides and fungicides were only occasionally found in biota samples: Linuron was 15.4 μg $kg^{-1}$ d.w. in *Mytilus trossulus* at station 8; Diuron concentration in *Mya arenaria* soft tissues was 11.1 μg $kg^{-1}$ d.w. at station 80. Fenarimol was only detected in *Mytilus trossulus* at station 32 (6.0 μg $kg^{-1}$ d.w.). Vinclozolin was never detected.

These compounds are water soluble and easily metabolisable, thus they do not bioaccumulate. The Linuron metabolite, DCPMU, was found in the mussel tissues where the parent compound was also detected, while traces of DCA were detected in *Mya arenaria* tissues polluted by Diuron. The occurrence of Linuron herbicide at the Dead Vistula River Canal outlet should have a land based origin.

Table 5
Organotin and organochlorine compound concentrations in biota from the Gulf of Gdańsk

| Station | Species | ng/g as Sn w.w. | | ng/g w.w. | | | |
|---|---|---|---|---|---|---|---|
| | | TBT | DBT | $PCB_{tot}$ | *p,p*′-DDE | *p,p*′-DDD | *p,p*′-DDT |
| 8 | *Mytilus trossulus* | 26 | <LOQ | 11.61 | 0.39 | 0.01 | 0.10 |
| 9a | *Platichthys flesus* | 106 | 34 | 7.19 | 2.23 | 0.35 | 0.33 |
| 9a | *Mytilus trossulus* | <LOQ | <LOQ | 4.28 | 0.72 | 0.07 | 0.16 |
| 9a | *Crangon crangon* | 205 | 43 | 3.05 | 1.42 | 0.04 | 0.12 |
| 9b | *Crangon crangon* | 203 | <LOQ | 4.50 | 1.31 | 0.03 | 0.10 |
| 12 | *Crangon crangon* | 203 | <LOQ | 1.83 | 1.70 | 0.08 | 0.11 |
| 12 | *Cardium glaucum* | <LOQ | <LOQ | 2.60 | 0.42 | 0.05 | 0.04 |
| 12 | *Mya arenaria* | 149 | <LOQ | 6.02 | 1.01 | 0.14 | 0.17 |
| 13 | *Crangon crangon* | 224 | <LOQ | 1.11 | 0.74 | 0.02 | 0.06 |
| 15 | *Platichthys flesus* | 46 | <LOQ | 5.22 | 1.00 | 0.06 | 0.23 |
| 15 | *Psetta maxima* | 253 | <LOQ | 7.71 | 2.13 | 0.13 | 0.46 |
| 15 | *Gobius microps* | 60 | <LOQ | 3.55 | 0.83 | 0.08 | 0.15 |
| 15 | *Gasterosteus aculeatus* | <LOQ | <LOQ | 27.87 | 5.23 | 0.10 | 2.07 |
| 15 | *Crangon crangon* | 288 | <LOQ | 1.36 | 0.75 | 0.01 | 0.04 |
| 20 | *Crangon crangon* | 84 | <LOQ | 5.89 | 5.10 | 0.19 | 0.30 |
| 20 | *Mya arenaria* | 1453 | 118 | 4.22 | 2.90 | 0.01 | 0.04 |
| 22 | *Platichthys flesus* | <LOQ | <LOQ | | | | |
| 22 | *Mya arenaria* | 1804 | 142 | | | | |
| 30 | *Mytilus trossulus* | <LOQ | <LOQ | | | | |
| 30 | *Mya arenaria* | 1425 | 140 | 4.88 | 3.23 | 0.22 | 0.15 |
| 32 | *Platichthys flesus* | 60 | <LOQ | 15.29 | 3.06 | 0.10 | 0.69 |
| 32 | *Psetta maxima* | 418 | 47 | 12.66 | 2.89 | 0.41 | 0.43 |
| 32 | *Crangon crangon* | 168 | <LOQ | 4.56 | 1.43 | 0.08 | 0.12 |
| 32 | *Mytilus trossulus* | 133 | 37 | 0.40 | 0.09 | 0.01 | 0.04 |
| 33 | *Crangon crangon* | 289 | <LOQ | | | | |
| 40 | *Platichthys flesus* | 93 | <LOQ | 8.79 | 2.16 | 0.27 | 0.32 |
| 40 | *Gobius microps* | 52 | <LOQ | | | | |
| 40 | *Psetta maxima* | 369 | <LOQ | 18.80 | 4.11 | 0.53 | 0.69 |
| 40 | *Crangon crangon* | 208 | <LOQ | 2.70 | 0.83 | 0.01 | 0.05 |
| 41 | *Platichthys flesus* | 77 | 41 | 9.16 | 1.48 | 0.54 | 0.30 |
| 41 | *Psetta maxima* | 340 | 38 | | | | |
| 41 | *Crangon crangon* | 169 | <LOQ | 5.94 | 4.97 | 0.12 | 0.26 |
| 42 | *Platichthys flesus* | 92 | <LOQ | | | | |
| 42 | *Crangon crangon* | 175 | <LOQ | 1.84 | 0.76 | 0.04 | 0.09 |
| 43 | *Platichthys flesus* | 47 | <LOQ | | | | |
| 43 | *Gobius microps* | 85 | <LOQ | | | | |
| 43 | *Crangon crangon* | 160 | <LOQ | 1.72 | 0.74 | 0.01 | 0.05 |
| 44 | *Platichthys flesus* | 95 | <LOQ | 6.84 | 4.79 | 0.19 | 0.50 |
| 44 | *Psetta maxima* | 355 | <LOQ | 15.86 | 4.13 | 0.53 | 0.76 |
| 44 | *Crangon crangon* | 144 | <LOQ | 2.28 | 0.93 | 0.03 | 0.04 |
| 44 | *Mytilus trossulus* | 13 | <LOQ | 3.35 | 0.88 | 0.06 | 0.15 |
| 60 | *Platichthys flesus* | 61 | 32 | 17.09 | 7.99 | 0.36 | 0.61 |
| 60 | *Mytilus trossulus* | 13 | <LOQ | 3.14 | 1.96 | 0.06 | 0.04 |
| 60 | *Crangon crangon* | 212 | 35 | 2.92 | 3.14 | 0.02 | 0.05 |
| 61 | *Platichthys flesus* | <LOQ | <LOQ | 13.18 | 7.92 | 0.78 | 0.18 |
| 61 | *Gasterosteus aculeatus* | 66 | <LOQ | | | | |
| 61 | *Eriocheir sinensis* | <LOQ | <LOQ | 292.16 | 144.42 | 6.29 | 7.00 |
| 61 | *Crangon crangon* | 222 | 65 | 5.42 | 2.54 | 0.06 | 0.19 |
| 61 | *Mya arenaria* | 1824 | 184 | 2.97 | 2.93 | 0.27 | 0.79 |
| 61 | *Mytilus trossulus* | 15 | <LOQ | 6.72 | 6.94 | 0.48 | 0.25 |
| 70 | *Platichthys flesus* | 114 | <LOQ | 10.04 | 2.87 | 0.16 | 0.36 |
| 70 | *Eriocheir sinensis* | 97 | <LOQ | 302.84 | 145.42 | 0.69 | 8.41 |
| 70 | *Crangon crangon* | 221 | 52 | 2.26 | 1.49 | 0.01 | 0.11 |
| 70 | *Mytilus trossulus* | 34 | <LOQ | 3.41 | 0.61 | 0.03 | 0.11 |
| 80 | *Platichthys flesus* | 88 | <LOQ | 7.50 | 5.03 | 0.35 | 0.56 |
| 80 | *Psetta maxima* | 321 | 41 | | | | |
| 80 | *Crangon crangon* | 224 | 106 | 2.67 | 1.94 | 0.11 | 0.11 |
| 80 | *Eriocheir sinensis* | <LOQ | <LOQ | 234.25 | 132.57 | 6.16 | 11.05 |
| 80 | *Mya arenaria* | 1262 | 111 | 2.27 | 0.94 | 0.12 | 0.16 |

Table 6
Lipid and water content (%) in the different aquatic species collected in the Gulf of Gdańsk

| % | *Mytilus trossulus* | *Mya arenaria* | *Crangon crangon* | *Eriocheir sinensis* | *Platichtys flesus* | *Psetta maxima* | *Gobius microps* | *Cerastonema glaucum* | *Gasterosteus aculeatus* |
|---|---|---|---|---|---|---|---|---|---|
| Lipid | 0.4 | 0.7 | 0.7 | 15.8 | 1.2 | 1.1 | 0.9 | 0.5 | 2.7 |
| Water | 91.7 | 86.2 | 77.6 | 60.6 | 80.0 | 79.3 | 77.2 | 90.3 | 72.6 |

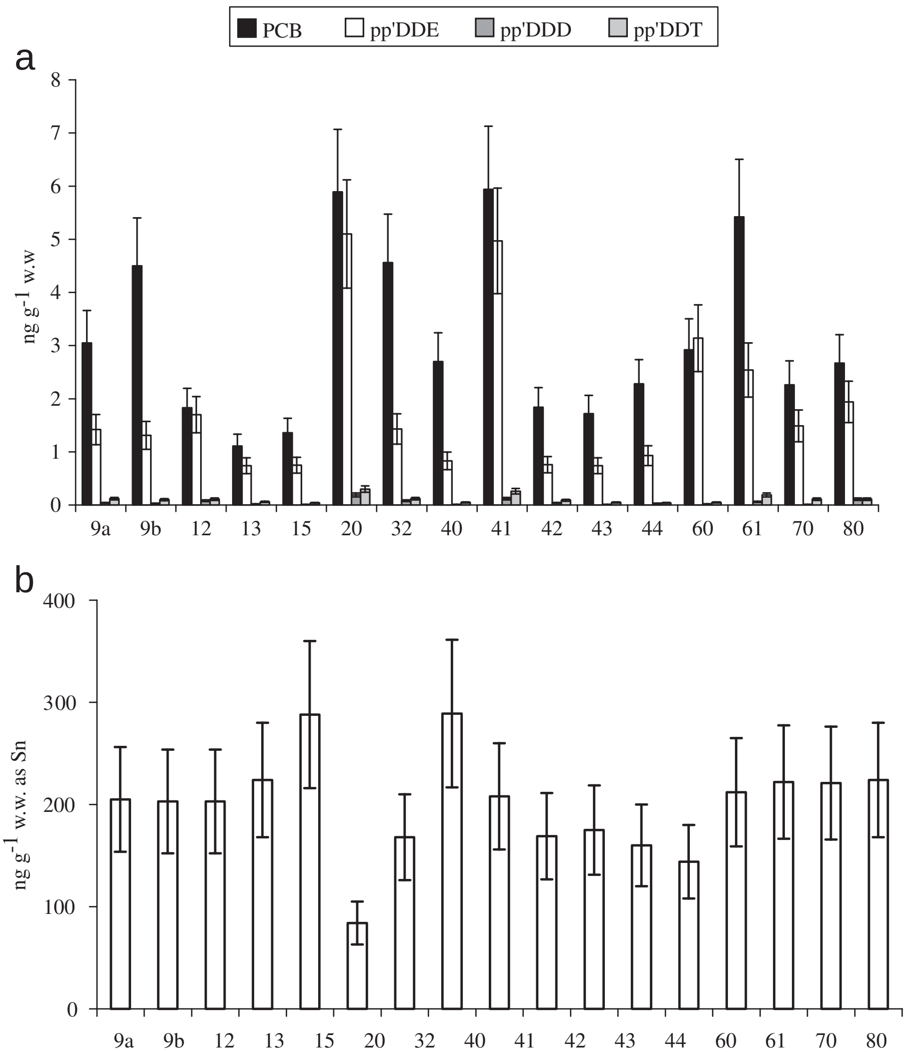


Fig. 2. Concentrations of organochlorine compounds (a) and TBT (b) in *Crangon crangon* (bars refer to total analytical variability-for the identification of the sampling station see Fig. 1).

## 4. Discussion

The absence of bottom fauna in certain areas of the Gulf is probably due to the coarse pollution in these aquatic environments for several years. Sediment analyses and aquatic animal experimentally exposed to the sediments where organisms were collected (Falandysz et al., 2006) confirm that micropollutants are present in the inner canals of the town of Gdańsk and in correspondence of the river inlets at much higher levels than along the coast.

It is widely recognized that not all the species are well-suited to become potential candidates as sentinel organisms; conversely, in the case of the Gulf of Gdańsk some potentially suitable species were not available at all the different sampling sites (Table 1). In fact, although the mussel is considered a useful bioindicator for the pollution of coastal areas, it can be absent along sandy shores when no human manufactured products are available for its attaching. As a consequence the use of other more widespread species, like *Crangon crangon*, as in our case, resulted particularly useful for the PTS characterization of the coastal areas, even in absence of the blue mussel.

Different pollutant classes do not exhibit the same trend in *Crangon crangon* tissues: OC compounds (Fig. 2a) seem to be much related to land-based sources of pollution since they are found close to the river mouths (Stations 9b, 20, 41, 61) or to urban discharges (Station 32). Actually, agricultural and barren land soil in Poland has been identified as a temporal reservoir of intensively OC use (Falandysz et al., 2000). The presence of the parent compound *p,p′*-DDT suggests a rather recent pollution origin.

In contrast, TBT (Fig. 2b) seems to be transported for long distances from the harbour zones or discharged directly in the sea.

The high variability between the contamination levels at the sampling sites shown by the results of the monitoring performed with *Crangon* does not enable to assess that the Gulf of Gdańsk is more polluted than the Pomeranian Bay (Potrykus et al., 2003): it looks a very complex ecosystem where PTS pollution along the coast line ranges from moderate levels (probably comparable to other coastal areas outside the Gulf) to very high levels, including a number of well known point sources.

However, the filter-feeding molluscs remain the most suitable sentinel species for the comparison on a larger environmental scale and for the evaluation of the pollution temporal trend as recommended by Bolognari et al. (1978), Gustavson and Jonsson (1999) and Midorikawa et al. (2004) given that exactly the same sampling sites are selected for the comparison.

Moreover, the multispecies approach used in the present work to investigate the contamination of the Gulf of Gdańsk pointed out the presence of species which can be considered target organisms of a particular kind of pollution.

In fact, aquatic organisms collected at the same sampling station (see, station 61 for instance, Table 5) reflect different contamination degrees for the pollutants considered in this study: TBT has the highest concentration in *Mya arenaria* while PCB and DDT homologues accumulate in *Eriocheir sinensis* to the greatest extent. OT compounds were not quantifiable both in *Eriocheir sinensis* and in *Platichthys flesus* tissues while *Mytilus trossulus* was able to bioaccumulate both OC and OT compounds.

Differences between OC concentrations in various species decrease drastically when data are normalised for lipid content (Table 6; Fig. 3); although it is well known that these hydrophobic pollutants are bioconcentrated in the aquatic organisms according to their lipid body burden, biomagnification should also be expected in carnivorous species. Nevertheless, physiological and ecological factors probably overwhelmed biomagnification contribution. For instance, *Platichthys flesus* specimens were at juvenile stages. If on one hand, this condition can guarantee that they are rather sedentary and therefore representative for the sampling area, on the other hand they were simply too young to reach the bioaccumulation equilibrium for hydrophobic chemicals. Conversely, the predator crab, *Eriocheir sinensis*, is not to be considered as a resident species because most of the specimens collected in this coastal area should be female migrating from the inner freshwaters for reproductive purposes.

The levels of TBT in *Mya arenaria* are much higher than in *Mytilus trossulus,* even if normalized on lipids (Fig. 3). The benthic clam *Mya arenaria* is recognized as a selective species able to extract OT compounds from bottom sediments (Harino et al., 2005). In fact, it lives buried in

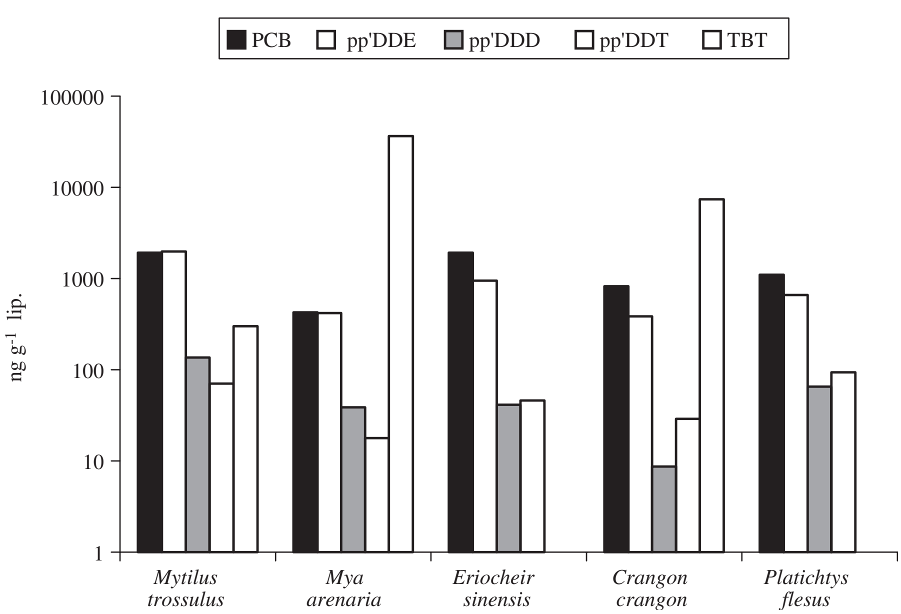


Fig. 3. PTS concentrations in different organisms collected at station 61, expressed on neutral lipids (for the identification of the sampling station see Fig. 1).

shallow water sand or mud from 20 to 75 cm of depth and is tolerant to pollution. As it takes oxygen and food from the water column by its long siphon, this mussel should consume more or less the same food as the sedentary *Mytilus* species, which lives attached to hard substrates. However, *Mya arenaria*, moving up and down in the sand or in the mud, re-suspends much more polluted deeper sediment that releases tributyltin adsorbed on its surface. PCB and DDE, which are much more hydrophobic than TBT, are likely less affected by *Mya arenaria* bioturbation because they are not easily released from the polluted sediments, being much more intensively bound to the organic matter.

## 5. Conclusions

The three different categories of contaminants monitored in this study present a different spatial trend and they certainly came into the Gulf of Gdańsk from different sources. OC compounds are mainly linked to the land-use, OT are directly discharged into the gulf, while fungicides are so little present in the aquatic fauna, probably because of their low persistence and bioaccumulation potential, that their origins remain unknown. A preferential bioaccumulation in the different species depending on their different habitat, feeding habits and physiology was observed; so it was not possible to find out only one species representative of the contamination of the whole area.

A first consequence of these observations is that in the evaluation of the ecological risk the organization of the monitoring programme and the choice of the target species representative of the area becomes crucial.

Another important consequence of the contamination of the aquatic animal living in coastal areas deals with the human health risk since clams, crabs and mussels are consumed as seafood in great part of Europe and US. Therefore, even if consumption of these species is probably negligible in the Gulf of Gdańsk, the capability of selective bioaccumulation of some classes of pollutants in edible species should be considered in further studies addressed to assess risk to human health through food consumption.

## Acknowledgments

This work has been funded by the European Union (COMPRENDO project, contract EVK1-CT-2002-00129). We would like to thank Prof. Efrain Halfon for reviewing this manuscript.

This study which involved the sample of wild animals living in the Gulf of Gdańsk was conducted in accordance with international guidelines for the protection of animal welfare.

## References

Albalat, A., Potrykus, J., Pempkowiak, J., Porte, C., 2002. Assessment of OT pollution along the Polish coast (Baltic sea) by using mussels and fish as sentinel organisms. Chemosphere 47, 165–171.

Bolognari, A., Ferro, R., Fossato, V.U., Piatelli, M., Renzoni, A., Viviani, R., 1978. Monitorage de l'état de la polllution marine le long des côtes Italiennes avec l'emploi des indicateurs biologiques IV, Journées Etud Pollutions, Antalya, C.I.E.S.M., 667–669.

CIPAIS Commissione Internazionale per la Protezione delle Acque Italo-Svizzere, 1999. Ricerche sull'evoluzione del Lago Maggiore, Aspetti limnologici, Programma quinquennale 1998–2002, Campagna 1999, CNR ISE Verbania Pallanza, p. 71.

Falandysz, J., Brudnowska, B., Iwata, H., Tanabe, S., 1999. Organochlorine pesticides and polychlorinated biphenyls in the Vistula River water (in Polish). Roczn. Panstw. Zakl. Hig. 50, 123–130.

Falandysz, J., Trzosińska, A., Szefer, P., Warzocha, J., Draganik, B., 2000. The Baltic Sea: especially southern and eastern regions. In: Sheppard, C.R. (Ed.), Seas at the millennium: an environmental evaluation. Vol. I Regional Chapters: Europe, the Americas and West Africa. Elsevier, Amsterdam, pp. 99–120.

Falandysz, J., Brzostowski, A., Szpunar, J., Rodriguez-Pereiro, I., 2002a. Butyltins in sediments and three-spined stickleback (*Gasterosteus aculleatus*) from the marinas of the Gulf of Gdańsk. Baltic Sea. J. Environ. Sci. Health A 37A (3), 353–363.

Falandysz, J., Wyrzykowska, B., Puzyn, T., Strandberg, L., Rappe, C., 2002b. Polychlorinated biphenyls (PCBs) and their congener-specific accumulation in edible fish from the Gulf of Gdańsk, Baltic Sea. Food Addit. Contam. 19, 779–795.

Falandysz, J., Albanis, T., Bachmann, T.J., Bettinetti, R., Bochentin, I., Boti, V., Bristeau, S., Daehne, B., Dagnac, T., Galassi, S., Jeannot, R., Oehlmann, J., Orlikowska, A., Sakkas, V., Valsamaki, V., Schulte-Oehlmann, U., 2006. Some chemical contaminant of surface sediments at the Baltic Sea coastal region with special emphasys on androgenic and anti-androgenic compounds. J. Environ. Sci. Health 41A (10 Part A), 2127–2162.

Gustavson, K., Jonsson, P., 1999. Some halogenated organic compounds in sediments and blue mussel (*Mytilus edulis*) in Nordic Seas. Mar. Poll. Bull. 38, 723–736.

Harino, H., O'Hara, S.C.M., Burt, G.R., Chesman, B.S., Langston, W.J., 2005. Distribution of organotin compounds in tissues of mussels *Mytilus edulis* and clams *Mya arenaria*. Chemosphere 58, 877–881.

HELCOM, 2003. The Baltic Marine Environment 1999–2002. Baltic Sea Environment Proceedings no. 87, Helsinki Commission, Baltic Marine Environment Protection Commission, p. 48.

Midorikawa, S., Takaomi, A., Harino, H., Ohji, M., Duc Cu, N., Miyazaki, N., 2004. Concentrations of organotin compounds in sediment and clams collected from coastal areas in Vietnam. Environ. Poll. 131, 401–408.

Münch, J., Axenfeld, F., 1999. Emission inventory of the German Baltic Sea catchment area. European Commission (DG XII). Environment and Climate project ENV4-CT96-0214. Research project no. 297 25 527.

Newman, J.W., Becker, J.S., Blondina, G., Tjeedrdema, R.S., 1998. Quantification of Aroclors using congener-specific results. Environ. Toxicol. 17 (11), 2159–2167.

Potrykus, J., Albalat, A., Pempkowiak, J., Porte, C., 2003. Content and pattern of organic pollutants (PAHs, PCBs and DDT) in blue mussel (*Mytilus trossulus*) from the southern Baltic Sea. Oceanologia 45, 337–355.

Senthilkumar, K., Duda, C.A., Villeneuve, D.L., Kannan, K., Falandysz, J., Giesy, J.P., 1999. Butyltin compounds in sediment and fish from the Polish coast of the Baltic Sea. Environ. Sci. Pollut. Res. 6 (4), 200–206.

Szpunar, J., Falandysz, V., Schmitt, O., Obrebska, E., 1997. Butyltins in marine and freshwater sediments of Poland. Bull. Environ. Contam. Toxicol. 58, 859–864.

Szumilas, T., Sobol, Z., 1990. Suggestion for a physical-chemical assessment of the sea coastal waters pollution on the basis of the analysis of the examination on the Gdańsk Bay waters. Bull. Inst. Marit. Trop. Med. Gdynia 41 (1-4), 157–166.