BONUS MICROPOLL PROJECT (1 July, 2017 – 30 June, 2020)

Deliverable 3.3 **Report evaluating MP capacity as carriers of organic pollutants to Baltic invertebrates and fish, with particular focus on developing principles for setting GES**

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Work package 3: **Impacts of MP, associated contaminants, and biofilms on Baltic biota**

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MP capacity as carriers of organic pollutants to Baltic invertebrates and fish, with particular focus on developing principles for setting GES

This deliverable is a contribution to task 3.2 (WP3). *Exposure, accumulation of organic pollutants and effects on the physiological and nutritional status in invertebrates*.

Summary

The focus of Deliverable 3.3 is on the transfer of organic pollutants to and from microplastic (MP) when in contact with biota. This report is based mainly on our recently published experimental and field studies, as well as and general considerations on the role of MP as a vector for organic contaminants, with particular focus on the Baltic Sea environment. For the latter, we used relevant literature and data presented in Deliverable 2.2 (MICROPOLL) [1].

Our objectives were:

- To evaluate the potential of MP as carriers of Hydrophobic Organic Contaminants (HOC) to Baltic Sea invertebrates and fish; and
- To assess whether this pathway can exacerbate HOC effects on biota in the Baltic Sea.

To address these objectives, we conducted experimental and field studies, which resulted in two publications that provided both proof-of-principle and empirical support for the thermodynamic modelling of contaminant transfer to and from plastic litter in the pelagic environment:

- Gerdes Z, Ogonowski M, Nybom I, Ek C, Adolfsson-Erici M, Barth A, Gorokhova E (2019) Microplastic-mediated transport of PCBs? A depuration study with Daphnia magna. PLoS ONE 14(2): e0205378. https://doi.org/10.1371/journal.pone.0205378

  Main findings: In zooplankton with a high body burden of PCBs, depuration of CB209 was enhanced 8-fold in the presence of less contaminated MP. However, the capacity of MP and algal cells to remove total PCB from the animal was largely similar. Given the great differences in the relative abundance of algae and MP *in situ*, this implies that as an environmental compartment, algae are more important in HOC transport.

- Ogonowski M, Wenman V, Barth A, Hamacher-Barth E, Danielsson S, Gorokhova E (2019) Microplastic Intake, Its Biotic Drivers, and Hydrophobic Organic Contaminant Levels in the Baltic Herring. Front. Environ. Sci. 7:134. https://doi.org/ 10.3389/fenvs.2019.00134

  Main findings: No correlation was found between MP and HOC concentrations in the Baltic herring from Bothnian Bay in the north to Bornholm in the south, suggesting no apparent impact of MP on HOC transfer and bioaccumulation in the secondary consumers of the pelagic food webs in the Baltic Sea.

BONUS MICROPOLL project has received funding from BONUS (Art 185), funded jointly by the EU and VINNOVA (The Swedish Governmental Agency for Innovation Systems).

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1 Background

1.1 MP as carriers of HOC to biota

Following the discovery of microplastic (MP) as an emerging environmental contaminant, the putative role of MP as carriers of pollutants to organisms via the so-called “Trojan horse” effect [2] has gained much attention, both public and within the research community. There are many reports in recent years concerning loads of organic pollutant adsorbed onto microplastics. The literature survey reveals substances that have received much attention including polyaromatic hydrocarbons (e.g. fluoranthene, phenanthrene, etc.) and persistent organic pollutants such as polychlorinated biphenyls (PCBs), the insecticide DDT and its degradation product p,p’-DDE, as well as hexachlorocyclohexane isomers (HCHs) [3].

There are two types of pollutant transfer from plastic to organisms. One is the transfer of chemicals added to the polymers and polymer-based materials during their production, e.g., various chemical additives affecting the polymer properties. Some of them can leach out of the polymer matrix because they are not covalently bound. The second type of transfer is via absorbance of pollutants from the environment and their subsequent release once inside a living organism or in the immediate surroundings. The hypothesis that *MP sorb HOC when in contact with water or sediment and then transfer them to biota following ingestion* initially took a firm hold on the views and research efforts evaluating MP impacts.

1.2 Some theoretical considerations

In the environment, chemicals are associated with different compartments (e.g., water, mineral solids and biological materials, live or dead, containing carbohydrates, proteins and lipids) depending on the physicochemical properties of these chemicals as well as the environmental compartments. The amount of a chemical in a specific compartment depends on its relative concentration in the other compartments. Passive transfer of chemicals between the compartments follow laws of thermodynamics, and so-called chemical equilibrium is reached when the overall transfer approaches zero. At chemical equilibrium, partitioning of a chemical between, e.g., plastic and lipid tissues of an organism is defined by the equilibrium partitioning coefficient (K) between the two phases:

$$C_{Plastic}/C_{Lipid} = K_{P\text{-}L},$$

where $C_{Plastic}$ and C_{Lipid} are the respective HOC concentrations in the plastic material and the organisms lipids. Note that HOC concentrations in biota are typically normalised their non-polar elements, i.e. lipids or organic carbon. Since many fossil-based polymers structurally resemble lipids and are similarly non-polar, $K_{P\text{-}L}$ approaches 1. In multimedia unequilibrated system, the relationship between the chemical concentrations and their equilibrium partitioning coefficient is essential for predicting the direction of HOC transport. The chemicals in question diffuse in the direction where the concentration ratio approaches K. For organisms ingesting microplastic, this means that when $C_{Plastic}/C_{Lipid} < 1$, the chemical will move from the organism’ lipids into the plastic.

In general, plastic is an excellent sorbent of HOCs and will accumulate high amounts of such substances from the environment [4–7]. Also, MP is especially efficient at taking up HOCs because of their large surface to volume ratio [8]. Mato et al. (2001) evaluated sorption of HOC on pristine MP deployed in marine environments. Based on the observed HOC concentrations in MP, which were 5-6 orders of magnitude higher than those in the surrounding water, they suggested that MP could transfer the pollutants to organisms and called for further studies [6].

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Several experimental studies have demonstrated proof-of-principle for HOC transfer from MP to biota [9–13]. However, for the interpretation of these findings, it is crucial to acknowledge the experimental settings. In general, these studies were designed as follows:

- *Step 1*: non-contaminated test organisms are exposed to contaminated MP;
- *Step 2*: the animal' body burden is measured after the exposure, and HOC uptake from MP is analysed [9,11–14].

Unsurprisingly, all such studies show that the transfer is possible. However, such experimental design is not environmentally relevant, and, thus, the transfer observed in the laboratory conditions does not imply that MP will affect body burden and bioaccumulation *in situ*. The distinction between the principal possibility, and environmental probability, of transfer to occur is highly important from a hazard assessment perspective.

In the environment, there are multiple uptake routes for HOCs to animals, plants and microorganisms. It is also highly probable that in environmentally realistic conditions, all compartments (e.g., MP, organism and environmental media, such as water or sediment) carry some amounts of the contaminants in question. This background contamination implies that physically adjacent compartments are already in equilibrium or approaching it, thus, contrasting the transfer scenario employed in the experimental studies.

An environmentally relevant study on MP bioaccumulation effects must, therefore, consider the existing equilibrium and contributions from all relevant compartments when microplastic is introduced in the system (Fig. 1). Also, one should keep in mind when designing such experiments that due to the long residence time of plastic in the environment, water- or sediment-born MP are likely to be in equilibrium with this media when they come in contact with consumers, such as invertebrates and fish [15].

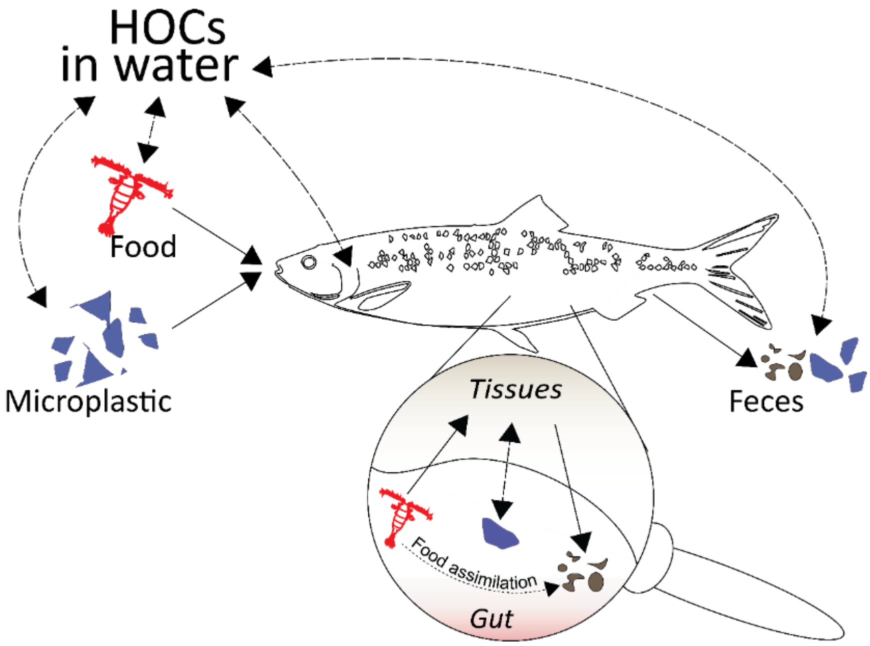

Figure 1. *A conceptual diagram showing main transfer pathways for HOC involving an organism (e.g., fish) and its immediate environment. The arrows indicate the expected direction of the chemical transfer, which can be bi-directional (dashed lines), depending on the concentration ratio between the compartments relative their K. Ingested food is assimilated in the gut and excreted as faeces. Unlike the food that is digested as it moves along the gut, MP remain unaltered during the gut passage.*

HOC uptake from food differs from its uptake via microplastic. In food, HOCs are associated with organic matter, particularly lipids. When food is digested during the gut passage, the amount of lipids decreases and, as a result, HOC concentration in this compartment increases [16], which favours the HOC uptake in the gut (so-called gastrointestinal magnification). By contrast, microplastic remains relatively intact during the gut passage, and HOC transfer between the MP and the gut wall is directly regulated by the initial concentrations in the plastic and the animal. Therefore, to evaluate the relative contributions of HOCs originating from different sources (e.g., MP, water and food) in aquatic consumers, it is not sufficient to compare their initial contaminant loads. Actually, to derive the relative contribution of MP to the bioaccumulation of HOCs, one must account for the partitioning between the compartments, food assimilation and physiological effects that might be excerted by MP and contaminants [17], which usually requires a modelling approach.

Put simply: thermodynamic models use the equilibrium partition coefficients between all different compartments to calculate transfer, contribution and rate of HOC exchange. Such modelling studies conducted to date suggest that the MP contribution to HOC bioaccumulation is negligible in comparison to the dietary intake [18,19]. At first, conclusions by experimental and modelling studies appeared to contradict each other; however, experimental studies designed to validate the model-based predictions are few [17]. Koelmans and co-workers abridged this discrepancy in a common framework, which states that transfer from MP to organisms will occur if the levels of HOCs are higher in the polymer phase than in the organism and $C_{Plastic}/C_{Lipids} > K_{P-L}$ [15]. Thermodynamically, this relationship implies that the opposite transport, from organism to polymer, is also possible. However, for this process, experimental validation has been lacking [20]; this is what motivated us to conduct the study described in the section 2.

1.3 HOC and MP pollution in the Baltic Sea

The contaminant load in the Baltic Sea biota is assessed by the Swedish National Monitoring Program for Contaminants in Marine Biota (SNMPC). This program conducts yearly measurements of pollutants, such as the legacy persistent organic pollutants, POPs (e.g. PCBs, dioxins and polybrominated diphenyl ethers), in fish and mussels along the Swedish coastline. Although levels of these contaminants significantly decreased over the last decades, the concentrations in the Baltic Sea remain relatively high compared to the North Sea, and further decreases are slow [21].

Data on MP abundance in the surface and water column are starting to accumulate, but so far they are limited to the Baltic Proper and the Gulf of Finland. Due to differences in sampling, particle size selection, and analytical methods, the variation between the reported concentrations is considerable (Fig. 2). In addition, there is no standard agreed way of reporting MP abundances. The data are typically reported as the number of particles per area or volume, but not the mass-based concentrations, which complicates the use of these data for creating a relevant MP exposure scenario in either experimental studies or models.

To estimate probabilities for the contaminant transfer from the MP to biota, we can combine MP abundance data with HOC concentrations measured in biota by SNMPC and in MP deployed for six weeks at three coastal sites, along the southern Baltic Proper (Deliverable 2.2 [1]). Contaminant groups analysed by both SNMPC and the deployed MP were PCBs, dioxins and PBDEs (polybrominated diphenyl ethers). Since no traces of the chemicals from these groups were found in the deployed MPs, whereas their concentrations were measurable in the fish and mussels analyzed within SNMC, the increased bioaccumulation in the Baltic Sea biota due to MP ingestion seems unlikely; this conclusion was supported by our experimental and field studies.

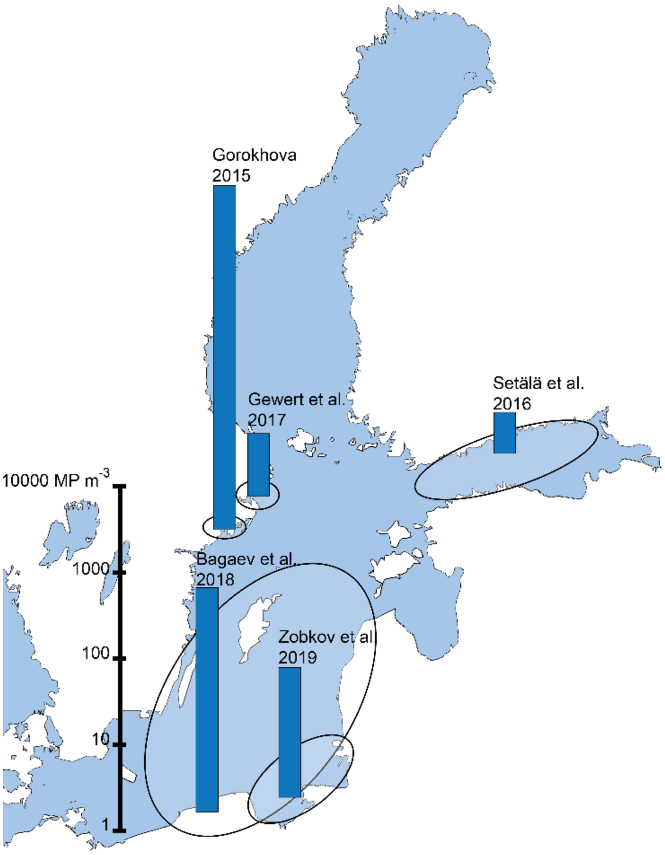

Figure 2. *MP abundance in the Baltic Sea reported by five different studies* [22–26]. *For each study, we extracted the average concentration (blue bars, items m^{-3}) and indicated the surveyed area (black ovals).*

2 Experimental study on PCB transfer

Depending on the relative concentrations of contaminants in MP/tissue and the equilibrium partition coefficient, MP may act as both a source and a sink of organic contaminants (see *section 1.1* for the theoretical rationale). The uptake of HOCs from plastic into biota is well documented, but the experimental evidence of the reverse process is scarce [15]. We designed an experiment to investigate the depuration capacity of pristine MP on the body burden of contaminated zooplankton. In parallel, the effects of MP exposure on the animal fitness-related parameters were evaluated to assess interactions between the contaminant and MP exposures in the presence of food, i.e. a realistic setting from the ecological perspective.

The accumulation-depuration experiment was carried out using *Daphnia magna*, a filter-feeding cladoceran, as a model zooplankton organism. First, during the accumulation phase, animals were fed HOC-loaded food (Fig. 3). The compounds used for the loading were polychlorinated biphenyl congeners (PCB 18, 40, 128, and 209); this range reflected PCB’ range of hydrophobicity. Then, during the depuration phase, the animals were moved to a clean media with or without MP exposure (Fig. 3). We exposed the animals to relatively high levels of plastic to increase the chance of measurable effects (7 × 10^5 particles mL^{-1}). As a result, the mass of MP relative to the total suspended solids (food and MP) was 77%, which is much higher than in ambient seston worldwide.

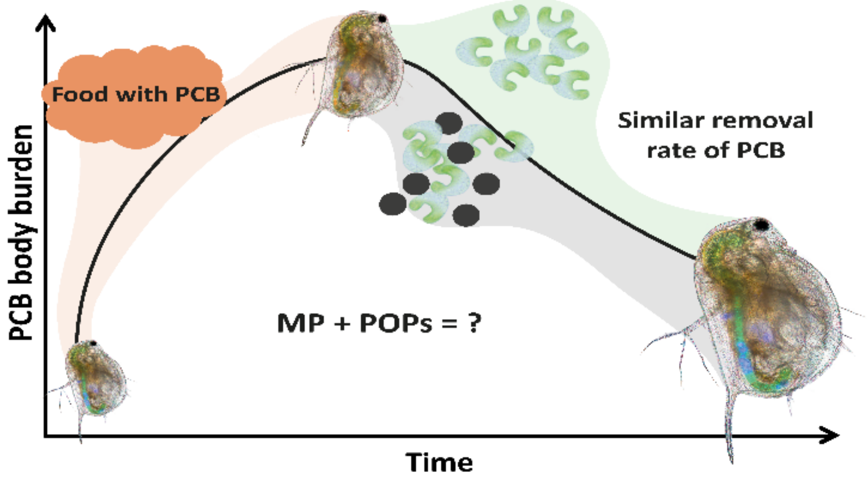

Figure 3. *Experimental outline of the accumulation-depuration experiment. Animals accumulated PCBs for 48 hours and were then depurated during the next 96 hours.*

At the end of the accumulation phase, the animals reached a contaminant level of 31.0 ± 3.6 µg total PCBs g^{-1} (mean ± SD), which declined ~10-fold post depuration. Only for PCB209, the elimination rate was 8-fold higher for the MP-exposed daphnids compared to those exposed to food only, suggesting an enhanced depuration induced by MP. Notably, the concentrations of both MP and PCBs, that produced this significant effect, were far greater than those typically observed in the pelagic waters (Table 1). No biologically significant effects were induced by the pristine MP, whereas exposure to both MP and PCB increased fecundity, in line with the expected effects of a relieved PCB burden in the biological tissues due to their migration to the polymers.

Table 1. *Summary of the relevant data from our studies as well as the sorption experiments reported in Deliverable 2.2 (MICROPOLL)* [1]. *In addition, examples of particulate carbon (POC) concentration and Baltic Sea contaminant levels are presented.*

Source	**Compartment**	**PCB concentration,** µg total PCBs g^{-1}	**Particle concentration in water**	
			µg mL^{-1}	particles mL^{-1}
Experiment [27]	Zooplankton, *Daphnia*	3.1×10^{1}, by dry weight		
	MP	0	3.4×10^{4}	7.0×10^{5}
	Algae/POC	0, by organic carbon	5.1×10^{3}	7.0×10^{5}
	Water	0		
Field data	Fish, *Clupea harengus*	1.7×10^{-1} [28], by lipid		
	MP	0 [1]		3×10^{-6}- 10^{-2} Fig.2
	Algae/POC	1.7×10^{-2} [29], by organic carbon	3.7×10^{-1} [30]	
	Water	1.0×10^{-8} [31]		

These results agree with the predicted role of MP in HOC transport. However, predicting environmental effects from laboratory results is always challenging, particularly when taking into account correlations between the levels of plastic additives, contaminants, plastic abundance and tissue concentrations in animals sampled in various environments [32,33].

3 Field study on MP body burden and HOC concentrations in fish

Comparative field studies on the levels of MP burden and contaminant concentrations are still rare. Exploring correlations between MP ingestion and HOC concentration *in situ* could provide insights into whether MP contributes to HOC bioaccumulation in complex environmental settings. We evaluated the relationship between the occurrence of MP in the gastrointestinal tract of herring (*Clupea harengus*), collected in the Baltic Sea and the HOC concentration in the same fish. Gastrointestinal tracts of herring were provided by SNMPC and consisted of samples collected along the entire West Coast of the Baltic Sea, from Bothnian Bay in the north to Bornholm in the south. Herring is a zooplanktivore, and the size of crustacean zooplankton overlaps with MP size range found in the herring (1-5 mm) [28]. Therefore, we consider this species representative for the secondary consumers in the pelagic food web of the Baltic Sea.

MP ingestion was examined in 130 individuals (both males and females, approximately 50:50) and related to the HOC concentrations measured in the muscle tissue of the same fish. In summary, 22% of the examined herring were found to contain 3.9 MP ind^{-1} ± 4.4 (mean ± SD). When considering all examined individuals (i.e., also those with zero MP occurrence), which would represent the average MP burden in the population, the value was 0.9 MP ind^{-1}. Importantly, there was no correlation between the MP occurrence in the gut and HOC concentrations in the herring muscle tissue for any of the HOC group (Fig. 4). Additional comparative analyses of HOC concentrations and MP burden in gastrointestinal tract of Baltic Sea cod (*Gadus morhua*) and herring are underway (WP3). Preliminary results indicate no effect on the fish' fitness (B. Urban-Malinga 2019, personal communication, October 2019). In line with the current view and the experimental findings described in *section 2*, the lack of such correlations suggest that there is no direct effect of MP on HOC transfer and bioaccumulation in the secondary consumers of the Baltic Sea.

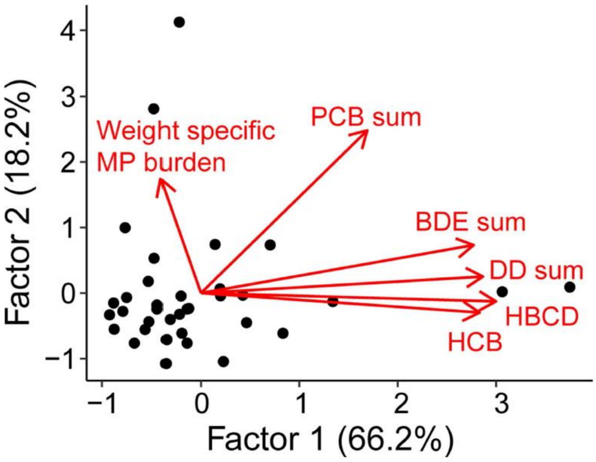

Figure 4. *Factor scores (axes) and loadings (arrows) of contaminants (HBCD, HCB and the sum of PCBs, BDEs, and DDs) and weight-specific MP burden in the Baltic herring. The data included in this analysis were weighted MP burden and HOC concentrations in herring muscle tissue determined in (1) individual fish specimens, and (2) pooled fish samples (10 ind. per sample); see publication by Ogonowski and co-workers (2019) for details.*

4 Implications for effect assessment in the Baltic Sea

The capacity of MP as vector of HOC for consumers is a function of MP availability and the HOC concentration in these MP compared to the concentrations in other ingested particles. In the Baltic Sea, reported abundances of MP vary between 3 and 10^4 MP m^{-3} (Fig. 2); moreover, MP was found to carry low levels of pollutants [1]. Our field and experimental studies, together with other published reports [1,27,28]**,** suggest that under these exposure conditions, no measurable facilitation of HOC transport from the environment to biota can be expected. However, the levels of HOC (e.g., total PCBs) in MP reported in the Deliverable 2.2 [1] were unexpectedly low, considering that these contaminants occur in the water phase (Table 1). One explanation is that the experimental MP had not equilibrated during the six week deployment period meaning that the values reported may underestimate the actual HOC levels in MP from the Baltic Sea. However, sorption of HOCs to MP have been observed at shorter time frames [5] calling for more analytical work to measure environmental contaminants in both field-collected and experimentally exposed MP in diferent basins of this system.

If we follow a precautionary principle and consider HOC levels reported for MP collected from beaches around the world as plausible for the Baltic Sea environment, MP may contain up to 2.3 µg total PCBs g^{-3} plastic [34,35]. This concentration of total PCBs in MP is higher compared to herring and suggests that passive diffusion from MP, into the organism, is thermodynamically possible. Still, the possibility of hazardous impact by contaminated MP is questionable. First, the release rate of HOC from ingested plastic in the gastrointestinal tract would be too slow considering the gut passage time (hours, [36]) to contribute to the uptake. After all, no sorption was observed in MP after six weeks in the Baltic Sea. Second, the food bolus far exceeds the amount of ingested MP as we observed in the field study, which means that zooplankton prey would be responsible for the bulk HOC uptake via ingestion. Third, although the food may contain lower HOC levels compared to the organism, the food assimilation and gastrointestinal magnification potentiate the uptake. For MP, there is no expected assimilation or gastrointestinal magnification and, therefore, the probability of HOC uptake is lower. Nevertheless, more controlled experiments are necessary to establish the conditions and concentrations that would be of concern for the risk assessment of microplastic as vector of organic contaminants to biota.

The abundance of particulate matter in the water column is also important to consider in the risk assessment of MP as HOC vectors because any suspended solids can sorb HOC. In the estuaries, such as the Baltic Sea, the riverine inflow brings large amounts of mineral and organic particulates, with typical levels of particulate organic matter (POM) ranging 0.05 to 1.4 mg C L^{-1} [30], which is orders of magnitude higher than the reported MP quantities (Table 1; Fig. 2). Moreover, the indigenous plankton, including bacterioplankton, phytoplankton and zooplankton contribute even higher POM concentrations, while posessing a high sorption capacity for HOC. Therefore, even though MP can accumulate high amounts of HOC, their vector capacity in natural pelagic systems is likely to be negligible compared to other sources and pathways. More studies are needed to evaluate HOC transport mediated by MP in environmental settings.

Another area of concern with respect to MP-mediated HOC transfer is the fact that high concentrations of suspended solids in the water column promote aggregation and sedimentation of MP, which further decreases their role in the HOC transport in the pelagic system. In theory, the MP capacity as a HOC vector would be most apparent in an extremely oligotrophic system with low concentrations of both mineral and organic suspended solids, high HOC levels in the water, and very high MP load. The Baltic Sea pelagia is different from such a system; therefore, the vector capacity of MP for HOC transport in this system is expected to be negligible compared to other routes.

5 Relevance for MSFD and GES targets

The EU Marine Strategy Framework Directive (MSFD, 2008/56/EC) establishes a framework where within 2020 – EU Member States shall take action to achieve, or maintain, good environmental status (GES) of their marine waters. GES is based on 11 qualitative descriptors, as listed in Annex I of the MSFD. Marine litter, including microplastic, is addressed by Descriptor 10, which states that for GES to be achieved *Properties and quantities of marine litter do not cause harm to the coastal and marine environment*.

As a part of Descriptor 10/MSFD, properties and quantities of microplastic should be determined for each system in question, and GES targets are needed to facilitate the environmental assessment with regard to marine litter. To this end, it is important to identify the hazardous potential of specific microplastic properties and consider them when setting the GES targets. Potentially, such properties may include a capacity of microplastic to act as a contaminant vector. Microplastic quantities can be monitored both in habitats (water or sediment) and its inhabitants, e.g. selective or filter-feeding consumers that are acting as active or passive MP collectors, respectively.

In the marine environment, including the Baltic Sea, MP is ingested by fish [28] and other animals, such as mussels and crustaceans (Deliverable 3.2; [37]). Animal guts could, therefore, provide an accurate indication of MP levels in the marine environment and have, as such, been named as possible indicators for MP pollution under Descriptor 10/MSFD [38–40]. In our field studies, we have identified both fish [28] and invertebrates (Deliverable 3.2; [37]) that can be used as such indicators for MP pollution in the Baltic Sea. Moreover, for herring, we have evaluated biotic factors, such as age, stomach fullness and reproductive status that can be used to standardize specimen selection for MP monitoring. Setting the GES targets has not been attempted yet, although, the discussion on what species/functional groups would be the most efficient indicators is on-going in the research community. Once the indicator species are selected, it will be necessary to establish a baseline to gauge whether or not trends in plastic are increasing or decreasing, leading to possible management actions.

Our findings support the view based on the thermodynamic modelling [18,19,41,42] that the MP-mediated transfer of HOC from water to pelagic animals can be considered negligible. Therefore, there is no empirical evidence that GES values should account for MP posing additional risk of increased bioaccumulation in biota. Thus, based on current knowledge, the future development of GES targets can be solely based on the baseline determined for the indicators once they are established and the data for various time and spatial scales become available.

6 Conclusions

- MP may act as carriers of HOC to and from biota as evidenced by laboratory experiments. However, our results do not support the idea that this transfer occurs in field conditions and under environmentally relevant levels of MP and HOC in marine systems, including the Baltic Sea.
- The reported *in situ* MP abundances (absolute and relative to other suspended solids in the pelagia) and HOC levels observed in the field experiments (Deliverable 2.2, MICROPOLL) are at the levels that make it unlikely for MP to act as a significant vector for HOC transport from water to biota in the Baltic Sea.
- Toward the implementation of MSFD in the Baltic Sea, we have identified possible indicator species and established biotic factors that can be used to standardize selection of specimens should herring be used for MP monitoring and development of GES targets in the water column.
- The observed lack of the realized capacity for MP to transport HOC implies that future development of GES targets for plastic litter (Descriptor 10/MSFD) can be solely based on the baselines determined for the indicators once they are established and the data for various time and spatial scales become available.

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