Human health risk assessment for consumption of microplastics and plasticizing substances through marine species

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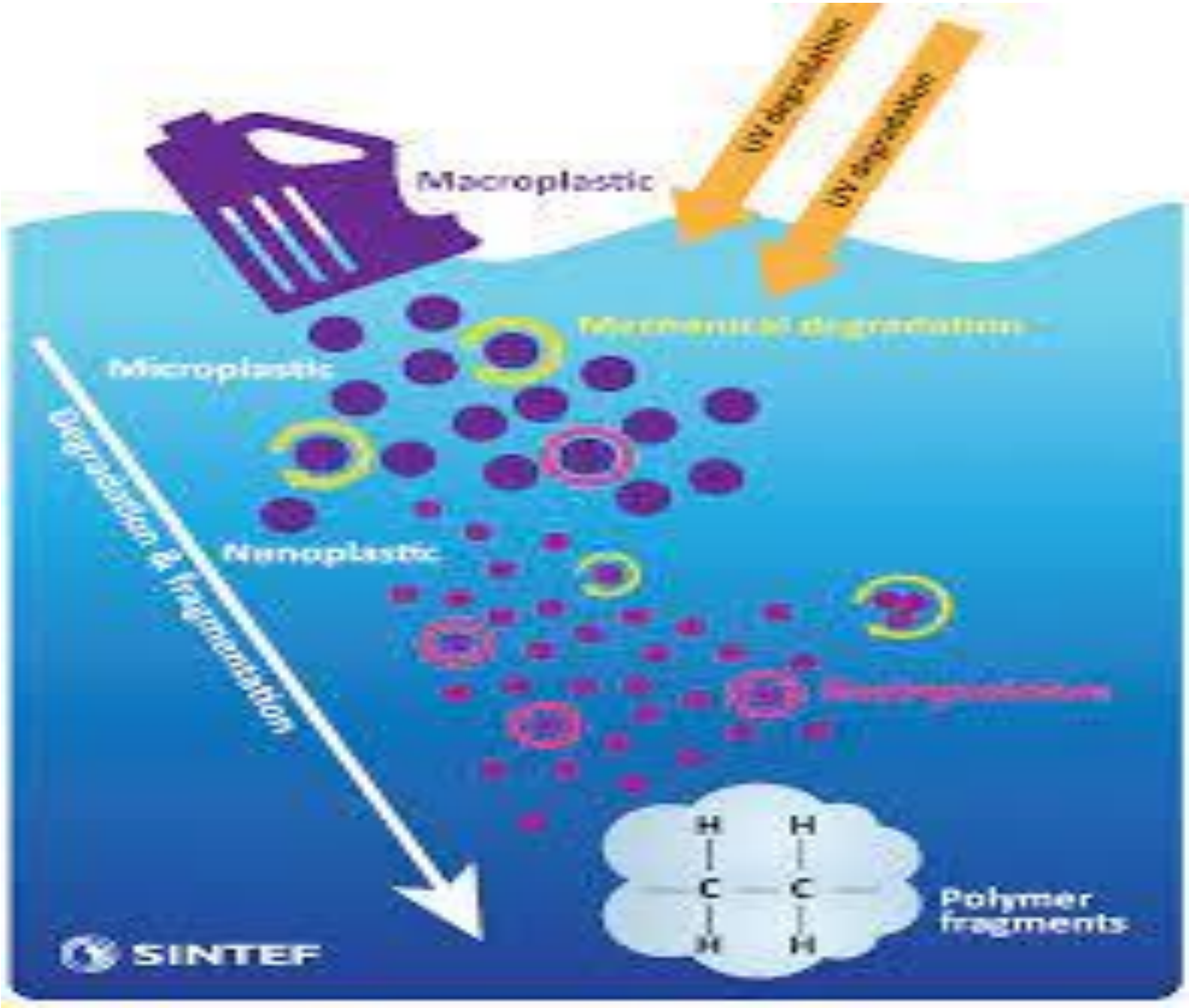
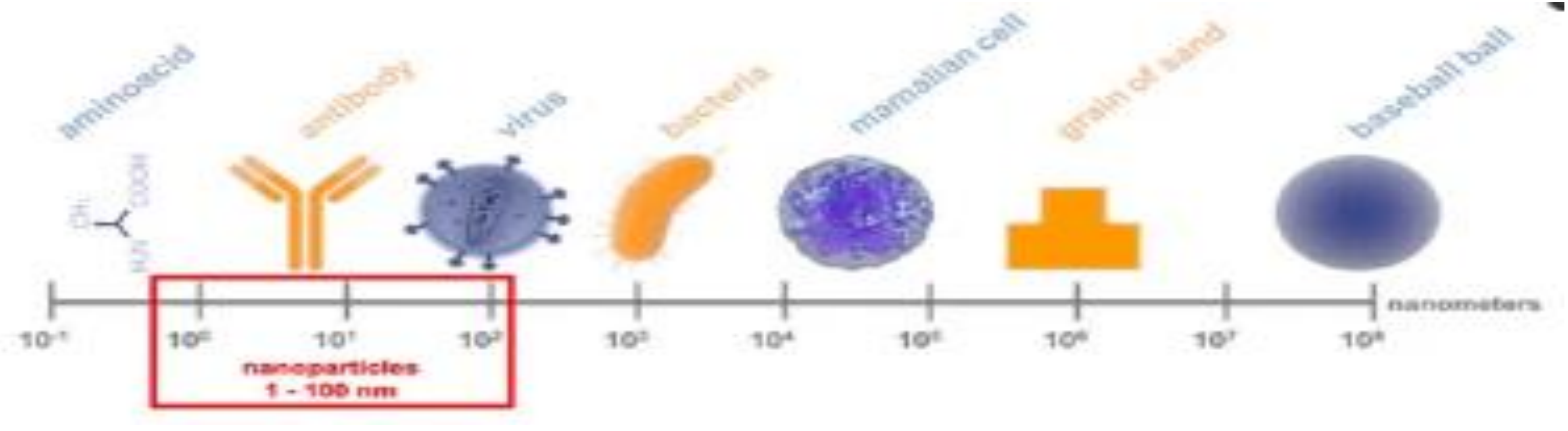
Microplastics (MP)

- Over time and with the external factors, these plastics start to degrade until reaching sizes in the scale of micrometers and nanometers, this is smaller-size structures, but do not disappear from the environment.

- In the ocean they may act as carriers of additives specific to the plastic materials used in their manufacture, such as plasticizers, among which Bisphenol-A (BPA), bis (2-ethylhexyl) phthalate (DEHP), dybuthyl phthalate (DBP).



❖Plastic particles, sized under 5 mm are known as microplastics (MP) they are not water-soluble, and their degradability is low, lasting from weeks to thousands of years to decompose, depending on the conditions to which they are exposed.



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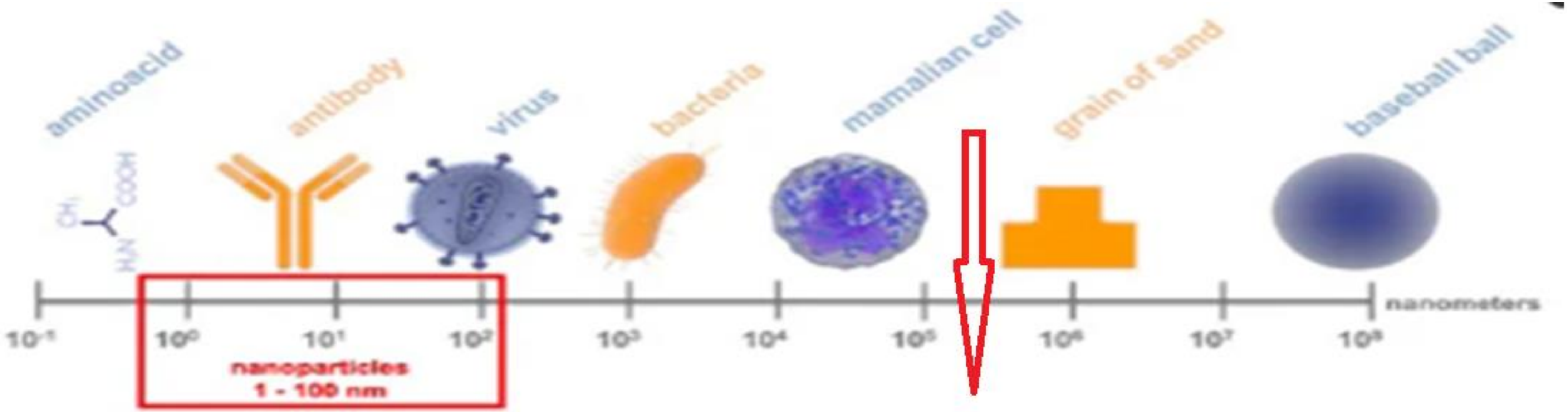
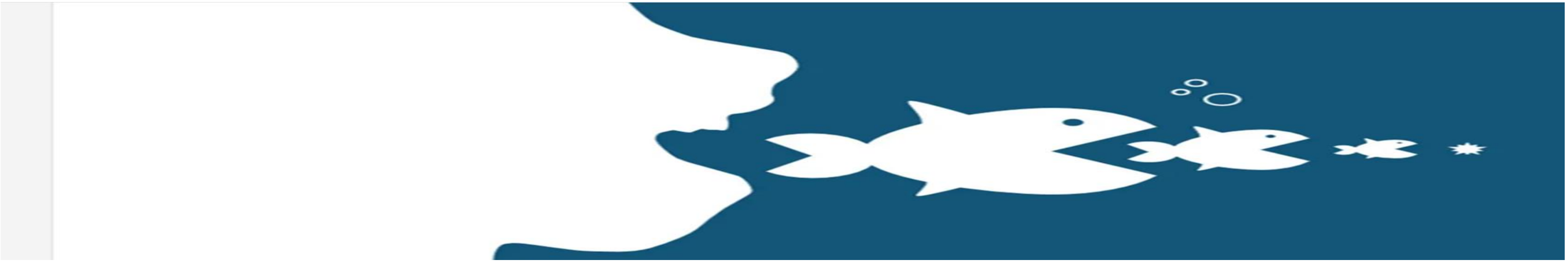
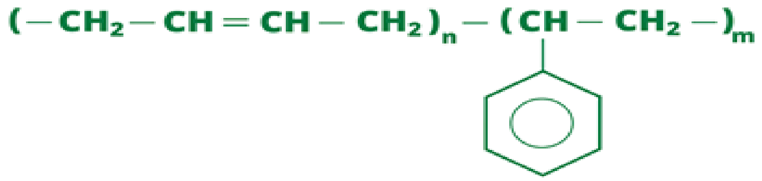


- MP consumption can generate bioaccumulation and biomagnification as well; this has generated interest, because of the possibility these particles (and their harmful substances) reaching higher trophic levels, that they reach human beings.
- Microplastics' intake by marine species may induce release of the additives used for their manufacture, such as plasticizing substances.

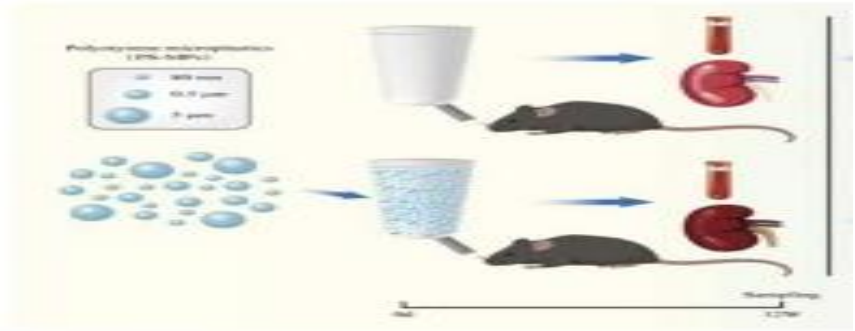


- The toxicity on human health caused by MNP may be due to the nature of plastic as such; such is the case of styrene-butadiene rubber (Gruber et al., 2022); the other one is the size of the particles (De Felice et al., 2019).

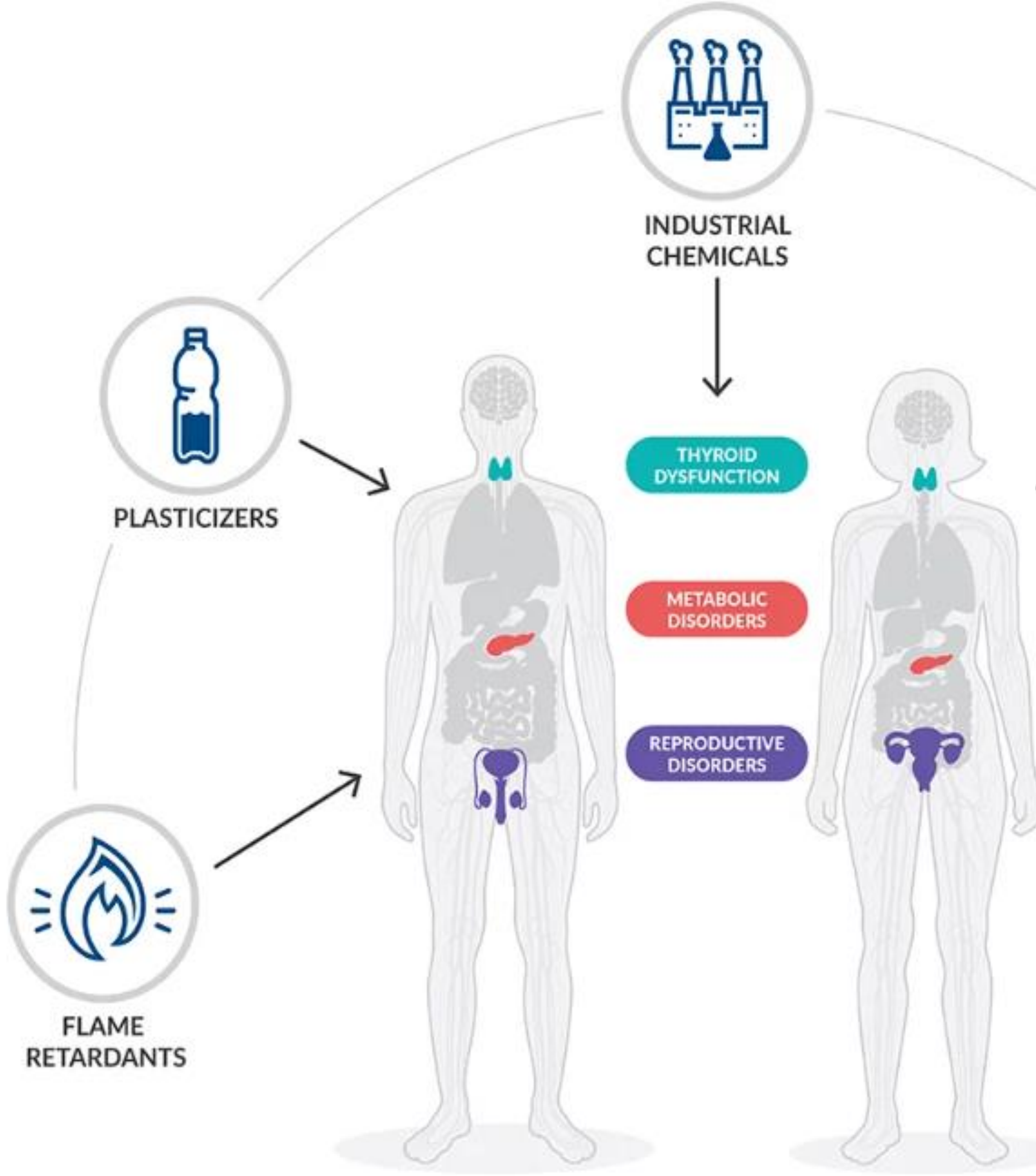
Styrene-Butadiene Rubber (SBR)



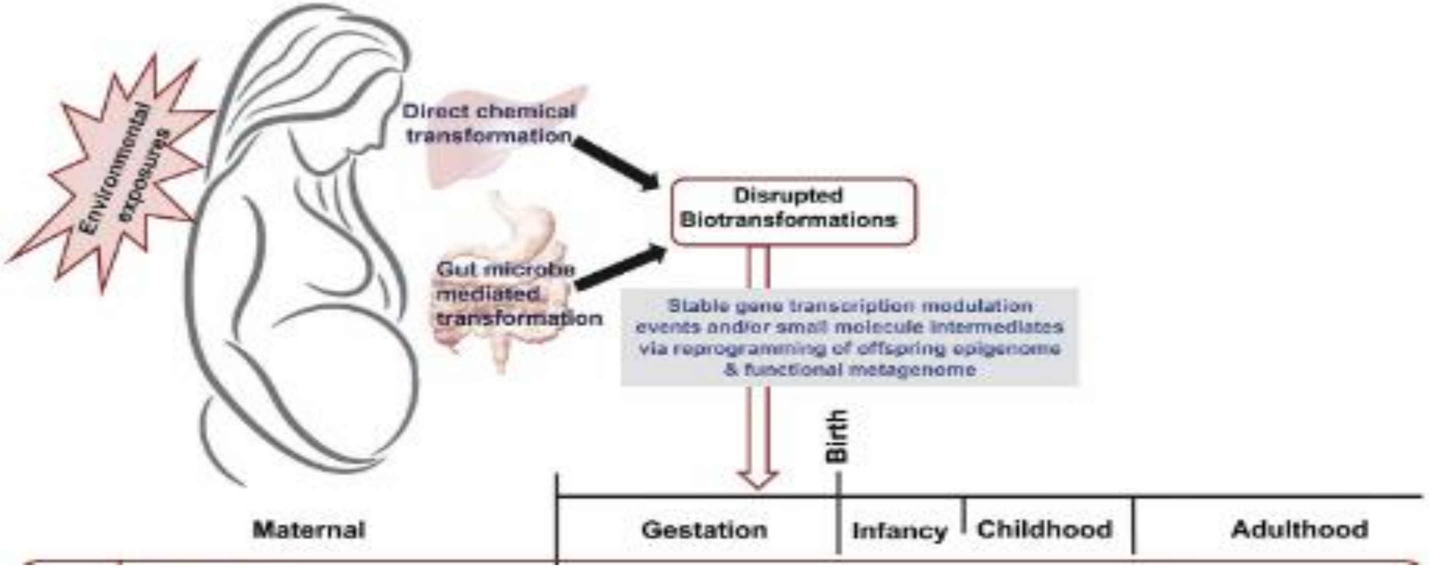
- The use of animal models with mammals to predict the potentially harmful impact of MNP on human health is increasingly being studied; it has been found that mice exposed to MP with diameters of 5 and 20 µm for 28 days, showed the presence of MP in the liver, kidney and gut



- The plastic additives can have a variety of toxic effects, including potential carcinogenic and epigenotoxicity effects. BPA and Phthalates among other environmental contaminants, cause a wide range of disruptive effects in the body, partly because they interfere, at very low doses, with the function of various hormones; these compounds are known as EDCs – ”endocrine disrupting chemicals”.



- EDCs can alter fetal programming at an epigenetic level, which can be passed down through generations and may play a role in the development of various chronic disorders later in life, such as metabolic, reproductive, and degenerative diseases, as well as some forms of cancer. In utero exposure to antiandrogenic EDCs, particularly at a sensitive period of fetal testicular development, the so-called ‘masculinization programming window (MPW)’, can disturb testicular development and function. Low androgen effect during the MPW can cause both short- and long-term reproductive disorders (Rodprasert et al., 2021).



- In the present investigation, isolation of MP was conducted as well as quantification of plasticizing substances in the same samples of marine species: mangrove cockle (Anadara tuberculosa), Stolzmann’s weakfish (Cynoscion stolzmanni) and arched swimming crab (Callinectes arcuatus).

- Finally, a risk assessment was carried out and the dietary exposure to BPA to which consumers would be exposed with these foods was calculated; these values were compared with the tolerable intake dose (TDI) established by European Food Safety Authority (EFSA).

Materials and Methods

MP Extraction, quantification, and visualization

- The marine species were collected directly from fishermen of the Gulf of Nicoya, Costa Rica during the year 2020. Work was done with 86 specimens of composite sample, (these samples were also used for the determination of plasticizing substances.
- Extraction of MP was done through physical-chemical techniques and identification was carried out employing the techniques of light microscopy, energy dispersive spectrometer (EDS), Scanning Electron Microscope (SEM) and Raman Spectroscopy.

Quantification of BPA and Pthalates (DEHP and DBP).

- It was performed identification and quantification using Gas Mass Chromatography Equipment (GC-MS with a spitless injection system (Thermo Scientific Trace 1310) with Mass Spectrometry Detector, triple quadrupole.

Toxicological risk analysis associated to consumption of arched swimming crab, mangrove cockle and Stolzmann's weakfish.

- For risk evaluation, dietary exposure to which consumers would be submitted was calculated. The result of food consumption in kg multiplied by concentration of the toxic found in µg/kg between the adult's body weights in kg, thus obtaining a dietary exposure as µg/kg bw/d. These values are compared to TDI established for each case by recommendation of EFSA (European Foods Safety Authority).

Calculation:

$$\text{Dietary Exposure} = \frac{\text{Food consumption} \times \text{Substance concentration}}{\text{Body weight}}$$

Results

MP amount found

- For this investigation, work was done with sieves allowing the passage of particles whose size oscillated between 0.5 µm - 106 µm, thus obtaining the following average results: for Arched swimming crab 4.0 ± 1.0 (SD) MP/g; in the case of the mangrove cockle the value corresponded to 3.3 ± 2.9 (SD) MP/g; in the case of Stolzmann's weakfish, an average of 2.4 ± 1.3 (SD) MP/g was obtained.

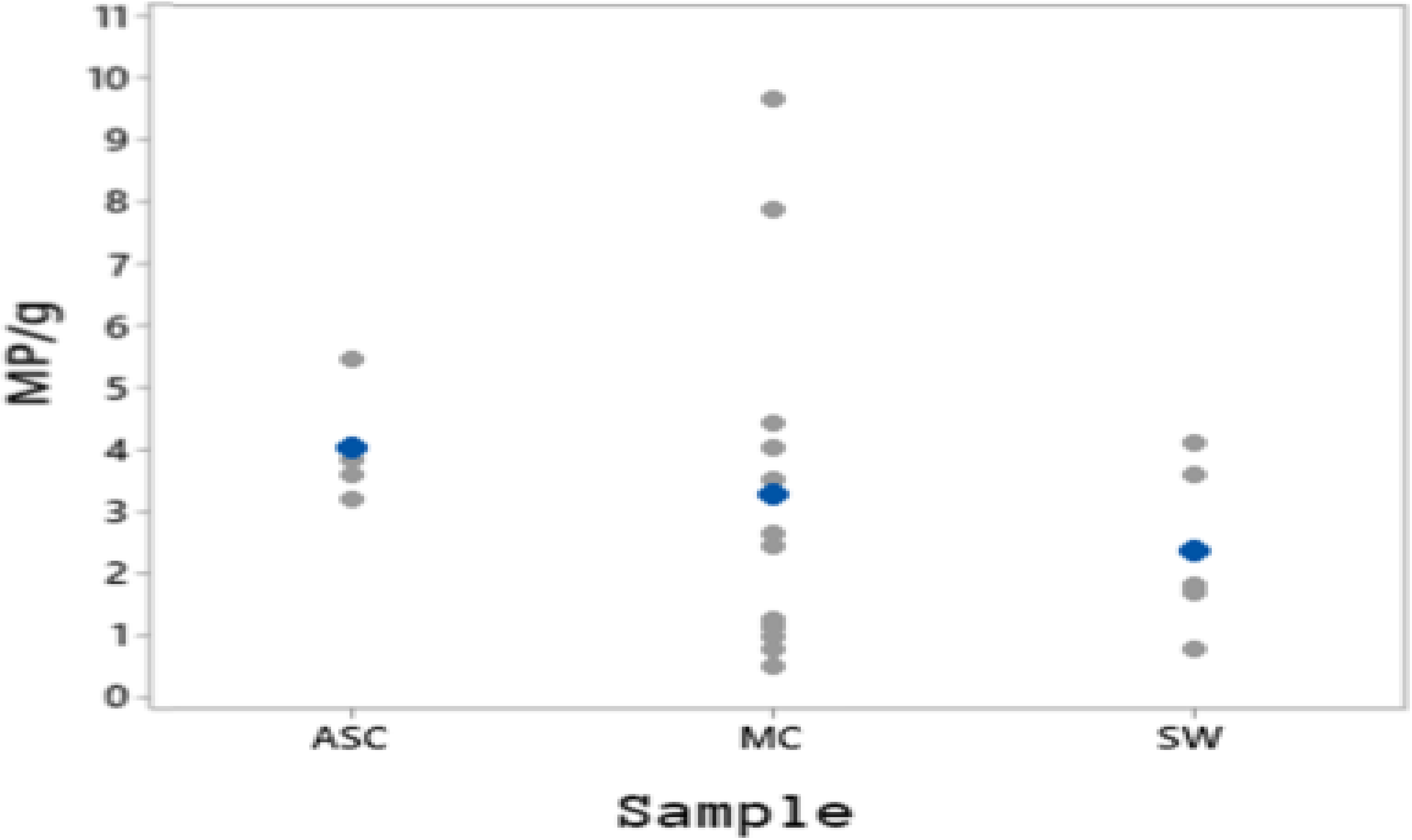


Fig 1 Amount of MP in composite samples; ASC: arched swimming crab, MC: mangrove cockle, SW: Stolzmann's weakfish. The blue points represent the average value of each marine species.

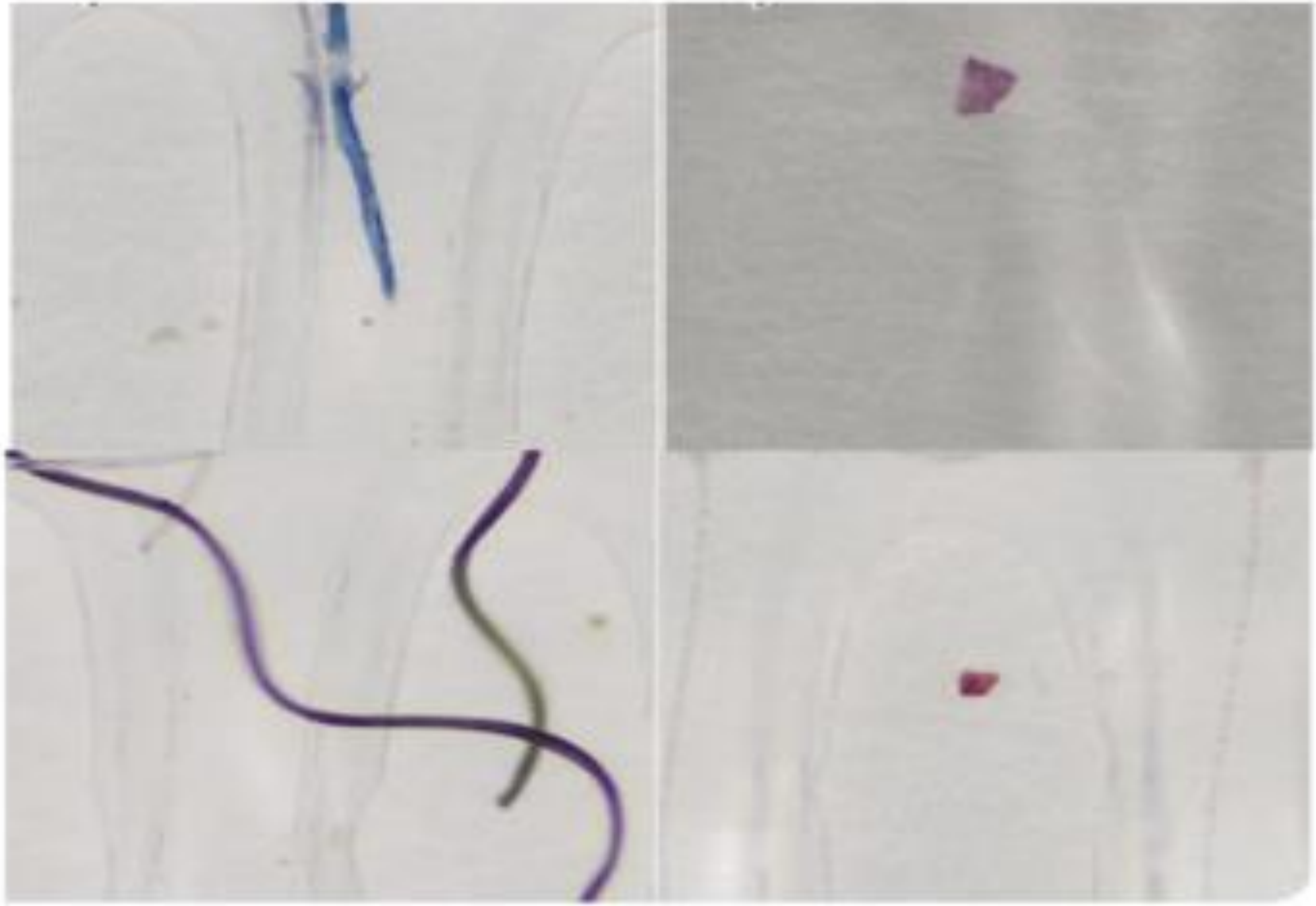


Fig 2. Pictures of isolated MP from marine species seen on the optical microscope at 4x; the different colors are shown.

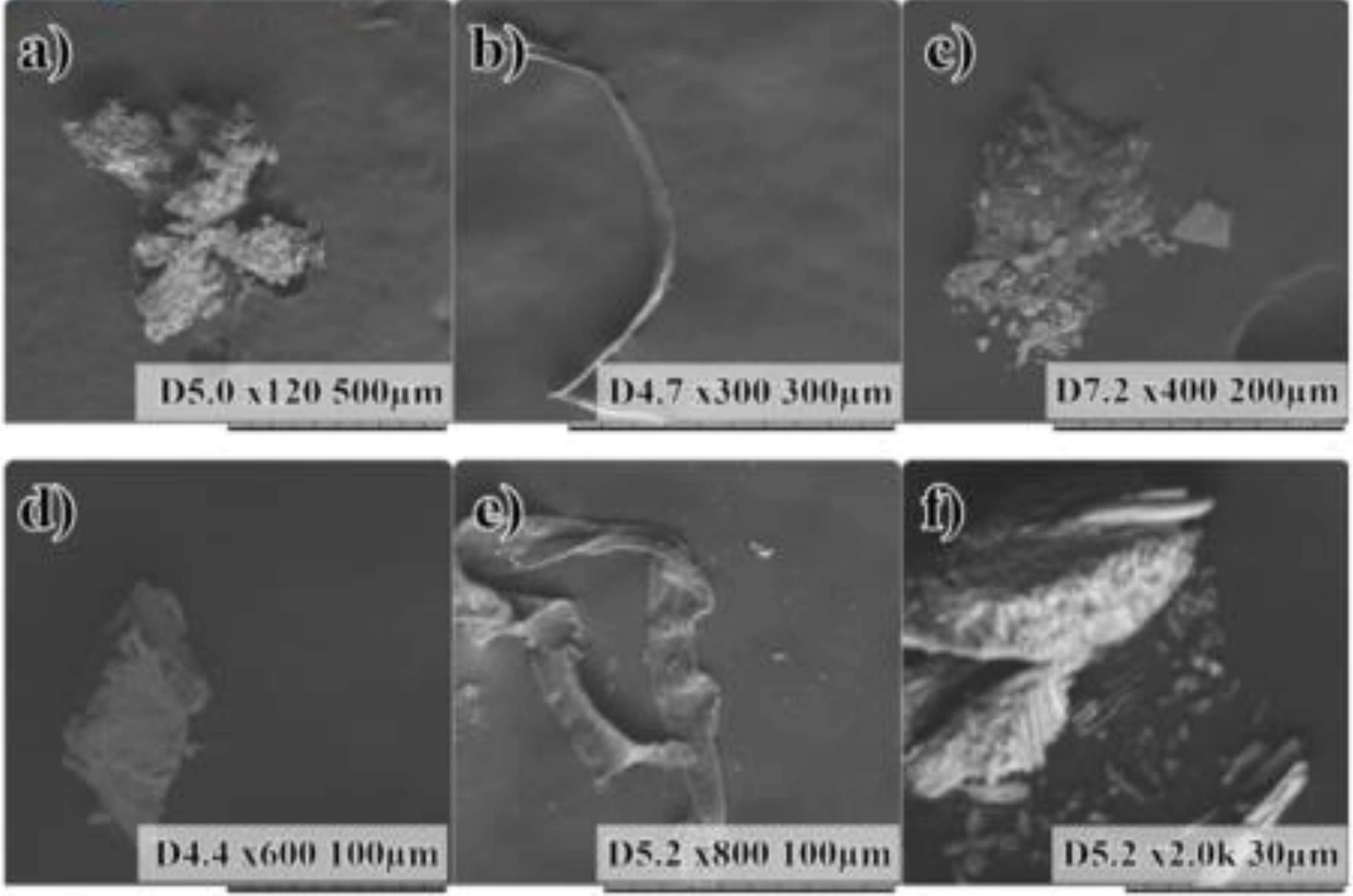


Fig 3. Micrographs of MP found on samples of mangrove cockle. a, c, d, f particles; b, e fibers.

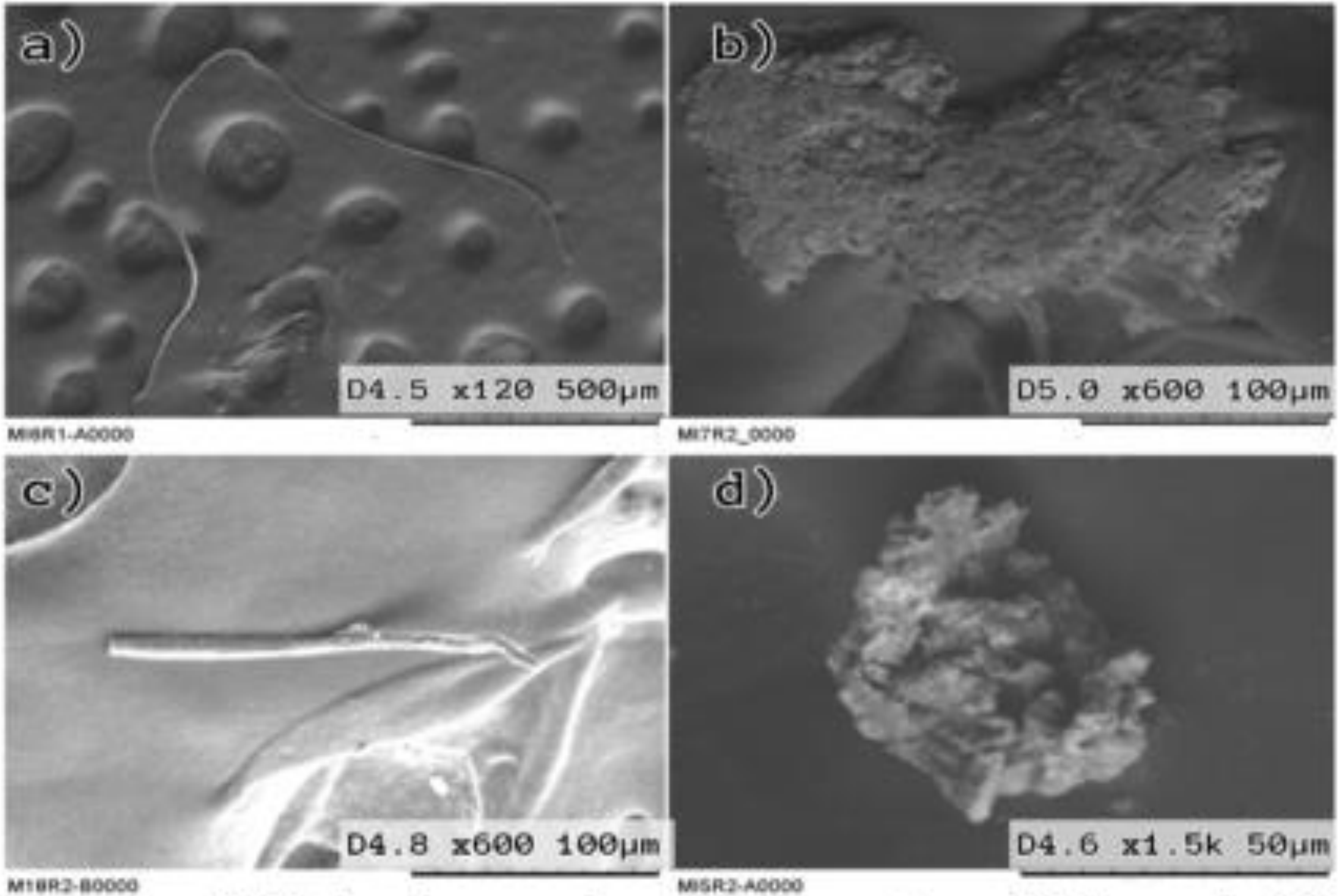


Fig 4. Micrographs of MP found on samples of Stolzmann's weakfish: a and c) fibers; b and d) particles.

Table 1. MP characterization by SEM/EDS and Raman of identified marine species.

Species	SEM/EDS	Wavelength (cm^{-1}) Raman peaks	Reference (Rebollar et al., 2014)	Possible identification
Arched swimming crab	Ideal EDS	1720 1611 1287	C=O C-C benzene ring C-O	PET
Mangrove cockle	Ideal EDS	1572 1311	ND C-H	PA or PE
Mangrove cockle	Ideal EDS	1611 1486	C-C C-H, C-OH	PA
Mangrove cockle	EDS with 7.7% Al.	1720 1605 1281	C=O C-C benzene ring C-O	PET
Mangrove cockle	Ideal EDS	605 435	ND ND	ND
Mangrove cockle	EDS with 5.6% Ca.	1076 639	C-C C-Cl	PVC
Mangrove cockle	Ideal EDS	1568 534	ND ND	ND
Stolzmann's weakfish	Ideal EDS	1590 1315	N-H C-H	PA
Stolzmann's weakfish	Ideal EDS	1723 1609 1283 853	C=O C-C benzene ring C-O C-H	PET
Stolzmann's weakfish	Ideal EDS	1533 1332 739	C-O-C C-H ND	ND

*ND: Not defined

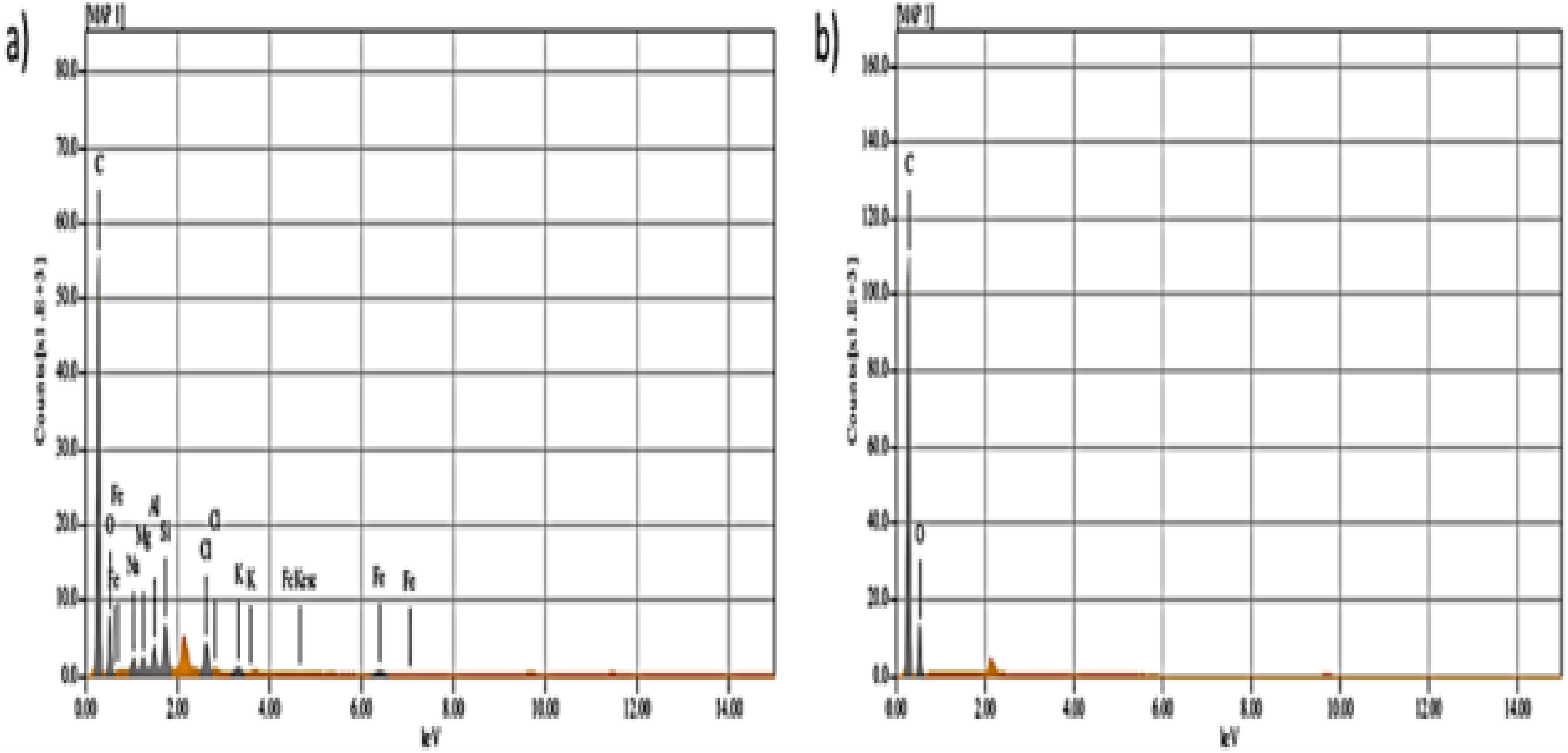


Fig 5. Graphs of EDS samples from MPs corresponding to a) polyethylene and b) polypropylene found in arched swimming crabs.

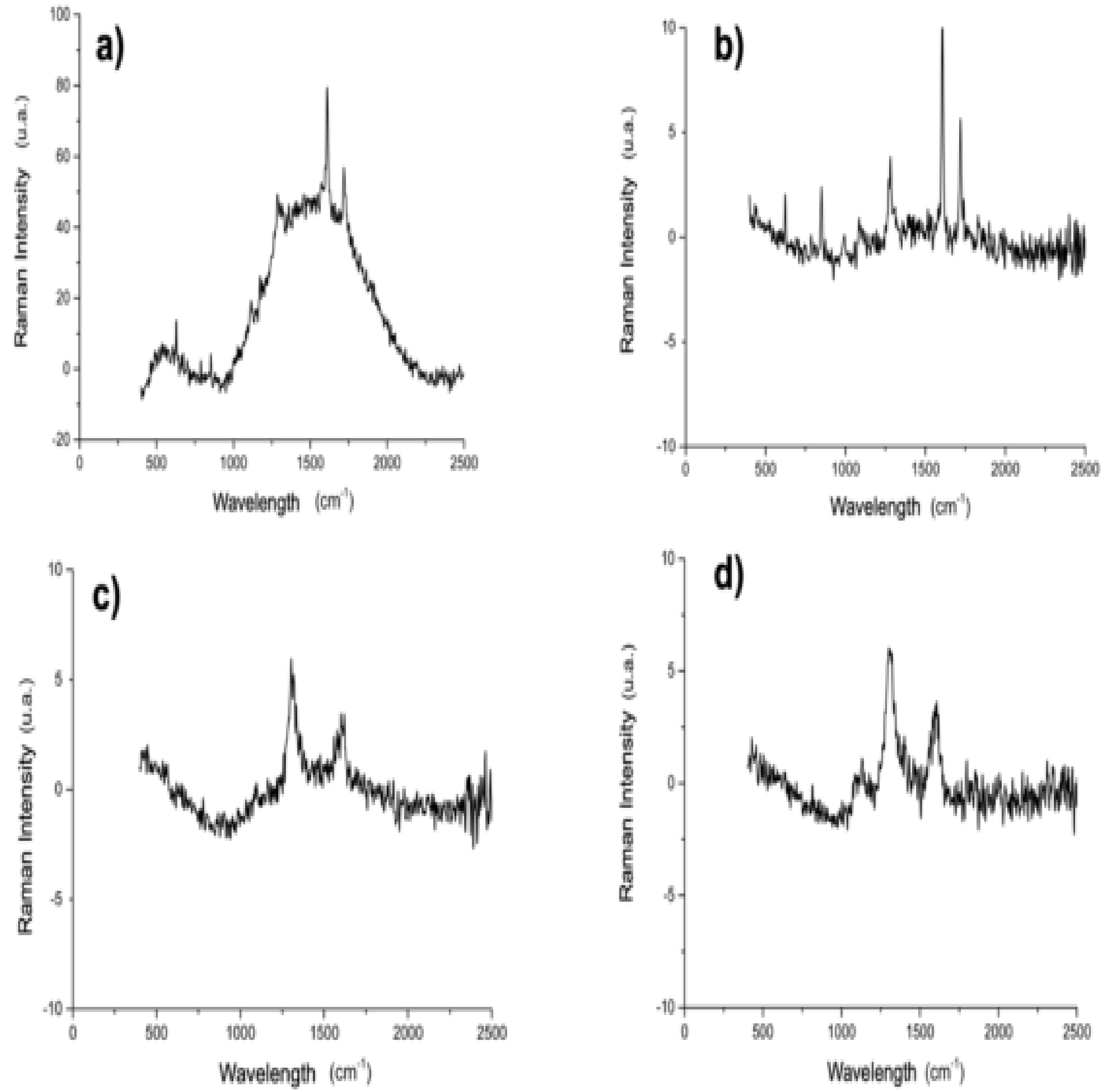


Fig 6. Raman spectra of MP, corresponding to MP found in marine species, a) arched swimming crab, b) mangrove cockle, c) and d) Stolzmann's weakfish. 780nm Laser and 3mW power.

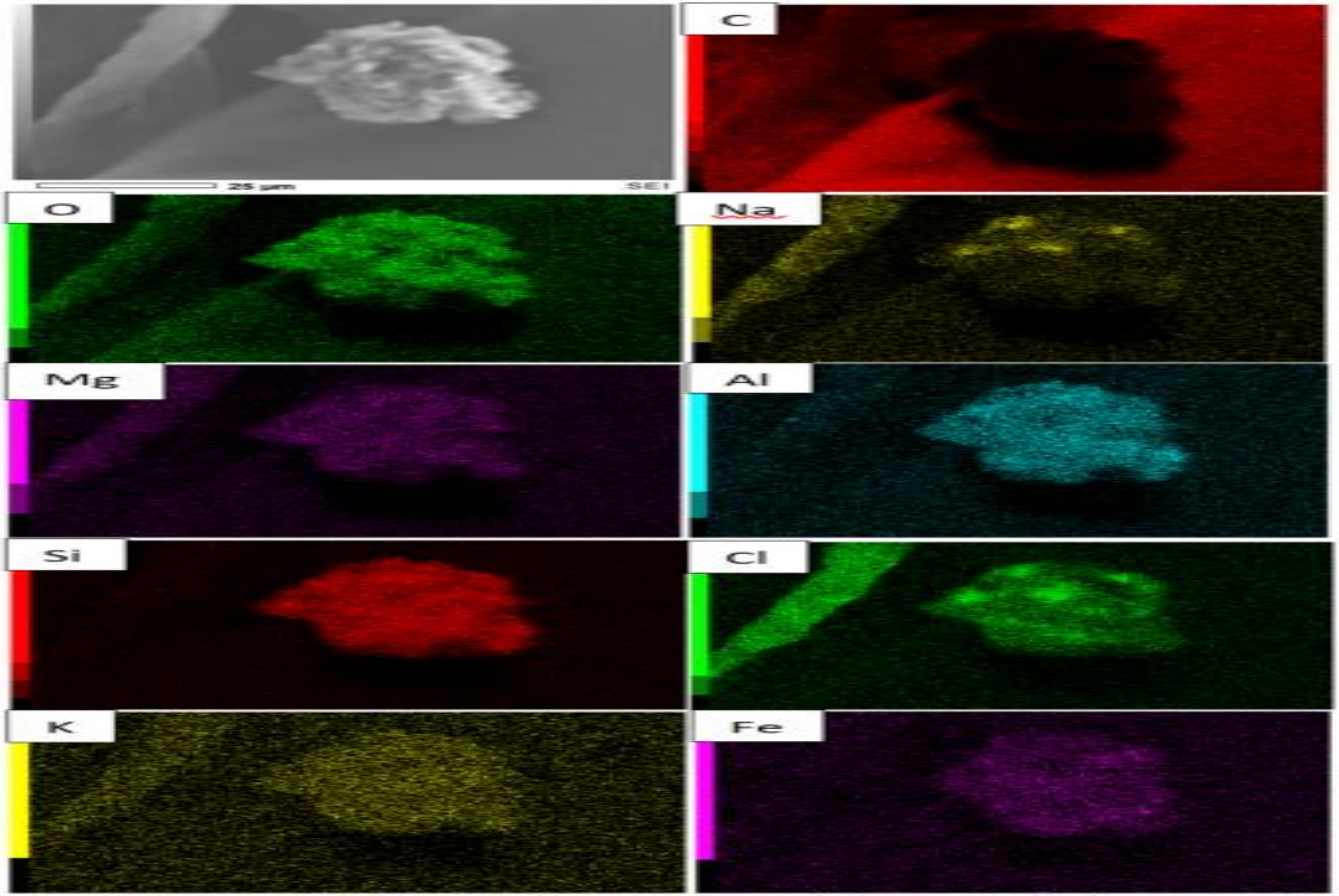


Fig 7. Elemental mapping of a particle correlating to microplasti

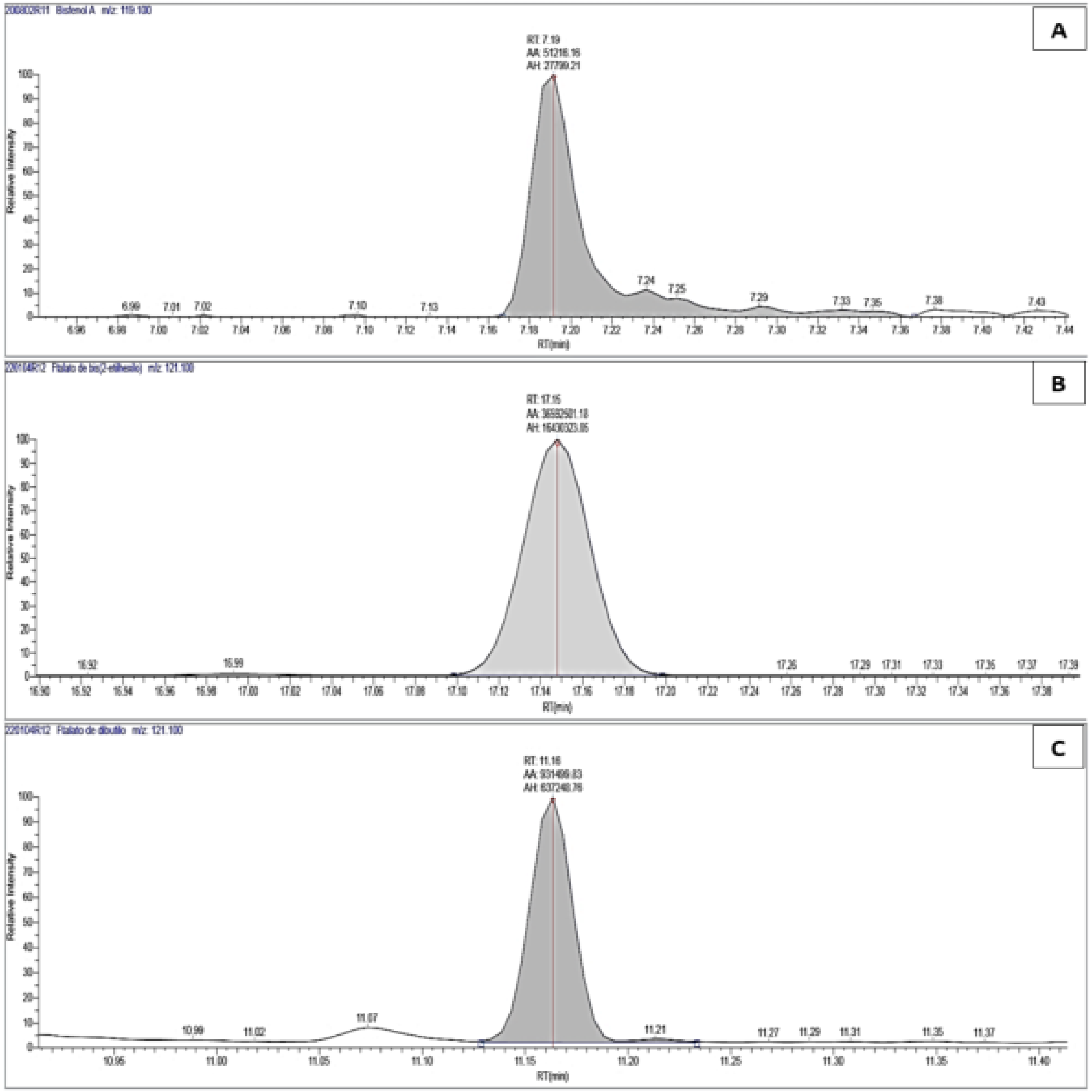


Fig 8. A: corresponds to a detected BPA signal in a sample of arched swimming crab; the signal is triggered in 7.19 minutes; B: corresponds to a DEHP signal in a sample of mangrove cockle; signal is triggered in 17.15 minutes and C: corresponds to a DBP signal in mangrove cockle; the signal is triggered in 11.16 minutes.

Table 2. Plasticizers found in positive composite samples for marine species.

Marine species	Composite samples	Analytes	Average concentration (µg /g sample) *	Range of concentrations (µg /g)
Mangrove cockle	15	BPA	0.014 ± 0.006	0.008 - 0.039
Mangrove cockle	15	DEHP	0.13 ± 0.01	0.01 – 1.41
Mangrove cockle	7	DBP	0.017 ± 0.002	0.013 – 0.025
Arched swimming crab	5	BPA	0.028 ± 0.006	0.007 – 0.076
Arched swimming crab	5	DEHP	0.03 ± 0.01	0.01 – 0.1
Arched swimming crab	1	DBP	0.059 ± 0.002	0.059 – 0.059
Stolzmann's weakfish	7	BPA	0.009 ± 0.006	0.006 – 0.018
Stolzmann's weakfish	12	DEHP	0.04 ± 0.01	0.01 – 0.08
Stolzmann's weakfish	8	DBP	0.019 ± 0.002	0.013 – 0.022

* LD: 0,002 µg/g, LC: 0.006 µg/g for BPA & DEHP and 0.008 µg/g for DBP

Table 3. Dietary exposure and TDI relation for adults according to values obtained in the study.

Species	Analytes	Average concentration (µg/kg sample)	Dietary exposure (D. exp) per portion µg/kg/ bw/d	TDI food (EFSA, 2015) µg/kg/ bw/d	Relationship D.exp -TDI a Pregnant women	TDI n b (New value proposed EFSA) 2021
Mangrove cockle	BPA	14	0.015	4	D.exp < TDI	**D.exp >>TDI**
Mangrove cockle	DEHP	130	0.139	50	N.D c	N.D
Mangrove cockle	DBP	17	0.018	10	N.D	N.D
Stolzmann's weakfish	BPA	9	0.023	4	D.exp < TDI	**D.exp >>TDI**
Stolzmann's weakfish	DEHP	40	0.10	50	N.D	N.D
Stolzmann's weakfish	DBP	19	0.049	10	N.D	N.D

aTDI pregnant women for BPA 0.33 µg/kg/bw/d (ANSES, 2014)
b TDI n New value proposed 0.004 ng/ kg bw/d of BPA (European Food Safety Authority, 2021) for EDCs damages.
c N.D: No updated data available (EFSA Panel on Food Contact Materials, Enzymes and Processing Aids (CEP) et al., 2022).

Conclusions

- According to the data obtained it can be stated that from the plasticizers found, if well it can’t be affirmed coming exclusively from plastic residues found as (MP), their origin may not be dismissed; since the work consisted of determining microparticles and plasticizing substances in the same samples, such samples were acquired directly from artisan fishermen.

- Beyond doubt if can be affirmed that the plasticizers, hazardous substances and especially important such as BPA, DEHP and DBP, have reached the highest levels of the trophic chain, It must be expressed that 34% of the samples were positive for the 3 plasticizers, which suggests a toxic mixing effect, in a synergistic way; urgent measures should be established to protect the marine resource from pollution by plastics, and hence, public health.

- Although it is true that plasticizers have a ubiquitous global distribution, it takes special importance respect to the risk when these substances are present in food or water for human consumption, being especially sensitive population children and pregnant women. Other, especially prospective studies, can be carried out to establish safe doses of these substances in these population.

- According to the type of substance found, it is suggested pregnant women do not ingest seafood during the gestation period, considering the low quantity suggested for BPAs in food, currently being under the scientific criteria of EFSA; under acceptance by TDI of 0.004 ng/ kg bw d; given the estrogenic effect of this substance in the fetus' gestation period and consumption by children be also limited.

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Thank you !!