Universidade do Minho

DIPARTIMENTO DI MEDICINA VETERINARIA E SCIENZE ANIMALI

EUROPEAN COMMISSION

75th EAAP Annual Meeting

1/5 September 2024 - Florence, Italy

TRACEABILITY AND CHARACTERIZATION OF NANOPLASTICS ON NOVEL FEED/FOOD SOURCES: THE CASE OF MICROALGAE

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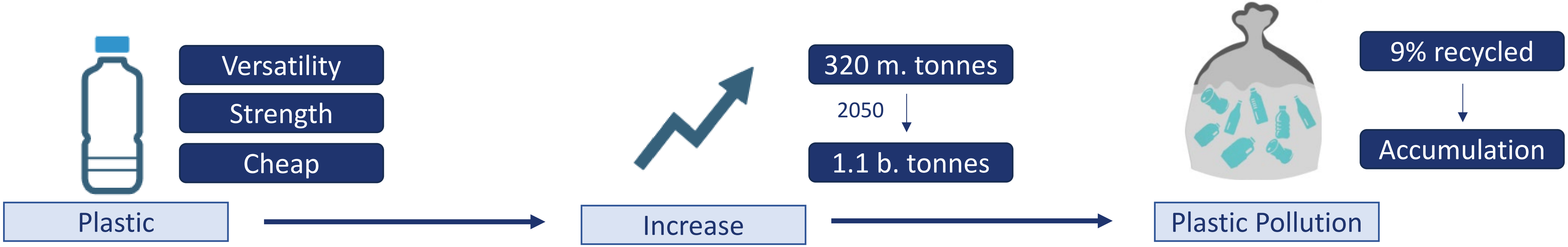
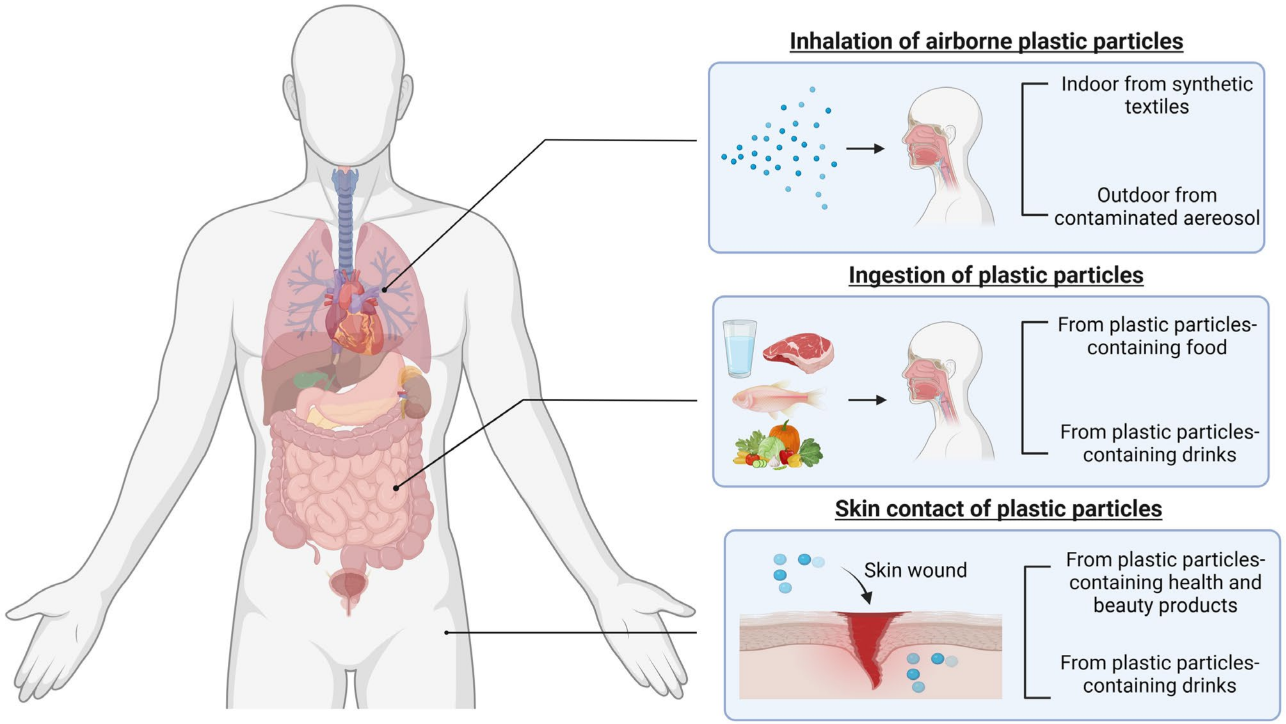
INTRODUCTION



Key routes for microplastics and nanoplastics in human body



The annual intake of plastic particles per person is approximately 39.000-52.000

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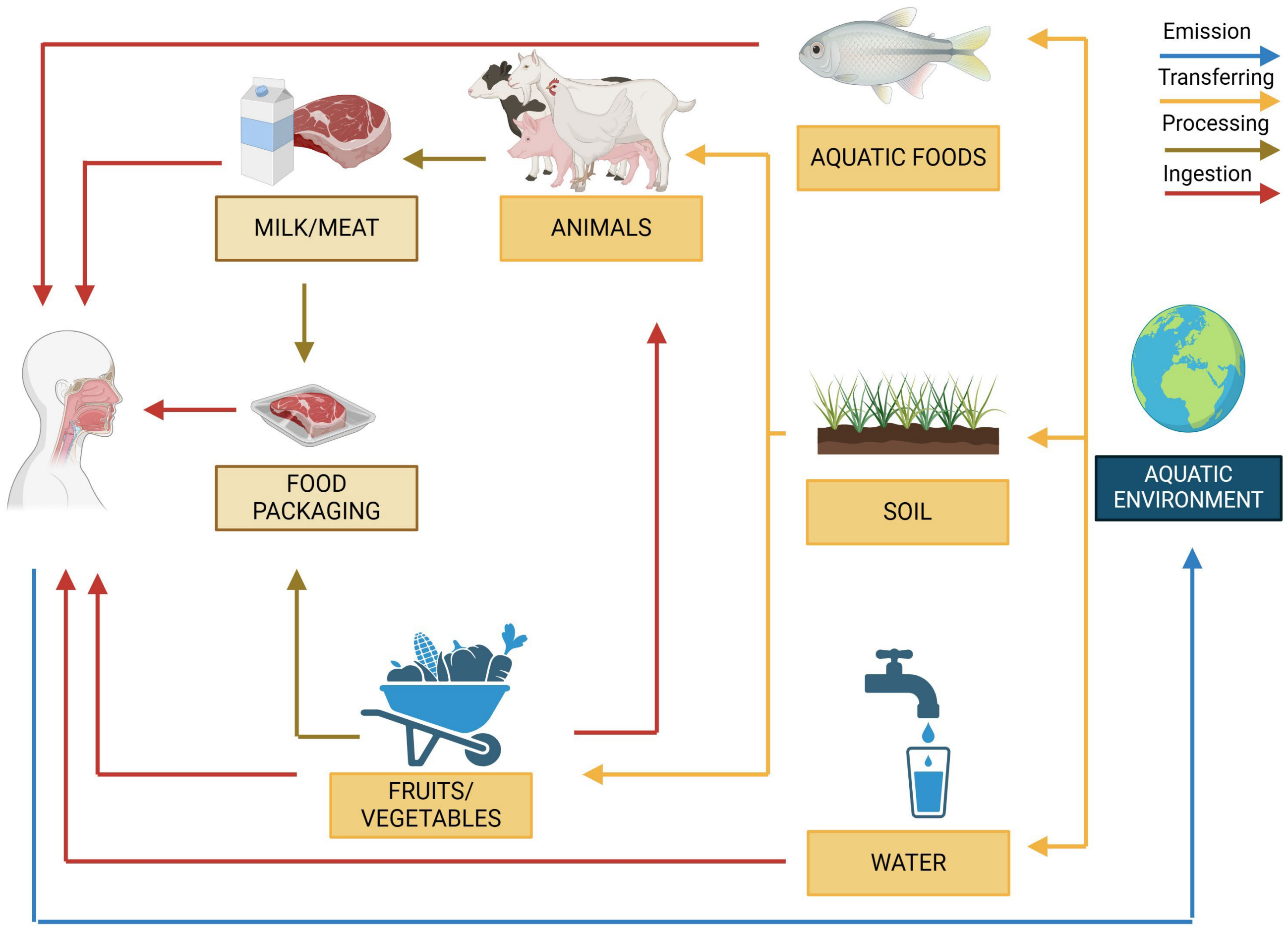
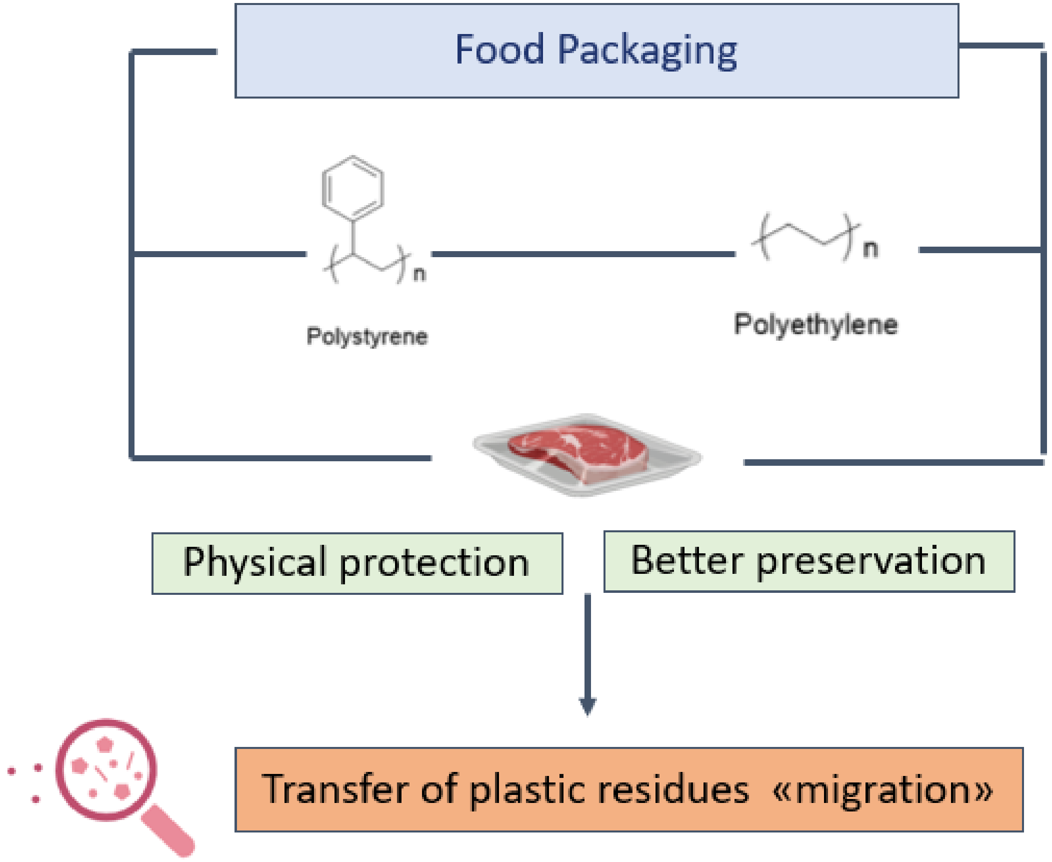
Figure created using Biorender.com

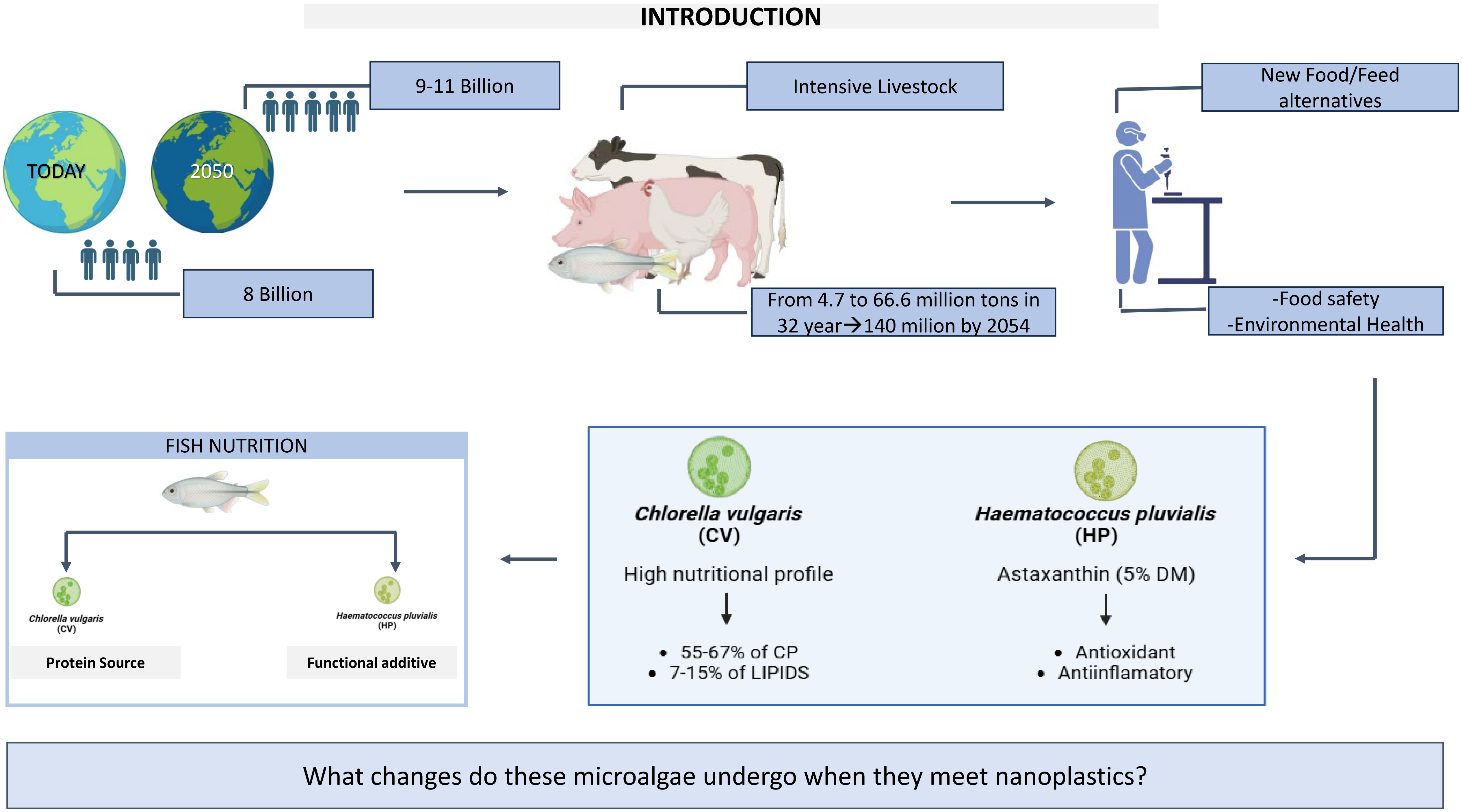
INTRODUCTION



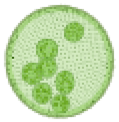
Contamination sources of microplastics and nanoplastics in the food chain. Figure created with bioreder.com and adapted from Liu et al. (2021).



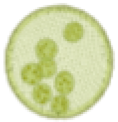
What happens to these nanoplastics during the digestion process? What changes occur?


INTRODUCTION
9-11 Billion
TODAY
2050
8 Billion
Intensive Livestock
From 4.7 to 66.6 million tons in 32 year→140 milion by 2054
New Food/Feed alternatives
-Food safety
-Environmental Health
FISH NUTRITION
Chlorella vulgaris (CV)
Haematococcus pluvialis (HP)
Protein Source
Functional additive
Chlorella vulgaris (CV)
High nutritional profile
55-67% of CP
7-15% of LIPIDS
Haematococcus pluvialis (HP)
Astaxanthin (5% DM)
Antioxidant
Antiinflamatory
What changes do these microalgae undergo when they meet nanoplastics?

AIM



Chlorella vulgaris
(CV)

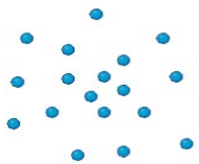


Haematococcus pluvialis
(HP)

In this work we aimed to understand the effects on the **functional profile** of two microalgae species (**CV and H**P) relevant as food/feed supplements, upon single (PS, PE) and NPs mixture (PS+PE) exposure, having in mind a pre-assumption of potential microalgae contamination via migration of plastics from packaging to the food stuff, before and after ***in vitro*** and ***ex vivo*** digestion.

MATERIALS

POLYSTYRENE



Polysciences (Polybead® Microspheres, 00876-15)

Diameter 100.0 (± 15) nm

POLYETHYLENE

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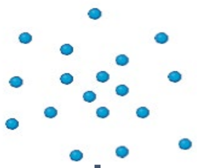
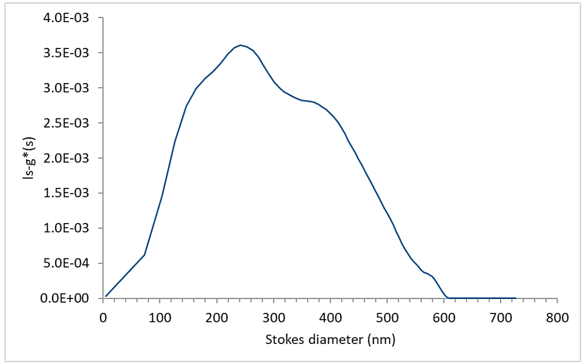
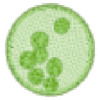


in house synthetised (oil-in-water emulsion precipitation protocol). **Raman spectroscopy** was conducted to characterize the colloidal PE plastic particles chemical composition. PE NPs size distribution and particles density were determined using a centrifugal sedimentation method



Chlorella vulgaris



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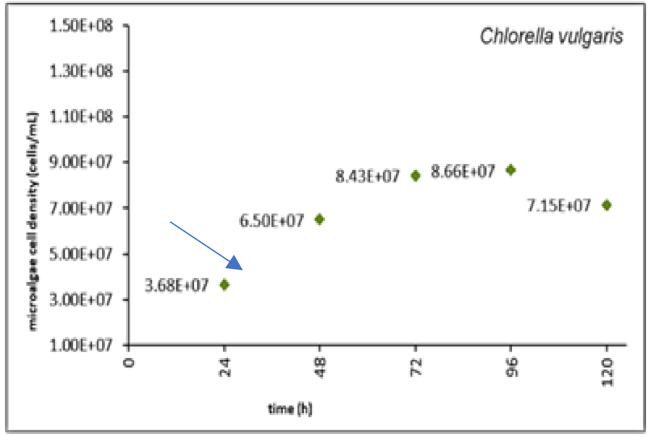
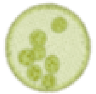
Chlorella vulgaris **(CV)**

Mixotrophic conditions using dairy waste as carbon source (**hydrolysed cheese whey**).

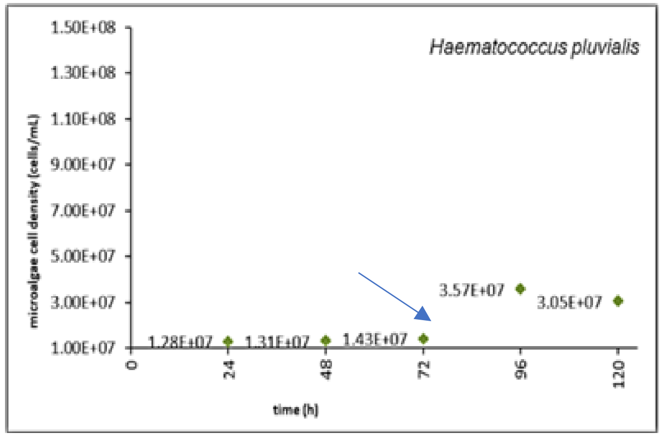


Haematococcus Pluvialis



Haematococcus pluvialis **(HP)**

autotrophic conditions using the Blue Green 11 (BG-11) medium, a widely reported mainstream medium for microalgal biomass

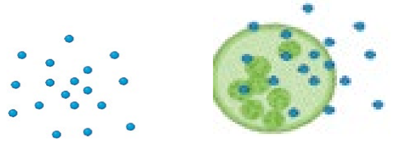


- ***CV*** **at 3.80 x 10^7 cells mL^{-1}**
- **HP at 1.75 x 10^7 cells mL^{-1}**
- **1 x 10^{12} part mL^{-1} of standard PS**
- **5 x 10^9 part mL^{-1} of PE**

under stirring at the same environmental chamber as the microalgae mother strain cultures, **for 24 h**.

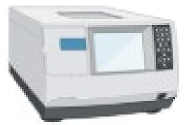
METHODS

Dynamic Light Scattering (DLS)



Before digestion

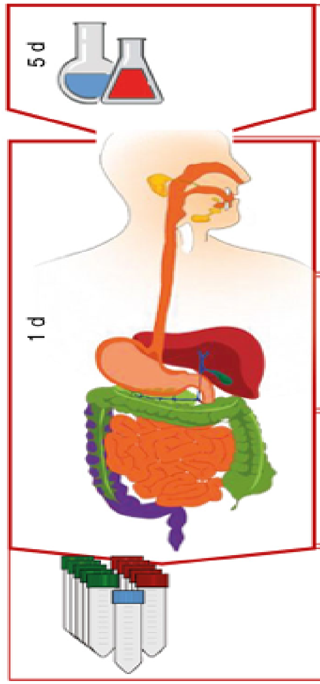
After digestion



Size, PdI (Batch Mode)

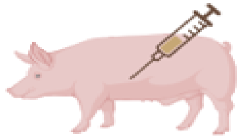
ζ potential (Electrophoretic Mode)

Infogest 2.0 (Brodkorb et al., 2019)

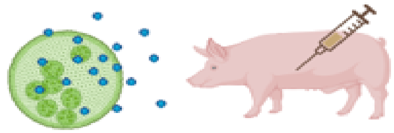


		Step
Preparation	• Perform enzyme activity and bile assays	1
	• Prepare SSF, SGF and SIF stock solutions	2
	• Perform pH-test adjustment experiment	4
Oral phase	• Mix Food with SSF (1:1, (wt/wt))	7–12
	• Include $CaCl_2$ (1.5 mM in SSF)	13
	• Add salivary amylase, if necessary (75 U/mL)	14
	• Incubate while mixing (2 min, 37 °C, pH 7)	15, 16
Gastric phase	• Mix oral bolus with SGF (1:1 (vol/vol))	17, 18
	• Include $CaCl_2$ (0.15 mM in SGF)	19
	• Add pepsin, gastric lipase (2,000, 60 U/mL)	20, 21
	• Incubate while mixing (2 h, 37 °C, pH 3.0)	22–24
Intestinal phase	• Mix gastric chyme with SIF (1:1 (vol/vol))	25, 26
	• Include bile (10 mM bile salts)	27
	• Include $CaCl_2$ (0.6 mM in SIF)	28
	• Add pancreatin (trypsin activity 100 U/mL)	29
	• Incubate while mixing (2 h, 37 °C, pH 7.0)	30–32
Sampling	• Sampling procedure and sample treatment (Table 1)	

Ex vivo digestion (Devle et al., 2014; Lanzoni et al., 2024)

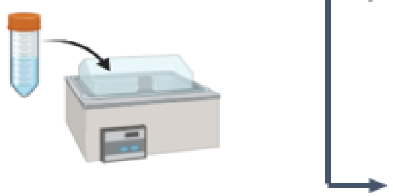


Fluids taken at slaughterhouse (50-110 days, 24 animals).



CV: 9.5 x 10^6 cells mL^{-1}

HP: 4.4 x 10^6 cells mL^{-1}



GASTRIC PHASE

39 C 2 h 2 ± 0.05

39 C 2 h 6.7 ± 0.05

INTESTINAL PHASE

Total Phenolic Content (Attard, 2014) (pre and post digestion)

RESULTS: NPs PRE DIGESTION WITHOUT MICROALGAE

Sample	Z-average (d.nm)	PdI	ζ-potential (mV)
PS in *CV* growth medium	85.32 ± 0.32[a]	0.05 ± 0.01[a]	-10.60 ± 0.26[a], pH 7.65
PS in *HP* growth medium	87.86 ± 0.09[a]	0.10 ± 0.01[a]	-11.93 ± 1.09[a], pH 7.36
PE in *CV* growth medium	207.50 ± 1.18[b]	0.13 ± 0.01[b]	-15.57 ± 0.29[b], pH 7.68
PE in *HP* growth medium	205.30 ± 1.45[b]	0.13 ± 0.00[b]	-24.60 ± 0.95[c], pH 7.17
PS + PE in *CV* growth medium	267.20 ± 4.84[c]	0.33 ± 0.02[c]	-19.03 ± 0.17[b], pH 7.73
PS + PE in *HP* growth medium	204.67 ± 1.77[b]	0.17 ± 0.00[b]	-17.23 ± 0.98[b], pH 7.13

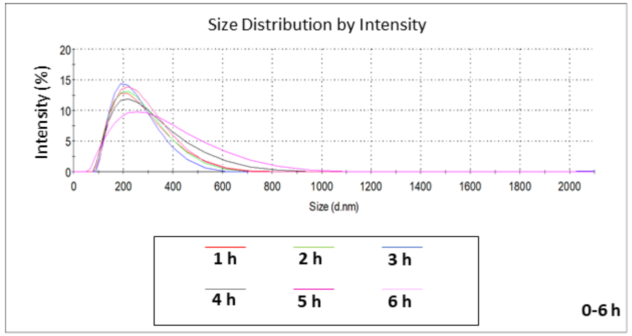
Values in the range of the expected standard particles nominal size

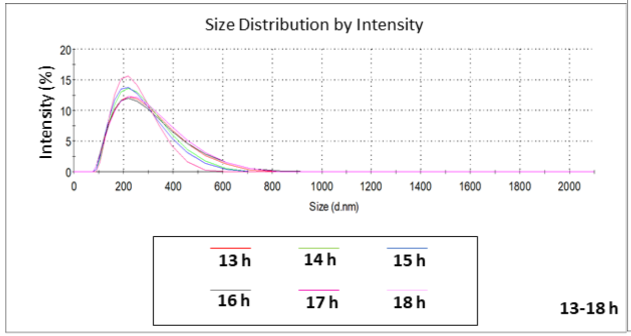
Larger particles are detected more readily than smaller ones.

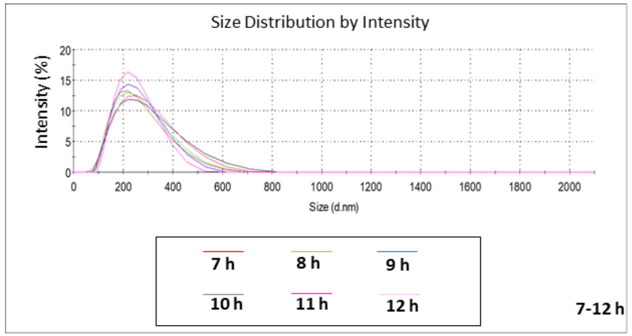
Agglomeration or aggregation?

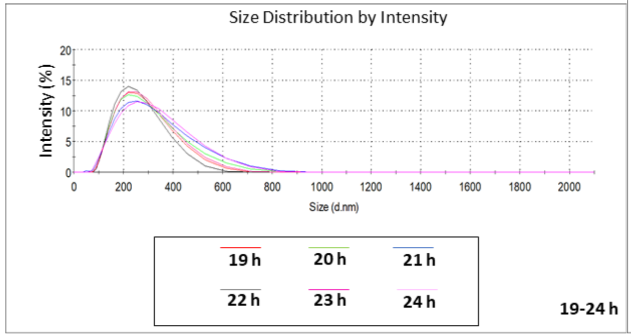
Size (Z-average), PdI and ζ-potential of PS, PE and PS + PE nanoplastics in microalgae growth media. Different superscript letters indicate statistically significant differences among groups ($P < 0.05$). Results are reported mean ± SEM.

PS+PE in HP growth medium after 24 h

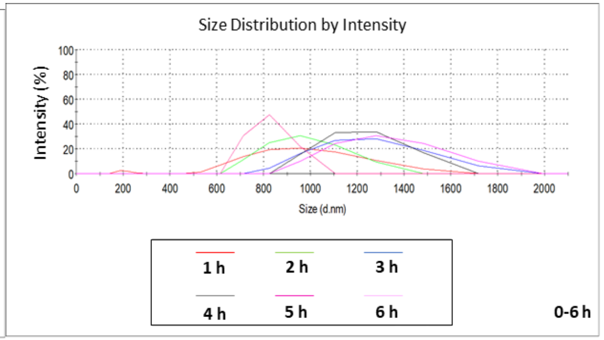


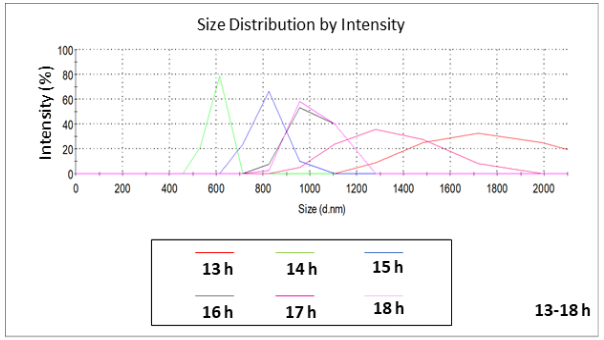


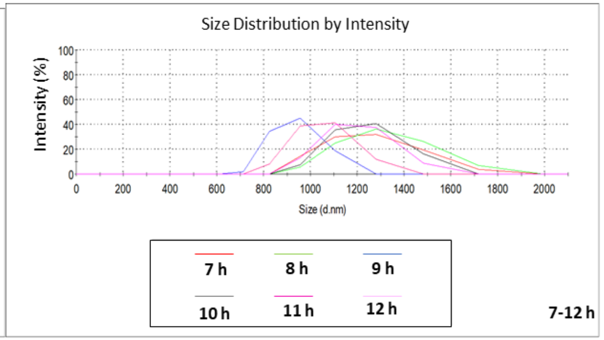


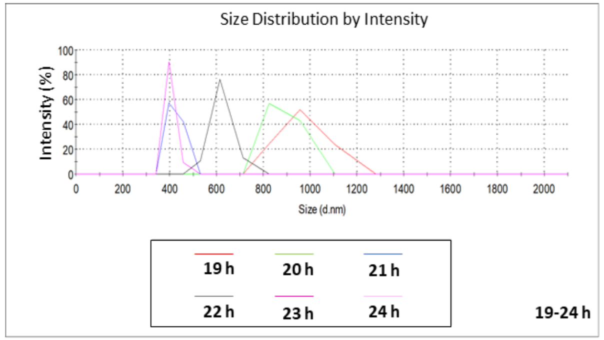


PS+PE in CV growth medium after 24 h









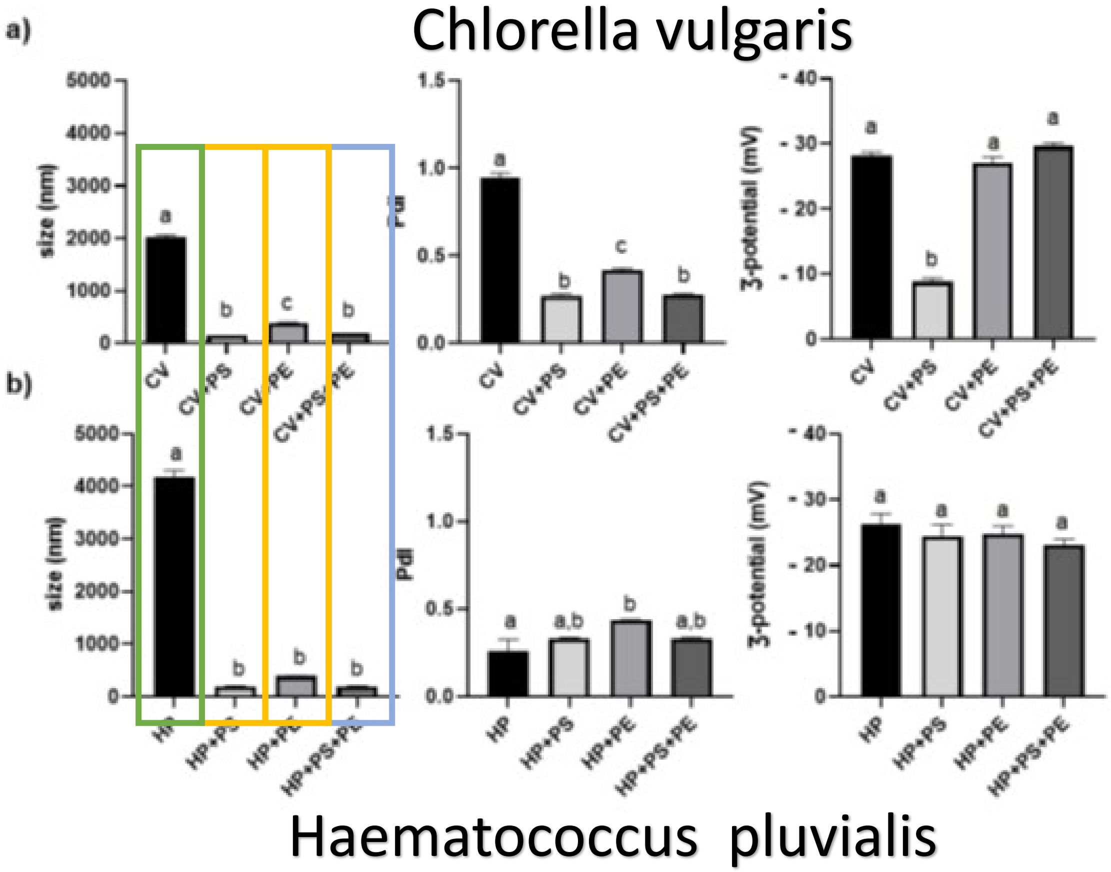
RESULTS: INTERACTIONS NPS and MICROALGAE BEFORE DIGESTION

CV= 2040 ± 27,43 nm
HP= 4169 ± 133,67 nm

CV+PS= 146,17 ± 0,32 nm
HP+PS= 197,30 ± 5,18 nm

CV+PE= 399,03 ± 9,23 nm
HP+PE= 390,27 ± 9,52 nm

CV+PS+PE= 206,13 ± 0,64 nm
HP+PS+PE= 199,20 ± 2,81 nm



Size (Z-average) detected for microalgae suspensions of NPs post (a) *in vitro* and (b) *ex vivo* digestion. Different superscript letters indicate statistically significant differences among groups ($P < 0.05$). PS = Polystyrene; PE = Polyethylene; CV = *Chlorella vulgaris*; HP = *Haematococcus pluvialis*

CV= Confirmed the values reported in the literature
HP= *green vegetative palmella*

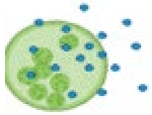
Extracellular polymeric substances (EPS) → Natural biopolymers secreted in response to stress that act as a protective layer against external agents. EPS consist of polysaccharides, enzymes and structural proteins which can coat the surface of NPS (bio-corona) influencing physicochemical properties

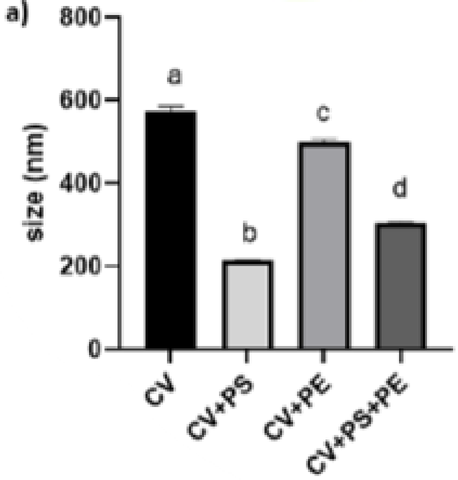
CV: Agglomeration of PS + PE NPs decreases the contact area and interaction strength between the NPs and biomolecules,
Furthermore, bio-corona formation can counteract ionic effects and stabilise NPs, thus reducing agglomeration through steric interactions.

HP: In the neutral microalgae growth media, weaker electrostatic interactions lead to lower overall agglomeration. PE NPs, being larger, again dominate the size distribution, stabilising the mixture around their weighted average hydrodynamic diameter

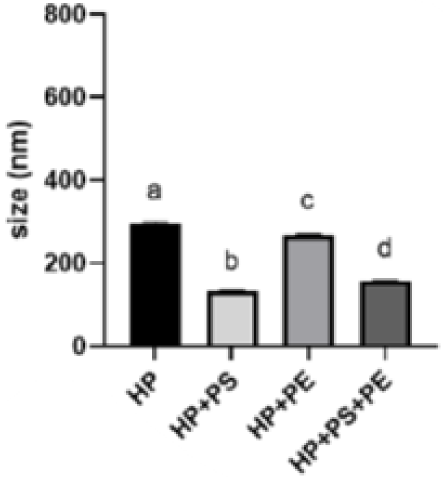
RESULTS: MICROALGAE+NANOPLASTICS AFTER DIGESTION

IN VITRO



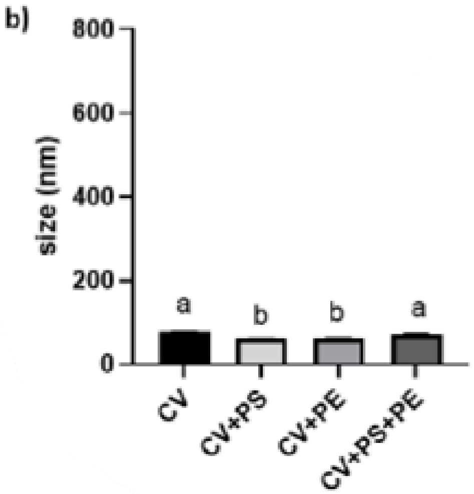


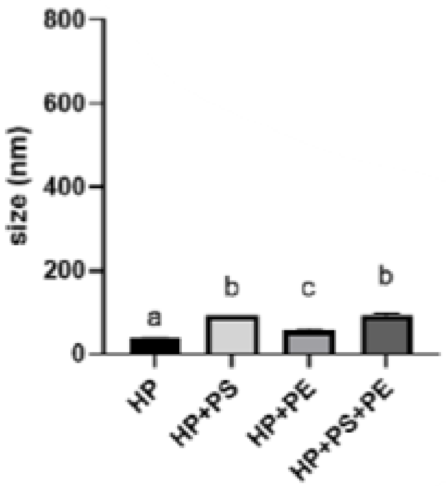
CHLORELLA VULGARIS



HAEMATOCOCCUS PLUVIALIS

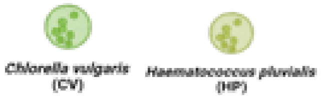
EX VIVO





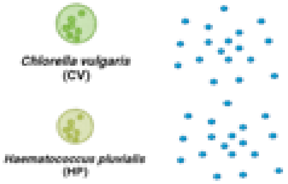
Size (Z-average) detected for microalgae suspensions of NPs post (a) *in vitro* and (b) *ex vivo* digestion. Different superscript letters indicate statistically significant differences among groups ($P < 0.05$). PS = Polystyrene; PE = Polyethylene; CV = *Chlorella vulgaris*; HP = *Haematococcus pluvialis*.

IN VITRO



CV (574,83 ± 11,04 nm) **> HP** (296,77 ± 2,48 nm)

- **CV**: Complex 3-layer structure. The last layer resistant to enzymatic action except for chitinases
- **HP**: Only in the final stage do they acquire a structure that is difficult to degrade. Moreover, the walls are highly sensitive to the action of hydrochloric acid



Increased size

Reduced size

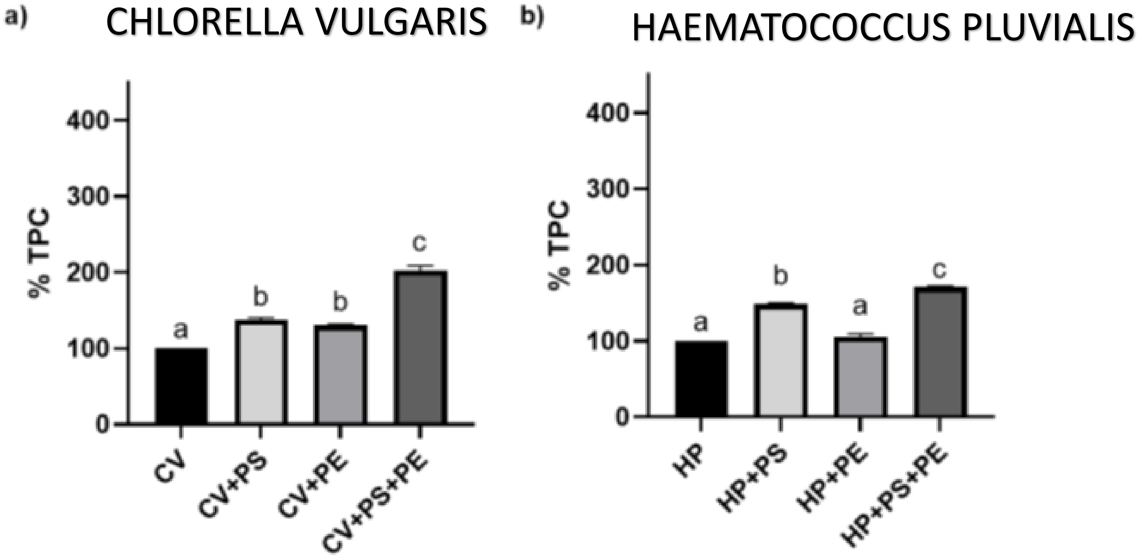
Polymer | **Digestion** | **Agglomeration** | **Bio-corona**

Ability of NPs to persist in the digested fraction, making thus these available for absorption by intestinal cells

EX VIVO

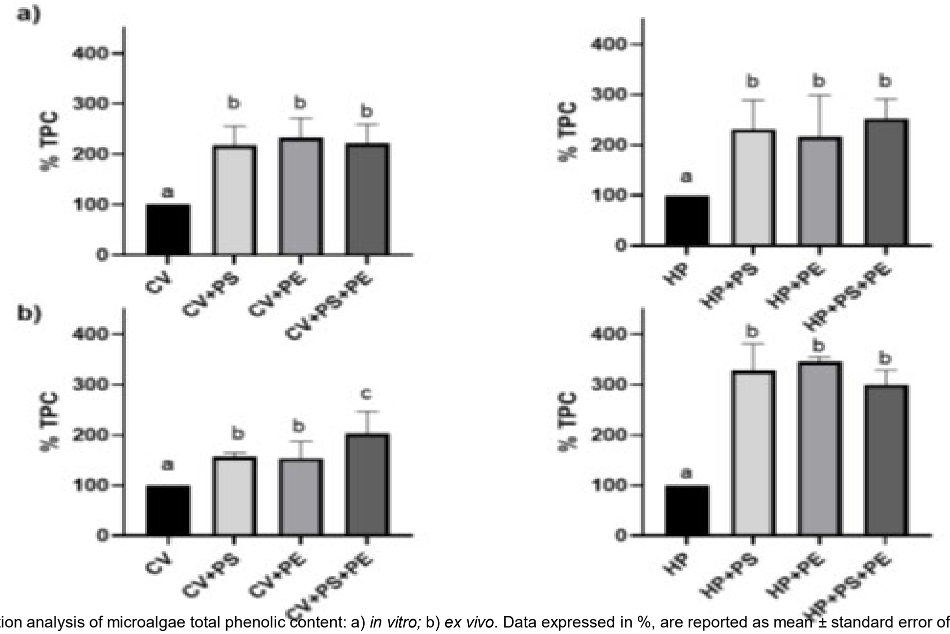
CV (di 76,89 ± 3,12 nm) **> HP** (38,74 ± 1,23 nm) → Higher digestion (Pdi>0,90)

TOTAL PHENOLIC CONTENT PRE DIGESTION



Pre-digestion analysis of microalgae total phenolic content: a) *Chlorella vulgaris*; b) *Haematococcus pluvialis*. Data expressed in %, are reported as mean ± SEM (n = 3), and are standardised towards experimental control group (microalgae in growth media only; no NPs co-exposure). Different superscript letters indicate statistically significant differences among groups ($P < 0.05$). PS = Polystyrene; PE = Polyethylene; CV = *Chlorella vulgaris*; HP = *Haematococcus pluvialis*.

TOTAL PHENOLIC CONTENT POST DIGESTION



Post-digestion analysis of microalgae total phenolic content: a) *in vitro;* b) *ex vivo*. Data expressed in %, are reported as mean ± standard error of the mean (SEM) (n = 3), and are standardised towards experimental control group (microalgae in growth media only; no NPs co-exposure). Different superscript letters indicate statistically significant differences among groups ($P < 0.05$). PS = Polystyrene; PE = Polyethylene; CV = *Chlorella vulgaris*; HP = *Haematococcus pluvialis*.

RESULTS

Parameter not investigated in the literature

↓

Trend confirmed in the literature (2022)

↓

NPs increased TPC in a seagrass

ORAL PHASE

Partial Digestion of phenols in the oral cavity

GASTRIC PHASE

Low pH → High release of phenols

INTESTINAL PHASE

Instability at alkaline environment→ Transformation of phenols

CONCLUSIONS

Nanoplastics

NPs behaviour was influenced by the microalgae growth media

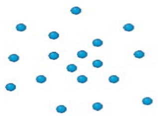
Nanoplastics and digestion

Post digestion studies revealed that NPs were detected in the digested fraction indicating a potential risk to human and animal intestinal health

Total phenolic content

Increased TPC → complex interplay between the polymer particles and microalgae

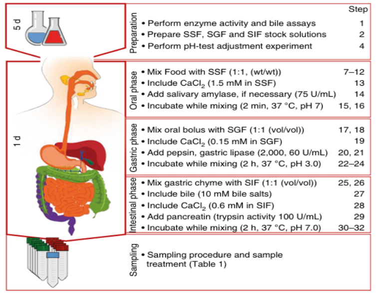
FUTURE PROSPECTS



PS 350 nm

PS 500 nm

PS 350 + PS 500 nm



Pre digestion

Post digestion

DLS

SPES

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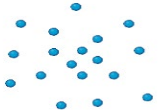
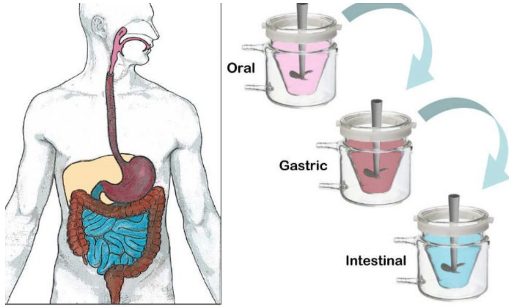


PS 100 nm

PP 180 nm

PE 350 nm

PE + PS + PP



Post digestion

Effects on cells

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THANKS FOR YOUR ATTENTION

Traceability and characterization of nanoplastics on novel feed/food sources: the case of microalgae

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