Science of the Total Environment 631–632 (2018) 778–788

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Evaluation of uptake and chronic toxicity of virgin polystyrene microbeads in freshwater zebra mussel *Dreissena polymorpha* (Mollusca: Bivalvia)

Stefano Magni [a,*], François Gagné [b], Chantale André [b], Camilla Della Torre [a], Joëlle Auclair [b], Houda Hanana [b], Camilla Carla Parenti [a], Francesco Bonasoro [c], Andrea Binelli [a,*]

[a] Department of Biosciences, University of Milan, Via Celoria 26, 20133 Milan, Italy
[b] Aquatic Contaminants Research Division, Environment and Climate Change Canada, 105 McGill, H2Y 2E7 Montréal, Québec, Canada
[c] Department of Environmental Science and Policy, University of Milan, Via Celoria 2, 20133 Milan, Italy

HIGHLIGHTS

- Few information about microplastic toxicity in freshwaters were available.
- Uptake/toxicity of virgin polystyrene microbeads (PMs) were evaluated in zebra mussel.
- PMs are concentrated in the gut lumen, tissues and hemolymph of zebra mussel.
- PMs induce a low alteration of oxidative status and dopamine level in zebra mussel.
- A considerable uptake and low toxicity summarize the effects of PMs in zebra mussel.

GRAPHICAL ABSTRACT

ARTICLE INFO

Article history:
Received 2 February 2018
Received in revised form 7 March 2018
Accepted 7 March 2018
Available online xxxx

Editor: D. Barcelo

Keywords:
Microplastics
Zebra mussel *Dreissena polymorpha*
Uptake
Confocal microscopy
Chronic toxicity
Biomarkers

ABSTRACT

Microplastics (MPs), plastic debris smaller than 5 mm, are widely found in both marine and freshwater ecosystems. However, few studies regarding their hazardous effects on inland water organisms, have been conducted. For this reason, the aim of our research was the evaluation of uptake and chronic toxicity of two mixtures (MIXs) of virgin polystyrene microbeads (PMs) of 10 μm and 1 μm in size (MIX 1, with 5×10^5 of 1 μm size PMs/L and 5×10^5 of 10 μm size PMs/L, and MIX 2 with 2×10^6 of 1 μm size PMs/L and 2×10^6 of 10 μm size PMs/L) on freshwater zebra mussel *Dreissena polymorpha* (Mollusca: Bivalvia) during 6 exposure days. The PM uptake in the mussel body and hemolymph was assessed using confocal microscopy, while the chronic toxicity of PMs was evaluated on exposed mussels using a comprehensive battery of biomarkers of cellular stress, oxidative damage and neuro- genotoxicity. Confocal microscopy analyses showed that MPs concentrated in the gut lumen of exposed mussels, absorbed and transferred firstly in the tissues and then in the hemolymph. The results revealed that PMs do not produce oxidative stress and genetic damage, with the exception of a significant modulation of catalase and glutathione peroxidase activities in mussels exposed to MIX 1. Regarding neurotoxicity, we observed only a significant increase of dopamine concentration in mussels exposed to both MIXs, suggesting a possible implication of this neurotransmitter in an elimination process of accumulated PMs. This research represents a first study about the evaluation of virgin MP toxicity in zebra mussel and more research is warranted concerning the long term neurological effects of virgin MPs.

* Corresponding authors.
E-mail addresses: stefano.magni@unimi.it (S. Magni), andrea.binelli@unimi.it (A. Binelli).

https://doi.org/10.1016/j.scitotenv.2018.03.075
0048-9697/

1. Introduction

The problem of plastics as emerging environmental pollutants is a growing concern because the global plastic production has risen exponentially since the 1950's, reaching >320 millions of tons in 2014 (PlasticsEurope, 2016). China is the largest producer of plastic materials (26%), followed by Europe (20%) and NAFTA (North American Free Trade Agreement) countries (19%). It is interesting that two third of plastic demand in Europe is concentrated in only five countries: Germany (24.8%), Italy (14.3%), France (9.6%), UK (7.7%) and Spain (7.4%; PlasticsEurope, 2016). Therefore, the so-called "plastic age" carries negative consequences for aquatic and terrestrial ecosystems, biota and human health. Sutherland et al. (2010) suggested that the problem due to plastic debris must be considered, along with the climate change, as the issue which could affect the conservation of biological diversity in the short to medium-term.

Microplastics (MPs) are operationally defined as plastic fragments <5 mm in diameter down to the μm range (Thompson et al., 2009). The release of fragments down to the nanometer range is also considered given that nanomaterials could be become more reactive owing to their increased surface area/volume ratio and readily available towards cells. They are produced by primary and secondary sources: the first one includes manufactured plastic products, such as scrubbers in cleaning and cosmetic products or pellets used in feedstock or plastic production (Fendall and Sewell, 2009; Cole et al., 2011), while the secondary origin of MPs results from the breakdown of larger plastic items, such as fishing nets, line fibers, films, industrial raw materials, consumer products and pellets and polymer fragments from degradable plastic (Hidalgo-Ruz et al., 2012; Free et al., 2014). Although there is little information about degradation rates of plastic items and their fragmentation in the environment, the spread and abundance of MPs is raising worldwide (Browne et al., 2011; Law and Thompson, 2014).

The studies regarding the impact of MPs on aquatic environments have been focused mainly on marine ecosystems, while a more limited amount of studies have been conducted on freshwater habitats (Wagner et al., 2014; Horton et al., 2017). For instance, MPs have been recently found in Europe in surface waters or sediments of Lake Geneva (Switzerland; Faure et al., 2012), Lake Garda (Italy, Imhof et al., 2013, 2018), Danube River (Austria; Lechner et al., 2014), Tamar estuary (UK; Sadri and Thompson, 2014) and in the Elbe, Mosel, Neckar and Rhine rivers (Germany; Wagner et al., 2014). Other surveys were carried out on freshwater ecosystems of North America, Asia and Africa (Free et al., 2014; Su et al., 2016; Wang et al., 2016, 2017; Anderson et al., 2017; Di and Wang, 2018; Nel et al., 2018). The gap in the knowledge of MPs' distribution between marine and freshwater ecosystems is also reflected in the knowledge of their potential toxic effects on biota.

Indeed, while several studies were carried out in field and under laboratory conditions to evaluate the ingestion and effects of MPs in marine organisms, studies regarding the impact of MPs on freshwater species are wanting (e.g. Wagner et al., 2014; Guilhermino et al., 2018; Lei et al., 2018). While the available results showed the ingestion capability of MPs in all the examined freshwater taxa (fish, crustaceans, ostracods, gastropods and chironomids; Imhof et al., 2013; Nel et al., 2018), their ecotoxicological effects remain largely unknown. However, the few available studies seemed to suggest physical impacts similar to those observed for marine organisms (Eerkes-Medrano et al., 2015).

Another problem of MPs is related to their composition and large surface area, which make them prone to adsorb waterborne organic contaminants (Cole et al., 2011) that can be then transported in the aquatic organism trough a "Trojan-horse mechanism" as with products from nanotechnology. Moreover, also the leaching of the plasticizers (e.g., phthalates and bisphenol A) can increase the toxicity of MPs when in the organism. A review by Wagner et al. (2014) underlined several gaps of knowledge about monitoring, source, fate, exposure and effects of MPs, that need to be addressed by the near future studies on freshwater ecosystems and biota.

Therefore, in the present study, we investigated the gaps related to the evaluation of the MP exposure and effects in freshwater organisms. In particular, we choose as biological model the freshwater zebra mussel *Dreissena polymorpha* (Mollusca: Bivalvia), considering its physiological characteristics, as the high filtration rate (Binelli et al., 2014, 2015; Magni et al., 2015), its easiness in stabulation, and its key role in the European and American freshwater ecosystems, being a species that links the littoral and benthic habitats. We exposed for 6 days in static conditions zebra mussel specimens to two different mixtures (MIXs), at different concentration, of virgin polystyrene microbeads (PMs), one of the main MP class detected in the environment, with size of 10 μm and 1 μm, respectively. After the exposures, we investigated the MP ingestion and their eventual uptake and infiltration in the mussel tissues through the use of cryostat and confocal microscopy, while a wide battery of biomarkers was used to assess the potential chronic toxicity of selected contaminants. In particular, on the basis of other evidences of MP effects on oxidative status and neuro-enzyme activity on aquatic organisms (Oliveira et al., 2013; Avio et al., 2015; Ribeiro et al., 2017; Barboza et al., 2018), in the present work we choose to investigate more profoundly these aspects evaluating end-points of cellular stress, oxidative damage and neuro- genotoxicity. To the best of our knowledge, the present study represents an innovative attempt to simultaneously investigate both the fate of MPs and their toxicological impact on freshwater mussels by a multiple biomarker approach.

2. Materials and methods

2.1. Mussel collection

We collected zebra mussel specimens from Lake Iseo (Lovere, North Italy) in January 2017. Mussels were collected from the rocks and transported in containers filled with lake water to the laboratory. Before the exposure, mussels were acclimated for a period of two weeks in 15 L tanks with tap and deionized water (50:50 v/v) and maintained at 20 ± 1 °C in oxygen saturation conditions, with natural photoperiod. Mussels were fed three times *per* week with phytoplankton (*Spirulina* sp.), as reported in our previous work (Magni et al., 2016, 2017).

2.2. Concentration selection and mussel exposure to polystyrene microbeads

The two standard aqueous suspensions (5%) of virgin PMs with a size of 10 μm and 1 μm were purchased from Sigma-Aldrich (Italy). Selected standards were diluted in ultrapure water to obtain the 2 PM working suspensions of 50 mg/L. Since we decided to perform the exposures by considering the real number of beads (and not a simple mass/volume ratio), we quantified the number of 10 μm and 1 μm PMs in the 50 mg/L working suspensions using the Bürker chambers (neutral beads were not subjected to aggregation phenomena), obtaining the following bead numbers (mean ± SD): $116 \times 10^6 \pm 33 \times 10^6$ of 10 μm PMs/L and $23 \times 10^9 \pm 530 \times 10^6$ of 1 μm PMs/L. Because of the great release of MPs in the freshwater environment from Wastewater Treatment Plants (WWTPs) of about 65 millions of MPs/day (Murphy et al., 2016), we chose to test the toxicity of these two different PM MIXs: MIX 1, with 5×10^5 of 1 μm size PMs/L and 5×10^5 of 10 μm size PMs/L, and MIX 2 with 2×10^6 of 1 μm size PMs/L and 2×10^6 of 10 μm size PMs/L. The PM exposures were conducted in triplicate (three tanks for control, three tanks both for MIX 1 and MIX 2), placing in each tank (4 L) 70 mussels under static conditions for 6 days (from $t = 0$ to $t = 6$ days), feeding the animals two times with phytoplankton (*Spirulina* sp.), and maintaining a low stirring to avoid PM sedimentation. Considering the high number of animals required to carry out both microscopy analyses and biomarker measurements, we conducted three different PM exposures in the same conditions. Before the treatment we assessed the baseline levels ($t = 0$) of biomarkers on mussels collected from the acclimation tanks, as reported by Magni