



Preliminary data indicates the importance of depuration in oysters for microfibers contamination

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Abstract. The aim of this study is to investigate the presence of microfibers in oysters after depuration with an experimental filtering approach. The control didn't show microfibers and the treatment presented microfibers in oysters' soft parts, suggesting the importance of depuration.

Key words: Contamination, Marine debris, Bivalve, *Crassostrea*, Seafood.

Resumo: Dados preliminares indicam a importância da depuração em ostras para contaminação de microfibras. O objetivo deste estudo é investigar a presença de microfibras em ostras depuradas com um ensaio experimental de filtração. O controle não apresentou microfibras e o tratamento apresentou microfibras nas partes moles das ostras, sugerindo a importância da depuração.

Palavras-chave: Contaminação, detritos marinhos, bivalves, *Crassostrea*, frutos do mar.

Plastic pollution is a global issue, mainly in marine regions (Lusher; Hollman; Mendoza, 2017; Thompson et al., 2004; Wagner; Lambert, 2018). Pollution caused by microplastics, mainly by synthetic microfiber products associated with the textile industry is an alarming factor (Thompson et al., 2004; Van Amber; Niven; Wilson, 2010). Synthetic clothes used by thousands of individuals worldwide account for the release of microfibers (smaller than 1mm in diameter) during washing procedures, which can be deposited in coastal and oceanic regions (Absher et al., 2019; Cesa et al., 2017; Ladewig; Bao, Chow, 2015; Napper; Thompson, 2016). According to Mintenig et al. (2017), water treatment stations cannot retain microfibers that end up accumulating in aquatic environments such as lakes, bays, beaches and oceans. Microfibers are easily ingested by aquatic

organisms due to their small dimensions (Lusher; Hollman; Mendoza, 2017; Staichak et al, 2021). They have been found in the stomach of fish (Vidal et al., 2021) and invertebrates (Cole et al., 2014; Moreschi et al, 2020; Taylor et al., 2016; Vieira et al, 2021). Moreover, they may lead to bioaccumulation and biomagnification and be introduced in humans' diet (Li et al., 2015; Rochman et al., 2015; Teuten et al., 2009). Problem gets when it comes to cultivable and commercialized bivalve filter animals such as oysters and mussels, since microfibers can be filtered and assimilated by tissues of these mollusks and, consequently, be consumed by humans (Cho et al., 2019; Lusher; Hollman; Mendoza, 2017; Nobre et al., 2020; Rochman, 2015). Purification systems used to clean biological contaminants deriving from microorganisms are an alternative to help mitigate contamination caused by microfibers in bivalves

(Leal Diego et al., 2013). Birnstiel et al. (2019) reported that depuration in the mussel *Perna perna* (Linnaeus, 1758) helped reducing microplastic incidence in wild and farmed mussels. The aim of this study is to investigate the incidence of microfibers in depurated and sold individuals of the native oyster species *Crassostrea brasiliiana* (Lamarck, 1819) in Paranaguá city.

Twenty-four alive oysters (*C. brasiliiana*) were acquired in Paranaguá Municipal Market (25.522°S; 48.507°W), Parana State coastal region, Brazil, in 2017. Paranaguá is the largest city in the Paraná coast, with a population of 150 thousand inhabitants. Cargo transport is the main economic activity in Paranaguá City, which has the second largest harbor in the country. Prior to their commercialization, the oysters were subjected to depuration in running seawater for 24 hours, then filtered and treated with UV light, according to the Paranaguá Municipal Law (Decrees n° 2027/2007 and Wording given by Decree n° 758/2009). Oysters were transported to the Zoology Laboratory at Universidade Estadual de Ponta Grossa (UEPG), where they were weighed (gross weight) and had their shells measured in height (umbonal region at the opposite end of the valve), length (from the anterior to the posterior region of the valve) and thickness (dimension between right and left valves) (Supplementary material FS1). Subsequently, an experiment was performed with depuration oysters sold. The oysters were placed in two trays with 12 individuals (each) and stored in filtered seawater in cellulose filter at 28‰ salinity and room temperature (25 °C) for 24 hours. The experimental time was set according to the depuration time often used for oysters (Leal Diego et al., 2013; Phuong et al., 2018; Rochman et al., 2015). The control (C) tray was filled with filtered seawater without microfibers, whereas the treatment (T) tray was filled with filtered seawater in and 15 mg (3 mg.L⁻¹) of green and red microfibers (average diameters of 17.35 ± 4.17 µm) from known synthetic clothing (Fig. 1, Supplementary material Fig. S2). After the end of the experiment, the oysters were mechanically opened and had their adductor muscle sectioned to determine the presence, or absence, of microfibers in soft tissues. Mann-Whitney U test (nonparametric) was applied to assess the differences (p < 0.05) in biomass and shell biometry between the control and treatment groups; it was carried out in STATISTICA 10 demo software.

Microfiber samples were washed with ultrapure water, left to dry at room temperature and placed on a glass slide. Microfiber identification was

performed with the aid of a spectrometer (Confocal Raman Horiba / Xplora Plus model) at C-LABMU/UEPG. Spectra were excited at a wavelength of 532 nm, under a 200X microscope objective lens and 200-µm laser penetration. Readings were performed five times based on a three-second exposure time. Spectra were recorded at a wavenumber ranging from 600 cm⁻¹ to 3400 cm⁻¹. Samples were fixed with carbon adhesive tape on metallic supports and metallized with gold to be analyzed under a scanning electron microscope (FEG - model Mira 3 / Tescan) at C-LABMU/UEPG.

Oysters were 72.54 mm in height (± 7.03), 50.78 mm (± 6.83) in length, 20.86 mm (± 3.84) in thickness and 51.22 g (± 13.04) in weight. There were differences in height (p = 0.002) and length (p < 0.001) between the control and treatment groups (Supplementary material Table I). However, all oysters presented the commercial size (shell height) set for Brazilian species *Crassostrea gigas* (Thunberg, 1793), *Crassostrea rhizophorae* (Guilding, 1828) and *C. brasiliiana* (Christo; Absher, 2006; Christo et al., 2015; Sampaio et al., 2019; Sühnel et al., 2017). Birnstiel et al (2019) did not observe difference in the amount of microplastics, regardless of shell size in *Perna perna* mussels cultivated in, and extracted from, natural banks.

The current study did not find microplastics in depurated oysters (control) at the end of the experiment (24 hours); this outcome has indicated that commercialized depurated oysters did not have microfibers (Supplementary material Table II and Fig.S2). The microfibers were found in the stomach content, gills, lip palp, feces, pseudofeces (Supplementary material Table II, Fig.S2 and Fig. 1), mantle and gonads of oysters in the treatment group (Supplementary material Table III), thus indicating that the species has the ability to filter microfibers. Cho et al. (2009) have analyzed non-depurated bivalves and found microplastics in soft parts of four different species (*Crassostrea gigas*, *Mytilus edulis*, *Ruditapes philippinarum* and *Mizuhopecten yessoensis*) that have been sold in fish markets in South Korea. Ribeiro et al. (2021) found the presence of microplastics evaluating 10 specimens of the oyster *Saccostrea glomerata* in Australia. Paranaguá Municipal Market sells non-depurated individuals of the mussel *Mytella guyanensis*, the cockle *Anomalocardia brasiliiana* and oyster species *C. rhizophorae* at non-depurated state. Only part of *C. brasiliiana* oysters were sold at depurated state. Microfibers were observed in the soft parts of non-depurated *Argopecten purpuratus*

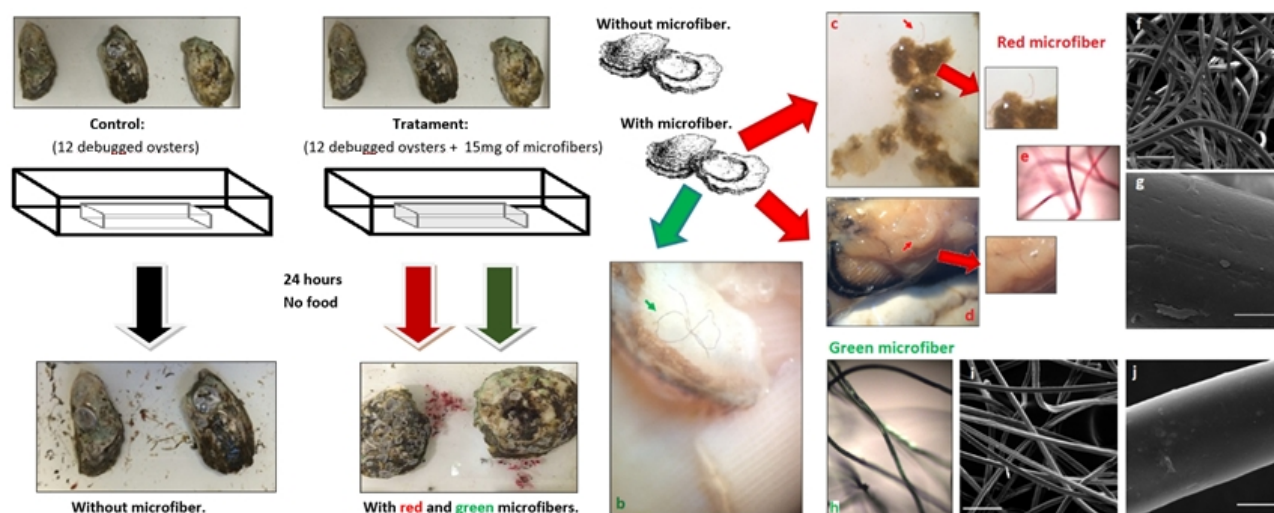


Figure 1. Experimental design scheme (left) and Microfibers in soft tissues (right - b, c and d) of commercialised oyster. a – oyster *Crassostrea brasiliiana* and microfibers in feces and pseudofeces; b- lip palp with green microfibers; c – stomach contents with red microfibers; d – gills with red microfibers; e, f and g – red microfibers; h, i and j – green microfibers. Scales: f – 500 μm ; i - 100 μm ; g and j – 5 μm .

sold in a fish market in Peru (De La-Torre; Mendoza-Castilla; Pilar, 2019).

Oyster species *Saccostrea cucullata* extracted from natural banks on the coast of Thailand presented microfibers in their soft parts (Thushari et al., 2017). Studies conducted with mussel species *P. perna* have indicated that the depuration system has effectively reduced the incidence of microplastics in the soft parts of specimens grown in, and extracted from, natural banks (Birnstiel; Soares-Gomes; Da Gama, 2019). Depuration is applied to extracted and cultivated oysters to eliminate biological contamination associated with bacteria, viruses, and other microorganisms (Leal Diego et al., 2013).

Microfibers found in the environment can comprise polyester, nylon and rayon, mainly the ones deriving from synthetic fabric (Absher et al., 2019; Faria et al., 2021) and ropes (Birnstiel; Soares-Gomes; Da Gama, 2019). The diameter of microfibers (deriving from fabric) used in the current experiment ranged from 13.06 μm to 21.73 μm ; red fibers had the largest diameter (Supplementary material Table III). Red microfibers presented a mix of synthetic (acrylic) and natural fibers, which were identified as animal hair and vegetable fibers (Schaffer, 1981; Tungol; Bartick; Montaser, 1993; Lamichhane, 2018; Supplementary material Fig. S3 and Fig. S5). Contamination can derive from laundry and from natural fibers that are part of the it - even “acrylic fiber” fabric can have some natural fibers in it (Souza; Pacheco, 2016; Lamichhane, 2018). Only polyester was found in

green microfibers (Supplementary material and Fig. S5), these microfibers were similarly observed by Saini et al. (2016) and Silva et al. (2020). Thus confirming the green microfibers like polyester. Birnstiel et al. (2019) observed sizes ranging from 16.6 ($\pm$ 6.6) to 31.2 ($\pm$ 17.8) μm in blue, red and transparent nylon (polyamide) microfiber deriving from ropes. Studies have reported the incidence of microfibers in aquatic systems associated with urban centers (Li; Zheng; Liu, 2010), since water treatment stations are unable to retain them (Minténig et al., 2017). A large number of microfilaments are often released when synthetic clothes are washed with different products, as reported by Lamichhane (2018). Nobre et al. (2020) have found combined effects of microplastics associated with triclosan on the physiology and biochemistry of cultivated oyster species *C. brasiliiana*, as well as on lysosomal membrane stability in this bivalve. This issue gets even worse in estuarine regions where oyster extraction and cultivation for human consumption takes place in spite of to the presence of microfibers in the environment (Cho et al., 2019; Van Cauwenberghe; Janssen, 2014; Vieira et al., 2021). Depuration can be used as alternative to mitigate this issue (Leal Diego et al., 2013). Lack of microfibers in the oysters from the control group suggests that this technique is an alternative that can be applied along the Brazilian coast, at a larger scale. Estuarine regions close to urban centers on Paraná coast are the environments accounting for most oyster production in the region (Christo; Absher, 2006;

Noernberg et al., 2008; Christo et al., 2015). Thus, it is of paramount importance to provide incentive and subsidy for the implementation and maintenance of local oyster depuration systems to improve oyster farming.

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Supplementary Material

Tables S.I – S.III

Figures S.1 – S.5

Supplementary material

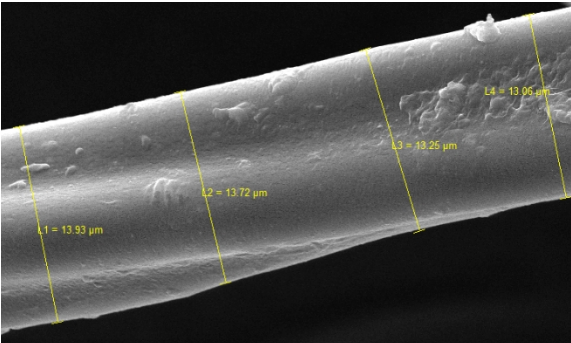
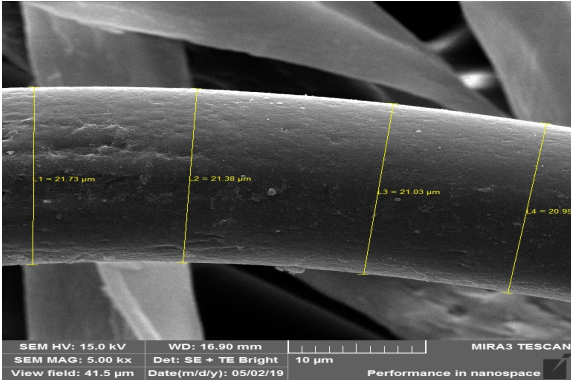
Table I. Biometrics of the shell and biomass of the oyster *Crassostrea brasiliana*. U - Mann-Whitney U test (nonparametric); * statistical differences ($p < 0.05$).

Biometrics (mm)	Total	Control	Treatment	U	p
Height ($\pm$ SD)	72.54 ($\pm$ 7.03)	76.77 ($\pm$ 3.39)	68.31 ($\pm$ 7.26)	18.0	0.001 *
Length ($\pm$ SD)	50.78 ($\pm$ 6.83)	45.99 ($\pm$ 3.82)	55.54 ($\pm$ 5.74)	13.0	<0.001 *
Width ($\pm$ SD)	20.86 ($\pm$ 3.84)	21.81 ($\pm$ 4.59)	19.90 ($\pm$ 2.80)	48.0	0.166
Biomass (g)					
Total weight ($\pm$ SD)	51.22 ($\pm$ 13.04)	49.20 ($\pm$ 10.83)	53.24 ($\pm$ 15.14)	63.0	0.603

Table II. Characterization of the presence of microfibers in the soft parts of the oyster *Crassostrea brasiliana*. R –red microfiber; G –green microfiber.

Tissue	Control	Treatment
Stomach contents	-	R
Gills	-	R
Lip palp	-	G
Feces and pseudofeces	-	R and G
Mantle and gonads	-	R

Table III. Microfibers diameters from the Scanning Electron Microscopy (SEM). n= diameters of microfibers; SD - standard deviation.

Microfiber	Lines (μm)				n	Average (± SD)
	L1	L2	L3	L4		
Green					04	13.49 (± 0.40)
Red					04	21.27 (± 0.36)
Total	-	-	-	-	08	17.38 (± 4.17)

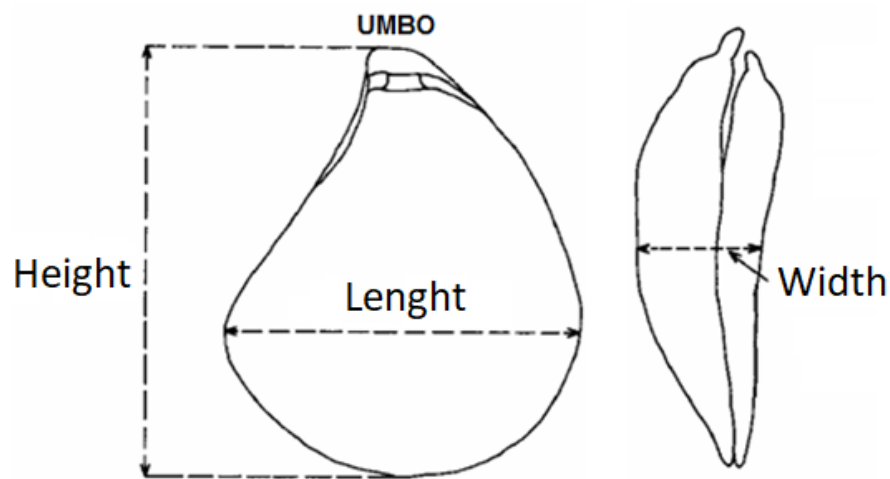


Figure S1. Biometrics of the oyster shell of the genus *Crassostrea*. Adapted from Galtsoff (1964).

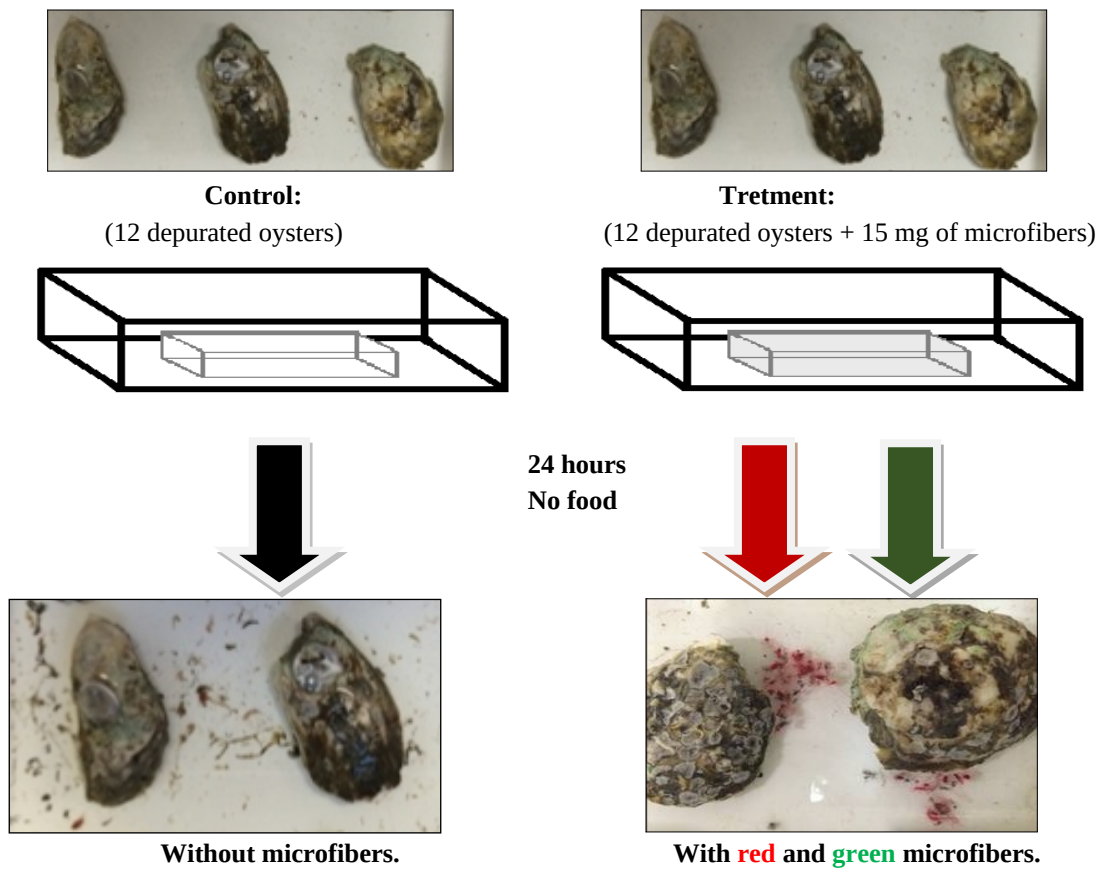


Figure S2. Experimental design.

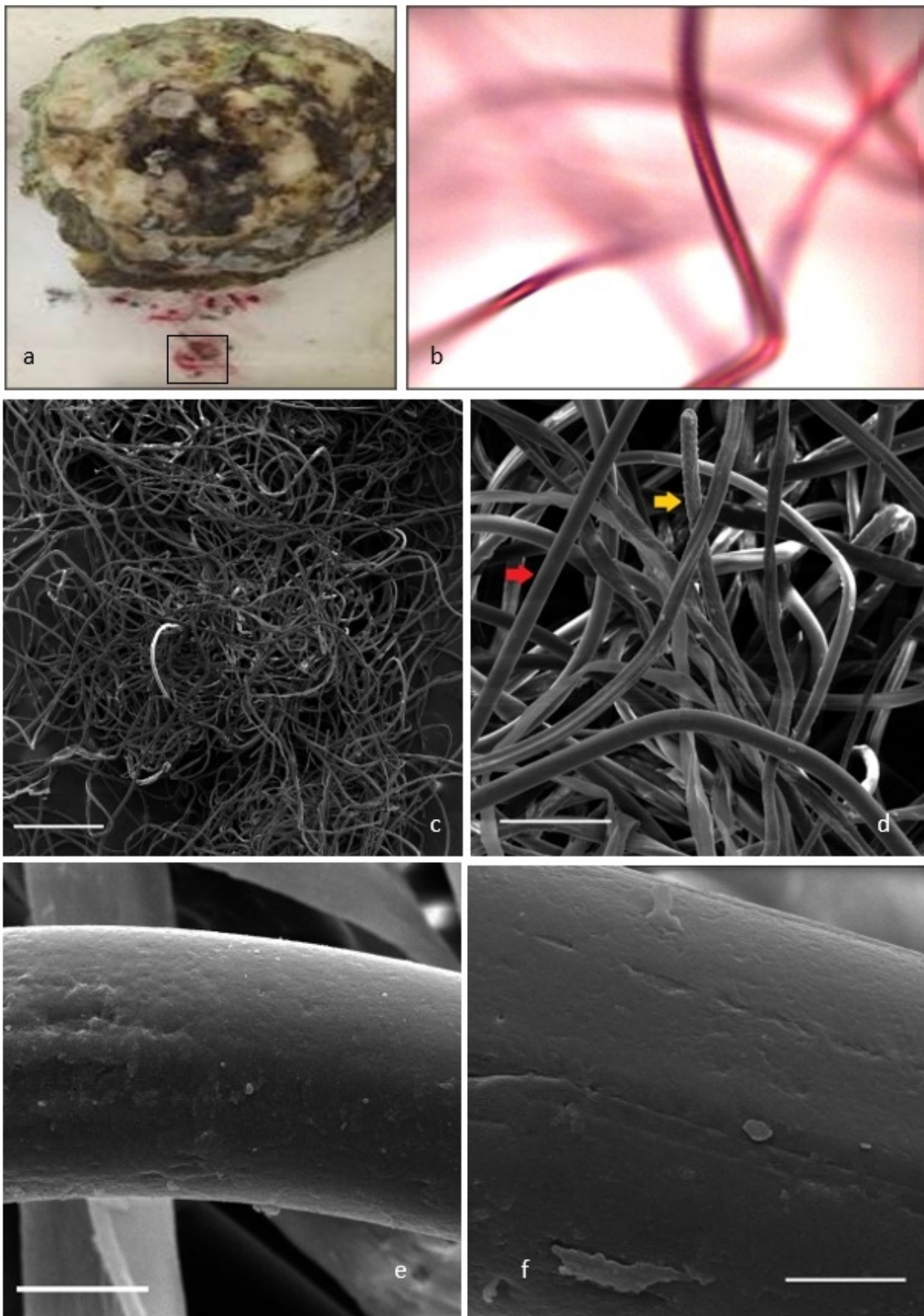


Figure S3. Red microfibers. a - microfibers in feces and pseudofeces in *Crassostrea brasiliana*; b - synthetic red microfibers in microscopy optica; c to f – synthetic red microfibers in Scanning Electron Microscopy (SEM). Red arrow - synthetic red microfibers; yellow arrow - natural red microfibers. Scales: c – 500 μm ; d - 100 μm ; e – 10 μm ; f – 5 μm .

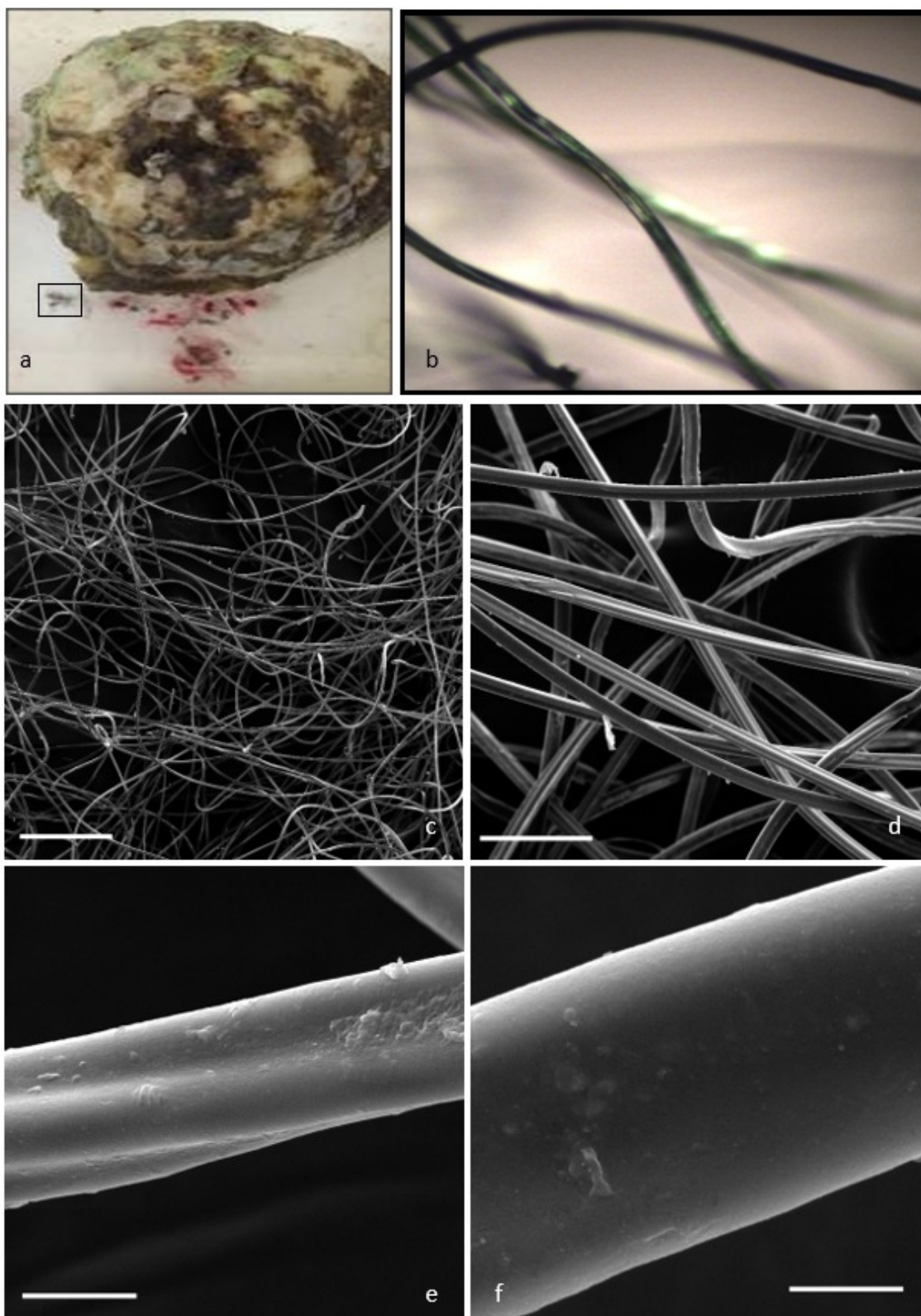


Figure S4. Green microfibers. a - microfibers in feces and pseudofeces in *Crassostrea brasiliana*; b - synthetic green microfibers in microscopy optica; c to f – synthetic green microfibers in Scanning Electron Microscopy (SEM). Scales: c – 500 μm ; d - 100 μm ; e – 10 μm ; f – 5 μm .

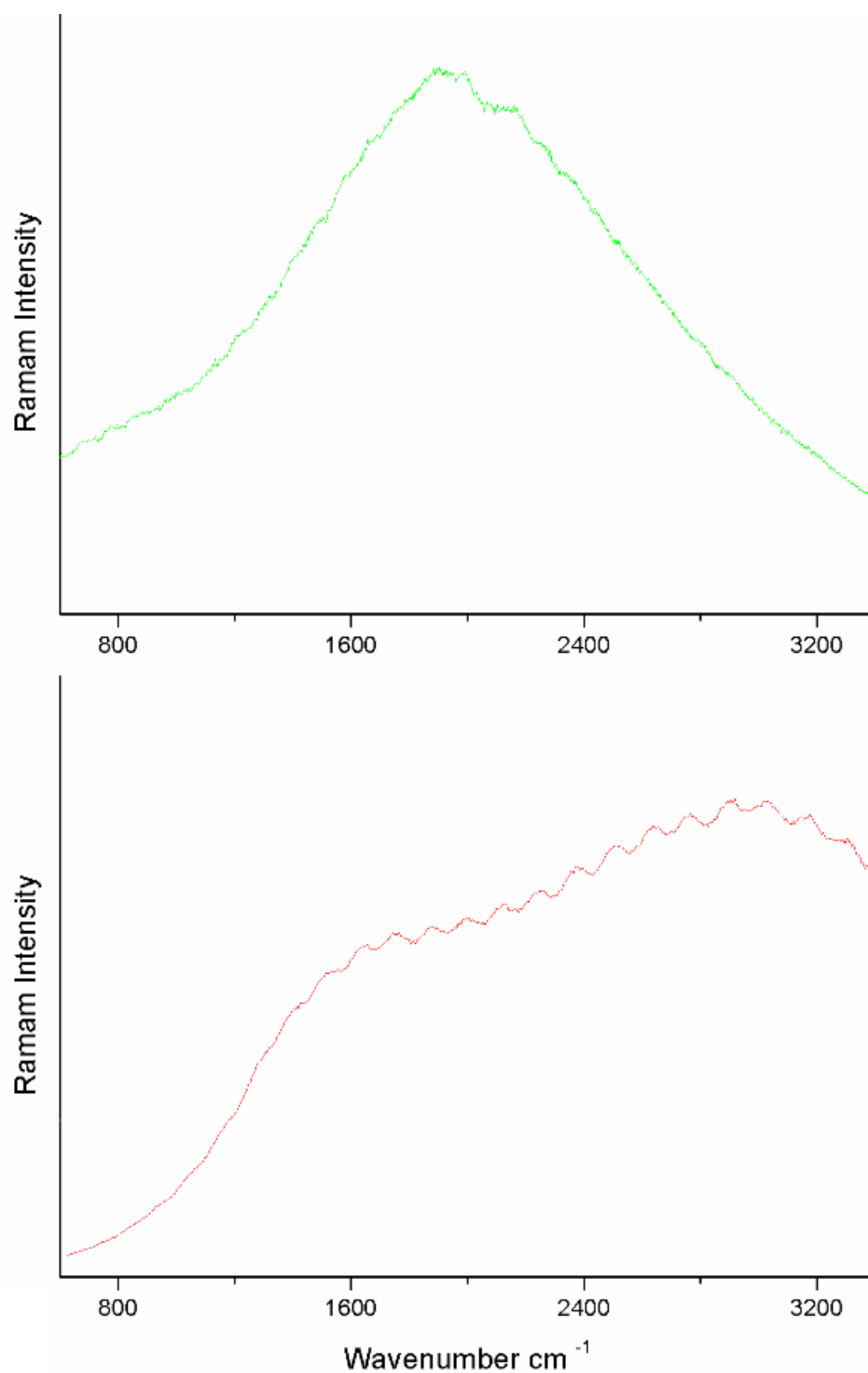


Figure S5. RAMAN microscopic spectra of the microfibers. Green line - Green microfibers; Red line - Red microfibers.