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Trophic Transfer of Mercury in a Temperate Marine Food Web, Southern Baltic Sea

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Abstract **-** Mercury (Hg) is considered to be one of the most dangerous global environmental pollutants. The toxicity and bioavailability of Hg depend on its chemical form, and the greatest hazard is posed by the organomercurial compounds. Hg is introduced into the human body primarily through the consumption of fish and seafood. Despite numerous studies on Hg in marine organisms, there is still a gap in the knowledge on Hg uptake and accumulation by primary producers and animals that occupy a low trophic position, such as benthic invertebrates. The main aim of this study was to investigate the trophodynamics of Hg at the base of the marine food pyramid. The research was carried out in a shallow bay located in the southern Baltic Sea, which is considered a good model of a temperate marine ecosystem. The results showed that the level of Hg in a benthic fauna was strongly affected by the amount and origin of organic matter, both suspended in the water and deposited in bottom sediments. The bioaccumulation of Hg was dependent on the environmental conditions and biomass of the primary producers and consumers. A similar trend occurred in the case of biomagnification of Hg along the trophic chain. The increase in the trophic position of the studied organisms was accompanied by an increase in Hg concentration and an increase in the organic Hg percentage. Despite the differences in the distribution of individual Hg forms in the studied organisms, the bioavailable labile Hg fractions represented more than 90% of total Hg. This indicates an effective transfer of Hg between successive links of the trophic chain and is particularly important because of exposure of Hg to marine organisms but also to humans consuming them.

Keywords**:** Mercury; Trophic transfer; Biomagnification; Aquatic plants; Benthic invertebrates; Baltic Sea

1. Introduction

Mercury (Hg) is one of the most dangerous elements in the environment and has been an important topic of scientific interest for years. This is mainly related to the chemical and biological activity of Hg, its high mobility, and rapid spread in its environment, as well as its ability to bioaccumulate and biomagnify [1]. 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 disease. Hg also contributes to impaired kidney function and circulatory system; it can also cross the placental barrier, causing fetal damage and miscarriage [2]. 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 primarily through the digestive system, especially through the consumption of fish and other seafood [3]. 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 [4, 5]. A significant amount of Hg reaching the sea accumulates in the coastal zone, mainly through sedimentation at the bottom, from which it can be included in the trophic chain by benthic organisms [6-8]. This is of particular importance, given that the coastal zone is a region characterized by high density and rich species composition of the flora and benthic fauna, in relation to open-sea regions [9]. Organisms that inhabit 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 at high trophic levels, such as predatory fish, birds, and mammals [10-15]. 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) [6, 16-19]. 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. Research material was collected only during one season (mainly summer). Consequently, 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. Furthermore, only comprehensive studies of many elements of the marine ecosystem, taking into account the food preferences of organisms and their position in the trophic network, allow the correct determination of the biomagnification factor [20].

Considering the 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 biomagnification they can reach elevated concentrations in higher-order consumers [1]. Previous studies on organisms with low trophic status mainly concerned the level of total Hg concentration [5-7, 18, 21] or MeHg [22-27]. 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 [29, 30]. The scarcity of research on individual Hg forms in organisms form the basis of the trophic pyramid is mainly due to analytical difficulties: the most popular methods of determination of Hg speciation 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 [31, 32].

The essence of this work was to fill the gap in studies on the accumulation of Hg in zoobenthic organisms that are an important initial link of the marine trophic chain. Therefore, the following research objectives have been formulated: i) indication of the origin of organic matter in the marine coastal zone and determination of Hg concentration in the dietary elements of zoobenthic organisms, ii) the recognition of factors affecting the spatial and temporal variability of Hg concentration in the bottom fauna, iii) the determination of the role of zoobenthic organisms in the Hg transfer in the trophic marine chain. Taking into account the current state of knowledge, including studies in the literature and the results of research previously conducted by the author, the research hypotheses verified in this work were formulated as follows: i) Hg concentration in zoobenthic organisms depends not only on the taxonomy and 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 Hg 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 throughout the year, iii) zoobenthic organisms play an important role in the transfer of bioavailable Hg in the marine trophic chain.

2. Materials and Methods

2.1. Study area

The scope of the research has been limited to the Gulf of Gdańsk located in the southern part of the Baltic Sea. Due to the high biodiversity and biomass of benthic organisms, research stations have been located in the western part of the gulf, 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* [33, 34]. 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 by the Hel Peninsula. However, 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, relative to areas far from the shore. In addition, benthic organisms that inhabit coastal areas are an important source of food for birds and many fish, including species consumed by humans, such as flounder, perch, or goby [9].

2.2. Sample collection

The research material collected to determine the Hg burden of benthic organisms was sampled in 2011-2013, once a month, at two sampling stations located approximately 10 m from the shore. The location of the stations took into account the differences in the ecohydrodynamic conditions of the bottom zone, as well as the intensity of surface runoff and anthropogenic pressure. The research material consisted of 20 macrozoobenthos taxa (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., *Jaera* sp., *Rhithropanopeus harrisii*, *Lekanesphaera hookeri*), snails (*Peringia* sp., *Peregriana 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. In addition, other components of the marine ecosystem were analysed, those that are important sources 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 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. Selected benthic fish, which are consumers of zoobenthos (the European flounder *Platichthys flesus*, the sand goby *Pomatoschistus minutus*, and the nine-spine 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. A detailed description of the collection of research material can be found in the author's previous work [35-37].

The additional material collected to identify the sources of allochthonous organic matter and Hg in the Gulf of Gdańsk, 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), rainfall (Gdynia), water from the storm sewer outlets in the urbanized area (Gdynia), and sediments of the cliffs that are crumbling into the sea due to the coastal erosion (cliffs in Gdynia Orłowo and Osłonino).

To determine the contribution of labile forms of Hg in zoobenthos compared to those of organisms located at the top of the trophic pyramid, the conducted research has been supplemented by the analysis of additional organisms. These 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 grey seal *Halichoerus grypus*.

2.3. Laboratory analysis

The 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). Importantly, in the case of zoobenthic organisms, the Hg_{TOT} concentration was analyzed in individual species, and if the sample mass was sufficient in individual organisms. In addition to the determination of the Hg_{TOT} concentration, several additional analyses were performed on the collected material to help interpret the results. The determination of contribution of the individual Hg forms was carried out using the thermodesorption method [38], using the DMA-80 analyzer (Milestone). This technique allows to separate five Hg fractions, taking into account its bioavailability in the environment – three labile fractions: i) $Hg_{labile\ 1a}$ (Hg halides), ii) $Hg_{labile\ 1b}$ (mainly 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). Importantly, the Hg fractionation method by thermodesorption used in this research for the analysis of animal tissues was the first such case in the world literature. To verify the correctness of the method, some samples were also subjected to analysis of the MeHg concentration, which was performed using the gas chromatography-atomic fluorescence spectrum combination instrument MERX-M (Brooks Rand) [38].

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 using the Flash EA 1112 elemental analyzer (Thermo Scientific) combined with the mass spectrometer IRMS Thermo Delta V Advantage (Thermo Electron) was used. 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). In surface sediments, the content of water, the granulometric composition, and the share of organic matter expressed as the loss of ignition at 550°C (LOI) were also analyzed.

3. Results and Discussion

3.1. Hg level at the base of the marine food pyramid

The first step of this study was to indicate the origin of organic matter in Puck Bay together with the determination the Hg concentration in the elements of the zoobenthos diet. The results of this study showed that the research stations Puck Bay, despite the small distance that separates them (about 10 km in a straight line), differed in terms of the amount quality of organic matter [35]. Suspended matter (SPM) in the seawater at the station located on the west coast of Puck 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 tributaries, such as Reda, Gizdepka or Zagórska Struga, but also the proximity 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 a marine origin, which was related to the station's distance from the land. The main source of SPM in this region was the primary production in situ – phytoplankton, microalgae and their degradation products. Additionally, a significant impact on the organic matter composition at this station had 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 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. The diversified Hg load introduced into individual regions of Puck Bay also resulted in a different Hg concentration in other components of the environment [35]. This study showed that, similarly as in the case of SPM, the concentration of Hg 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 near-bottom suspended matter (FLSM), in which higher concentrations of Hg were measured at a station far from the land, which may indicate the remobilization of Hg from sediments, as a result of resuspension and diffusion. Among the investigated food sources of benthic macrofauna, the highest concentration of Hg was determined in zooplankton and among the primary producers, in phytoplankton and epiphytes. The Hg level measured in macrophytobenthos (vascular plants and macroalgae) was several times lower than in the microflora.

3.2. Factors affecting Hg uptake and accumulation

The next step of the discourse was to recognize the factors determining the spatial and temporal variability of the Hg concentration in the bottom fauna. The results indicated that the level of Hg concentration in the zoobenthic organisms depended on their feeding preferences. The highest concentration of Hg was measured at both research stations in grazing macrofauna organisms that feed mainly on microphytobenthos but also on phytoplankton, characterized by a 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 [36, 37]. In the coastal zone with increased surface runoff, the 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. However, the elevated concentration of Hg in suspended matter at the water-sediment interface (FLSM) at the station under the influence of the open sea led to an increase in the concentration of Hg in deposit feeders. The dependence of the level of Hg concentration in dietary components on the Hg concentration in zoobenthos was also confirmed by studies conducted in individual months of the research period. Furthermore, 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 the biomass of organisms in the environment leads to a decrease in the 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 the warm months, in which its biomass was the largest. Hg accumulation of Hg in zoobenthos was also conditioned by several environmental parameters, such as the salinity and ionic composition of the pore waters [36, 37]. This was visible especially in the case of the 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 the periodic appearance of hydrogen sulfide and low pH influenced the limited accumulation of Hg in zoobenthos. An important factor that affects the concentration of Hg in benthic fauna and its variability throughout 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 the benthic fauna, indicating that SPM is an important element of their diet, even for species that prefer other sources of food.

3.3. Trophic transfer of Hg

The results of the study allowed the recognition of the role of zoobenthic organisms in Hg transfer in the marine trophic chain. Hg transfer was estimated using a model based on the function of the ln normalized Hg concentration in the studied elements of the ecosystem studied and their position in the trophic web determined by the value of $\delta^{15}N$. The slope of this function in the literature is defined as TMS (trophic magnification slope) and is currently considered one of the best indicators of the trophic transfer of pollutants, including Hg, in the aquatic environment [20]. The TMS in the research area was slightly higher than the world average calculated for marine trophic webs, but was typical for the seas of the temperate zone [20]. However, TMS does not take into account the structure of the studied trophic web, which affects the flow of energy and chemical substances. 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 described in the earlier work [36]. The results obtained showed that the trophic transfer of Hg in Puck Bay was more efficient (2.2) compared to the world average (1.8). The 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 of Hg varied according to the studied region. In the case of a station under land influence, the calculated BMF was approximately 30% lower than in a case of the station with limited surface runoff. This is important, given the fact that the Hg concentration measured in the elements of the zoobenthos diet in the region under land influence was higher than at the station under marine influence. As in the case of Hg accumulation in zoobenthos, its trophic transfer was influenced by the structure of the trophic web (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). It was found that along with the increase in the position of the organism in the trophic pyramid, not only did 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. Furthermore, 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 approximately twice as high as the share of MeHg in benthic macrofauna according to the data from the literature [23, 39]. 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 [38].

4. Conclusion

The results obtained in this research allowed to formulate the following conclusions: i) the organic matter reaching the Puck Bay was characterized by a different origin, depending on the region of the basin. This indicates a different source of Hg for benthic organisms that feed on this matter. The quality of organic matter in a given region of Puck Bay depended on the intensity of surface runoff, the amount of primary production, and the dynamics of the environment. It is crucial, terrestrial organic matter was characterized by enrichment in Hg compared to the matter of marine origin, 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 these primary producers were characterized by an elevated Hg concentration compared to other food sources of macrozoobenthos as a result of the large surface area. Hg concentrations in suspension and/or deposit feeders, as well as in omnivores were on average 40% lower than in herbivorous grazers, 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 the level of Hg in suspended matter and microphytobenthos and consequently in the filtering fauna that feeds on them. The inflow of marine matter from deeper regions, including Hg-rich fine-grained sediments, influenced the increase in Hg concentration in deposit feeders, 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 seasonal biomass fluctuations, together with changes of environmental conditions and diversified Hg inflow of Hg along with surface runoff, 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, 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 most of the Hg accumulated in benthos can be transferred to higher trophic levels and that the role in this process is substantial.

The results presented in this study significantly enrich the knowledge about the cycle of Hg in the environment. They also enable a better understanding of the processes that affect the trophodynamics of this toxic element. The level of Hg and the form in which it occurs in benthic fauna are also important because seafood is a significant component of a human diet. Therefore, it is a valid aspect that should be taken into account in assessing the potential risk to human health resulting from increased consumption of marine invertebrates. Determination of factors influencing the accumulation of Hg in benthic fauna may also be used in the fishing and mariculture industry, e.g., 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 methodical difficulties, such studies have not been conducted so far. The proposed method for determining labile and stable forms of Hg by fractionation using thermodesorption allows reducing 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 [32]. This technique is also characterized by a short analysis time and is also cost-effective because it does not require the use of reagents. It can also be used as a tool for preselection of samples for MeHg analysis. This gives the thermodesorption method the potential for wide application in studies on the biogeochemical Hg cycle in the environment.

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