SUMMARY

Mercury (Hg) is one of the most dangerous elements in the environment and has been an important subject of scientific interest for years. This is mainly related to the chemical and biological activity of Hg, its high mobility and rapid spread in the environment as well as the ability to bioaccumulate and biomagnify (Fröstner and Wittmann, 1981; Jackson, 1998). Hg is a highly toxic element – it causes irreversible damage to the brain and nervous system, leading, among others, to Alzheimer's and Parkinson's diseases. Hg also contributes to the impaired function of the kidneys and circulatory system, it can also pass the placental barrier, causing fetal damage and miscarriage (Bose-O'Reilly et al., 2010; Hong et al., 2012). Both Hg toxicity and its bioavailability depend on the form in which it occurs in the environment, with the most dangerous of its organic compounds, especially methylmercury (MeHg).

Hg is introduced into the human body mainly *via* the digestive system, especially through the consumption of fish and other seafood (Boeing, 2000). This is related to the fact that the marine environment was exposed for many years to an uncontrolled inflow of Hg-containing pollutants, both from the land (including surface runoff and industrial wastewater), and atmospheric deposition (Jackson, 1998; Bełdowska et al., 2014; 2016a; Saniewska et al., 2014; Kwasigroch et al., 2018; Zaborska et al., 2019). A significant amount of Hg reaching the sea is accumulated in the coastal zone, mainly through sedimentation on the bottom (Bełdowska et al., 2013; Jędruch et al., 2013; 2015), from where it can be included in the trophic chain by plant and animal benthic organisms (Falandysz, 1994; Boszke et al., 2003; Bełdowska et al., 2015; 2016b). This is of particular importance, given the fact that the coastal zone is a region characterized by high density and rich species composition of the flora and benthic fauna, in relation to the open sea regions (Warzocha, 2009). Organisms inhabiting the seabed are an important element of the marine ecosystem – they are the initial link in the trophic chain, being a significant ingredient in the diet of many fish, birds, and marine mammals, as well as humans.

The level of Hg in marine organisms has been the subject of scientific studies for many years, however, most of them concerned organisms from high trophic levels, such as predatory fish, birds and mammals (Braune, 1987; Skaare et al., 1994; Falkowska et al. 2010; Bełdowska and Falkowska, 2016; Szumiło-Pilarska et al., 2016; Nehring et

al., 2017; Polak-Juszczak, 2017; 2018). In the case of organisms representing the initial links of the food chain, previous research focused mainly on a selected group (e.g. zooplankton, mussels) (Falandysz, 1994; Gutiérrez et al., 2006; Di Leo et al., 2010; Bełdowska and Kobos, 2016; Bełdowska and Mudrak-Cegiołka, 2017). In most cases, these studies also did not take into account Hg levels in the diet of organisms or only one of the potential food sources was analyzed. The research material was mostly collected only during one season (mostly summer) – thus the differences in the metabolic processes of organisms and fluctuation of environmental parameters during the year were not taken into account, as well as changes in the auto- and allochthonous organic matter loads, and thus changes in the inflow and bioavailability of Hg. Moreover, only comprehensive studies of many elements of the marine ecosystem, taking into account the food preferences of organisms and their position in the trophic web, enable the correct determination of the Hg biomagnification factor (Lavoie et al., 2013).

Considering the Hg trophic transfer, it is also important to determine the chemical form of this element – only labile Hg compounds can be absorbed in the tissues of organisms, and as a result of the biomagnification they can reach elevated concentrations in higher-order consumers (Jackson, 1998). Previous studies on organisms with low trophic status mainly concerned the level of total Hg concentration (Falandysz, 1994; Boszke et al., 2003; Meng et al., 2015; Bełdowska et al., 2015; 2016b; Bełdowska and Kobos, 2016) or MeHg (Mikac et al., 1996; Andersen and Depledge, 1997; Loukmas et al., 2006; Molina et al., 2010; Hammerschmidt et al., 2013; Chen et al., 2014). MeHg, although it is the most toxic, is not the only form of Hg that poses a threat to organisms and can be included in the trophic chain. The remaining organic forms of Hg also undergo biomagnification, although their percentage share is much smaller than MeHg (Broussard et al., 2002; Bradley et al., 2017). The scarcity of research on individual Hg forms in the organisms form the basis of the trophic pyramid is mainly due to analytical difficulties – the most popular methods of Hg speciation determination are labor- and cost-intensive due to their multistep character, and require a relatively large sample mass, which is difficult to obtain in the case of organisms of small sizes, such as zoobenthos (Wallschläger et al., 1998; Taylor et al., 2008).

The essence of this work was to fill the gap in studies on the accumulation of Hg in zoobenthic organisms which are an important, initial link of the marine trophic chain. **Therefore, the following research objectives have been formulated**:

i. the indication of the origin of organic matter in the marine coastal zone and determination of mercury concentration in the dietary elements of zoobenthic organisms,
ii. the recognition of factors affecting the spatial and temporal variability of mercury concentration in the bottom fauna,
iii. the determination of the role of zoobenthic organisms in the mercury transfer in the trophic marine chain.

Taking into account the current state of knowledge, including literature studies and the results of research previously conducted by the author, **the research hypotheses verified in this work were formulated as follows**:

i. mercury concentration in zoobenthic organisms depends not only on taxonomy and the feeding mode but also on the origin of organic matter which is important element of their diet and the intensity of primary production in the environment,
ii. accumulation of mercury in organisms representing the initial links of the marine trophic web is related to the environmental conditions of the bottom zone, the character of bottom sediments and the structure of benthic communities, and thus is a subject to variability through the year,
iii. zoobenthic organisms play an important role in the transfer of bioavailable mercury in the marine trophic chain.

The scope of research has been limited to the Gulf of Gdańsk area, however, due to the high biodiversity and biomass of benthic organisms, research stations have been located in its western part – the inner Puck Bay. It is an area of exceptional ecological value, which is associated primarily with the occurrence of underwater meadows of vascular plants, such as the seagrass *Zostera marina* (Węsławski et al., 2013; Jankowska et al., 2019). The intense growth of bottom vegetation in this region is possible mainly due to the small depth (3 m on average) and the gently sloping bottom, as well as its sheltering from the open sea with the Hel Peninsula (Nowacki, 1993). On the other hand, these conditions are conducive to the accumulation of organic matter and pollutants, including Hg, in the inner part of the Puck Bay. The research stations were located in the coastal zone, which was associated with high biomass and biodiversity of benthic flora and fauna, in relation to areas distant from the shore. In addition, benthic organisms inhabiting coastal areas are important source of food for birds and many fish, including species consumed by humans, such as flounder, perch or goby (Warzocha, 2009).

The research material was collected in the years 2011 – 2013, once a month, at two sampling stations located approximately 10 m from the shore (**publication 1**, **publication 2**, **publication 3**). The location of the stations took into account the differences in the eco-hydrodynamic conditions of the bottom zone, as well as the intensity of surface runoff and anthropogenic pressure. The research material consisted of 20 taxa of macrozoobenthos (organisms greater than 0.5 mm in size), represented by mussels (*Cerastoderma glaucum*, *Limecola balthica*, *Mya arenaria*), crustaceans (*Amphibalanus improvisus*, *Bathyporeia pilosa*, *Corophium* sp., *Gammarus* sp., *Idotea* sp., *Jera* sp., *Rhithropanopeus harrisii*, *Lekanesphaera hookeri*), snails (*Peringia* sp., *Radix labiata*, *Theodoxus fluviatilis*), polychaetes (*Hediste diversicolor*, *Marenzelleria* sp., *Streblospio shrubsolii*), oligochaetes, ribbon worms (Nemertea) and insect larvae. In addition to the taxonomic composition, the biomass and abundance of zoobenthos were determined. Also the other components of the marine ecosystem were analyzed – those that are important source of food for benthic fauna and their living environment: suspended particulate matter (SPM) in seawater (surface microlayer, subsurface water), phytoplankton (organisms greater than 20 μm in size), zooplankton (organisms greater than 50 μm in size), macrophytobenthos together with the microflora on its surface (epiphyton), plant biofilm from the surface of stones (epilithon), surface sediments, suspended matter at the water-sediment interface (FLSM), and interstitial waters (Fig. 1). Selected benthic fish, which are the consumers of zoobenthos (the European flounder *Platichthys flesus*, the sand goby *Pomatoschistus minutus*, and the ninespine stickleback *Pungitius pungitius*), were also taken for analysis. Each time, the basic environmental parameters of the bottom zone - temperature, salinity and pH of the near bottom water as well as the oxidation and reduction potential of the sediments were also measured.

Analysis of the total mercury concentration (Hg_{TOT}) in the collected samples was carried out by the thermodesorption method using atomic absorption spectrometry on the AMA-254 analyzer (Altec). What is important, in the case of zoobenthic organisms, the Hg_{TOT} concentration was analyzed in individual species, and if the sample mass was sufficient also in individual organisms. In total, in this part of the study (**publication 1**, **publication 2**, **publication 3**), the Hg_{TOT} concentration analysis was performed in over 1 500 samples (Fig. 1).

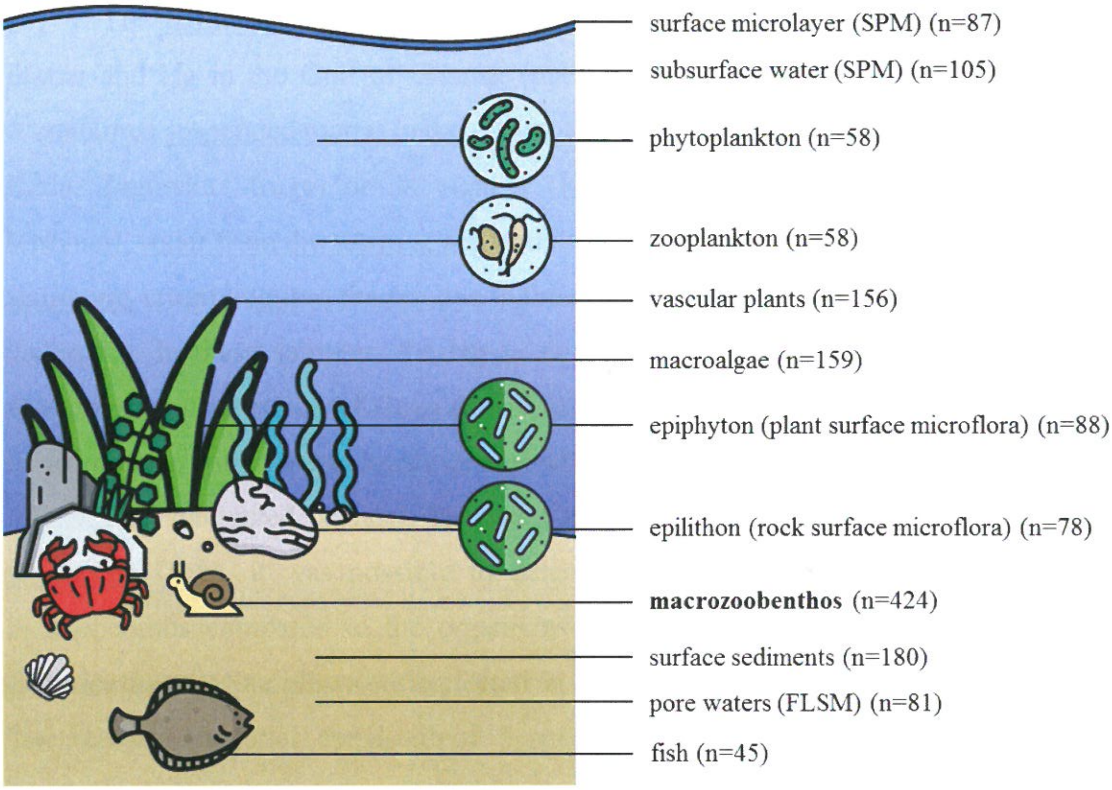

Fig. 1 Investigated components of the marine ecosystem of the coastal zone and the number of samples (n) in which the analysis of total mercury (Hg_{TOT}) was conducted

In addition to the determination of Hg_{TOT} concentration, a number of additional analyses were performed in the collected material in order to help interpret the results. The concentration of suspended particulate matter (SPM) filtered from samples of the surface microlayer and subsurface water was determined. In the obtained SPM the elemental composition (concentration of organic carbon C_{ORG} and total nitrogen N_{TOT}) and the ratios of stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopes were analyzed. For this purpose, the Flash EA 1112 elemental analyzer (Thermo Scientific) combined with the mass spectrometer IRMS Thermo Delta V Advantage (Thermo Electron) was used (**publication 1**). These analyses were carried out at the Marine Biogeochemistry Laboratory of the Institute of Oceanology, Polish Academy of Sciences. Additionally, the ionic composition (Na^{+}, K^{+}, Ca^{2+}, Mg^{2+}, Cl^{-}, SO_4^{2-}) of the interstitial waters was determined using the 850 Professional IC ion chromatography system (Metrohm) (**publication 3**). In the samples of surface sediments, the content of water, the granulometric composition, and the share of organic matter expressed as the loss on ignition at 550°C (LOI) (Santisteban et al., 2004) were also analysed (**publication 2, publication 3**).

The additional material, collected to identify the sources of allochthonous organic matter and Hg in the Gulf of Gdańsk (publication 1), was sampled in 2012 – 2013. It contained suspended matter in the water of rivers flowing into the Puck Bay (Gizdepka, Reda, Zagórska Struga) or its vicinity (Kacza, Wisła), wet atmospheric deposition (Gdynia), water from the storm sewer outlets in the urbanized area (Gdynia), and the cliff sediments crumbling into the sea due to the coastal erosion (cliffs in Gdynia Orłowo and Osłonino). In these samples, the Hg_{TOT} concentration was determined, as well as the elemental (concentration of C_{ORG} and N_{TOT}) and isotopic ($\delta^{13}C$ and $\delta^{15}N$) composition, in accordance with the previously described methods (**publication 1**).

The conducted research has been supplemented by the analysis of additional organisms. Thus, it was possible to determine the contribution of labile Hg forms in zoobenthos compared to the organisms located at the top of the trophic pyramid (**publication 4**). Samples were collected in 2016 – 2017 in the Gulf of Gdańsk region. The research material consisted of 7 species of benthic macrofauna (*Peringia* sp., *Limecola balthica*, *Gammarus* sp., *Idotea* sp., *Saduria entomon*, *Eriocheir sinensis*, *Hediste diversicolor*), 3 species of fish (herring *Clupea harengus*, cod *Gadus morhua*, salmon *Salmo salar*), and gray seal *Halichoerus grypus*. The Hg_{TOT} concentration analysis and determination of contribution of the individual Hg forms were performed by the thermodesorption method (Saniewska and Bełdowska, 2017; Bełdowska et al., 2018), using the DMA-80 analyzer (Milestone). This technique allows to separate five Hg fractions, taking into account its bioavailability for the environment – three labile fractions: i. $Hg_{labile\ 1a}$ (mainly halides of Hg), ii. $Hg_{labile\ 1b}$ (organic Hg, including MeHg), iii. $Hg_{labile\ 2}$ (Hg sulfate and oxide), and two stable forms: iv. HgS (Hg sulfide), v. Hg_{res} (residual Hg). What is important, the Hg fractionation method by thermodesorption used in this research for analysis of animal tissues was the first such case in the world literature. In order to verify the correctness of the method, some samples were also subjected to analysis of MeHg concentration, which was carried out using the gas chromatography-atomic fluorescence spectrum combination instrument MERX-M (Brooks Rand) (**publication 4**).

The **obtained results** allowed for the implementation of the objectives of this dissertation. To achieve the first research aim, which was **the indication of the origin of organic matter in the Puck Bay and determination of the mercury concentration in the elements of zoobenthos diet**, **publication 1** and **publication 2** were dedicated.

In the **publication 1**, it was shown that the research stations in the inner Puck Bay, despite the small distance separating them (about 10 km in a straight line), differed in terms of the amount and quality of organic matter. Suspended matter (SPM) in the seawater at the station located on the west coast of the Puck Bay was typically terrestrial, which was confirmed by the results of the elemental and isotopic composition analysis. It was connected with the intensive surface runoff in this region, caused by the proximity of river outlets, such as Reda, Gizdepka or Zagórska Struga, but also the vicinity of eroded cliff sections, including the Osłonino cliff. In the case of a research station located near the Hel Peninsula the SPM was characterized by marine origin, which was related to the station's distance from the land. The main source of the SPM in this region was primary production *in situ* – phytoplankton, microalgae, and their degradation products. Also a significant impact on the organic matter composition at this station had the material from the open parts of the sea transported with the currents along the Hel Peninsula. The intensity of surface runoff also determined the concentration of SPM, as well as the level of Hg in the coastal zone. In the area of the station under the influence of the land, the Hg concentration in the SPM was more than twice as high as the values measured in the area of the station with limited land impact. This is important, considering the fact that SPM is a significant food source for zoobenthic organisms in the research area (Jankowska et al., 2018). Diversified Hg load introduced into individual regions of Puck Bay also resulted in a different Hg concentration in other components of the environment, as described in **publication 2**. It showed that, similarly as in the case of SPM, Hg concentration in other food sources of benthic fauna (phyto- and zooplankton, micro- and macrophytobenthos) was higher in the region with increased surface runoff. However, the largest differences were observed in the case of Hg concentration in surface sediments – in the area under the influence of land, the measured values were more than three times higher than in the area influenced by the open sea. It was related to the sedimentation of Hg-rich suspended matter, which was possible due to the small water dynamics in the area of the station located on the western shore of the Puck Bay. The exception was the near-bottom suspended matter (FLSM), in which higher concentrations of Hg were measured at a station distant from the land, which may indicate the remobilization of Hg from sediments, as a result of resuspension and diffusion (Bełdowski and Pempkowiak, 2007). Among the investigated food sources of benthic macrofauna, the highest concentration of Hg was determined in zooplankton, and among primary producers, in phytoplankton and epiphytes. The level of Hg measured in macrophytobenthos (vascular plants and macroalgae) was several times lower than in the representatives of the microflora.

The next step of the discourse was the **recognition of factors determining the spatial and temporal variability of mercury concentration in the bottom fauna**, which was the second goal of this dissertation. The results presented in **publication 2** and **publication 3** indicated that the level of Hg concentration in zoobenthic organisms depended on their feeding preferences. The highest concentration of Hg, at both research stations, was measured in grazing macrofauna, organisms feeding mainly on microphytobenthos but also on phytoplankton, characterized by high level of Hg. In the case of macrozoobenthos with other feeding modes, the accumulation of Hg in their tissues depended on the area of research. In the coastal zone with increased surface runoff, enrichment of suspended matter in Hg resulted in an increased concentration of this element in filter-feeders, compared to the area with limited land influence. On the other hand, the elevated concentration of Hg in the suspended matter at the water-sediment interface (FLSM) at the station under the influence of the open sea, led to an increase in Hg concentration in deposit-feeders. The dependence of the Hg concentration level in dietary components on Hg concentration in zoobenthos was also confirmed by the studies conducted in individual months of the research period. In addition, these studies have shown that biotic factors, such as primary production volume and zoobenthos biomass, play an important role in the accumulation of Hg in benthic organisms during the year. It was found that the increase in biomass of organisms in the environment leads to a decrease in Hg concentration in zoobenthos, which is associated with the "biodilution" of Hg. This had a significant effect on the seasonal variability of the Hg concentration in the studied organisms – the lowest concentration of Hg in the bottom fauna was measured in warm months, in which their biomass was the largest. Hg accumulation in zoobenthos was also conditioned by a number of environmental parameters, such as salinity and ionic composition of pore waters. This was visible especially in the case of region affected by increased surface runoff – together with the inflow of fresh water and Hg-rich riverine suspended matter, the concentration of Hg in zoobenthos increased. The level of Hg in the benthic fauna was also shaped by the oxygen conditions of the bottom zone. It was found that oxygen deficiency together with periodically occurring hydrogen sulfide and low pH influenced the limited accumulation of Hg in zoobenthos. An important factor affecting the Hg concentration in benthic fauna and its variability during the year was the intensity of the surface runoff, and hence the fluctuations of the SPM load entering the marine environment. This relationship was observed regardless of the feeding habits of benthic fauna, which indicates that SPM is an important element of their diet, even for species that prefer other sources of food.

The results of the research also enabled the implementation of the third study aim, which was **to determine the role of zoobenthic organisms in the mercury transfer in the marine trophic chain**. In **publication 2**, the Hg transfer was estimated using a model based on the function of ln-normalized Hg concentration in the studied elements of the ecosystem and their position in the trophic web determined by the value of $\delta^{15}N$ (Broman et al., 1992; Rolff et al., 1993). The slope of this function in the literature is defined as TMS (trophic magnification slope) and is currently considered as one of the best indicators of the trophic transfer of pollutants, including Hg, in the aquatic environment (Lavoie et al., 2010; 2013; Kim et al., 2012; Riyadi et al., 2015; Chouvelon et al., 2018). The TMS in the research area was slightly higher than the world average calculated for marine trophic webs (Lavoie et al., 2013). It was also a typical value for the seas of the temperate zone. However TMS does not take into account the structure of the studied trophic web, which affects the flow of energy and chemical substances (Sokołowski et al., 2012). The length of the food chain was taken into account in the case of the BMF (biomagnification factor) of Hg, calculated according to the method of Hobson and Welch (1992). The obtained results showed that the trophic transfer of Hg in the Puck Bay was more efficient (2.2) in the relation to the world average (1.8). Calculated BMF value means that the Hg concentration increased more than twice in each subsequent link of the trophic chain. It was also found that the effectiveness of trophic transfer Hg varied, depending on the studied region. In the case of a station under land influence, the calculated BMF was about 30% lower than in the case of the station with limited surface runoff. This is important, given the fact that the Hg concentration measured in the zoobenthos diet elements in the region under the land influence was higher than at the station under the marine influence. As in the case of Hg accumulation in zoobenthos, its trophic transfer was influenced by the trophic web structure (producers and consumers biomass), macrofauna feeding preferences, and the environmental parameters of the bottom zone (quantity and quality organic matter, pH, temperature and oxygen conditions). In **publication 4** it was found that along with the increase of the organism position in the trophic pyramid, not only the Hg concentration increased but also the share of bioavailable, labile Hg forms ($Hg_{labile\ 1a}$, $Hg_{labile\ 1b}$, $Hg_{labile\ 2}$). The total share of these forms exceeded 90% in all studied zoobenthos species and was close to the values specified for organisms with high trophic status, such as predatory fish and seals. What is more, the most dangerous form of Hg ($Hg_{labile\ 1b}$) – organic Hg, including MeHg, also had a significant proportion. The share of this fraction in zoobenthic organisms was on average 60%, which is about twice as high as the share of MeHg in benthic macrofauna according to the literature data (Andersen and Depledge, 1997; Margetinova et al., 2008). It was also found that MeHg accounted for 90% of organic Hg ($Hg_{labile\ 1b}$) accumulated in tissues of zoobenthic organisms, which is a similar result to the values obtained for apex consumers.

The results obtained in this research allowed to formulate the following conclusions:

i. Organic matter reaching the Puck Bay was characterized by a different origin, depending on the region of the basin. This indicates a different sources of Hg for benthic organisms that feed on this matter. The quality of organic matter in a given region of the Puck Bay depended on the intensity of surface runoff, the amount of primary production and the dynamics of the environment. What is crucial, terrestrial organic matter was characterized by enrichment in Hg in comparison to the matter of marine origin (**publication 1**).

ii. The level of Hg in the bottom fauna was related to the feeding preferences of the organisms. The highest concentration and the highest bioconcentration of Hg were determined in grazing organisms feeding on microphytobenthos and phytoplankton. It is related to the fact, that those primary producers were characterized by an elevated Hg concentration compared to other food sources of macrozoobenthos due to the large adsorption surface area. Hg concentrations in suspension and/or deposit feeders, as well as in omnivores were on average 40% lower than in herbivorous grazers (**publication 2**, **publication 3**).

iii. The Hg concentration in zoobenthos in the coastal zone was shaped not only by the quantity but also by the quality of organic matter. The environmental dynamics influencing the accumulation and transport of Hg in the study area was also particularly important. Intense surface runoff together with limited water exchange favored the increase in Hg level in suspended matter and microphytobenthos and consequently in the filtering fauna that feeds on them. The inflow of the marine matter from deeper regions, including Hg-rich fine-grained sediments, influenced the increase in Hg concentration in deposit feeders (**publication 2**, **publication 3**).

iv. The Hg concentration in zoobenthos varied throughout the year, which was not only related to Hg concentration in the dietary components but also to the seasonal biomass fluctuations, together with changes of environmental conditions and diversified inflow of Hg along with surface runoff (**publication 3**).

v. The Hg concentration in the benthic food web increased more than twice in each subsequent trophic level. The Hg biomagnification factor was about 40% lower in the area under the strong influence of land, compared to the area with limited surface runoff, characterized by lower primary production and smaller biomass of benthic organisms. It was related to the "biodilution" of Hg in the initial links of the food chain (**publication 2**).

vi. Over 90% of Hg in the tissues of zoobenthic organisms was in a bioavailable form, with the dominant share (on average: 60%) of the most dangerous organic Hg. This means that the majority of Hg accumulated in benthic organisms can be transferred to the higher trophic levels. Thus, the investigated organisms play an important role in the trophic transfer of Hg in the marine environment (**publication 4**).

The results presented in this dissertation significantly enrich the knowledge about the cycle of Hg in the environment. They also enable a better understanding of the processes affecting the trophodynamic of this toxic element. The level of Hg and the form in which it occurs in benthic fauna is also important due to the fact that seafood is a significant component of a human diet. Therefore, it is a valid aspect that should be taken into account in the assessment of the potential risk to human health resulting from the increased consumption of marine invertebrates (FAO, 2014). Determination of factors influencing the accumulation of Hg in benthic fauna may also be used in the fishing and mariculture industry, for example in determining fishing areas or planning the location of farms of mollusks or crustaceans.

The results also showed new research perspectives – areas requiring more detailed analyses. An important but poorly recognized aspect, is the determination of the Hg level and the share of its individual forms in the elements of the macrozoobenthos diet, such as phytoplankton or microphytobenthos. An interesting issue is also the concentration of Hg and its forms in meio- and microzoobentic organisms. However, due to the methodical difficulties, such studies have not been conducted so far. The proposed method of determining labile and stable forms of Hg by fractionation using thermodesorption allows to reduce the mass of the analysed sample, as well as to minimize the risk of contamination. This is particularly important in the case of a material with low Hg concentration (Taylor et al., 2008). This technique is also characterized by a short time of analysis, and is also cost-effective because it does not require the use of reagents. It can be also used as a tool for preselection of samples for MeHg analysis. This gives the thermodesorption method the potential for wide application in the studies on the biogeochemical Hg cycle in the environment.