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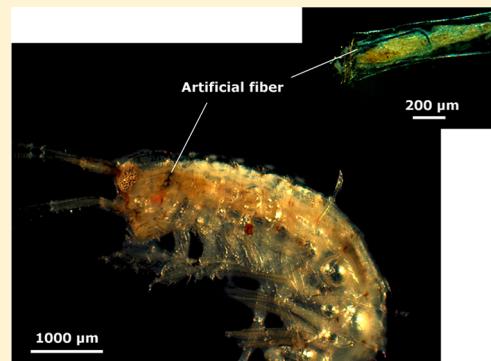
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When Microplastic Is Not Plastic: The Ingestion of Artificial Cellulose Fibers by Macrofauna Living in Seagrass Macrophytodebris

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ABSTRACT: Dead leaves of the Neptune grass, *Posidonia oceanica* (L.) Delile, in the Mediterranean coastal zone, are colonized by an abundant “detritivorous” invertebrate community that is heavily predated by fishes. This community was sampled in August 2011, November 2011, and March 2012 at two different sites in the Calvi Bay (Corsica). Ingested artificial fibers (AFs) of various sizes and colors were found in 27.6% of the digestive tracts of the nine dominant species regardless of their trophic level or taxon. No seasonal, spatial, size, or species-specific significant differences were revealed; suggesting that invertebrates ingest AFs at constant rates. Results showed that, in the gut contents of invertebrates, varying by trophic level, and across trophic levels, the overall ingestion of AFs was low (approximately 1 fiber per organism). Raman spectroscopy revealed that the ingested AFs were composed of viscose, an artificial, cellulose-based polymer. Most of these AFs also appeared to have been colored by industrial dyes. Two dyes were identified: Direct Blue 22 and Direct Red 28. The latter is known for being carcinogenic for vertebrates, potentially causing environmental problems for the *P. oceanica* litter community. Techniques such as Raman spectroscopy are necessary to investigate the particles composition, instead of relying on fragment size or color to identify the particles ingested by animals.



1. INTRODUCTION

Constituting up to 60–80% of all marine debris,¹ plastic detritus in the littoral areas have long been observed and recorded.^{2–5} However, in the few past years, an increasing number of studies and environmental concerns deal with a very particular type of plastic debris: “microplastics”. These microplastics are fragments less than 5 mm in size, as defined by the GESAMP (Group of Experts on the Scientific Aspects of Marine Environmental Protection) working group. They accumulate in surface waters, on beaches, and on the sea bottom.⁶ A large variety of microplastics find their way into the coastal shallow benthic environment.⁷ Among the variety of microplastics, fibers are one of the most abundant shapes encountered in the marine environment. They could adsorb organic pollutants, transport them through the marine environment,⁸ and, when ingested, could release pollutants in living organisms.⁹

Microplastic fibers are found in sediments worldwide.^{10–12} In surface samples taken from the North Pacific central Gyre, monofilaments were by far the most abundant plastic type found in the largest size range analyzed (>4.76 mm), and the second most abundant type found in the range from 2.80 to 4.76 mm.¹³ Colored microplastic fibers were also the predominant form in the intertidal samples found in beach environments.¹⁴ The main marine source of fibers appears to be the wastewater of washing machines: a single clothing garment could release more than 1900 fibers per wash.¹⁵

Studies dealing with the ingestion of artificial (manufactured from natural material) or synthetic (oil-based chemically manufactured) fibers by benthic invertebrates in natural conditions are not frequent although they are emerging.^{16–18} For example, in 2011 Murray and Cowie¹⁶ showed that benthic crustaceans (*Nephrops norvegicus*) ingest microplastics (83% of all individuals sampled) mainly composed of strands and monofilaments. Fibers ingested by marine organisms are not always made of plastic. Lusher et al.¹⁹ found that over half of the polymers ingested by fish in the English Channel were made of rayon, an artificial textile material made of reconstituted cellulose compounds.

In the Mediterranean Sea, the Neptune grass *Posidonia oceanica* (L.) Delile, an endemic seagrass, covers vast coastal areas from 0 to 40 m depth²⁰ forming extensive “meadows”. A significant part of its primary production (50 to 90% according to Cebrian and Duarte²¹) decays inside the meadow or is exported to adjacent sand patches. On these sand patches, dead *P. oceanica* leaves associated with living shoots, drifted macroalgae, and micro-organisms form exported litter accumulations.²² The animal community colonizing macrophytodebris (MPD) is largely dominated by crustaceans.²³ As

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anthropogenic debris end up in benthic shallow coastal zones, these MPD accumulations constitute a potential accumulation compartment for artificial or synthetic microfibers.

Owing to their small size, these microparticles can be ingested by an array of organisms^{2,17,19} including those from the vagile macrofauna associated with exported litter accumulations of *P. oceanica*, as it has been demonstrated for a supralittoral talitrid amphipod, *Talitrus saltator*.¹⁷ Litter macro-invertebrates constitute a potential food source for a variety of fishes and shrimps (Bell and Harmelin-Vivien²⁴ and personal data) and ingestion of artificial or synthetic fibers represent a potential contamination pathway to higher trophic levels and finally to other coastal ecosystems.^{17,19}

Various previous research on microplastic rely on visual identification and/or separation of plastic material from organic detritus using digestion (i.e., acid or concentrated hydrogen peroxide) and density separation.^{25–27} While some studies pair this approach with tools to identify the chemical composition of the material,^{25,28} many studies do not.^{29,30}

The lack of physicochemical characterization techniques could lead to a wrong estimation of the particles composition in some published papers. Particles categorized as plastic could be made of other material.

The main purpose of this study was to bring out the need of analytical techniques to assess that particles are made of plastic. In this case, fibers looked like plastic but spectroscopic analyses by Raman revealed another composition. The occurrence of such fibers in vagile macrocrustaceans living in *P. oceanica* detritus accumulations were also investigated.

2. MATERIAL AND METHODS

2.1. Sampling. Adult macrocrustaceans ($>500\ \mu\text{m}$) along with *P. oceanica* MPD were collected during field campaigns via scuba diving in summer 2011, autumn 2011, and winter 2012 at two 10 m deep sampling sites near the STARESO (STAtion de REcherches Sous-marines et Océanographiques) oceanographic station in the Bay of Calvi (Corsica, $8^{\circ}45'\text{E}$ $42^{\circ}35'\text{N}$). Litter samples, including *P. oceanica* rhizomes, were collected by hand in 50 L plastic bags closed under water to avoid contamination. Samples were subsequently sieved in laboratory on a 10 mm and 500 μm mesh in order to separate the MPD from macrocrustaceans. The living specimens were identified to specific level in laboratory prior to freezing ($T = -28\ ^\circ\text{C}$) for storing. After thawing, the digestive tract of each individual ($N = 235$ sampled invertebrates) was manually removed from its body and spread on a microscope slide with 99.5% bidistilled glycerin for later observation. *P. oceanica* reference fibers observed with SEM and used for Raman analysis (sections 2.4 and 2.5) were coming from rhizomes sampled at 10 m deep near the STARESO oceanographic station, in winter 2012. As a contamination control, samples of a blue paper towel were taken from the lab of STARESO for comparisons with ingested blue fibers.

2.2. Preventing Contamination. To avoid contamination the following steps were taken: a 100% cotton, white laboratory coat was worn, Petri dishes and microscopic slides were cleaned prior to dissection with 99% ethanol; gloves were worn throughout the preparation, dissection, and observation of the slides; and all instruments were cleaned with 99% ethanol in between each specimen. Glycerin was dropped on the microscopic slides at the last moment to avoid dust and airborne fibers to potentially contaminate the samples. As a contamination control, a blue paper towel frequently used in

the lab was sampled for SEM and Raman spectroscopy analyses and compared with blue fibers found in the invertebrates gut. Procedural controls were performed as well. Ten slides were cleaned with ethanol and prepared with nitrile gloves and 100% cotton laboratory coat. Glycerin was dropped on the 10 slides and left uncovered for 15 min (the average duration of a dissection) under the stereomicroscope. Slides were then observed during 5 min each under microscope at 40 \times and 100 \times magnification.

2.3. Light Microscopy. Microscopic slides were observed under a Zeiss microscope (magnification: $\times 40$), and the comparative proportions of gut content items were evaluated using the adapted technique described by Wilson and Bellwood³¹ to account for the small size of the invertebrate gut contents. A 4 cm^2 grid containing 100 small areas of 4 mm^2 was built, and 25 of these areas were randomly marked. For each marked area, the dominant item was identified, and values for each category of item were expressed as the percentage of squares in which that item was dominant. To deal with very small and rare items like artificial fibers, their number was simply counted over the entire gut content, separately from the semiquantitative method described above. The mean number of fibers, and the proportion of invertebrates with more than 1 fiber in the gut contents ("average global rate of ingestion") were calculated for each species. The artificial fragments were counted, described (shape, color), and photographed using a MOC-510 Mueller-Optronic 5 megapixel CMOS camera. Pictures taken were used to measure observed fibers with Tucsen-TS View 7 software.

2.4. Scanning Electron Microscopy. The stomach contents of each specimen were left to air-dry at room temperature on glass slides, covered to prevent contamination, for 24 h, and set on an aluminum support. The samples were sputter-coated with 20 nm Pt in BALZERS SCD 030 unit. The stomach contents were then observed in a JEOL JSM 840A scanning electron microscope (SEM) under 20 kV accelerating voltage. The same procedure was carried out for the observation of reference fibers from cotton and *P. oceanica* fibers.

2.5. Raman Spectroscopy. Analyses were performed on 15 fibers using a LabRam spectrometer (Jobin-Yvon) with an Olympus confocal microscope and an Andor BRDD Du401 CCD detector. To choose the best excitation wavelength, we used two lasers selected according to fiber color: a Spectraphysics argon-ion laser (488.0 or 514.5 nm) or a Torsana diode laser (784.7 nm). Due to low signal/noise ratio, various neutral density filters were used, but, as an average, the laser power on the sample was about 2 mW (blue laser) and 40 mW (red laser). The integration times ranged from 10 to 50 s per spectral portion, depending on the sample. The laser spot was focused on the target using a CCD camera. When necessary, a baseline correction was applied to the recorded spectra using a polynomial regression model and homemade software. The recorded spectra were matched with references available from our own libraries with the help of Thermo Spectra (v2.0) software. A total of 15 fibers were analyzed: 11 from stomach contents, 3 from *P. oceanica* rhizomes, and 1 from a commonly used blue paper towel used in the STARESO laboratories near the sampling sites (as a contamination control).

2.6. Statistics. AF ingestion data were tested for normality and for homogeneity of variance using Shapiro-Wilk's test and Levene's test, respectively. These showed the non-normality

and the heterogeneity of variance; therefore, ingestion data were tested using the nonparametric Kruskal–Wallis test for both seasonal and species specific variability and the nonparametric Mann–Whitney test for the spatial variability. All statistical analyses were conducted using Graphpad Prism (version 5.03) and Statsoft STATISTICA (version 10). Statistical significance was fixed at $p \leq 0.05$. Results are expressed in mean \pm standard deviation.

3. RESULTS

3.1. Airborne Contamination. The 10 control glass slides showed no fiber of any kind within the glycerin. Airborne contamination was therefore considered nonexistent.

3.2. Particles in the Stomach of Macroinvertebrates. A total of 17 species were identified (Table 1), among which

Table 1. Taxonomic List of Species Including Their Phylum, Order, Species, and Feeding Type

phylum	order	species	feeding type
Arthropoda	Amphipoda	<i>Gammarella fucicola</i>	detrivorous
		<i>Gammarus aequicauda</i>	detrivorous
		<i>Melita hergensis</i>	detrivorous
		<i>Nototropis guttatus</i>	detrivorous
	Nebaliacea	<i>Nebalia strausi</i>	omnivorous
	Decapoda	<i>Palaemon xiphias</i>	predatory
		<i>Liocarcinus navigator</i>	omnivorous
		<i>Athanas nitescens</i>	omnivorous
		<i>Galathea intermedia</i>	detrivorous

dominated the amphipods *Gammarella fucicola* (Leach, 1814), *Gammarus aequicauda* (Martynov, 1931), *Melita hergensis* (Reid, 1939), and *Nototropis guttatus* (Costa, 1853) together with the leptostracean *Nebalia strausi* (Risso, 1826) and the decapods *Athanas nitescens* (Leach, 1813), *Palaemon xiphias* (Risso, 1816), *Liocarcinus navigator* (Herbst, 1794), and *Galathea intermedia* (Liljeborg, 1851). The eight remaining species were pooled together into one single category, hereafter referred to as “others”. Diet of this “others” category was considered irrelevant as this pool is constituted of invertebrates species presenting very different diets. Therefore, it was not shown in Table 1.

The gut content items were sorted into five categories: dead *P. oceanica*, living *P. oceanica*, other vegetal material, animal tissues, and unknown material. Among the 235 gut contents examined, 65 exhibited at least one artificial fiber, representing a total of 91 fibers (Figure 1): 40 blue and 51 red. The digestive tract-contents revealed a diversity of feeding patterns among the macroinvertebrate community that includes detritivorous species (*G. aequicauda*) ingesting large amounts of *P. oceanica* detritus, primary consumers (*G. fucicola*, *M. hergensis*, *N. strausi*) eating macro- and/or microepiphytic algae growing on detritus, omnivorous species (*L. navigator*, *A. nitescens*, *G. intermedia*), and predators (*P. xiphias*) (Figure 2A).

The average global level of ingestion of fibers (Figure 2B) for the community was of 27.6%. The lowest relative percentage of invertebrates with more than 1 fiber in the gut contents was observed for the decapod *Galathea intermedia* (11.1%), and the highest one was observed for the amphipod *Gammarella fucicola* (33.3%). Despite the apparently diverse trophic preferences of the species, no significant difference in fiber ingestion (Figure 2B) was observed between them.

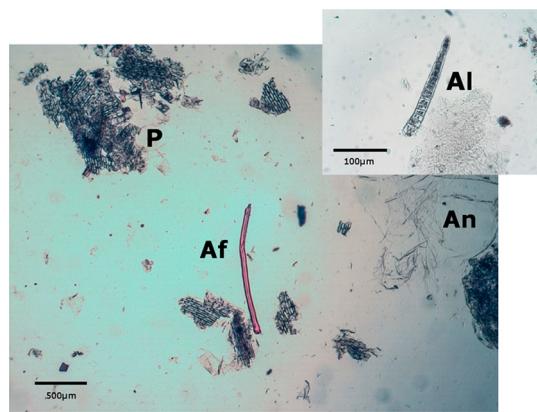


Figure 1. Photograph of a partial gut contents of specimen (*Gammarus aequicauda*) as viewed in light microscopy, showing an artificial fiber (Af), animal material (An), and fragments of dead *Posidonia oceanica* leaves (P) and algal tissues (Al).

Among the invertebrates that ingested fibers, we recorded an average of 1.38 (± 0.79) ingested fiber per organism, and the number of ingested fibers ranged from one to a maximum of six per individual (6 fragments encountered only once in *Gammarus aequicauda* in March 2012). Observed fibers were either positioned longitudinally inside the posterior digestive tract or folded inside the anterior digestive tract. The average fiber length was of 1.23 mm (± 0.66), ranging from 0.1 mm to 6 mm (Figure 3). No significant difference in the number of ingested fibers was observed between seasons or sites.

3.3. SEM Observations. The morphology of the fibers found inside the stomachs was compared with reference fibers which could potentially be ingested, such as textile fibers (represented by cotton) and *P. oceanica* fibers (Figure 4). AFs ingested were cylindrical and composed of several smaller cylinders. Their morphology differs in size and external aspect from *P. oceanica* and from cotton. *P. oceanica* fibers (vascular bundles) are much thicker ($\pm 250 \mu\text{m}$) than both AFs (60 μm) and cotton (40 μm). Second, the aspect of the *P. oceanica* fibers is irregular, with stripes and cavities (Figure 4 E,F), while cotton fibers are smooth and flattened. Consequently, it can be concluded that the fibers ingested were not cotton fibers and did not come from the seagrass *P. oceanica* despite the latter being part of the diet of benthic macroinvertebrates sampled.

3.4. Fiber Identification by Raman Spectroscopy. Raman spectroscopy analyses of the fibers found in macrocrustaceans' stomachs revealed the main component of these blue and red fibers to be cellulose. Comparing the Raman spectra of such fibers (Figure 5A,B) with that of a natural cotton fiber of pure cellulose (Figure 5C) also revealed the presence of an additional component in the AFs.

Subtracting the cellulose spectrum to 6 spectra from blue fibers (Figure 6A) provided 6 very similar subtraction-spectra of the additional component and allowed its identification (Thermo Spectra program) as being assigned to a blue coloring agent called Direct Blue 22 (DR 22) (Figure 6B). Once the cellulose spectrum is subtracted, the match between one blue fiber and the DR 22 spectrum was 43%. This match is actually not very high, but one has to consider that 1) the spectra intensity is weak because a low laser power had to be used to avoid damage to the sample and the noise is high, 2) the subtraction procedure induced additional noise, and 3) the fibers could be altered by their stay in seawater and in the invertebrate stomach for unknown duration. Considering these

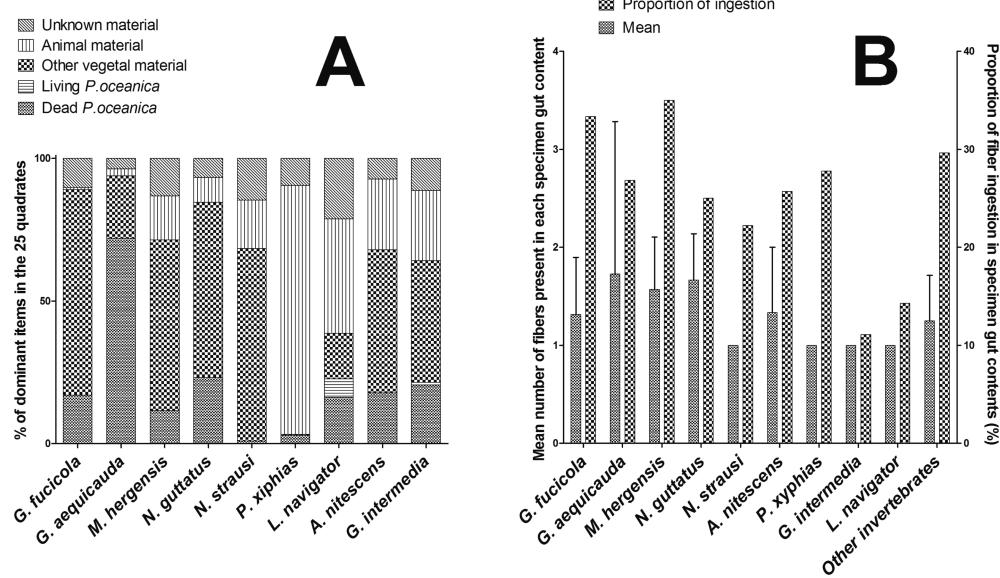


Figure 2. (A) Proportion (%) of dominant items observed in the 25 random quadrates for the nine most dominant vagile invertebrates species; (B) relative proportion of invertebrates with ≥ 1 fiber in the gut contents, observed in the 9 most dominant vagile invertebrates species. Mean values are expressed with standard deviation bars.

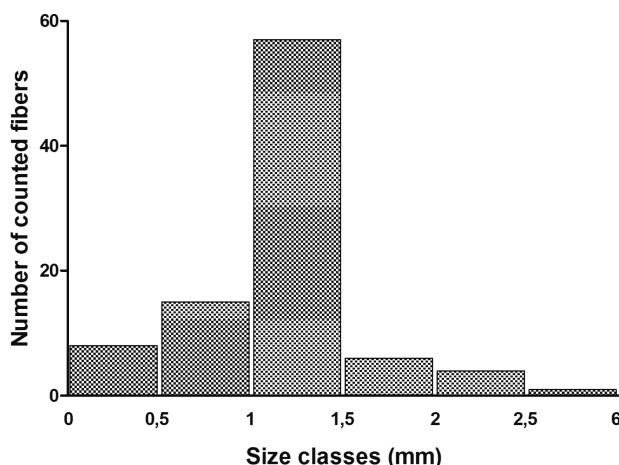


Figure 3. Length classes (mm) of the 91 artificial fibers found in the 17 vagile macrocrustacean species.

potential problems, the result of the comparison is satisfactory and as shown in Figure 6B, the DR 22 spectrum and the fiber one fit reasonably well.

The spectra of five red fibers were also identified as a mix of two spectra. Despite the five red fibers not showing exactly the same spectrum, they were interpreted as a mix of cellulose and a red coloring agent called Direct Red 28 (DR 28, also known as “congo red”). Most of the peaks were present in each spectrum, but they varied in intensity. The fourth Raman spectrum nearly matched the one of the cellulose (Figure 6C). It might be related to the difference in coloring agent concentration between fibers and between regions of a same fiber. The match between one red fiber and DR 28 was 46%. The other peaks came from the cellulose (Figure 6D).

The *P. oceanica* fiber was analyzed, and three identical spectra were obtained. The typical peak pattern of cellulose (a large peak at 1400 cm^{-1} , a high and double peak at 1100 cm^{-1} , and a sharp peak at 900 cm^{-1}) was not observed (Figure 6E). It has

been assumed that the peak at 1600 cm^{-1} came from lignin³² and not from cellulose.

The spectrum obtained for the blue paper towel used in the laboratory of STARESO, which could potentially contaminate sampling sites, showed that it was not made of cellulose. Again, the three characteristic peaks of cellulose did not appear (Figure 6F). Unfortunately, no match was found between this spectrum and any other from the library. Moreover, the blue grains coloring the paper were not identified as Direct Blue 22 but as Ingrain Blue (data not shown).

4. DISCUSSION

This study reveals that, at a small spatial scale, the vagile litter macrofauna community at the basis of food webs is contaminated by artificial, but not synthetic, fibers. Indeed, the presence of artificial coloring agents proved that the stomach contains AFs which did not have a natural origin. As composition of only 11 out of 91 fibers have been confirmed by Raman spectroscopy, results and interpretations must be taken with care. The analyzed AFs were not plastic, which was the most anticipated possibility after observation in light microscopy, but were made of viscose. It cannot be ensured that all the 91 fibers were viscose, but it is likely that the majority of them are made of artificial cellulose. Only 11 out of 91 potentially artificial fibers have been analyzed with Raman spectroscopy, which could appear quite low. The only fibers actually available for Raman analysis were those observed during dissection, before gut content spreading in glycerin. Fibers observed under microscope in glycerin were contaminated for Raman spectroscopy, and washing techniques to eliminate glycerin contamination are destructive. As fibers were not easily distinguishable before spreading in glycerin, only a small fraction of the identified fibers was available for Raman analysis.

SEM views clearly showed that the ingested fibers originated from neither *Posidonia oceanica* nor cotton. These pictures were analyzed with the criteria defined by Noren³³ for plastic fibers, and it appeared that it was not plastic. That was confirmed by

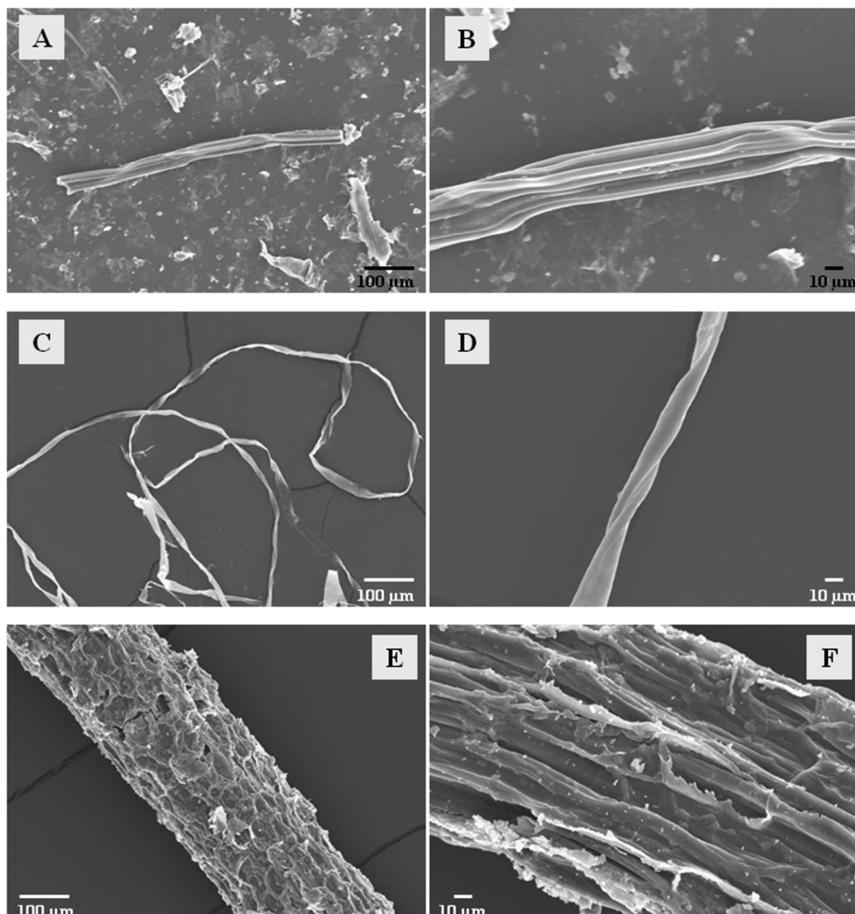


Figure 4. SEM images of AFs found in amphipod (*G. fucicola*) stomach (A, B), cotton fibers (C, D), and *P. oceanica* fiber (E, F).

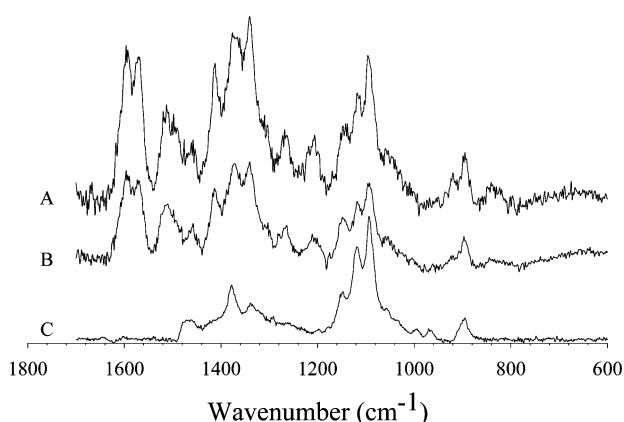


Figure 5. Raman spectra of two fibers (A, B) found in macrocrustaceans stomachs and of pure cellulose from a cotton fiber (C).

Raman spectroscopy analyses that demonstrated their cellulose composition. The comparison of the analyzed AFs morphology with photographs of fibers from the literature revealed a close similarity with viscose fibers from the previous studies of Kramar et al.³⁴ (Figure 7), Rojo et al.,³⁵ Suñol et al.,³⁶ and Xu et al.³⁷ In accordance with our Raman spectroscopy data, the sampled benthic macrocrustaceans ingested artificial fibers, such as dyed cellulose fibers, or viscose as termed by the textile industry. Cellulose xanthate (also called “viscose”, “rayon”, or “artificial silk”) was the first artificial manmade textile fiber family, invented in 1884 by the French scientist and

industrialist, Hilaire de Chardonnet (1838–1924). This fiber family is made of wood pulp from various trees or plants (e.g., bamboo) which is chemically converted into a soluble compound. It is then dissolved and forced to an extruding spinneret to produce filaments which are nearly pure cellulose.³⁸ The properties of viscose are close to those of cotton: poorly elastic, highly absorbent, and easily dyed. Viscose production was developed in 1891 and used extensively in the textile and tire industries, representing up to 25% of the world manmade fibers production in 1978.³⁸ According to the “PCI Fibers Red Book 2012”, global worldwide viscose production for 2012 was ~4.9 million tons, still representing 5.7% of 2012 global worldwide fiber production (~85 million tons). The production of viscose has increased in recent years due to an increasing interest in “natural” or “wood-made” textile tissues.

Nonsynthetic materials such as viscose can easily be mistaken for plastic due to its color, shape, or buoyancy. In this study, the fibers found in the stomach contents of macrocrustaceans were not plastic, despite their color and shape. Few studies analyzed plastic-like particles with spectroscopic techniques. Some studies used chemical digestion to eliminate organic-based material such as hydrochloric acid digestion³⁹ or hydrogen peroxide,²⁸ and others relied only on visual criteria.^{40,41} A number of studies could therefore have underestimated or overestimated the number of plastics due to their physical characteristics or their external aspect potentially misleading.

Our study results emphasized the need to use Raman spectroscopy or other chemical analyses to explore and confirm

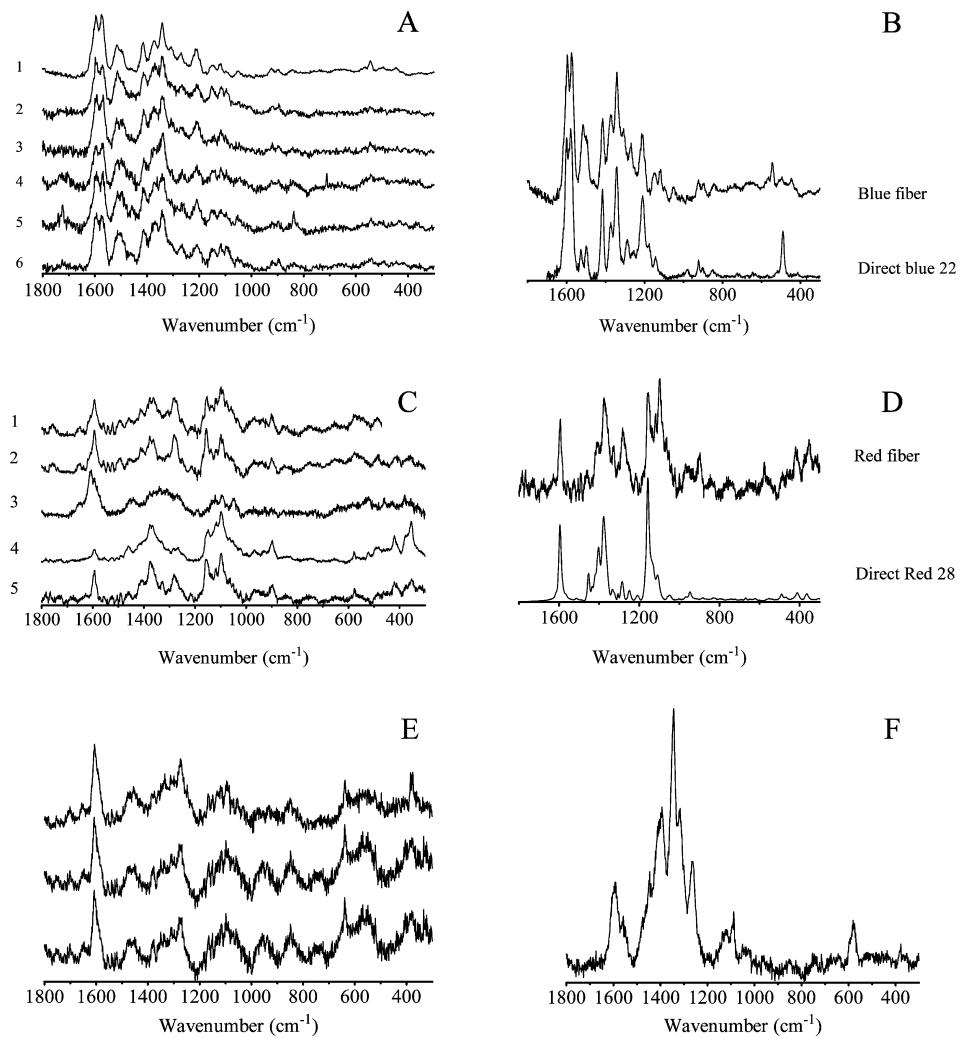


Figure 6. Raman cellulose-subtracted spectra of (A) the six blue fibers found in macrocrustaceans stomachs, (B) the first blue fiber and the Direct Blue 22 colorant (personal library, Inorganic Analytical Chemistry, ULg), (C) the five red fibers found in macrocrustaceans stomachs; (D) the red fiber no. 5 and the Direct Red 28 colorant (personal library, Inorganic Analytical Chemistry, ULg); (E) three different zones in the same *P. oceanica* fiber; (F) one fiber of a common blue paper towel used in STARESO.

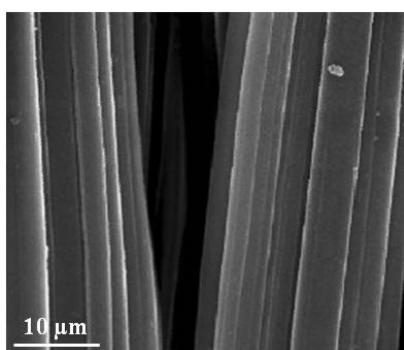


Figure 7. SEM photographs of viscose fiber, modified from Kramar et al.³⁴

the fiber composition. Moreover, it is also important not to only focus on colorants or pigments, since artificial colorants can be used for dyeing natural fibers (e.g., cotton) or artificial fibers (e.g., viscose) as well as plastic. In our study, the two colorants found are artificial and used, *inter alia*, in the textile industry. This study demonstrates that artificial colorants are not reliable indicators of microparticles of plastic.

Cellulose may not be an environmental issue in itself, but associated dyes or additives could be potentially harmful for the studied macroinvertebrates population. Indeed, while Direct Blue 22 is not considered harmful for humans, Direct Red 28 is classified as a carcinogenic, mutagenic, or toxic to reproduction coloring agent (Sustainable Production and Consumption (SUSPROC), 2006).⁴² Its negative effect on marine invertebrates remains uncertain but is clearly proven in the case of mammals and fishes. It is known that human intestinal bacteria are able to reduce the azo-linkage of Direct Red 28, which results in benzidine molecules which have been classified as carcinogenic for humans and cause bladder cancer (International Agency for Research on Cancer (IARC), 2012),⁴³ as well as inducing malformations of the telencephalon region in zebrafish.⁴⁴ The harmful effect of the Direct Red 28 coloring agent for the studied macroinvertebrates is only a hypothesis for the reason that its cleavage in benzidine molecules or its assimilation in crustaceans has not been proven yet. Terrestrial and marine invertebrates are potentially capable of digesting and degrading cellulose,^{45–47} consequently accelerating a coloring agent leaking in the invertebrates' digestive tracts and inducing a higher contamination. However, as fibers

ingestion appears to be low, the retention time seems to be short, and the colorant concentration in fibers is unknown, the impacts on invertebrates should be minor.

Vagile *P. oceanica* litter macroinvertebrates show no significant seasonal, spatial, or color trends in the ingestion of viscose fibers. Even though 27% of sampled organisms contained 1 or more artificial fibers, the average amount of artificial fibers in each individual digestive tract was small (1.38 fiber) which is relatively low and could therefore indicate the small retention time of these fibers in the guts of the sampled invertebrates. According to several studies,^{16,48,49} microplastic particles could remain in the digestive tract of a crustacean from several hours to 14 days. The retention time depends on the microplastic shape, the internal morphology of the foregut, and the presence or absence of food in the digestive tract.

Invertebrates seem to ingest fragments in a wide range of sizes, but as we did not perform any controlled feeding experiments it is tricky to assess whether this represents an actual 1 to 1.5 mm fibers size selection or only a greater "natural" abundance of this size class in the environment and, consequently, in the gut contents. A controlled feeding experiment conducted with adult talitrid amphipods (*Talitrus saltator*) showed no size selectivity.¹⁷

It has recently been demonstrated by *in vitro* studies that microplastics can be transferred in invertebrates from one trophic level to another.^{50,51} Plastics can be translocated to consumer tissues and then be transmitted to the predator or directly be transmitted from the consumer's digestive tract to the predator's digestive tract.⁵¹ The observed viscose fibers thus do not seem to be transmitted from lower to higher trophic levels via predation. One of the main possible explanations could be related to the lower retention time of the nonplastic observed fibers here in the gut. Indeed, cellulose, even of artificial origin like viscose, is more digestible and degradable⁵² than plastic. Some marine invertebrates are known to be able to digest cellulose, and this could explain both the faster digestive transit of the fibers^{45–47} and the absence of accumulation. The small average amount of AFs found in the invertebrates' gut contents also seems to favor this nonaccumulation or transmission.

Another major observation from this study is that AFs from macrocrustacean guts did not show any sign of significant degradation in SEM, since every part remained smooth and quite regular. In addition, viscose fibers are known to degrade more rapidly (100% in 8 weeks) than cotton fibers, both by sunlight or in soil when buried.^{52–54} There is however a lack of literature describing the degradation of AFs (primarily viscose) in seawater.

Finally, the precise origin of these AFs is unknown, but as the blue and red coloring agents were similar for all the fiber found in the macrocrustacean guts, it can be hypothesized that the same sources produced all of the sampled fibers present year round. As marine currents can be strong and very variable in the Calvi Bay, the precise location of the source(s) is unknown.

These AFs, associated with toxic industrial coloring agent, may cause a potential environmental and health issue. Sampling heavily impacted sites seems crucial for a better understanding of this contamination. However, the real impact of these AFs on the macroinvertebrates digestive tracts, as well as the impact of the toxic coloring agents on them and their predators, currently remains unknown.

Most importantly, this research demonstrates the need to use very specific analytical methods such as Raman spectroscopy.

Other suitable physicochemical techniques are also promoted; pyrolysis-gas chromatography (GC) in combination with mass spectrometry (MS), infrared (IR), or Fourier-transform infrared (FTIR) spectroscopy allow an accurate identification of polymers. However, while Raman spectroscopy and FTIR are conservative techniques, pyrolysis has the disadvantage of destroying the particle. To fully characterize particles' composition, accurate chemical identification is needed in order to avoid erroneous and misuse of the term "microplastic".

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Author Contributions

#F.R. and F.C. contributed equally and share first coauthorship. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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