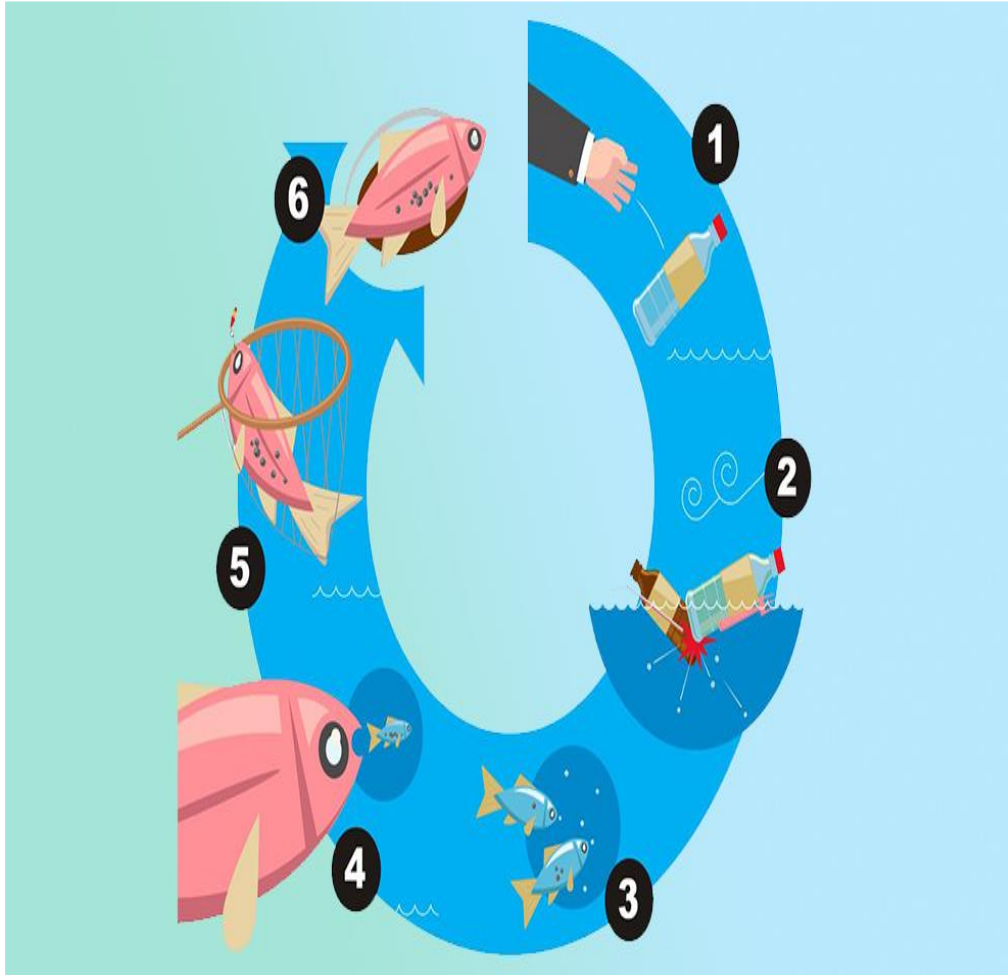


Análisis de Riesgo a la Salud Humana por el consumo de microplásticos y sustancias plastificantes mediante el consumo de especies marinas.

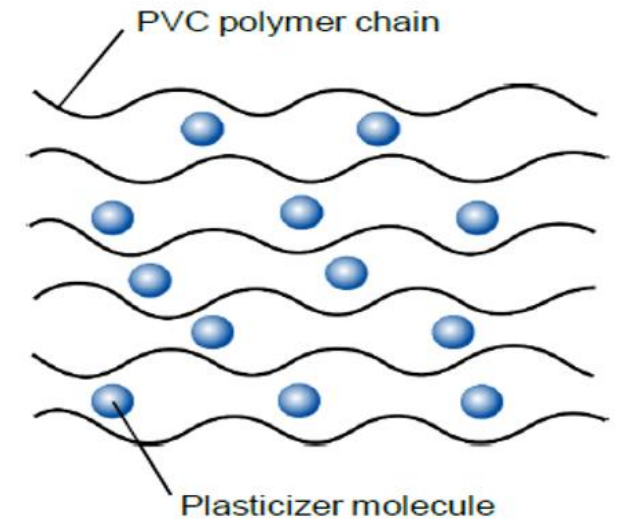
Virginia Montero, Yarenis Chinchilla, Luis Gómez, Adrián Flores,
Alejandro Medaglia, Rossy Guillén, Ernesto Montero.



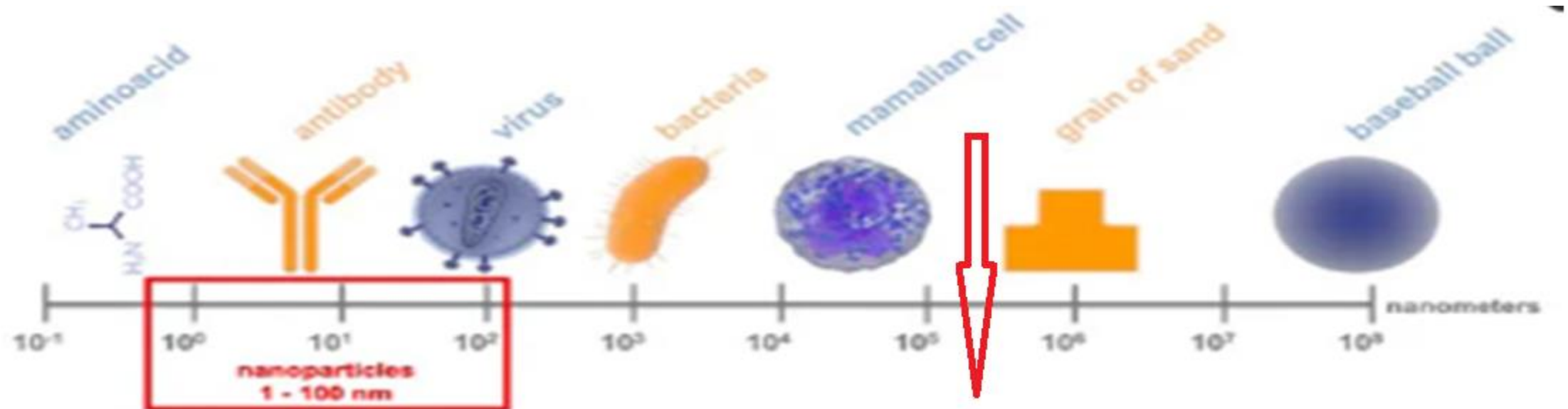
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In the ocean they may act as carriers of additives specific to the plastic materials used in their manufacture, such as plasticizers, among which Bisphenol-A (BPA), bis (2-ethylhexyl) phthalate (DEHP), dybuthyl phthalate (DBP).

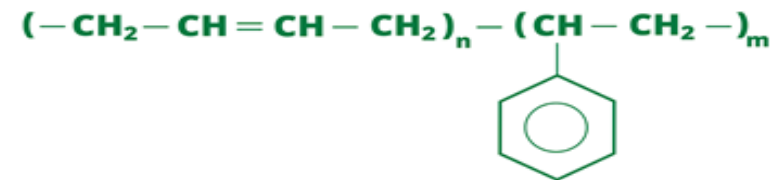


- ❖ Las partículas de plástico, de tamaño inferior a 5 mm, se conocen como microplásticos (MP), no son solubles en agua y su degradabilidad es baja, tardando desde semanas hasta miles de años en descomponerse, dependiendo de las condiciones a las que están expuestas.

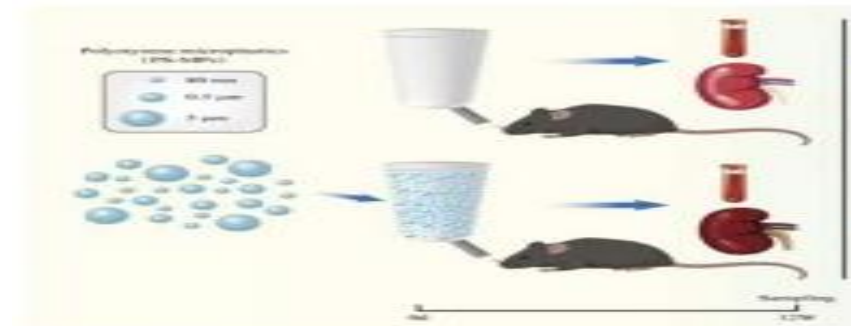


- La toxicidad para la salud humana causada por MNP puede deberse a la naturaleza del plástico como tal; tal es el caso del caucho de estireno-butadieno (Gruber et al., 2022); el otro es el tamaño de las partículas (De Felice et al., 2019).

Styrene-Butadiene Rubber (SBR)



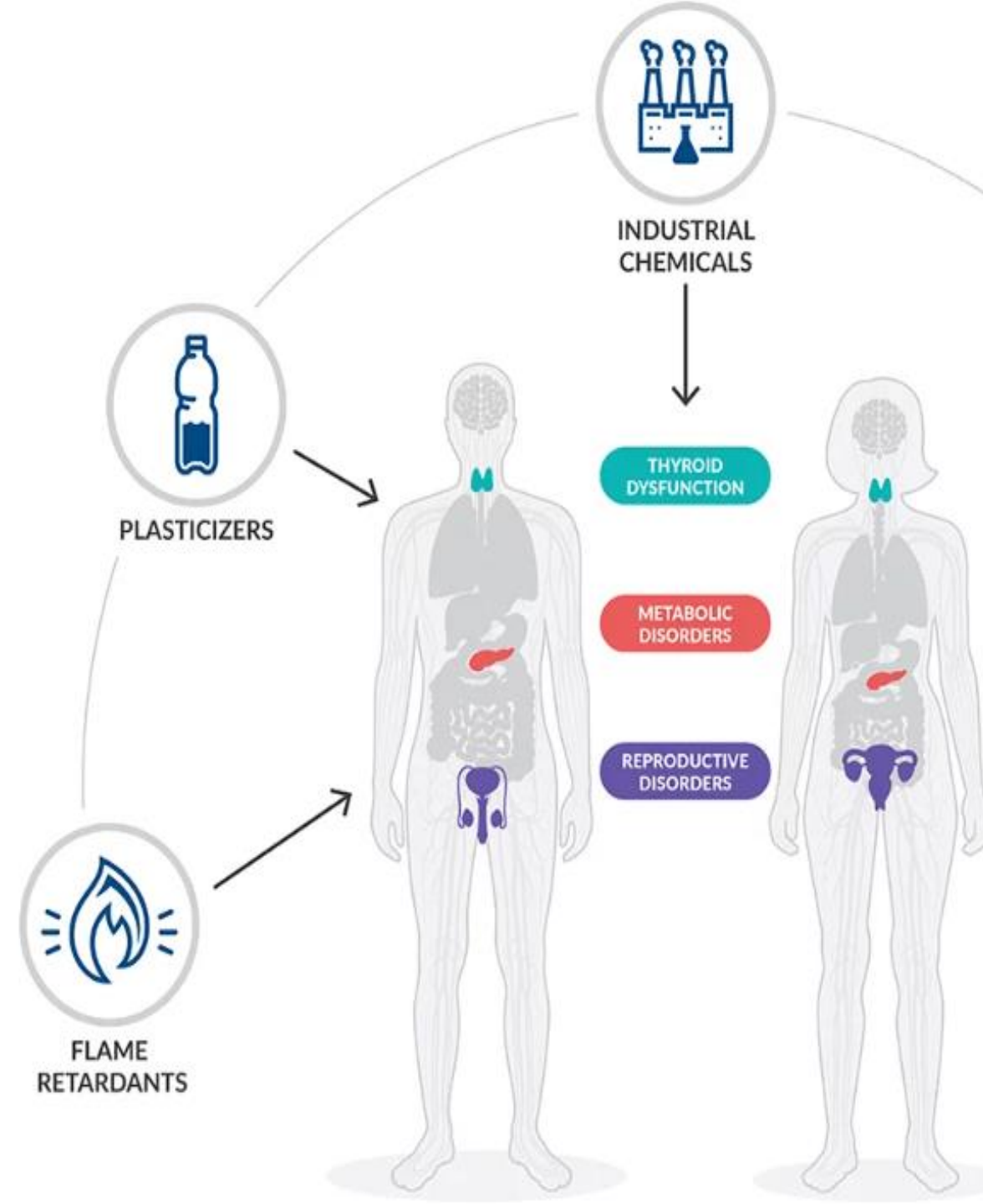
- Cada vez se estudia más el uso de modelos animales con mamíferos para predecir el impacto potencialmente dañino de las MNP en la salud humana; Se ha encontrado que ratones expuestos a MP con diámetros de 5 y 20 µm durante 28 días, mostraron presencia de MP en hígado, riñón e intestino.

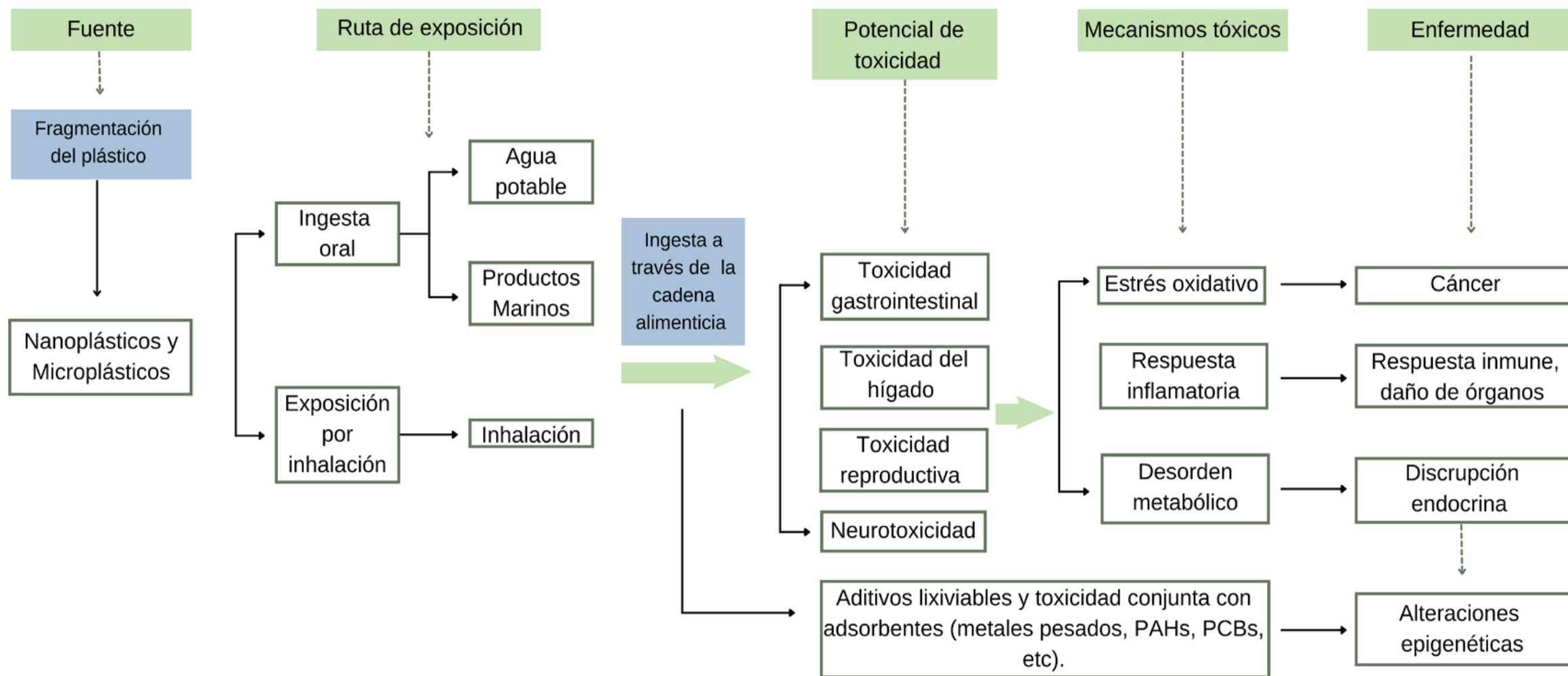


➤ Estos aditivos plásticos pueden tener una variedad de efectos tóxicos:

- potential carcinogénico
- Efectos epigenotóxicos
- El BPA y los ftalatos, entre otros contaminantes ambientales, causan una amplia gama de efectos perjudiciales en el organismo, en parte porque interfieren, en dosis muy bajas, con la función de diversas hormonas; Estos compuestos se conocen como EDC (*productos químicos que alteran el sistema endocrino*).

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- En la presente investigación se realizó el aislamiento de MP y la cuantificación de sustancias plastificantes en las mismas muestras de especies marinas: mangrove cockle (piangua) (*Anadara tuberculosa*), Stolzmann's weakfish (corvina) (*Cynoscion stolzmanni*) y arched swimming crab (cangrejo) (*Callinectes arcuatus*).

Stolzmann's weakfish



Arched swimming crab



Mangrove cockle

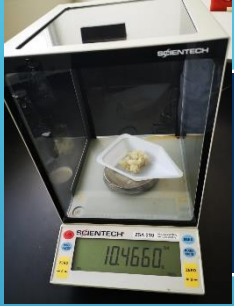


- Finalmente, se realizó una evaluación de riesgo y se calculó la exposición dietética al BPA a la que estarían expuestos los consumidores con estos alimentos. Estos valores se compararon con la dosis de ingesta tolerable (TDI) establecida por la Autoridad Europea de Seguridad Alimentaria (EFSA).

Materiales and Métodos

Extracción, cuantificación y visualización de MP.

- Las especies marinas fueron recolectadas directamente de pescadores del Golfo de Nicoya, Costa Rica durante el año 2020. Se trabajó con 86 ejemplares de muestras compuestas, (estas muestras también se utilizaron para la determinación de sustancias plastificantes).
- La extracción de MP se realizó mediante técnicas físico-químicas y la identificación se realizó empleando las técnicas de microscopía óptica, espectrómetro de energía dispersiva (EDS), microscopio electrónico de barrido (SEM) y espectroscopia Raman.



Pesaje



Filtración tamiz
acero 106 μm
en cristizador

Enjuague
matraz 100 ml
 H_2O tipo I y
trasvase



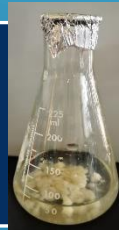
50 ml H_2O
tipo I

Sonicar
10 min



Enjuague
cristizador 50
ml agua tipo I

6 ml H_2O_2
al 45%



OXI 2 horas
agitación 107
rpm

Calentamiento
baño térmico 15
min 60°C



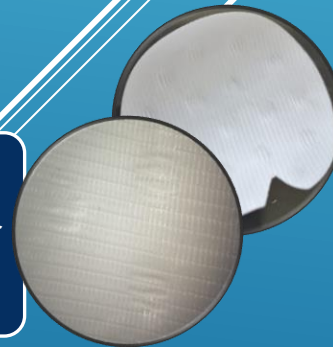
OXI 18 horas
agitación 107
rpm

6 ml KOH al
9%

Filtración al vacío
membrana
politetrafluoroetile
no 0,5 μm



Secado filtros
en desecador
silica gel



Cuantificación de BPA y Pthalates (DEHP and DBP).

- La identificación y cuantificación se realizó mediante un equipo de cromatografía de gases masas (GC-MS con sistema de inyección (Thermo Scientific Trace 1310) con detector de espectrometría de masas, triple cuadrupolo.



Análisis de riesgo toxicológico asociado al consumo de arched swimming crab, mangrove cockle y Stolzmann's weakfish.

Exposición Dietaria:

Data needed:

- Data on substance concentration (μg substance/g food)
- Data on food consumption (g food/day)
- Body weight BW (kg)

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

Cantidad de MP encontradas:

- Para esta investigación se trabajó con tamices que permitieron el paso de partículas cuyo tamaño osciló entre $0.5\ \mu\text{m}$ - $106\ \mu\text{m}$, obteniendo así los siguientes resultados promedio: para Arched swimming crab 4.0 ± 1.0 (SD) MP/g; en el caso mangrove cockle el valor correspondió a $3,3 \pm 2,9$ (SD) MP/g; en el caso de Stolzmann's weakfish se obtuvo una media de $2,4 \pm 1,3$ (DE) MP/g.

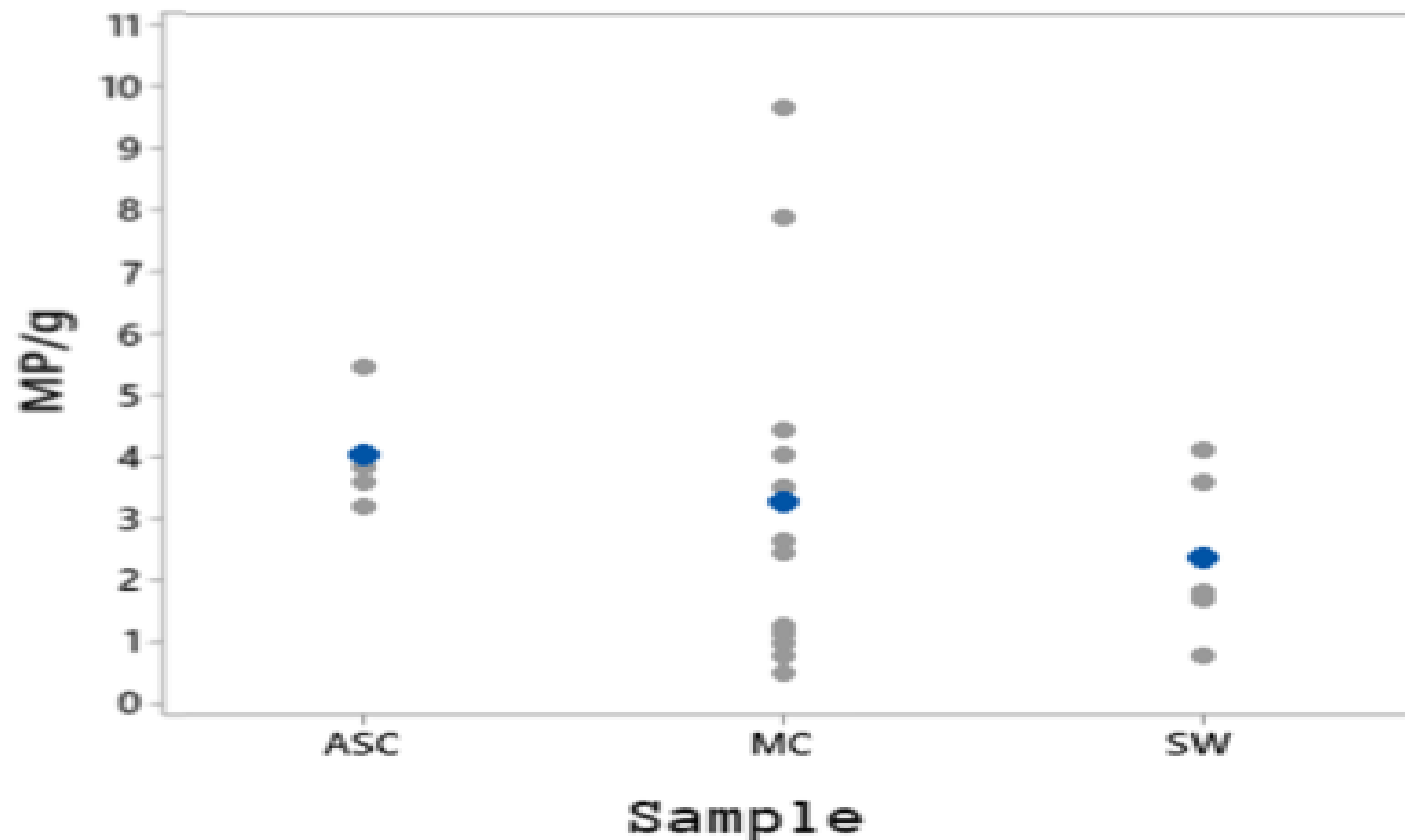


Fig 1 Amount of MP in composite samples; ASC: arched swimming crab, MC: mangrove cockle, SW: Stolzmann's weakfish. The blue points represent the average value of each marine species.

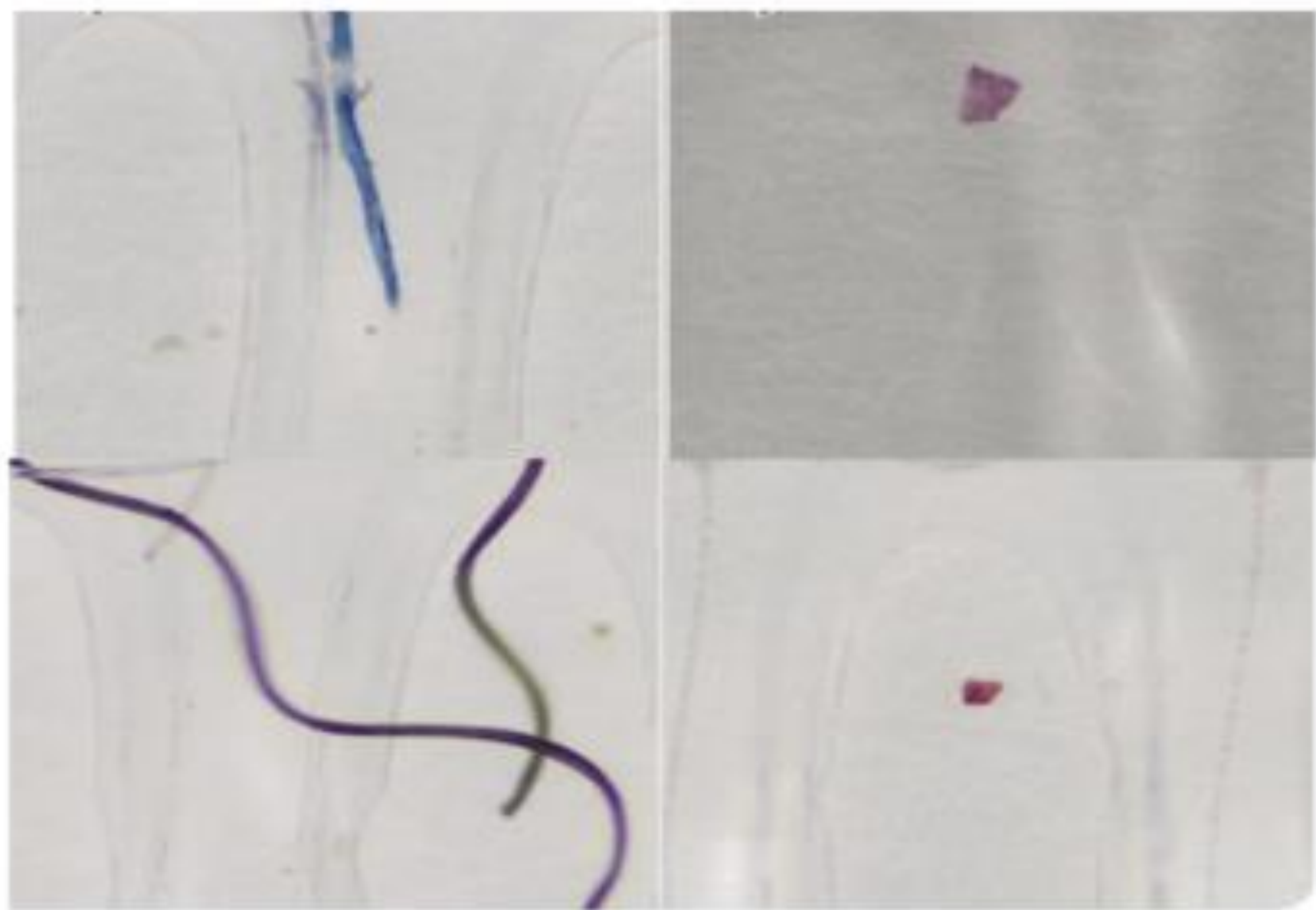


Fig 2. Pictures of isolated MP from marine species seen on the optical microscope at 4x; the different colors are shown.

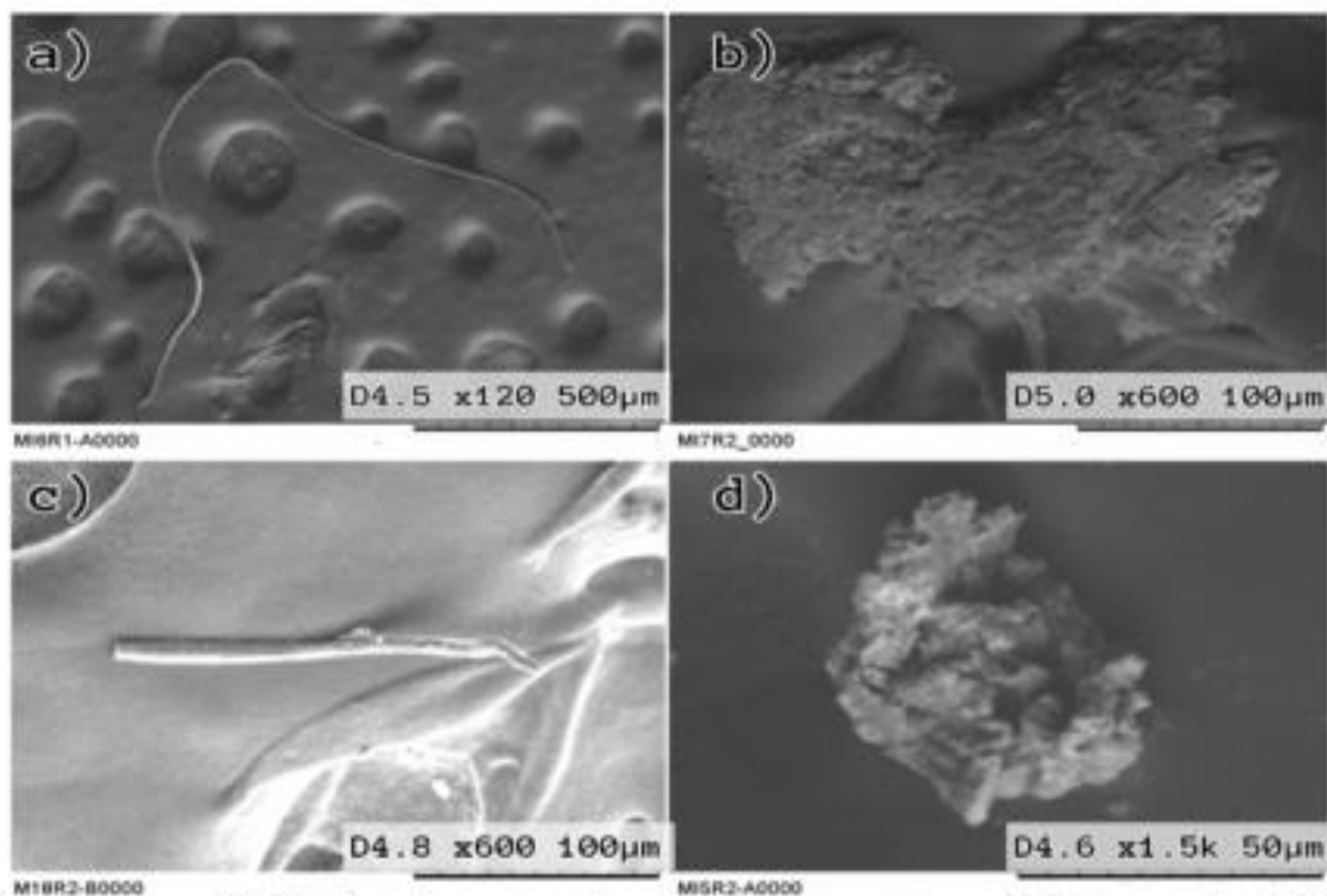


Fig 4. Micrographs of MP found on samples of Stolzmann's weakfish: a and c) fibers; b and d) particles.

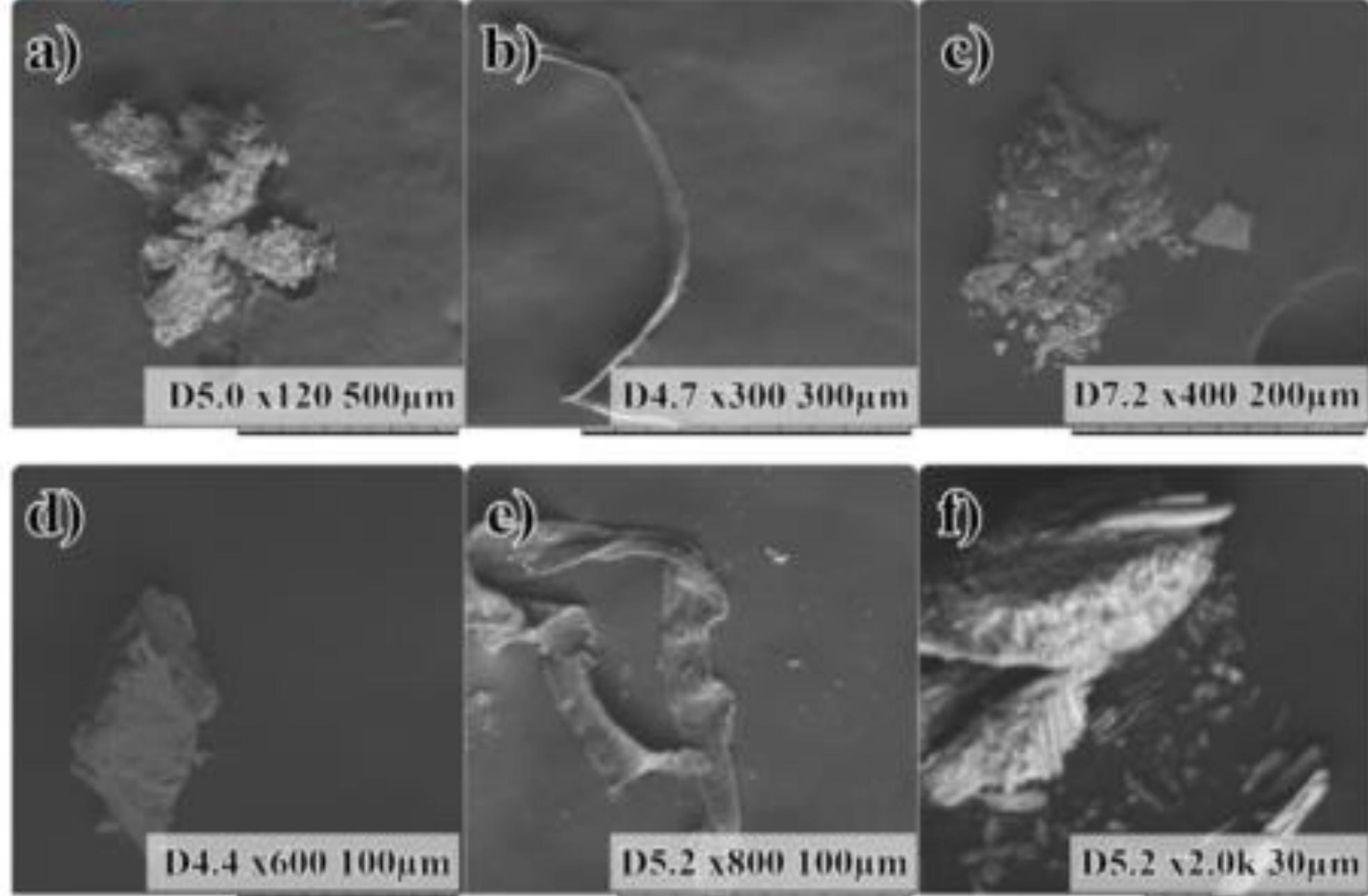


Fig 3. Micrographs of MP found on samples of mangrove cockle. a, c, d, f particles; b, e fibers.

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 <u>benzene</u> 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 <u>benzene</u> 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
<u>Stolzmann's weakfish</u>	Ideal EDS	1590 1315	N-H C-H	PA
<u>Stolzmann's weakfish</u>	Ideal EDS	1723 1609 1283 853	C=O C-C <u>benzene</u> ring C-O C-H	PET
<u>Stolzmann's weakfish</u>	Ideal EDS	1533 1332 739	C-O-C C-H ND	ND

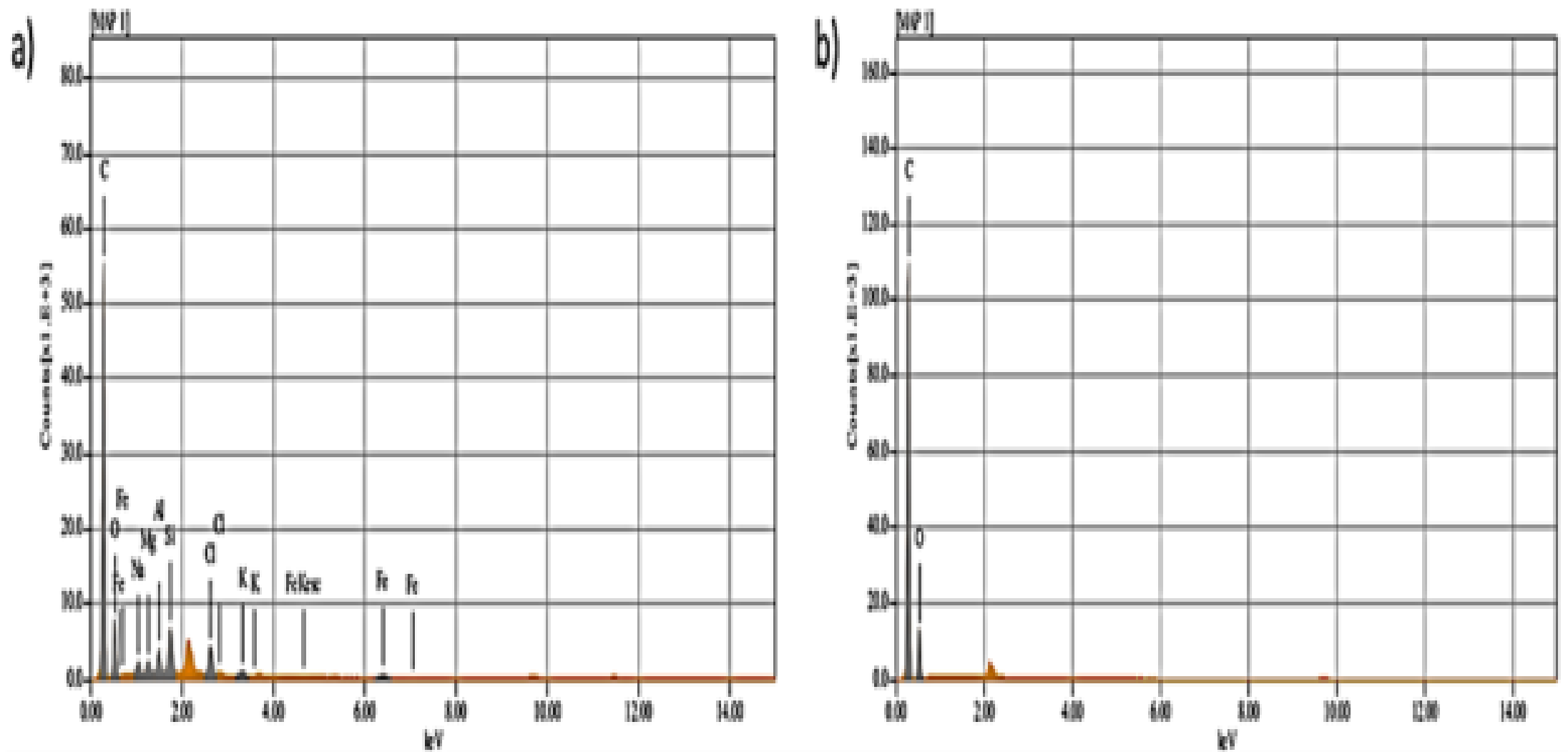


Fig 5. Graphs of EDS samples from MPs corresponding to a) polyethylene and b) polypropylene found in arched swimming crabs.

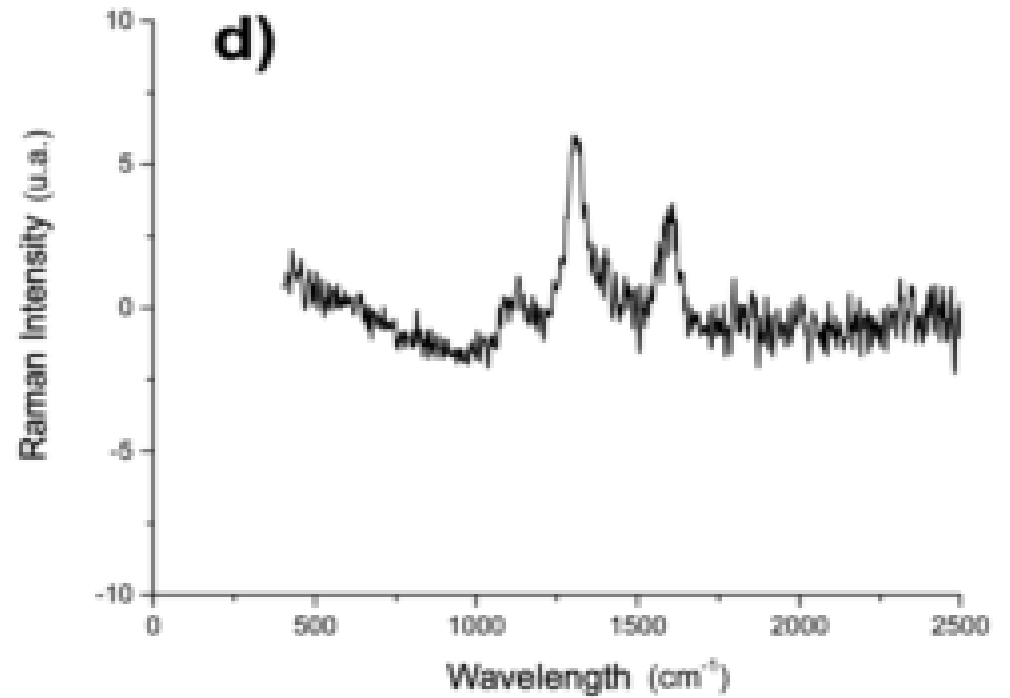
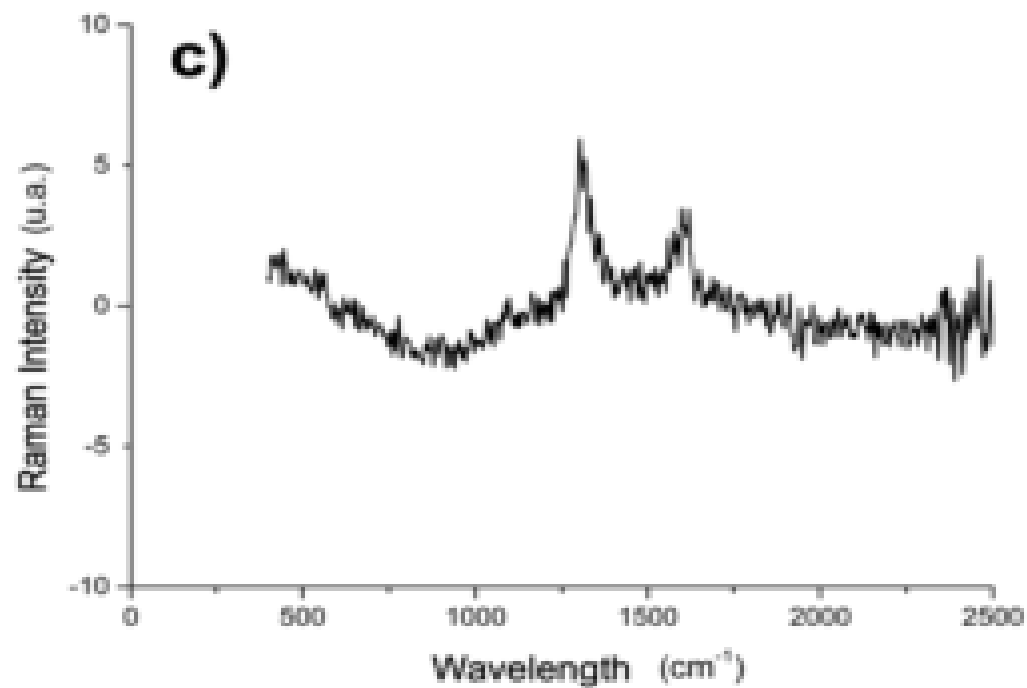
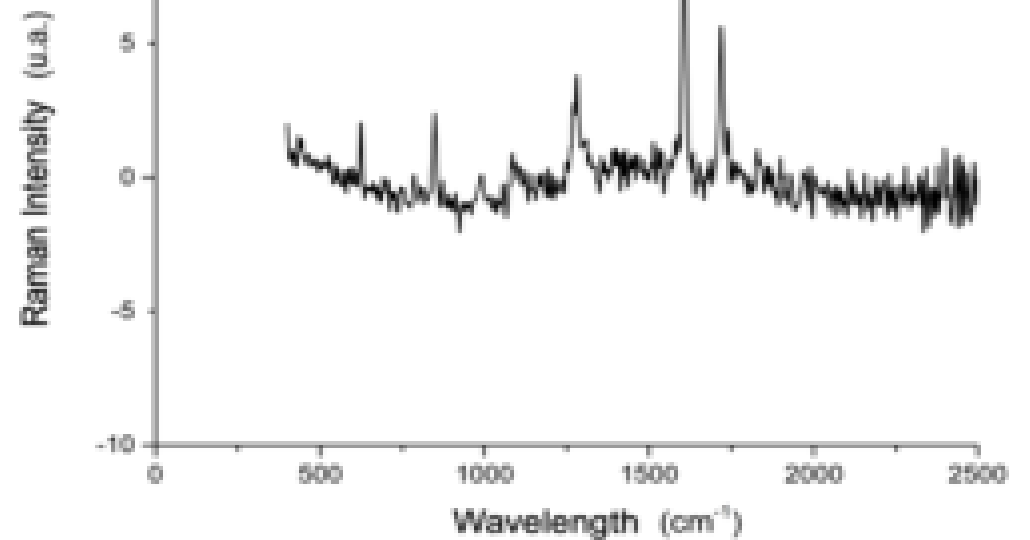
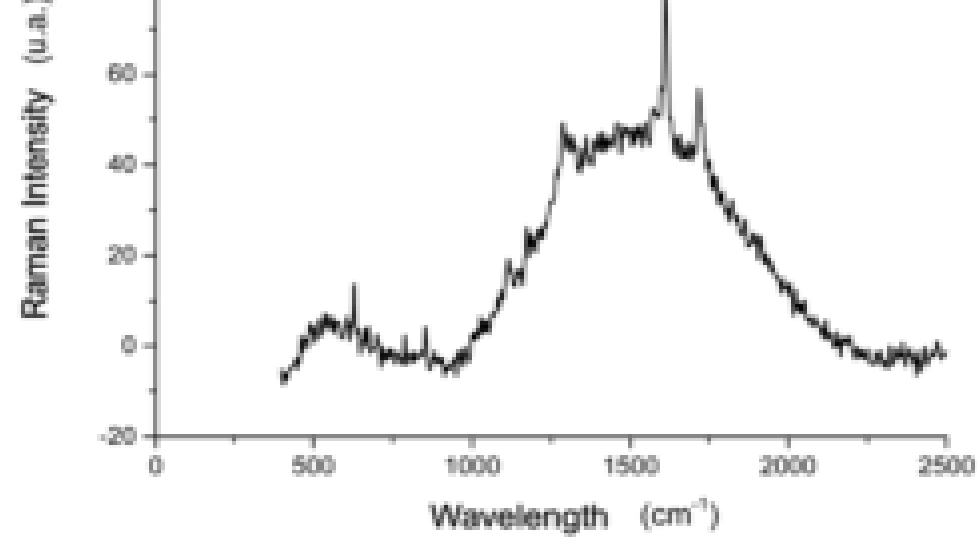


Fig 6. Raman spectra of MP, corresponding to MP found in marine species, a) arched swimming crab, b) mangrove cockle, c) and d) Stolzmann's weakfish. 780nm Laser and 3mW power.

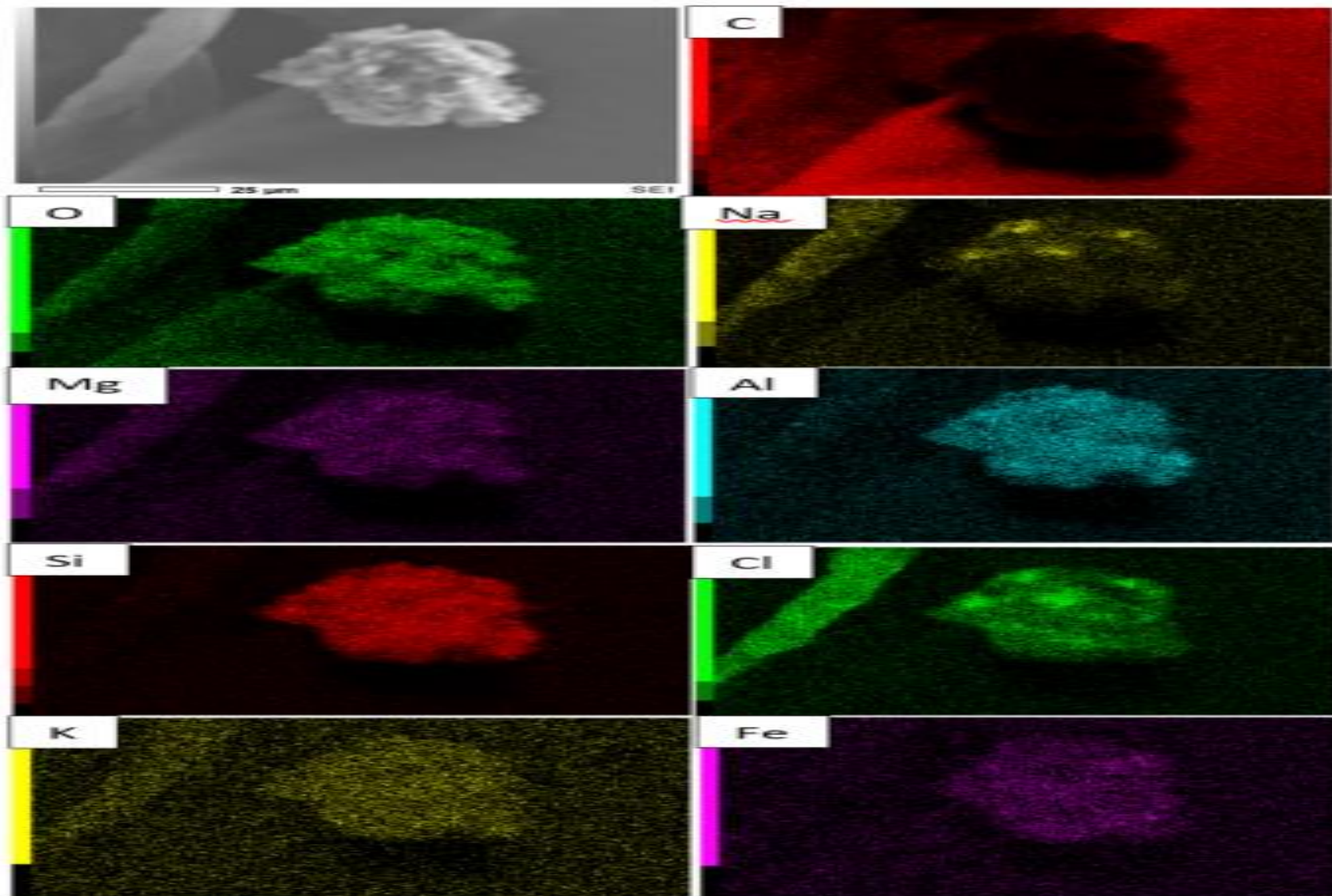


Fig 7. Elemental mapping of a particle correlating to microplastic

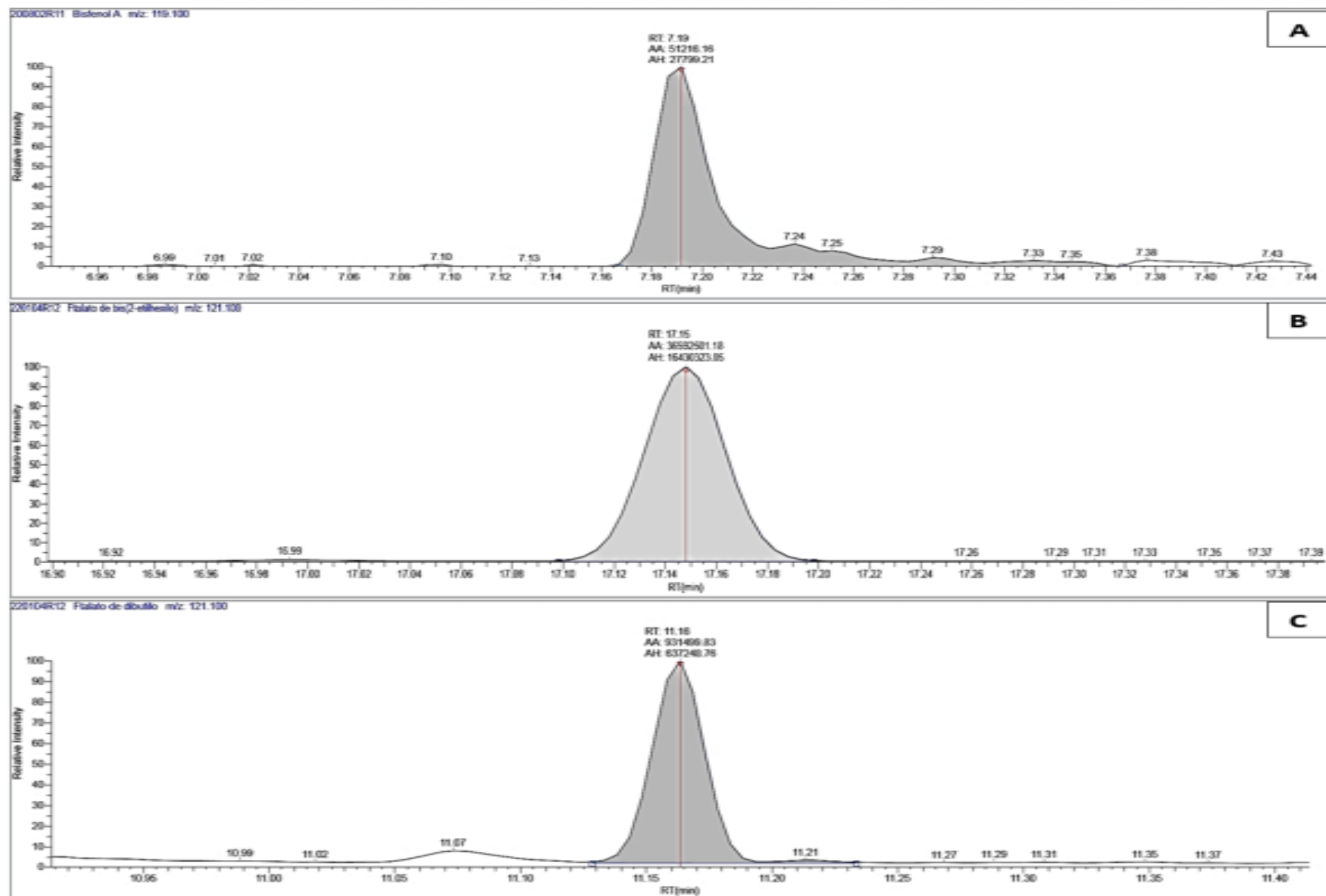


Fig 8. A: corresponds to a detected BPA signal in a sample of arched swimming crab; the signal is triggered in 7.19 minutes; B: corresponds to a DEHP signal in a sample of mangrove cockle; signal is triggered in 17.15 minutes and C: corresponds to a DBP signal in mangrove cockle; the signal is triggered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

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

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

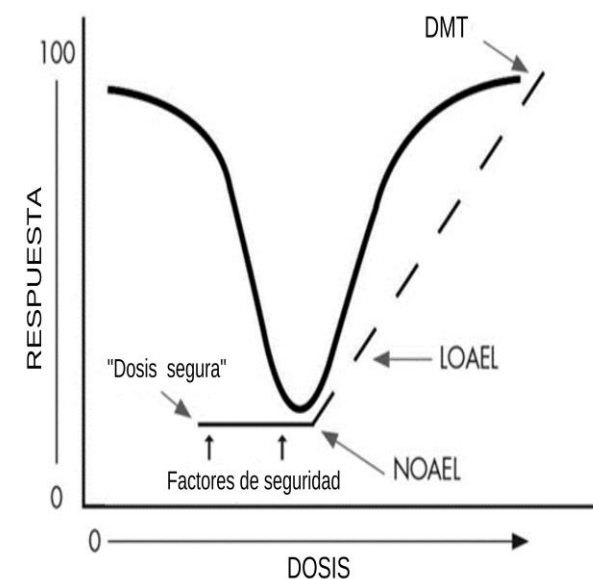
Table 3. Dietary exposure and TDI relation for adults according to values obtained in the study.

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

^a TDI 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).





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

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ABSTRACT

A special characteristic of MP (microplastics) in the ocean is 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), dibutyl phthalate (DBP). Both MP as the plasticizers were searched in composite samples of mangrove cockle (*Anadara tuberculosa*), Stolzmann's weakfish (*Cynoscion stolzmanni*) and arched swimming crab (*Callinectes arcuatus*). 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; the sizes of MP obtained were between 0.5 μ m and 106 μ m, the following average results being obtained: for Arched swimming crab 4.0 ± 1.0 MP/g; mangrove cockle 3.3 ± 2.9 MP/g; and for Stolzmann's weakfish, the average was 2.4 ± 1.3 MP/g; the most observed shapes were fibers and irregular segments; the most identified MP was polyethylene terephthalate (PET). Regarding extraction and quantification of plasticizers, the extraction stage was carried out using QuEChERS tubes; and the identification and quantification with gas chromatography coupled to a mass spectrometer (GC-MS). Regarding the plasticizing substances, DEHP was found in detectable levels in all the samples; BPA was found in 84% of the composite samples analyzed; DBP was found in 50% of them, of the analyzed samples 34% were positive for the 3 analytes. The dietary exposure of people to plasticizers was calculated and for BPA the exposure obtained was compared with respect to the TDI (tolerable intake dose) for pregnant women and the new TDI proposed by EFSA in 2021 according to the estrogenic effect of this substance in the fetus. The objective of the work was to determine if a relationship could be established between both PM and plasticizers, which gave a positive relationship.

Conclusiones

- De acuerdo a los datos obtenidos se puede afirmar que de los plastificantes encontrados, si bien no se puede afirmar que provengan exclusivamente de residuos plásticos encontrados como (MP), no se puede descartar su procedencia; ya que el trabajo consistió en determinar micropartículas y sustancias plastificantes en las mismas muestras, dichas muestras fueron adquiridas directamente de los pescadores artesanales.
- Sin duda si se puede afirmar que los plastificantes, sustancias peligrosas y especialmente importantes como el BPA, DEHP y DBP, han alcanzado los niveles más altos de la cadena trófica, cabe expresar que el 34% de las muestras fueron positivas para los 3 plastificantes, lo que sugiere un efecto de mezcla tóxica, de forma sinérgica; se deben establecer medidas urgentes para proteger el recurso marino de la contaminación por plásticos, y por ende, la salud pública.

- Si bien es cierto que los plastificantes tienen una distribución global ubicua, cobra especial importancia el riesgo que supone la presencia de estas sustancias en alimentos o agua de consumo humano, siendo especialmente sensibles los niños y las mujeres embarazadas. Se pueden realizar otros estudios, especialmente prospectivos, para establecer dosis seguras de estas sustancias en estas poblaciones vulnerables.
- De acuerdo al tipo de sustancia encontrada, se sugiere a las mujeres embarazadas no ingerir mariscos durante el periodo de gestación, considerando la baja cantidad sugerida de BPAs en alimentos, estando actualmente bajo el criterio científico de EFSA; bajo aceptación por TDI de 0.004 ng/kg pc d; dado el efecto estrogénico de esta sustancia en el periodo de gestación del feto y también se debe limitar su consumo por parte de los niños.

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MUITO
Obrigada

The word "Obrigada" is written in a large, elegant, black cursive script. Above it, the word "MUITO" is written in a smaller, black, sans-serif font. The text is surrounded by decorative elements: several small pink hearts are scattered around the letters, and a line-art illustration of a bouquet of flowers with long stems and small blossoms is positioned at the bottom right.