Title page

Microplastic moves pollutants and additives to worms reducing functions linked to health and biodiversity

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Running head: Microplastic transfers chemicals reducing health

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Summary

Plastic debris can contain sorbed persistent and bioaccumulative pollutants. Although animals across trophic levels ingest micrometer-sized plastic debris (microplastic), it is unknown whether eating microplastic transfers pollutants to animals. Despite positive correlations reported for concentrations of ingested plastic and pollutants in tissues of animals, few, if any, controlled experiments have examined whether ingestion of microplastic transfers pollutants and additives to animals. We exposed lugworms (*Arenicola marina*) to polyvinylchloride (PVC; 5%) microplastic with common pollutants (nonylphenol, phenanthrene) and additive chemicals (Triclosan, PBDE-47) presorbed onto PVC. Ingested microplastic transferred pollutants and additives into gut-tissues of lugworms, at concentrations causing some biological effects, although clean sand transferred larger concentrations of pollutants into their tissues. Uptake of nonylphenol from PVC or sand reduced the ability of coelomocytes to remove pathogenic bacteria by >60%. Uptake of Triclosan from PVC diminished the ability of worms to engineer sediments and caused mortality, each by >55%, whilst PVC alone made the worms >30% more susceptible to oxidative stress. As global microplastic contamination accelerates, our findings that large concentrations of microplastic and additives can harm ecophysiological functions identify possible ecological impacts. It, however, remains to be seen whether these problems are significant at smaller concentrations of pollutants and microplastic.

Highlights

(i) Ingestion of microplastic by animals can transfer pollutants and additives to their tissues.

(ii) Biochemically clean sand transferred more pollutants into tissues than microplastic.

(iii) Uptake of pollutants, additives and microplastic damaged ecophysiological functions.

Results

By 2050, an extra 33 B.tonnes of plastic is anticipated to be added to our planet (1) This will place a larger burden on inadequate systems of management and policy that are struggling to prevent plastic waste infiltrating ecosystems [1, 2]. Disintegration into smaller pieces means the abundance of microplastic in marine habitats has increased [3] and now outnumbers larger debris [2, 4]. Once ingested by animals, microplastic provides a feasible pathway to transfer sorbed pollutants and additives into their tissues [7-14]. Here we unravel the hazards of microplastic as a multiple stressor to sedimentary organisms. Using single-concentration experiments (an approach endorsed by the EPA, [25]) we examined whether microplastic (PVC) sorbed and then released, pollutants (nonylphenol, phenanthrene) and additives (Triclosan, PBDE-47) to tissues of lugworms and if so, whether this altered the ability of lugworms to perform important ecophysiological functions. Animals from sedimentary habitats are vulnerable because plastic can accumulate concentrations of pollutants a hundred times greater than sediments [9] and there is 250% more microplastic in habitats that receive sewage [2] or are down-wind [3].

Surprisingly, the relative importance of ingesting microplastic versus sediments as vectors for pollutants to the tissues of animals is poorly understood. *In vitro* experiments simulating guts of lugworms indicate that pollutants which accumulate from seawater to microplastic would desorb into the gut, with more transfer from microplastic in sediments with less organic carbon [9]. In contrast, theoretical studies for fish predict eating microplastic would not increase burdens of pollutants because concentrations of pollutants will be at equilibrium with their environment [26]. Equilibrium scenarios are, however, problematic [27] because they assume pollutants and organisms are evenly distributed around the world and discount the (i) kinetic effects of gastric surfactants, pH and temperature on the desorption of pollutants from plastic; (ii) transfer and storage of microplastics in tissues of animals. To understand the actual chemical and biological impacts of microplastic experiments are required [9, 27, 28]. But experiments have been unable to determine whether microplastic transfers greater concentrations of

pollutants to tissues than sediments, or if microplastic is capable of transferring large enough concentrations of pollutants and additives to impair functions of animals that sustain health and biodiversity.

Transfer of chemicals from microplastic and from sand to the tissues of lugworms. Plastics discarded into habitats accumulate pollutants, such as nonylphenol and phenanthrene [8, 24] and could release these by desorption. To reproduce conditions in habitats, pollutants were sorbed separately onto particles of PVC or sand (Fig 1A-B). PVC was chosen because it comprises >25% of microplastic in estuaries inhabited by lugworms [3]. Total amounts of plastics and pollutants in treatments were large, but not without precedent in sedimentary habitats and experiments [28, 30, S1]. Here we show nonylphenol and phenanthrene desorbed from PVC and transferred into tissues (Figure 1 C-D). This provides the first conclusive evidence showing transfer from microplastic and supports predictions from modelling of desorption of phenanthrene that has been pre-sorbed to polyethylene [9, 12, 13]. Despite particles of PVC containing 135% (nonylphenol) and 5860% (phenanthrene) larger concentrations than those on sand (Figure 1A, B), worms exposed to sand (with smaller concentrations of pollutants) accumulated >250% more phenanthrene and nonylphenol in their tissues than when PVC transferred the pollutants. Each day worms ingested 47-74% of their mass in sediments and gastric concentrations of pollutants (ingestion) were >180% greater than those found in their body-wall (sorption) irrespective of whether they ingested contaminated PVC or sand (Figure 1C, D).

Do chemicals used as additives in plastic manufacture transfer from microplastics in a similar way to pollutants? To test this, worms were exposed to microplastic with the presorbed PBDE-47 (flame retardant) and Triclosan (antimicrobial) (Text S1). These chemicals are thought to improve the safety of plastic articles by reducing the risks of fires and microbial growth, respectively. Quantities of additives added to plastic were realistic to proportions of PBDE-47 (5-30%; [10]) and Triclosan (0-5%) used by industry [15]. We did not use a sand-only treatment as we were examining the potential for additives used in plastic manufacture to transfer from plastics rather than the role of particles of sand or plastic as

vectors for pollutants. As with pollutants, additives transferred from microplastics into tissues, leading to consistent patterns of bioconcentration (Figure 2). Again, the main route of uptake for chemicals into worms was sorption into the gut via ingestion. Relative to concentrations in experimental sediments, the body-walls of worms accumulated up to 950% greater concentrations, and the gut up to 3500% greater concentrations, of each additive (Figure 2C-D).

Biological consequences of microplastic and chemical transfer to lugworms. To determine whether microplastic is capable of transferring large enough concentrations of pollutants and additives to impair functions of worms that help maintain health and biodiversity, we used established bioassays for mortality, feeding, immune-function and oxidative status. Previous work showed animals exposed to nonylphenol and phenanthrene feed/burrow less [32-34] and are more susceptible to oxidative stress, pathogens and mortality [35-38], whilst additives (e.g. Triclosan) can also be toxic [39-42].

Because lugworms structure faunal assemblages by removing phytoplankton and silt from sediments [43, 44], we measured feeding and survival to determine if ingestion of microplastic reduced this ability. Here we show exposure to PVC in clean sand with and without nonylphenol, phenanthrene and Triclosan, in some cases, disrupted feeding. In treatments containing PVC with Triclosan, over 55% of worms died ($F_{1,10} = 22.73$, $P < 0.001$***, Figure 3D), but exposure to PVC with nonylphenol or phenanthrene had no effect (Figure 2A-C). Exposure to PBDE sorbed onto PVC reduced feeding although not significantly (Figure 3C). Likewise, ingestion of Triclosan from PVC, reduced feeding in *A. marina* by >65% ($F_{1,11}$= 19.94, $P < 0.01$**) and although not significant, worms ingesting PBDE from PVC fed 30% less (Figure 4C-D). Exposure to sand and/or microplastic with nonylphenol and phenanthrene did not reduce feeding (Figure 5A-B).

Previous experimental work had showed lugworms use phagocytosis to clear pathogenic bacteria from their coelomic fluid [45], so we used an established immunoassay [6] to measure the ability of coelomycytes to engulf particles of zymosan. Ingesting either sand or microplastic with nonylphenol reduced the phagocytic activity of coelomocytes by >60% ($F_{1,19}$= 6.70, $P < 0.05$*; Figure 5A), similar,

but non-significant patterns were shown for Triclosan, whilst phenanthrene and PBDE-47 had no effect (Figure 5B-D).

Because mammalian cells exposed to nanometer-sized plastic produce reactive oxygen species [46, 47] and lugworms use antioxidants in their tissues to buffer the oxidative damage caused by hydrogen peroxide that accumulates in tissues during summer low-tides [48, 49], we measured the oxidative status of lugworms. Here we show the coelomic fluid of lugworms that ingested sediment with PVC had >30% smaller capacity to deal with oxidative stress (Figure 6B), while exposure to pollutants and additives through desorption from PVC, had no effect.

Discussion

Here we show pollutants and additives transfer via desorption from both sand and from microplastics to the tissues of an important bioengineer. This is the first suitably controlled experimental evidence showing that eating plastics can move pollutants and additives into the tissues of animals.

The principal route by which chemicals transferred was ingestion via the gut, rather than sorption through the body-wall. In our experiments despite the considerable capacity of plastics to sorb chemicals and the fact that only 1% of the experimental sediments were ingested during our experiments, pollutants and additives readily desorbed from microplastic and accumulated in the gut of worms at concentrations 326-3770% larger than experimental sediments within 10 days. When the bioavailability of pollutants from sand and PVC were compared, larger concentrations transferred from the sand to lugworms. Thus the extent and rate of desorption from sand was much greater than from plastic, which retains more of each pollutant than clean sand. It is, however, premature to conclude that sediment from habitats is likely to transfer more pollutants into animals upon ingestion. Further experiments are needed to compare retention in natural sediments with more clay and organic carbon, with that of smaller-sized polymers. For instance, polyethylene, polypropylene and polystyrene debris in habitats have larger concentrations of organic pollutants than PVC [24], while smaller (e.g. <10 μm)

140 microplastic translocate and accumulate in cells and tissues of animals [6]. Thus certain plastics, at
141 smaller sizes, could transfer chemicals into the tissues directly, without the need for gastric desorption.
142 To determine the relative importance of desorption of chemicals from ingested and translocated [6]
143 debris as vectors for pollutants into animals requires carefully designed experiments underpinned by
144 better quality information from programmes of monitoring. This includes adequate replication and
145 quantitation at smaller scales, improved methods of detection (debris <330 μm, dull in colour and/or
146 granular [2]) and clearly articulated hypotheses. Over time this will provide the necessary information to
147 design more complex manipulative experiments (field and laboratory) that expose more taxa to the
148 sizes, types, mixtures and concentrations of microplastic, natural particulates, pollutants and additives
149 found in habitats. It will also shed light on important factors that influence the distribution and
150 abundance of microplastic and allow us to estimate the frequency at which sedimentary habitats contain
151 quantities of microplastic that exceed 5% of the sediments.

152 For now our short-term experiments with large proportions of PVC (5%) show worms eating
153 microplastic accumulated large enough concentrations of pollutants or additives to reduce survival
154 (Triclosan), feeding (Triclosan, PBDE), immunity (nonylphenol) and antioxidant capacity (PVC).
155 Reductions in the phagocytic activity of coelomocytes were caused by nonylphenol and such pollutant-
156 induced reductions in immunity can reduce resistance to diseases in terrestrial worms [50]. Because
157 mammalian cells exposed to nanometer-sized plastic also produce reactive oxygen species and less
158 protein [46, 47], we suggest the smaller capacity of worms to deal with oxidative stress could be
159 indicative of proteolysis or reductions in synthesis of their antioxidants. For Triclosan, concentrations
160 were orders of magnitude smaller than those causing mortality in crustaceans [32]. Previously it was
161 thought that sorbed pollutants are more likely to transfer to tissues of organisms than additives from
162 plastic [27], however, our findings are consistent with the concerns that some additives may be more
163 problematic [51]. Our results also agree with correlative evidence from studies in which lugworms
164 exposed to micrometer-sized polystyrene and polychlorinated biphenyls fed less and lost weight [28].

Given that experimental exclusion of lugworms changes the structure and functioning of soft-sediment habitats [43, 44] our work raises concerns for habitats where plastics in sediments exceed 5% by mass. Our experimental work advances this field by showing that ingestion of microplastic by organisms can transfer pollutants and additives to their tissues at concentrations sufficient to disrupt ecophysiological functions linked to health and biodiversity.

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Figure legends

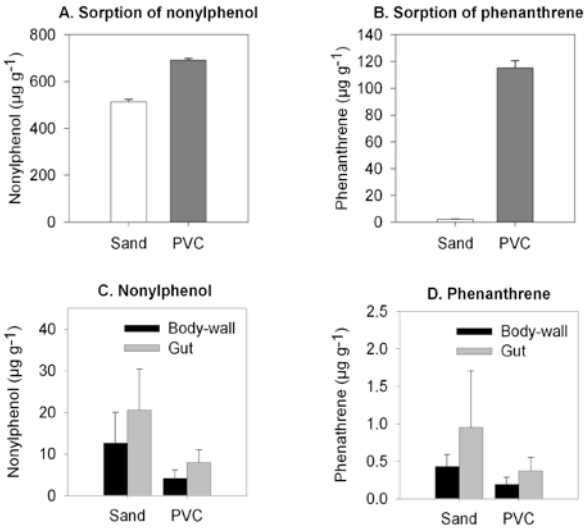

Figure 1. Pre-sorbed concentrations of pollutants nonylphenol (A) or phenanthrene (B) on clean particles of sand or PVC. Biological uptake of pollutants from either sand or micrometer-sized PVC to the tissues of lugworms (C, D). Transfer of pollutants from the microplastic PVC is demonstrated clearly. As expected, sand with pre-sorbed pollutants transported more phenanthrene and nonylphenol into tissues than microplastic due to the smaller retentive properties of clean sand. Interestingly, more of each pollutant accumulated in the gut than in the body-wall, irrespective of whether the worms ingested pollutants from desorption from PVC or sand. Data are means ± S.E. (n=5).

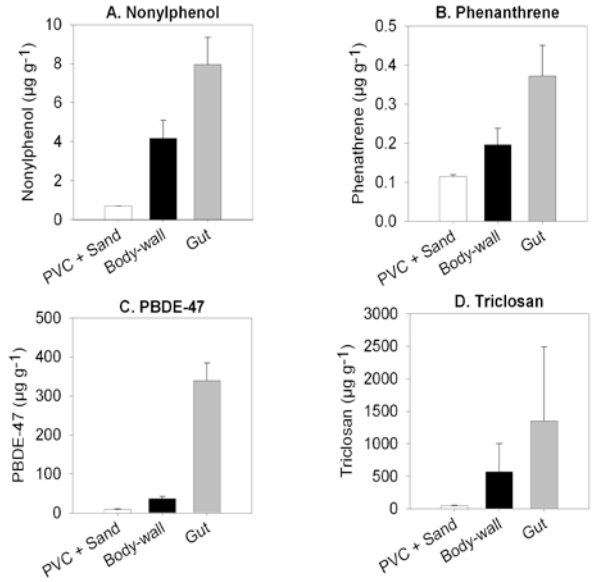

Figure 2. Bioconcentration of pollutants and additives within the tissues of lugworms from treatments with pollutants pre-sorbed onto PVC and mixed into clean sand (A-D). The body-walls of worms accumulated up to 950% greater concentrations, and the gut up to 3500% greater concentrations of each chemical. Data are means ± S.E. (pollutants n=5, PBDE-47 n = 6, Triclosan = 2-6). Figure 2A-B is different to Figure 1A-B because it provides the total concentrations of the pollutants that the worms were exposed to in the experimental sediments, whilst Figure 1A-B provides concentrations on the particles of sand or PVC alone.

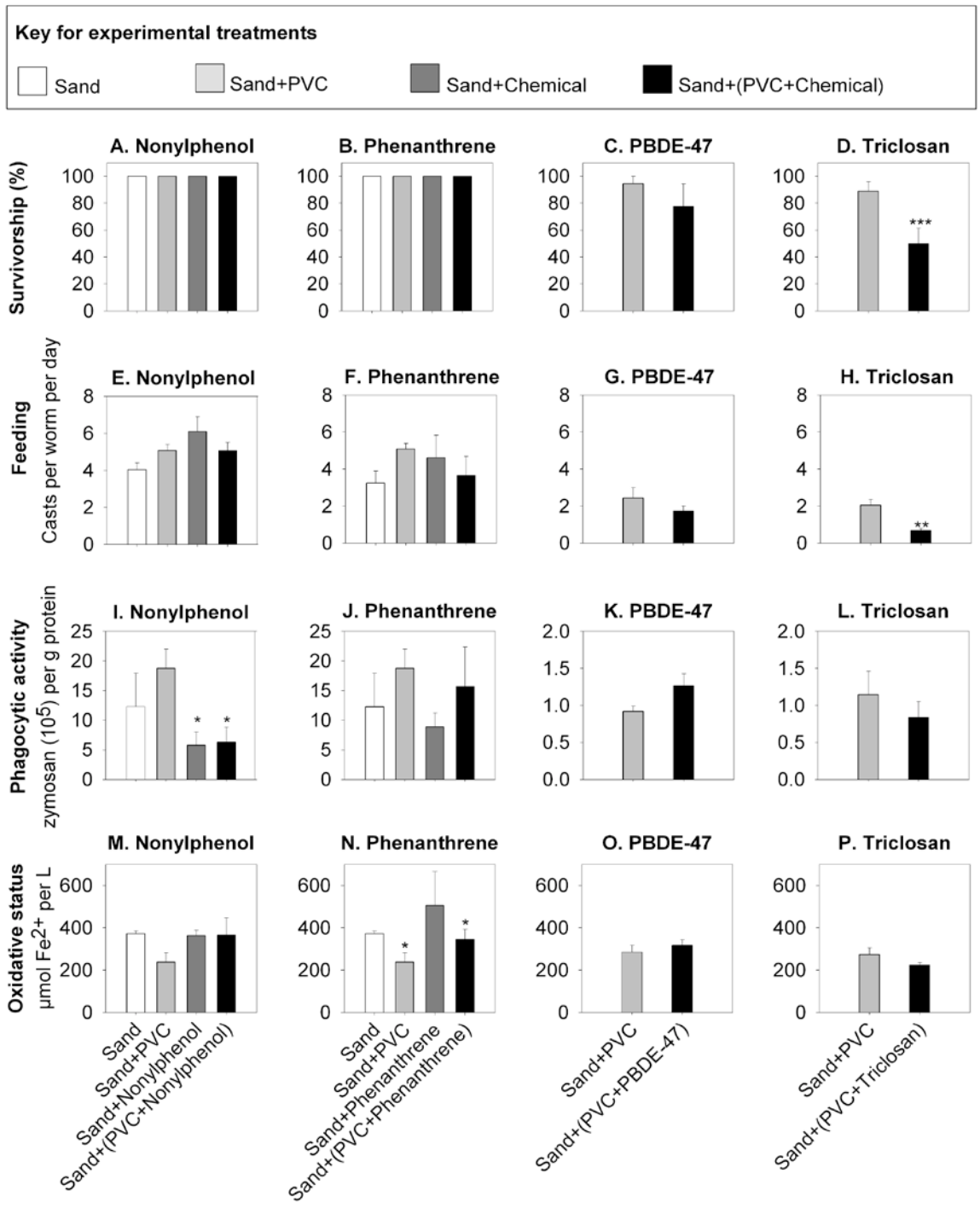

Figure 3. Effect of microplastic, sand, pollutants and additives on the survivorship (A-D), feeding (e-H), immunological (I-L)and oxidative functions (M-P)of lugworms. Triclosan reduced survival (D), and PBDE-47 (G) and Triclosan (H) reduced feeding. Ingesting either sand or microplastic with sorbed nonylphenol reduced the phagocytic activity of coelomocytes (I), similar, but non-significant patterns were shown for Triclosan (L). The coelomic fluid of worms that ingested sediment with PVC had a smaller capacity to deal with oxidative stress (M, N), while exposure to pollutants and additives through desorption from PVC, had no effect (O, P). Data are means ± S.E. Statistical significance at $P< 0.05$*, $P< 0.01$**, $P< 0.001$*** with n=5 (experiments with pollutants) and n=6 (experiments with additives). Large numbers of worms died in treatments with Triclosan so there were fewer animals to measure their phagocytic activity and oxidative status of their coelomic fluid (n=2).

Supplemental Information

Microplastic moves pollutants and additives to worms reducing functions linked to health and biodiversity

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Supplemental Inventory

Supplemental Experimental Procedures

Supplemental References

Supplemental Experimental Procedures

1. Sorption of chemicals onto microplastic and sand. We produced microplastic with similar concentrations of nonylphenol and phenanthrene to those found on plastic debris in the environment [8, 56]. 375 g of virgin PVC (230 µm; Goodfellow Cambridge Ltd) or certified clean sand (260 µm quartz silica sea sand; Fisher Scientific) was added to solutions of phenanthrene (7.524 mg) or nonylphenol (7.429 mg) dissolved in 400 mL absolute ethanol (Fisher Scientific). Particles of sand were within the size-range found on shores worldwide (114-697 µm) contaminated with microplastic [2-4]. Ethanol was evaporated in a fume cupboard and then contaminated PVC or sand was washed three times in Milli-Q-purified water to remove ethanol and unbound pollutants. Preliminary work showed that PVC treated in this way did not reduce the survival, feeding or immunity of worms (see Pilot study below, Fig. S1). For experiments with additives (Triclosan or PBDE-47) used in plastic manufacture was sorbed onto PVC by adding 375g to separate solutions of ethanol (400 mL; Fisher Scientific) with either Triclosan (411.7 mg) or PBDE-47 (60.5 mg), allowed to evaporate in a fume cupboard at room temperature and then washed as before to remove additives not bound to PVC. Chemical analyses confirmed 98 and 100% of PBDE-47 and Triclosan sorbed to PVC. Quantities of additives added to plastic were realistic to proportions of PBDE-47 (5-30%; [10]) and Triclosan (0-5%) used as an antimicrobial by industry [S1]**.** Although this does not mimic exactly the manner in which all additives are incorporated during manufacture, our approach is pragmatic since plastic with known concentrations of additives could not be sourced from suppliers due to issues of confidentiality.

Table S1. Concentrations of additives and pollutants on experimental particles (sand and PVC) and in treatments (sand+PVC), in relation to concentrations found on plastic articles and debris, and sediments from habitats.

Chemical	Source		Experimental concentrations (µg g^{-1})			Environmental concentration (µg g^{-1})			
	Additive	Pollutant	Sand	PVC	Sand+ PVC	Plastic article	Plastic debris	Sewage	Sediment
Nonylphenol	Stabilizer	Detergents	512.60 ±12.86	692.00 ±7.09	0.69±0 .01	<500-3300[S2]	0.13-16.00[S3]	450–2530(S4]	0.05-30[S5]
Phenanthrene	n/a	Combustion	1.97 ±0.34	115.32 ±5.53	0.11±0 .01	n/a	ΣPAH <1.90[S6]	0.25-1.76[S7]	<0.13 [S8]
PBDE-47	Flame Retardant	n/a	n/a	158.11	9.49±1 .94	50000-300000[S9]	0.59-1.13[S10]	<0.05-0.21[S11]	n/a
Triclosan	Antimicrobial	n/a	n/a	1097.8 7	57.30± 6.01	1000-50000[S12]	0.55-12.8[S13]	0.49± 13.87 [S14]	n/a

2. Plastic debris from sedimentary habitats.

Five replicate samples of debris were collected from the strandline at Plym Estuary (UK) and placed into a 500 mL foil containers. Material was separated, dried, identified and mass recorded using published methods [1] and the amount of plastic ranged from 13-29%.

3. Bioavailability and toxicity experiments.

Model organism. Lugworms were used because (i) they alter the physical and ecological structure of mud-flats by ingesting sediments [43, 44]; (ii) populations can comprise up to 32% of the biomass in food webs [52] and they provide food for predatory fish and birds [53, 54]; (iii) suffer large mortalities [55]; (iv) governments use studies of lugworms to evaluate the bioavailability and toxicity of pollutants [56, 57]; (v) ingest microplastics [4, 28]; (vi) the physico-chemical properties affecting gastric transfer pollutants from particulates to tissues are well studied [57, 58]; and (vii) tissues can be dissected [59].

Experiments. Two experiments examined the bioavailability and toxicity of presorbed chemicals from PVC or sand (Text S2). This first investigated the potential for microplastic to transport phenanthrene and nonylphenol from the environment to the tissues of worms, using the following replicated

420 treatments (n=5): sand only, sand+PVC, sand+contaminant, sand+PVC+contaminant. The second
421 examined whether ingested microplastic transfers additives (PBDE-47, Triclosan) to tissues with
422 replicated treatments (n=6) consisting of sand+PVC, sand+PVC+additive (see S3 for more details).
423 After 10 d worms were transferred into clean glass beakers containing clean seawater, so they could
424 remove sediment from their guts. On day 11, worms were removed from beakers for bioassays and
425 chemical analyses. For both experiments, each replicate was prepared in acid washed 2 L Pyrex©
426 beakers by adding 1500 g of the appropriate sediment mixture. Earlier attempts to quantify the route by
427 which pollutants transfer from habitats into animals rely on experimental designs that expose infauna to
428 pollutants with and without sediment [S15], however, maintaining animals without sediment is likely to
429 stress them confounding the comparison. We fed worms by mixing 750 µL *Isochrysis galbana* (Reed
430 Mariculture) into the sand with 500 mL of clean filtered seawater to form homogenous slurry.
431 Controlled amounts of food were used because animals deprived of food are more sensitive to pollutants
432 [7]. A further 1 L of seawater was added over a clean steel spoon to avoid disturbing the homogenous
433 mixture of sediment. Beakers were randomly arranged in the laboratory to remove bias associated with
434 environmental gradients. Tanks were covered with pre-cleaned (acetone/dichloromethane) ceramic tiles
435 and aerated, via glass pipettes inserted through a hole in each tile. Treatments were kept at 15 ºC under
436 12 hr light/dark cycle. Salinity was maintained, via addition of Mili-Q-water to a pre-marked level. For
437 each replicate, three lugworms were randomly chosen, mass recorded and carefully added to each
438 beaker. The mass of individual worms for experiments with pollutants was 2.9±0.4 g, whilst for
439 experiments with additives it was 4.6±0.8 g (mean ± S.D.). Dissolved oxygen, pH and salinity were
440 measured daily and faecal casts counted and collected each day, freeze-dried and weighed.

441 *Bioassays.* Feeding (number of casts, their mass) [56] and mortality were recorded. Coelomic fluid was
442 used to quantify the phagocytic activity of constituent coelomyctes and the ability of antioxidants to
443 reduce ferric ions (or "antioxidant power", [6]). This antioxidant assay provided an inexpensive tool for
444 measuring antioxidant status that did not require an understanding of the genetic and protein

composition of worms. The FRAP assay determines the antioxidant capacity of coelomic fluid to cope with oxidative stress, e.g. reactive oxygen species that can reduce survivorship potential. Coelomic fluid (10 µL) was pipetted in triplicate in microtitreplate wells. Aqueous solutions of known Fe^{II} concentrations in the range of 0-600 µmol L^{-1}, were used for calibration. 200 µL of reagent (300 mM acetate buffer, TBTZ (2,4,6-tripyridyl-s-triazine) was placed into each well. The plate was incubated at 25 ºC for 10 min and read at 593 nm. The phagocytosis assay [6] measures the ability of coelomocytes to clear bacteria through phagocytosis of particles of zymosan to give an indication of whether the immune-function has been damaged. For this haemolymph containing coelomocytes (10 µL) were transferred in triplicate into a microtitreplate and agitated using a plate shaker (1400 rpm for 60 sec). The plate was covered with a plate-sealer and incubated at 10 ºC for 50 min. Aliquots (50 µL) of suspended neutral-red-stained and heat stabilized zymosan suspension (containing 1 x 10^5 particles mL^{-1} in phosphate buffer) were added to each well and the plate incubated for 2.5 hr (10 ºC). The cells were washed to remove residual coelomocytes using 100 µL phosphate buffer (pH 7.4) and a series of zymosan standards were added. The dye was resolublized via addition of 100 µL of acetic acid in 50 % ethanol. The microtitreplate was covered with a plate-sealer and incubated for 10 min at 20 ºC, and then read at 550 nm. 190 µL was removed from each well and series of protein-standards (0, 0.2, 0.6, 1.0, 1.4, 2 g L^{-1}) added. BSA protein reagent (200 µL) was added to each well and left for 20-30 min. Protein assays were used to determine the number of zymosan particles phagoctyosed per g^{-1}coelomocyte protein [6]. Both assays have been used to measure changes in immune-function and oxidative stress in experiments with microplastic [6]. For the exposures involving phenanthrene and nonylphenol, formal comparisons of toxicity were made using two-factor ANOVA, where “toxicant” had two levels (present and absent) and “sediment” had two levels (sand and PVC). These were treated as fixed orthogonal factors. Formal comparisons of toxicity for PBDE-47 and Triclosan were made using one-factor ANOVA. For both Triclosan and PBDE-47 here “toxicant” had two levels (present or absent). Were necessary data were transformed to achieve Statistical analysis was done using GMAV (General Models of Analysis of Variance; EICC, University of Sydney, Australia). *Post-hoc* analysis of

significant interactions was done using SNK tests. Prior to experiments, worms, sediment and food did not contain detectable concentrations of the pollutants and additives. Worms were dissected using published techniques [59] to provide samples of body wall and gut (alimentary tract from pharynx to anus, including cecum, esophagus, stomachs, intestine, rectum). Coelomic fluids were removed from body-wall and sediment was removed from gut by rinsing with milipore water. Preliminary histological work showed microplastic was not attached to tissue samples.

Chemical analysis. Quantities of pollutants and additives in sediments, gut and body-wall were quantified using GC-MS. This was done using published methods [S17, S18], using nitrogen-dried extracts of Triclosan and nonylphenol, whilst PDBE-47 and phenanthrenewere done by re-dissolving samples in 1 mL dichloromethane prior to analysis by GC-MS. The efficiency of the extraction was >80% and was determined using standards and spiked sediments (Triclosan 95%, PBDE-47 82%, nonylphenol 90% and phenanthrene 91%).

4. Pilot study. Experimental treatments consisted of either sand+ PVC (control), and sand + PVC which was treated with ethanol and then washed three times. For both experiments each replicate was prepared in acid washed 2 L Pyrex© beakers by adding 1500 g of the appropriate sediment mixture. To maintain the animals throughout the exposure, 750 µL of *Isochrysis galbana* (4-7 µm; 8% dry mass); was mixed into the sand with 500 mL of clean filtered seawater to form a homogenous slurry. A further 1000 mL of seawater was added over a clean stainless steel spoon to avoid disturbing the mixture of sediment. For each experiment, the spatial arrangement was randomized to remove bias associated with possible environmental gradients in the temperature-controlled room. Tanks were then covered with pre-cleaned (acetone/dichloromethane) ceramic tiles and aerated, via a glass pipette inserted through a hole in the center of each tile. Treatments were kept at 15 ºC under 12 hr light/dark cycle for 10 d. Salinity was maintained, via addition of Mili-Q-purified water to a pre-marked level. For each replicate, three lugworms were chosen at random, their mass recorded and carefully added to each beaker.

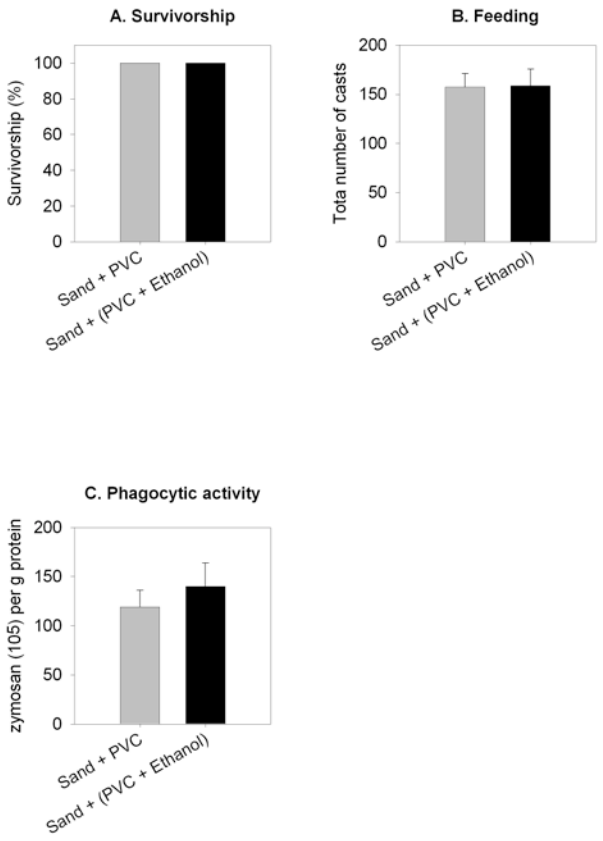

Fig. S2. Pilot study investigating whether treating PVC with ethanol and then washing it three times affected the survivorship (A) and feeding of worms (B), and the immunological functioning of their colemocytes (C). Data are means ± S.E. There were no significant differences and n = 5 for each.

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