ECOTOX BRASIL
SOCIEDADE BRASILEIRA DE ECOTOXICOLOGIA

Ecotoxicol. Environ. Contam.,v. 17, n. 1, 2022, 106-113
doi: 10.5132/eec.2022.01.10

EEC

Original Research

Can leachates of environmentally relevant concentrations of microplastics in marine sediments affect the reproduction of an epibenthic copepod?

Caio Rodrigues Nobre[1], Beatriz Barbosa Moreno[2], Aline Vechio Alves[2], Denis Moledo de Souza Abessa[1], Augusto Cesar[2], Rodrigo Brasil Choueri[2], Paloma Kachel Gusso Choueri[3*], Camilo Dias Seabra Pereira[2,3]

[1] Biosciences Institute, São Paulo State University - UNESP. Praça Infante Dom Henrique s/n. PC 11330-900, São Vicente, São Paulo, Brazil.

[2] Department of Marine Sciences, Federal University of São Paulo. Maria Máximo st. 168, PC 11030-100, Santos, SP, Brazil.

[3] Department of Ecotoxicology, Santa Cecília University. Oswaldo Cruz st. 266, PC 11045-907, Santos, São Paulo, Brazil.

Received May 04, 2022; Accepted July 11, 2022

Abstract

Most studies on the effects of microplastics on marine biota are carried out using unrealistically high concentrations. Moreover, although microplastics are capable of carrying toxic substances and thus can cause adverse effects even without coming into direct contact with organisms, little is known about the effects of not accessible for ingestion microplastics on benthic biota. Considering that the presence of microplastic particles can itself cause effects to the biota due to toxic substances leached, the present study evaluated the ecotoxicological effects of microplastic leachates of virgin and beach stranded in marine sediments (whole sediments and elutriates) on the epibenthic copepod *Nitokra sp.* (size 3 ± 1 mm). Effects on reproduction were evaluated using clean sediment enriched with environmentally relevant concentrations [1 pellet: 46.67g sediment (Low) and 1 pellet: 1.67g sediment (High)] of two types of plastic pellets (size 4 ± 1 mm) (i) virgin (obtained from the manufacturer) or (ii) stranded (collected from Santos beach, São Paulo, Brazil, a highly urbanized beach). The results of the present study showed that microplastics leachate (virgin or from the environment) did not cause an inhibiting effect on reproduction in *Nitokra sp.* in any of the scenarios tested. These results contribute to further risk assessments of plastic particles for marine biota.

Keywords: Marine debris; Plastic pellets; Benthic organism; Toxicity; Reproduction.

INTRODUCTION

Microplastics potentially interact with aquatic biota (including benthic organisms) through different ways, such as particle ingestion (Lusher *et al*., 2015); physical entanglement (Ziajahromi *et al.*, 2017); transport of microorganisms and introduction of exotic species (Wang *et al.*, 2018); and chemical exposure to substances adsorbed or released by plastic microparticles (Nobre *et al.*, 2015; Wang *et al.*, 2018; Izar *et al.*, 2019). It was reported that 78% of chemicals listed as priority pollutants by United States Environmental Protection Agency (USEPA) due to their ability to persist, bioaccumulate and produces toxic effects on biota was associated with marine pollution of plastic waste (Rochman *et al.*, 2013).

Plastic particles present in aquatic environments have their main origin on the continent, their presence has been reported in coastal and marine environments since the 1970s. in the last decades its introduction into ecosystems exceeds its production levels, with the occurrence and accumulation of MPs recorded from the coastal zone, shallow pelagic areas to the open ocean, ranging from the

*Corresponding author: Paloma Gusso Choveri. pguso@yahoo.com.br

tropics to the polar seas (Carpenter & Smith, 1972; Doyle *et al.*, 2011).

Microplastics are released into coastal ecosystems from different sources such as landfills, dumps, agriculture, hospitals, aquaculture, fisheries and mainly sewage treatment plants (ETEs), industries and harbour areas (Karbalai *et al.*, 2018).

Once in the environment, floating plastic microparticles end up stranding on beaches, making these ecosystems hotspots for microplastics (Panatier *et al.*, 2019). Furthermore, microplastics composed of low-density polymers tend to gain mass through the sorption of compounds and formation of biofilm on their surface, eventually depositing in sediments (Van Cauwenberghe *et al.*, 2015), increasing the bioaccessible of these particles to organisms that inhabit the sediments (Moore, 2008). In addition, sediment is an important environmental compartment that can act as both a source and sink for various biological and chemical wastes (Besseling *et al.*, 2017).

Understanding how microplastics present in the environment affect organisms and assessing potential risks is not an easy task, especially when it comes to the diversity of size, shape, density and charge of particles that are present in environments, and also considering that these properties can vary over time (Galloway *et al.*, 2017). Not only ingested microplastic particles can cause adverse effects to marine biota such as biochemical disturbances, cell death and inhibition of reproductive capacity, compromising ecological functions and leading to population decline (Anbumani & Kakkar, 2018; Alimba & Faggio, 2019), but considering that potentially toxic substances present in microplastics can be released into the environment, it is reasonable to hypothesize that only the presence of microplastic particles, in the role of carriers of toxic substances, can cause ecotoxicological effects (Huang *et al.*, 2021). However, there are few studies that have assessed the impacts of non-ingested plastic microparticles in marine sediments and their associated fauna.

In addition, to date, few studies have aimed to assess the exposure of organisms to concentrations of microplastics closer to those observed in the environment (e.g, Pannatier *et al.*, 2020; Izar *et al.*, 2019; Bour *et al.*, 2018). Most past studies aim to assess the biological effects of microplastics at concentrations much higher than those found in the marine environment (Lenz *et al.*, 2016; Van Cauwenberghe *et al.*, 2015). There is a need for more studies to be carried out evaluating ecotoxicological effects at environmentally relevant concentrations (Sá *et al.*, 2018), so that we can properly measure the ecological risk caused by microplastics to marine biota.

Benthic invertebrates play a key role in aquatic environments as nutrient recycling, structuring of environments, being indicators of ecosystem health (Reynoldson & Metacalfe-Smith, 1992; Alves *et al.*, 2010).

Among benthic organisms, *Nitokra* sp belonging to the phylum arthropoda, subphylum crustacea, class maxillopoda, subclass copepoda, order harpacticoid, have a size ranging from one to five mm in their adult phase, occurring between the surface and the first layer of the sediment, where feed on detritus and microorganisms (Lotufo & Abessa, 2002; Fenilli, 2012). Studies show that copepods of the genus *Nitokra* are model organism suitable for tests with whole sediment, being sensitive to contaminated sediments (Fenilli, 2012).

The effects of microplastics and associated contaminants in benthic populations can impact trophic energy transfer and/or trophic interactions in these environments (Haegerbaeumer *et al.*, 2019). However, there are still few studies that aim to assess the effects of microplastics on these organisms by not ingesting the particles, and the effects observed at the level of reproduction and development are restricted to the water column (Bjgarn *et al.*, 2015; Li *et al.*, 2015; Nobre *et al.*, 2015; Izar *et al.*, 2019).

The present study aimed to investigate the toxic effects on a epibenthic copepod species exposed to leachates of microplastic particles in two sediment matrices (whole sediments and sediment elutriates) enriched with environmentally relevant concentrations of virgin or beached stranded plastic pellets (collected on a highly urbanized beach). For this purpose, the harpacticoid copepod *Nitokra* sp. was used as a biological model, and reproductive parameters were evaluated after chronic exposure. Understanding the interaction between microplastics and benthic biota is necessary to inform ecological risk assessments and allow policymakers to take scientifically sound actions.

MATERIAL AND METHODS

The sediment used in the bioassays was sampled in a reference area (Ilhabela, São Paulo, Brazil). Previous studies on this site have shown low levels of contamination and toxicity (Torres *et al.*, 2009; Araujo et *al.*, 2013). Sediment grain size was analyzed based on the protocol proposed by Mccave & Syvitski (1991) and the results were classified based on the Wentworth scale. Calcium carbonate ($CaCO_3$) contents in each sample were measured using the method described by Hirota & Szyper (1976). Organic matter (OM) was analyzed according to ASTM (2000).

In this study, the biological effects on harpacticoid copepod *Nitokra* sp. (average size of three ± one mm) exposed to pellets in two sediment matrices were assessed: i) Sediment elutriates and ii) Whole sediment. According to Choueri *et al.* (2009), the sediment elutriate test aims to assess the transference of contaminants, and toxicity, from sediments to water after a resuspension process; the purpose of the whole sediment test is, in turn, to evaluate the effects caused by the direct contact with contaminated sediments, considering both the solid phase and the pore water. For each of these tests,

three treatments were carried out: (i) sediment without pellets (control); (ii) sediment spiked with polypropylene pellets with an approximate size of five mm obtained from industry (virgin pellet), and (iii) sediment containing beach stranded pellets with size varying four ± one mm and varied polymer composition. These stranded plastic pellets were collected in the Santos Estuarine System that encompasses the largest port in Latin America, one of the most important industrial complexes in the Brazilian coast, and presents a population of more than 1 million inhabitants (Taniguchi *et al.*, 2016).

The sediment used directly in the whole sediment bioassays, or in the elutriate process, were spiked in accordance with USEPA (2001). Two concentrations of plastic pellets were tested (low and high concentrations) for both whole sediment and elutriates tests. In the "Low concentration" treatment, proportionally 1 pellet were added to 46.67 g of sediment and 100 mL of seawater and mixed in a jar-rolling apparatus for 15 minutes to replicate the environmental concentration of plastic 25.000 pellets/m^3 found by Turra *et al.* (2014) at the same site where the sediments were sampled. In the "High concentration" treatment, proportionally one pellet was added to 1.67 g of sediment and 100mL of seawater mixed in a jar-rolling apparatus for 15 minutes. These pellet concentrations were similar to those observed by Izar *et al.* (2019) in Itaquitanduva beach, located in the municipality of São Vicente, São Paulo, Brazil. After the spiking, samples were left for seven days in a cold and dark chamber to allow the exchange of substances between the microplastic and the sediment.

For the whole sediment tests, first the plastics particles were removed by sieving, in order to expose the test organisms only to contaminants leached from the microplastic pellets. Sediment samples (control, and previously spiked with virgin pellets or beach stranded pellets) were then placed in four test tubes, each containing two mL of sediments and eight mL of clean seawater (salinity 17, filtrated through 0.45 µm membrane and autoclaved) - hereafter referred as "dilution seawater" - and allowed to stand for 24 hours in a cold chamber to equilibrate.

The elutriate treatment followed the protocol described by USEPA (2003). Sediment spiked samples (with pellets) were diluted in seawater in a beaker (one:four volume/volume), then agitated for 30 minutes, and finally left to stand for 24 hours. The test solutions consisted of the supernatant of each sample (control, virgin pellet, and beach stranded). Four replicates were used, each one consisting of glass test tubes containing 10 mL of sediment elutriate. The control treatment consisted of exposing the organisms only to dilution seawater.

The endpoint tested was the fecundity of the benthic copepod *Nitokra* sp. (Lotufo & Abessa, 2002), with photoperiod 16:8 h (light: dark), temperature 25 ± 2 °C, and salinity 17 kept throughout the test. After the exposure time (10 days) under static conditions, organisms were fixed with formaldehyde (4 %) and stained with rose Bengal. Adult females and their offspring (nauplii and copepodits) were counted using a stereomicroscope. Fecundity was calculated by the average offspring divided by the number of adult females.

Three rounds for each assay were performed (hereafter referred as trials one, two, and three), to evaluate the variability of the assay and the tested material although the virgin pellets belonged to the same lot and the beach stranded pellets were collected in a single sample.

The data obtained were analyzed for normality using the Shapiro-Wilk test (Shapiro & Wilk, 1965) and for homogeneity of variance using Bartlett's test. Since the prerequisites were met, Two-way analysis of variance (ANOVA) was applied separately for each treatment (elutriate and whole sediment) to compare possible differences between the samples (control, virgin pellet, and beach stranded pellet) and to identify treatments that differed significantly from the controls. The Bonferroni test was then used to compare means and identify differences between the samples ($p < 0.05$). Analyzes were performed using GraphPad Prism 5.0. software.

RESULTS

Sediment grain size analysis showed a predominance of fine sands (33.3 %) and very fine sands (49 %), and 13.69 % of silt and clay. Organic matter and calcium carbonate contents were 3 % and 8.10 %, respectively. The statistical analysis in the treatments carried out with the lowest concentrations of pellets (Low), showed a significant interaction effect between trial and treatments on the elutriate test (increased number of offspring in beach stranded treatment only in one trial), and significant differences between trials on the whole sediment test, although no effect of pellets of any origin (Table 1). Even so, the differences presented do not represent a reproduction inhibition effect, as shown in the Figures 1 A for elutriate and 1 B for whole sediment.

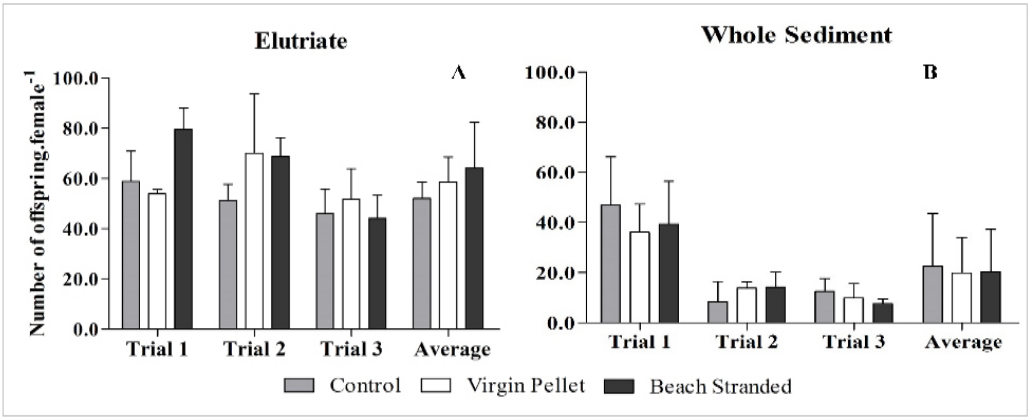

Figure 1: Mean individual fecundity of Nitokra sp. in "Low" after exposure to elutriated sediment (A) and whole sediment (B) (untreated control and sediment spiked with pellets) obtained in the 3 trials of tests (1, 2, and 3). Error bars represent standard deviation.

Table 1: Results of two-way ANOVA in assay "Low", comparing the (1) trial (1, 2 and 3) and (2) tests (control without pellets, virgin pellets, and beach stranded pellets) between treatments (elutriate and whole sediment).

Two - Way ANOVA	df	SS	MS	*F*	*p*
Elutriate					
Trial	2	2.183.282	1.091.641	8.200	0.002
Treatment	2	875.016	437.508	3.286	0.053
Trial x Treatment	4	1.612.018	403.004	3.027	0.035
Whole Sediment					
Trial	2	7.096.340	3.548.170	33.169	<0.001
Treatment	2	45.307	22.653	0.212	0.810
Trial x Treatment	4	333.793	83.448	0.780	0.548

In the tests carried out with the highest concentrations of pellets (High), there was a significant increasing effect on the reproduction of *Nitokra* sp. caused by elutriates of sediment spiked with beach stranded pellets (independent of the trial) (Table 2, Figure 2 A). In the test with whole sediment, there was a significant decrease in the number of offspring in females exposed to leachate from beach stranded pellets, although this was only observed in the first trial (Table 2, Figure 2 B).

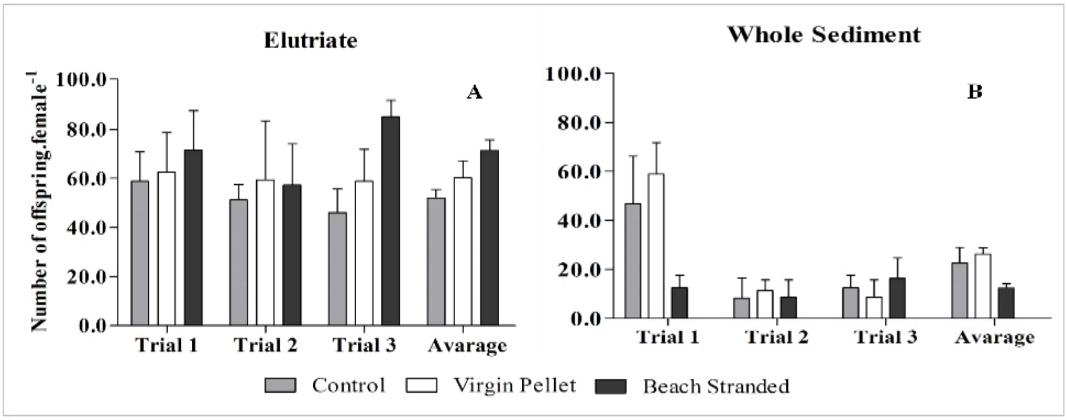

Figure 2: Mean individual fecundity of Nitokra sp. in assay "High" after exposure to elutriated sediment (A) and whole sediment (B) (untreated control and sediment spiked with pellets) obtained in the 3 trials of tests (1, 2, and 3). Error bars represent standard deviation.

Table 2: Results of two-way ANOVA in assay "High" comparing the (1) trial (1, 2 and 3) and (2) treatment (control without pellets, virgin pellets, and beach stranded pellets) between assays (elutriate and whole sediment assays).

Two - Way ANOVA	df	SS	MS	*F*	*p*
Elutriate					
Trial	2	491.482	245.741	1.180	0.323
Treatment	2	2.250.996	1.125.498	5.406	0.011
Trial x Treatment	4	1.423.111	355.778	1.709	0.177
Whole Sediment					
Trial	2	6.539.236	3.269.618	34.378	<0.001
Treatment	2	1.222.862	611.431	6.429	0.005
Trial x Treatment	4	3.557.318	889.329	9.351	<0.001

DISCUSSION

A major challenge in assessing the potential impacts of hydrophobic organic compounds and other contaminants on microplastics is understanding whether they are in equilibrium with other phases in the environment (Ziccardi *et al.*, 2016), making them available to biota or not. Several studies have shown that microplastics can act as chemical carriers of substances, both applied as additives in their manufacturing process, such as polybrominated diphenyl ethers (PBDE), polycyclic aromatic hydrocarbons (PAHs), nonylphenol and octylphenol, bisphenol-A, as well as adsorbed from the environment, such as dichlorodiphenyltrichloroethane (DDT) and related substances (DDE, DDD), polychlorinated biphenyls (PCBs), chlordanes and others (Hirai *et al.*, 2011; Van Moos *et al.*, 2012). All these substances, whether added to the composition of the plastic pellets, or adsorbed to their surface from the environment, can be leached into water and sediment in the marine environment (Nobre *et al.*, 2015).

The concentrations of organic compounds present in pellets collected in Santos Bay (Brazil) were measured in a previous study (Taniguchi *et al.*, 2016). These authors found high concentrations of PCBs (Σ51PCBs: 818 $ng.g^{-1}$ and Σ13PCBs: 551 $ng.g^{-1}$), PBDEs (ΣPBDEs: 2 $ng.g^{-1}$), PAHs (ΣPAHs: 8540 $ng.g^{-1}$ and Σ16HPAs: 1256 $ng.g^{-1}$), and organochlorine pesticides (ΣDDTs: 441 $ng.g^{-1}$, HCB: 44.4 $ng.g^{-1}$, Mirex: 55.8 $ng.g^{-1}$, ΣHCHs: 1.48 $ng.g^{-1}$, ΣChlordane: 22.9 $ng.g^{-1}$).

Even with the high levels of toxic substances that pellets can adsorb (Mato *et al.*, 2001) and the ability to release these compounds into the sediment, the lack of negative effects (i.e. reproduction impairment) observed in this study may be related to the results of sedimentological analyzes which demonstrated a predominance of very fine sediment and substantial levels of OM and $CaCO_3$.

The interaction capacity of microplastics is associated with several factors related to their polymeric aspects, such as composition, porosity, density, degree of crystallinity or rubbery degree, residence time of the plastic in the environment, degree of aging, photo oxidation, in addition to, environmental factors that directly influence the processes of sorption and desorption of substances from microplastics, and may interact with pollutants due to their degree of hydrophobicity or complexation, as they have more binding sites to immobilize toxic compounds, reducing their toxicity and availability (Wang *et al.*, 2016; Ferraz *et al*., 2020; Enyoh *et al.*, 2021).

Furthermore, the greater affinity of non-polar contaminants with pellets means that when these plastic particles are remobilized in the elutriation process, the contaminants end up having little or no interaction with the aqueous phase. Another factor to be considered is the formation of hydrophobic colloids (organic particles present in sediments associated with non-polar compounds), which have less interaction with the water column due to the stability of charges in its upper layer (Manahan, 2013).

The absence of toxicity observed in this study also may involve the biofilm formed in the surface of the plastic pellets. Plastic additives may act as a nutrient source for the microorganisms that comprise biofilm (Rummel *et al.*, 2017).

These organisms decrease the hydrophobicity of the compounds by their action, reducing the load of the substance (Lobelle & Cunliffe, 2011), promoting biotransformation through the metabolism of additives by the microbiota, making them more polar (e.g. the transformation of PAHs into NPAHs) (Netto *et al.*, 2000) and/or through biodegradation, reducing the release to the leachate.

In the sediment elutriation process, washed particles could have removed the biofilm, besides impeding direct contact with the additives.

Although no effects on the reproduction of *Nitokra* sp. have been observed, the current study is still relevant as there were few initiatives to test the toxicity of environmentally relevant concentrations of microplastics (Izar *et al.*, 2019; Bouer *et al.*, 2018; Redondo-Hasselerharm *et al.*, 2018; Ribeiro, *et al.*, 2017), especially regarding microplastics collected in the marine

environment (Rendell-Bhatti *et al.*, 2021; Pannetier *et al.*, 2020; Izar *et al.*, 2019). The few past studies reported that exposures to environmental concentrations have provoked effects mainly at low levels of biological organization, usually effects at a sub-individual level, such as changes in the modulation of energy reserve in clams (Bour *et al.*, 2018), and biomarkers responses (increased activity of enzymes for metabolization of organic xenobiotics, and DNA damage) in larvae and juveniles of Japanese Medaka (Pannetier *et al.*, 2020). At higher levels of biological organization (e.g. individual), effects were such as decreased growth of amphipods (Redondo-Hasselerharm *et al.*, 2018) and sea urchin abnormal embryolarval development (Rendell-Bhatti *et al.*, 2021).

On the other hand, studies evaluating the effects of microplastics at higher concentrations than those found in the environment showed acute biological responses. Bejgarn *et al.* (2015) observed mortality of the copepod *Nitokra spinipes* exposed to plastic leachates, which could be to the higher concentration of microplastics in the former, as such as the mode of exposure, since the compounds are not immobilized by binders when directly exposed to the leachates.

The results of the present study showed that microplastics (virgin or from the environment) did not cause an inhibiting effect on reproduction in *Nitokra* sp. in any of the scenarios tested. These results contribute to further risk assessments of plastic particles for marine biota. However, broader investigations involving other outcomes should be carried out to provide a better understanding of the environmental impacts of these pollutants on aquatic ecosystems. Furthermore, the change in the format of this primary microplastic and the improvement in the logistical process of the plastic pellet production chain tend to avoid its loss in the process, thus preventing its entry into ecosystems.

ACKNOWLEDGEMENTS

Nobre C. R. wishes to thank Coordenação de Aperfeiçoamento de Pessoal de Nível Superior and Fundação de Amparo à Pesquisa do Estado de São Paulo (Process #2017/12193-9). The authors would like to thank biologists Lucas B. Moreira, Ana C. F. Cruz, Karla Lucatelli, Jonas L. Rosa, Heloisa R. Franco, and Wagner Schule for their support in the laboratory. Abessa D.M.S., Cesar A., Choueri, R. B., and Pereira C.D.S. would like to thank CNPq for productivity fellowships.

AUTHOR'S CONTRIBUTIONS

CRN: Project administration, Conceptualization, Funding acquisition, Methodology, Writing - original draft, Data curation, Research, Visualization, Formal analysis, Writing - revision and editing. BBM: Writing - original draft, Investigation, Formal analysis, Writing - revision and editing. AVA: Writing - original draft, Investigation, Formal analysis, Writing - revision and editing. DMSA: Writing - original draft, laboratory support, Writing - revision and editing. AU: Writing - original draft, laboratory support, Writing - revision and editing. RBC: Conceptualization, Methodology, Data analysis, Writing - revision and editing. PKGC: Conceptualization, Methodology, Supervision, Writing - revision and editing. CDSP: Project administration, Funding acquisition, Conceptualization, Methodology, Writing - revision and editing, Supervision.

REFERENCES

ALIMBA, C.G. & FAGGIO, C. 2019. Microplastics in the marine environment: Current trends in environmental pollution and mechanisms of toxicological profile. *Environmental Toxicology and Pharmacology* 68, 61–74. https://doi.org/10.1016/j.etap.2019.03.001

ALVES, J.M., CALIMAN, A., GUARIENTO, R.D., FIGUEIREDO-BARROS, M.P., CARNEIRO, L.S., FARJALLA, V.F., BOZELLI, R.L. & ESTEVES, F.A. 2010. Stoichiometry of benthic invertebrate nutrient recycling: interspecific variation and the role of body mass. *Aquatic Ecology*, 44(2), 421-430.

ANBUMANI, S., & KAKKAR, P. 2018. Ecotoxicological effects of microplastics on biota: a review. *Environmental Science and Pollution Research*, 25(15), 14373-14396.

ARAUJO, G.S., MOREIRA, L.B., MORAIS, R.D., DAVANSO, M.B., GARCIA, T.F., CRUZ, A.C.F., ABESSA, D.M.S. 2013. Ecotoxicological assessment of sediments from an urban marine protected area (Xixová-Japuí State Park, SP, Brazil). *Marine Pollution Bulletin* 75(1-2):62-68.

ASTM. 2000. Standard test methods for moisture, ash, and organic matter of peat and other organic soils. Method D 2974-00. *American Society for Testing and Materials*. West Conshohocken, PA.

BEJGARN, S., MACLEOD, M., BOGDAL, C., BREITHOLTZ, M. 2015. Toxicity of leachate from weathering plastics: An exploratory screening study with *Nitokra spinipes*. Chemosphere 132:114-119.

BESSELING, E., QUIK, J.T., SUN, M., KOELMANS, A.A. 2017. Fate of nano-and microplastic in freshwater systems: A modeling study. *Environmental Pollution* 220:540-548.

BOUR; A., AVIO, C.G., GORBI, S., REGOLI, F., HYLLAND, K. 2018. Presence of microplastics in benthic and epibenthic organisms: Influence of habitat, feeding mode and trophic level. *Environmental Pollution* 243(Pt B):1217-1225.

BRANDON, J., GOLDSTEIN, M., OHMAN, M.D. 2016. Long-term aging and degradation of microplastic particles: Comparing in situ oceanic and experimental weathering patterns. *Marine Pollution Bulletin* 110(1): 299-308.

BROWNE, M.A., NIVEN, S.J., GALLOWAY, T.S., ROWLAND, S.J., THOMPSON, R.C. 2013. Microplastic moves pollutants

and additives to worms, reducing functions linked to health and biodiversity. *Current Biology* 23(23):2388-2392.

CARPENTER, E.J. & SMITH, K.L. 1972. Plastics on the Sargasso Sea surface. *Science*, 175(4027), 1240-1241.

CHOUERI, R.B., CESAR, A., TORRES, R.J., ABESSA, D.M., MORAIS, R.D., PEREIRA, C.D., NASCIMENTO, M.R., MOZETO, A.A., RIBA, I., DELVALLS, T.A. 2009. Integrated sediment quality assessment in Paranaguá Estuarine System, Southern Brazil. *Ecotoxicology and Environmental Safety* 17;72(7):1824-31.

DELLA TORRE. C., BERGAMI, E., SALVATI, A., FALERI, C., CIRINO, P., DAWSON, K.A., CORSI, I. 2014. Accumulation and embryotoxicity of polystyrene nanoparticles at early stage of development of sea urchin embryos *Paracentrotus lividus*. *Environmental Science & Technology.* 48(20): 2302–12311.

DOYLE, M.J., WATSON, W., BOWLIN, N.M. & SHEAVLY, S.B. 2011. Plastic particles in coastal pelagic ecosystems of the Northeast Pacific ocean. *Marine Environmental Research*, 71(1), 41-52.

ENYOH, C.E., OHIAGU, F. O., VERLA, A.W., WANG, Q., SHAFEA, L., VERLA, E. N., ISIUKO, B.O., CHOWDHURY, T. IBE, F.C. & CHOWDHURY, M.A.H. 2021. "Plasti-remediation": Advances in the potential use of environmental plastics for pollutant removal. *Environmental Technology & Innovation*, 101791. https://doi.org/10.1016/j.eti.2021.101791

FENILI, L.H. 2012. Qualidade do sedimento do canal de navegação do Porto de Santos (Santos, SP) após dragagem de aprofundamento: ensaios ecotoxicológicos com *Tiburonella viscana* e *Nitokra* sp. (Doctoral Thesis, São Paulo University). 112p.

FERRAZ, M.A., CHOUERI, R.B., CASTRO, Í.B., DA SILVA, C.S., GALLUCCI, F. 2020. Influence of sediment organic carbon on toxicity depends on organism's trophic ecology. *Environmental Pollution 261*, 114134.

GALLOWAY, T.S., COLE, M., LEWIS, C. 2017. Interactions of microplastic debris throughout the marine ecosystem. *Nature Ecology & Evolution*. 1:116. doi: 10.1038/s4s1559-017-0116

GOUIN, T., ROCHE, N., LOHMANN, R., HODGES, G. 2011. A thermodynamic approach for assessing the environmental exposure of chemicals absorbed to microplastic. *Environmental Science & Technology* 45(4):1466-1472.

HAEGERBAEUMER, A., MUELLER, M., FUESER, H., TRAUNSPURGER, W. 2019. Impacts of Micro- and Nano-Sized Plastic Particles on Benthic Invertebrates: A Literature Review and Gap Analysis. *Frontiers in Environmental Science* 7 (17) doi:10.3389/fenvs.2019.00017

HIROTA, J. & SZYPER, J.P. 1975. Separation of total particulate carbon into inorganic and organic components. *Limnology and Oceanography* 20(5):896-900.

HORTON, A.A., WALTON, A., SPURGEON, D.J., LAHIVE, E., SVENDSEN, C. 2017. Microplastics in freshwater and terrestrial environments: evaluating the current understanding to identify the knowledge gaps and future research priorities. *Science of the Total Environment* 586:127-141.

HUANG, S., PENG, C., WANG, Z., XIONG, X., BI, Y., LIU, Y., LI, D. 2021. Spatiotemporal distribution of microplastics in surface water, biofilms, and sediments in the world's largest drinking water diversion project. *Science of the Total Environment*, 148001.

IZAR, G.M., MORAIS, L.G., PEREIRA, C.D.S., CESAR, A., ABESSA, D.M.S., CHRISTOFOLETTI, R.A. 2019. Quantitative analysis of pellets on beaches of the São Paulo coast and associated non-ingested ecotoxicological effects on marine organisms. *Regional Studies in Marine Science* 29:100705.

KARBALAEI, S., HANACHI, P., WALKER, T.R., & COLE, M. 2018. Occurrence, sources, human health impacts and mitigation of microplastic pollution. *Environmental science and Pollution Research*, 25(36), 36046-36063.

KOELMANS, A.A., BESSELING, E., FOEKEMA, E., KOOI, M., MINTENIG, S., OSSENDORP, B.C., REDONDO-HASSELERHARM, P.E., VERSCHOOR, A., VAN WEZEL, A.P., SCHEFFER, M. 2017. Risks of Plastic Debris: Unravelling Fact, Opinion, Perception, and Belief. *Environmental Science & Technology* 51(20). 11513-11519.

LENZ, R., ENDERS, K., & NIELSEN, T.G. 2016. Microplastic exposure studies should be environmentally realistic. *Proceedings of the National Academy of Sciences*, *113*(29), E4121-E4122.

LOBELLE, D. & CUNLIFFE, M. 2011. Early microbial biofilm formation on marine plastic debris. *Marine Pollution Bulletin* 62(1):197-200.

LOTUFO, G.R. & ABESSA, D.M.S. 2002. Testes de toxicidade com sedimento total e água intersticial estuarinos utilizando copépodos bentônicos. Nascimento IA, Sousa, ECPM, Nipper M. *Métodos em ecotoxicologia marinha: aplicações para o Brasil*. São Paulo, Artes Gráficas e Industriais, pp151-162.

LUSHER, A. 2015. Microplastics in the marine environment: distribution, interactions and effects. Bergmann L, Gutow L, Klages M. (Eds.) In: *Marine Anthropogenic Litter*, Springer International Publishing, Cham, pp245-307.

MANAHAN, S.E. 2013. *Química ambiental*. Tradução Wilson de Figueiredo Jardim. Editora Bookman. 9ª Edição. 912p.

MATO, Y., ISOBE, T., TAKADA, H., KANEHIRO, H., OHTAKE, C., KAMINUMA, T. 2001. Plastic resin pellets as a transport medium for toxic chemicals in the marine environment. Environmental Science & Technology 35(2):318-324.

MCCAVE, I.N., SYVITSKI, J.P.M. 1991. Principles and methods of geological particle size analysis. In: J.P.M. Syvitski (ed.). Principles, Methods, and Application of Particle Size Analysis, Cambridge University Press, Cambridge. pp. 3-21.

MOORE, C.J. 2008. Synthetic polymers in the marine environment: a rapidly increasing, long-term threat. *Environmental Research* 108, 131–139. doi: 10.1016/j.envres.2008.07.025

NETTO, A.D.P., MOREIRA, J.C., DIAS, A. E.X., ARBILLA, G., FERREIRA, L.F.V., OLIVEIRA, A.S. & BAREK, J. 2000. Avaliação da contaminação humana por hidrocarbonetos policíclicos aromáticos (HPAs) e seus derivados nitrados (NHPAs): uma revisão metodológica. *Química nova*, 23, 765-773.

NOBRE, C.R., SANTANA, M.F.M., MALUF, A., CORTEZ, F.S., CESAR, A., PEREIRA, C.D.S., TURRA, A. 2015. Assessment of microplastic toxicity to embryonic development of the sea urchin *Lytechinus variegatus* (Echinodermata: Echinoidea). *Marine Pollution Bulletin* 92(1):99-104.

PANNETIER, P., CACHOT, J., CLÉRANDEAU, C., FAURE, F., VAN ARKEL, K., DE ALENCASTRO, L. F., LAVASSEUR, C., SCIACCA, F., BOURGEOIS, J.P. & MORIN, B. 2019. Toxicity assessment of pollutants sorbed on environmental sample microplastics collected on beaches: Part I-adverse effects

on fish cell line. *Environmental Pollution*. 248, 1088 - 1097. ff10.1016/j.envpol.2018.12.091ff. ffhal-03486962

PANNETIER, P., MORIN, B., LE BIHANIC, F.., DUBREIL, L., CLÉRANDEAU, C., CHOUVELLON, F., ARKEL, K.V., DANION, M., CACHOT, J. 2020. Environmental samples of microplastics induce significant toxic effects in fish larvae. *Environment International*, *134*, 105047.

REDONDO-HASSELERHARM, P.E., FALAHUDIN, D., PEETERS, E.T.H.M., KOELMANS, A.A. 2018. Microplastic Effect Thresholds for Freshwater Benthic Macroinvertebrates. *Environmental Science and Technology* 52(4):2278-2286.

RENDELL-BHATTI, F., PAGANOS, P., POUCH, A., MITCHELL, C., D'ANIELLO, S., GODLEY, B.J., PAZDRO, K., ARNONE, M.I., JIMENEZ-GURI, E. 2021. Developmental toxicity of plastic leachates on the sea urchin *Paracentrotus lividus*, *Environmental Pollution.* (269):115744

REYNOLDSON, T.B., & METCALFE-SMITH, J.L. 1992. An overview of the assessment of aquatic ecosystem health using benthic invertebrates. *Journal of aquatic ecosystem health*, 1(4), 295-308

RIBEIRO, F., GARCIA, A.F., PEREIRA, B.P., FONSECA, M., MESTRE, N.C., FONSECA, T.G., ILHARCO, L.M., BEBIANNO, M.J. 2017. Microplastics effects in *Scrobicularia plana Marine Pollution Bulletin* , 122 (1–2): 379-391

ROCHMAN, C. M., HOH, E., KUROBE, T. & TEH, S. J. 2013. Ingested plastic transfers hazardous chemicals to fish and induces hepatic stress. Scientific reports, 3(1), 1-7. https://doi.org/10.1038/srep03263

RUMMEL, C.D., JAHNKE, A., GOROKHOVA, E., KÜHNEL, D., SCHMITT-JANSEN, M. 2017. Impacts of biofilm formation on the fate and potential effects of microplastic in the aquatic environment. *Environmental Science and Technology* 4(7):258-267.

SÁ, L.C., OLIVEIRA, M., RIBEIRO, F., ROCHA, T.L., FUTTER, M.N. 2018. Studies of the effects of microplastics on aquatic organisms: What do we know and where should we focus our efforts in the future? *Science of the Total Environment* (645):1029-1039.

SHAPIRO, S.S. & WILK, M.B. 1965. An analysis of variance test for normality (complete samples). *Biometrika* 52, 591-611.

TANIGUCHI, S., COLABUONO, F.I., DIAS, P.S., OLIVEIRA, R., FISNER, M., TURRA, A., IZAR, G.M., ABESSA, D.M.S., SAHA, M., HOSODA, J., YAMASHITA, R., TAKADA, H., LOURENÇO, R.A., MAGALHÃES, C.A., BICEGO, M.C., MONTONE, R.C. 2016. Spatial variability in persistent organic pollutants and polycyclic aromatic hydrocarbons found in beach-stranded pellets along the coast of the state of São Paulo, southeastern Brazil. *Marine Pollution Bulletin* 106(1):87-94. https://doi.org/10.1016/j.marpolbul.2016.03.024

TORRES, R.J., ABESSA, D.M.S., SANTOS, F.C., MARANHO, L.A., DAVANSO, M.B., DO NASCIMENTO, M.R., MOZETO, A.A. 2009. Effects of dredging operations on sediment quality: contaminant mobilization in dredged sediments from the Port of Santos, SP, Brazil. *Journal of Soils and Sediments* 9(5):420-432.

TURRA, A., MANZANO, A.B., DIAS, R.J.S., MAHIQUES, M.M., BARBOSA, L., BALTHAZAR-SILVA, D., MOREIRA, F.T. 2014. Three-dimensional distribution of plastic pellets in sandy beaches: shifting paradigms. *Scientific Reports* 4:4435.

USEPA. 2001. Methods for Collection, Storage and Manipulation of Sediments for Chemical and Toxicological Analyses: Technical Manual. EPA 823-B-01-002. U.S. *Environmental Protection Agency*, Office of Water, Washington, DC.

USEPA. 2003. A Compendium of chemical, Physical and Biological Methods for Assessing and Monitoring the Remediation of Contaminated Sediment Sites. *EPA/68- W-99-033.* United States Enviromental Protection Agency. Duxbury.

VAN, A., ROCHMAN, C.M., FLORES, E.M., HILL, K.L., VARGAS, E., VARGAS, S.A., HOH, E. 2012. Persistent organic pollutants in plastic marine debris found on beaches in San Diego, California. *Chemosphere*, 86(3):258-263.

VAN CAUWENBERGHE, L., DEVRIESE, L., GALGANI, F., ROBBENS, J., JANSSEN, C.R. 2015. Microplastics in sediments: a review of techniques, occurrence and effects. *Marine Environmental Research* 111:5-17.

VAN MOOS, N., BURKHARDT-HOLM, P., KÖHLER, A. 2012. Uptake and effects of microplastics on cells and tissue of the blue mussel Mytilus edulis L. after an experimental exposure. *Environmental Science and Technology*, *46*(20), 11327-11335.

VECCHI, S., BIANCHI, J., SCALICI, M., FABRONI, F., & TOMASSETTI, P. 2021. Field evidence for microplastic interactions in marine benthic invertebrates. *Scientific Reports*, 11(1), 1-12.

WANG, J., TAN, Z., PENG, J., QIU, Q., & LI, M. 2016. The behaviors of microplastics in the marine environment. *Marine Environmental Research*, 113, 7-17.

WANG, F., WONG, C.S., CHEN, D., LU, X., WANG, F., ZENG, E.Y. 2018. Interaction of toxic chemicals with microplastics: a critical review. *Water Research* 139:208-219.

WENTWORTH, C.K. 1922. A scale of grade and class terms for clastic sediments. *The Journal of Geology* 30(5):377-392.

ZIAJAHROMI, S., KUMAR, A., NEALE, P.A., LEUSCH, F.D. 2017. Impact of Microplastic Beads and Fibers on Waterflea (*Ceriodaphnia dubia*) Survival, Growth, and Reproduction: Implications of Single and Mixture Exposures. *Environmental Science and Toxicology* 51(22):13397-13406.

ZICCARDI, L.M., EDGINGTON, A., HENTZ, K., KULACKI, K.J., KANE DRISCOLL, S. 2016. Microplastics as vectors for bioaccumulation of hydrophobic organic chemicals in the marine environment: A state-of-the-science review. *Environmental Toxicology and Chemistry* 35(7):1667-1676.