Plastics as vehicles of chemical compounds to marine organisms

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Abstract. Plastics in the marine environment are shown to be a source of toxic chemicals. To understand potential risk, we evaluated the toxicity of dirty and virgin plastic fragments on marine mussels Mytilus trossulus Gould, 1850. We simulated transfers of chemical compounds to marine water by air agitation. According to experiments, an exposure to plastic fragments induces oxidative stress and variations in the microelement composition in tissues of the mussels. A decrease in the activity of the antiradical link in mollusk tissues and a pronounced genotoxic effect of plastic are also reported.

1. Introduction

The abundance of drifting plastic debris in the oceans has become a global environmental issue due to the serious threat that it poses to marine life. After entering into the organism, plastic fragments cause not only mechanical damage to mucous tissues and clogging of the gastrointestinal tract, but also serious disturbances in physiological processes, including blockade of the digestive enzyme secretion, decreased level of steroid hormones, disturbance of ovulation and the reproductive function [1].

It has long been believed that synthetic polymers are chemically inert due to their high molecular weight (LMW) and cannot be considered materials hazardous to the health of animals and humans. However, relatively recent studies have shown that, in addition to a potential risk of physical damage, fragments of plastics and microplastic particles pose a health hazard due to chemicals they contain [2]. Having a relatively large hydrophobic surface, polymer fragments are capable of adsorbing and being impregnated with a variety of persistent and highly toxic chemicals (such as PCBs, DDT, NP, PAHs, etc.), thus, facilitating their distribution and transfer from various sources of contamination to coastal and open waters of the ocean [1, 3]. Besides adsorbed toxic pollutants, “own” (endogenous) chemicals used in the technology of polymer manufacture also pose an increased environmental hazard. According to estimates by Araújo [4] for different manufacturers, the content of monomers in various types of polymers can vary greatly from a very low, 0.01%, to more than 4%. Furthermore, LMW oligomer fragments, catalysts, synthetic stabilizers, as well as a wide range of chemical additives (phthalates, bisphenol A, PCBs, dyes, plasticizers, etc.) that provide polymers with desired consumer-attractive properties may be present in synthetic polymers [5].

Most of the surface-adsorbed and “own” (endogenous) LMW chemical compounds are associated with polymer structures rather physically, not chemically, and can be released (diffused) into the environment, thus, posing a threat to life of a wide variety of aquatic organisms.
The number of publications with assessments of the potential hazard from chemicals released from plastic fragments when they are ingested and enter the digestive system of various marine species is steadily increasing [6, 7]. However, these studies consider mainly organisms that are prone to ingesting plastic debris, and only few works draw attention to the possible toxic (negative) consequences for organisms that cannot ingest plastics but are immediately surrounded by [8]. There are concerns that plastic debris may release potentially toxic substances, which were used in the production of polymers or accumulated during exploitation and migration through various contaminated environmental niches, into the marine environment. The ecological consequences of these processes are quite obvious, since a much larger number of both micro- and macro-forms of marine organisms, including their planktonic developmental stages, become directly exposed to chemicals as a result of their desorption and diffusion into their marine habitat [9].

We believe that within the framework of the issue under consideration, experiments with large-sized plastic fragments and filter-feeding organisms will probably provide the most convincing assessment of the environmental hazard posed by plastics in marine ecosystems.

2. Materials and methods

2.1. Experiment description

We used adult individuals (5–7 cm) of the Pacific bay mussel Mytilus trossulus, collected from Severnaya Cove (Slavyansky Bay, Sea of Japan) in October, as study objects.

In this study, we set up several series of model experiments in which filter-feeding mollusks, after acclimation to the laboratory conditions, were kept in tanks (100 L) for a short time (72 h) exposed to two types of polyethylene (PE) fragments. In the first series, we used pieces of recently produced PE (bags) received directly from a manufacturer; the packaging bags met the requirements of GOST for low-density polyethylene (LDPE) film (16338-85) (we referred to them as “native” PE fragments). For the second series, we collected free-drifting fragments of PE in the form of sheets from the water column of Golden Horn Bay, Vladivostok, where the level of some pollutants in the water [10] exceeds the maximum permissible concentration (MPC) dozens of times (“dirty” PE fragments). We selected fragments of packaging bags of low-density polyethylene film (LDPE) (16338-85). In Russia, this type of PE is used for the manufacture of packaging bags. In the latter case, those were pieces of PE partially degraded under the effect of oxygen, sunlight (UV light), and temperature fluctuations, and having the characteristic smell of petroleum products and decomposing organics. The color of the fragments varied from light to dark brown.

The total area of plastic fragments in the experimental tanks was 0.05 m²/L, as recommended in the work of Li [11].

The experiments with bivalves were carried out in tanks at a constant temperature (18–19°C) and with intensive aeration to maintain a sufficient concentration of oxygen in the water. In addition, this created a flow of water that mimicked a current and wave fluctuations that contribute to the leaching of weakly bound endogenous and plastic-sorbed chemicals.

To determine the biochemical parameters, we used the digestive gland and the gills, which, after extraction from the bivalves, were quickly frozen in liquid nitrogen and stored at a temperature of −80°C.

2.2. Antioxidant activity and low-molecular weights antioxidant

Tissues were homogenized in a cooled 0.1 M phosphate buffer, pH 7.0, at 4°C. Supernatant was obtained by centrifugation at 10,000 rpm for 40 min. Content of the, glutathione (GSH), in tissues was measured using the method of Moron and co-authors [12]. Integrated antioxidant activity (IAA) was determined by inhibiting the reaction of ABTS [2,2′-azobis(2-aminopropane) dihydrochloride] oxidation [13,14]. Both parameters were then expressed per 1 mg protein [15].
2.3. Comet assay
The alkaline version of comet assay [16], adapted to marine organisms [17], was used to determine the degree of DNA damage. To process digital images, the Casp 1.2.2 software was used (CASPlab, Wroclaw, Poland).

2.4. Trace element analysis
For quantitative analysis of heavy metal content in mollusk tissues, the atomic absorption spectrometry method was used [18]. The methodology for the determination of heavy metals used in this study complies with the Russian national standard (GOST 30178-96 Raw material and food-stuffs. Atomic absorption method for determination of toxic elements).

Statistical processing of the results obtained was performed with the statistical tools available in the MS Office Excel and Statistica software packages. Significance of differences between samples was determined using the nonparametric Mann–Whitney test. Differences were considered statistically significant at p < 0.05.

3. Results
Despite the short duration of the experiments and the lack of contact between the animals and the plastics, we detected biochemical changes in tissues of the bivalves from both experimental groups, which indicates the development of oxidative stress.

Thus, in the experimental bivalves exposed to “native” plastics, the integrated antiradical activity (IAA) value in the gill and digestive gland cells decreased 1.5- and 1.3-fold, respectively; in those exposed to “dirty” PE fragments, the decrease in this parameter was more pronounced, 1.7- and 2.5-fold, respectively (figure 1 A). At the same time, the level of the LMW antioxidant, glutathione (GSH), decreased to varying degrees in the mussels of both experimental groups. In the bivalves from the tanks with conditionally “native” PE fragments, the GSH content reduced by ~25% (1.4-fold) in gill cells and only by ~3% in the digestive gland; a different (opposite) relationship was observed for the mussels exposed to PE fragments from the waters of Golden Horn Bay: the concentration of this antioxidant tripeptide in gill cells remained almost at the level of that in the control mussels, while in the digestive gland cells it decreased by more than 15% (figure 1 B).

Obviously, the significant reduction in the protective antioxidant (antiradical) potential in the mussels of both experimental groups caused a sharp intensification of pro-oxidative processes in tissues, which is confirmed by the increased oxidative destruction of DNA molecules. According to the comet assay, in the cells of gills and digestive glands of the mussels exposed to “native” PE fragments, the degree of DNA damage, which we estimated as the percentage of DNA migrating to the comet “tail” being formed in the electrophoretic field, increased compared to that in the control bivalves almost 2- and 1.5-fold, respectively. At the same time, in the experimental bivalves exposed to PE fragments from Golden Horn Bay the value of this parameter was even more pronounced and increased 2.5- and 4-fold, respectively (figure 1 C).

Simultaneously, taking into account the adsorption properties of polymers, we also measured the trace element composition of tissues of the experimental mussels (table 1).

In general, the analyses of tissues of the mussels from both experimental groups showed that the variations detected in the concentrations of most trace elements were insignificant. The exception was zinc (Zn), whose concentration increased in the gills of the mussels from the tanks with “native” PE fragments. Moreover, a decrease in the concentrations of lead (Pb) was recorded in the gills of bivalves exposed to “native” PE fragments. Also, a decrease in the concentration of manganese (Mn) was noted in the digestive gland of mussels exposed to “dirty” PE fragments. Obviously, to fully identify and evaluate the effect of polymers on the trace element composition of aquatic organisms, longer experiments are required. But, even based on the results obtained, we consider it relevant to highlight the following aspect.
Figure 1. Changes in molecular biomarkers exposed to “clean” and “dirty” PE fragments (mean ± SD, n = 15): (A) integrated antioxidant activity (IAA), nmol trolox/mg protein; (B) reduced glutathione, nmol/mg protein; (C) DNA damage in the tail, %.*significant difference vs. control (p < 0.05). Letters denote values significantly different from each other (p < 0.05).

Table 1. Changes in the trace element composition of tissues of the gills and digestive gland in M. trossulus exposed to plastic fragments (mean ± standard deviation, n = 15).

| Trace element | Gills | Digestive gland |
|---------------|-------|-----------------|
|               | Control | Clean PE fragments | Dirty PE fragments | Control | Clean PE fragments | Dirty PE fragments |
|               | µg/g d.w. | µg/g d.w. | µg/g d.w. | µg/g d.w. |
| Zn            | 158.55±15.07 | 177.52±7.82 | 102.50±4.36 | 109.40±0.87 | 91.06±4.85 | 98.77±2.942 |
|              | 7       | 7               | 1            | 1           | 4           | 5               |
| Fe            | 109.2±8.06 | 93.25±0.354 | 110.35±11.9 | 115.45±11.9 | 140.5±7.45 | 154.25±21.8 |
|              | 5       | 5               | 5            | 5           | 4           | 5               |
| Cu            | 5.67±0.555 | 5.32±0.036 | 5.75±0.534 | 8.23±0.384 | 8.14±0.095 | 11.45±1.248 |
|              | 5       | 5               | 5            | 5           | 5           | 5               |
| Cd            | 0.96±0.070 | 0.91±0.189 | 0.48±0.025 | 1.68±0.022 | 0.91±0.023 | 1.21±0.059 |
|              | 5       | 5               | 5            | 5           | 5           | 5               |
| Pb            | 1.40±0.152 | 0.70±0.034 | 0.959±0.080 | 0.78±0.054 | 0.87±0.021 | 0.96±0.182 |
|              | 5       | 5               | 5            | 5           | 5           | 5               |
| Mn            | 18.46±2.631 | 16.99±0.518 | 15.185±0.66 | 18.36±0.248 | 9.87±0.518 | 10.57±0.136 |
|              | 5       | 5               | 5            | 5           | 5           | 5               |
There is no doubt that the adsorption/desorption properties of polymers can affect the “bioavailability” of trace elements and, as a result, exert a direct influence on the level of these elements primarily in gill cells and an indirect one (through metabolic shifts) in other tissues, including digestive gland cells.

4. Discussion

Taking into account the characteristics of our model experiments (relatively large fragments of PE and filter-feeding organisms), there is every reason to believe that the key factor determining the biochemical shifts in the experimental mussels was the chemical factor. In other words, both types of plastic fragments are a source of chemicals that caused the development of oxidative stress processes in the animals and exhibit genotoxicity. When searching for explanations for the results obtained, we should emphasize the following aspects: in the former case, recently synthesized and not used previously PE fragments are a source of exclusively endogenous chemicals applied in the synthesis of this polymer, whereas PE fragments collected from the water of Golden Born Bay carry a wide range of chemical compounds contaminating this waterbody. Convincing experimental evidence in support of these ideas is available in the literature. Lithner with co-authors [2] were among the first researchers to draw attention to the environmental and health hazard posed by chemicals from synthetic polymers. After analyzing aqueous “extracts” from several dozen of common plastic household items, they showed that dangerous chemicals are leached from a number of polymers into the aquatic environment, which caused the mortality of the small planktonic crustacean Daphnia magna in short-term (48-hour) experiments. A similar pattern was observed by an American group of researchers [11]. In aqueous “extracts” from seven different types of polymers, they found a complex mixture of chemicals that in experimental conditions caused the mortality of adult and inhibited settling of larval acorn barnacles, Amphibalanus amphitrite. It was also reported that a number of highly toxic compounds migrating from plastics to the marine environment caused a sharp increase in the mortality rate of a marine coral-reef fish, Pseudochromis fridmani, both in short-term and chronic experiments. This is also confirmed by a study of Hermabessiere and co-authors [8] who showed that highly toxic additives (polybromides, diphenyls, phthalates, nonylphenol, bisphenol A, etc.), widely used in the polymer production, are found at various concentrations in waterbodies where plastic debris accumulates. Moreover, according to experimental data [6], the toxicity of aqueous “extracts” from certain types of polymers that have been previously exposed to sunlight increases substantially. It is obvious that exposure to the UV sunlight, causing the “ageing” of polymers, contributes to increased leaching of the additives, since an analysis of these “extracts”, according to the authors, did not reveal any products of polymer degradation among various chemical components present in the “extracts”.

Nevertheless, Martinez-Gomez and co-authors [9], when analyzing the inhibition of embryonic development in the sea urchin Paracentrotus lividus exposed to polystyrene microgranules and high-density polyethylene, concluded (assumed) that not only chemical additives but also residual (free) monomers, which are also attributed to the category of highly toxic. Thus, the level of residual (non-polymerized) monomers due to polymerization depends on many factors, including mainly the type of polymer, technology, and conditions of polymerization. Therefore, as Araujo [4] established, concentration of monomers in different types of polymers produced by different manufacturers can vary greatly, from a very low (0.01%, 100 ppm) to a very high (more than 4%, 40,000 ppm).

In recent years, researchers have paid special attention to the ability of polymers to adsorb and be impregnated by a variety of persistent and highly toxic chemicals, with not only hydrophobic xenobiotics (such as PCBs, DDT, NP, PAHs, etc.), but also a number of heavy metals found among them. It is believed that the ratio of the sorption/desorption properties of polymers towards various chemical compounds, depending on the environmental characteristics, underlies their distribution and transport from various sources of pollution to coastal and open ocean waters [1, 3].
During migration and “drifting” on the surface, plastic fragments are exposed to oxygen, temperature variations, and UV light, which causes a gradual physical and chemical degradation of their structure and, as a result, changes the sorption properties of the polymers. Therefore, there is a probability that floating plastic debris can act as a vector in the transfer of various highly toxic chemical compounds adsorbed on the surface of polymer fragments to aquatic organisms of various species. This point of view was confirmed in experimental works [7, 19]. These research groups have shown that chemicals that are toxic to developing embryos of the sea urchin Lytechinus variegates [19] and the mussel Perna perna [7] may pass from “native” microplastics (mPL), as well as from microplastics collected in a polluted zone of coastal waters, into the aqueous phase. At the same time, when analyzing the obtained results showing the pronounced differences in the degree of toxicity of these two mPL types, the authors emphasize that adsorbed chemicals are involved in the toxicity of mPL from the coastal zone, whereas “additives” are responsible for the toxicity in the case of “clean” mPL. This was particularly evident in experiments with P. perna embryos [7], where desorbed chemicals showed greater toxicity than endogenous compounds from the “native” mPL.

The above results are consistent with those obtained in our experiments. We also consider the pronounced inhibition of the antiradical link of the antioxidant system and the increased destruction of nuclear DNA in the mussels in the second series of our experiments as a response of these animals to the stress caused by chemical compounds desorbed from the surface of PL fragments in the polluted waters of Golden Horn Bay that were placed under the experimental conditions. This is indirectly confirmed by the systemic hydrochemical observations [10], according to which the water in Golden Horn Bay is categorized as highly polluted by a number of heavy metals (HM) and petroleum hydrocarbons (PH), with concentrations of the latter pollutant periodically exceeding the maximum permissible concentration (MPC) dozens of times.

Even such a short list of examples, along with our experimental results, suggests that synthetic polymers pose an actual danger not only directly to the biota that ingests them, but also indirectly, through a wide range of highly toxic chemicals of endogenous and exogenous origin.

It should also be noted that, due to the large scale of production and entry of plastic waste into various ecosystems, an analysis of the ecotoxicological situation should take into account not only the presence of “traditional” (common) pollutants in the environment, but also plastic debris of various sizes.

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