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**Identifying a quick and efficient method of removing organic matter  
without damaging microplastic samples**

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## Identifying a quick and efficient method of removing organic matter without damaging microplastic samples

### Abstract

Natural organic matter may confound the detection of microplastics, requiring a removal step. However, most available protocols are long and lack information on removal efficiency and polymer degradation. Thus, we have determined the digestion efficiency (%) for a pool of organic matter (algae, driftwood, feathers, fish muscle, paraffin, palm oil) for five digestion solutions, hydrogen peroxide ( $\text{H}_2\text{O}_2$ ), hydrogen peroxide with iron catalyst ( $\text{H}_2\text{O}_2+\text{Fe}$ ), potassium hydroxide (KOH), nitric acid ( $\text{HNO}_3$ ), and sodium dodecyl sulphate (SDS), under two temperatures (room temperature at  $25^\circ\text{C}$ ,  $50^\circ\text{C}$ ) and two periods (1, 6 h).  $\text{H}_2\text{O}_2+\text{Fe}$  and KOH at  $50^\circ\text{C}$  for 1 hour had the highest digestion efficiencies, of 65.9% and 58.3% respectively (mostly limited by driftwood and paraffin). Further testing revealed that  $\text{H}_2\text{O}_2+\text{Fe}$  is more appropriate for plant material and KOH for animal tissue. Weight loss (%), Fourier transform infrared spectrometry and carbonyl index of 9 virgin and 6 weathered polymers (polyethylene, polypropylene, polystyrene, polyethylene terephthalate, polyvinyl chloride, cellulose acetate, nylon) revealed that only identification of cellulose acetate was hindered. Filters were also tested revealing that quartz and glass fibre filters are resistant to these protocols. Thus, a digestion protocol based on  $\text{H}_2\text{O}_2+\text{Fe}$  or KOH at  $50^\circ\text{C}$  for 1 hour may be used on microplastic samples.

**Keywords:** Sample processing; digestion; organic matter removal; plastic integrity; microplastics

### 1. Introduction

Plastic production has reached 348 million tonnes in 2017 (PlasticsEurope 2018). This increase in demand, along with deficient waste management systems, has led to an accumulation of plastics in the environment. In the year 2010, 4.8 to 12.7 million tons of plastics reached the oceans (Jambeck et al. 2015), whereas Eriksen et al. estimates 250,000 tons of plastics pieces are already afloat (Eriksen et al. 2014). The accumulation of plastics in the environment is also a result of low biodegradation. However, radiation, heat and friction may cause fragmentation of plastics into smaller pieces <5 mm, called secondary microplastics (Andrady 2011). On the other hand, primary microplastics can also be intentionally produced to be used in products (e.g. cosmetics) or industries (Browne et al. 2011). Small sizes and high surface area of microplastics facilitates the interaction with a large range of organisms (Wright et al. 2013), as well as the adsorption and transport of persistent organic pollutants (Bakir et al. 2014) or microorganisms (Carson et al. 2013). Furthermore, plastics are known to release additives and monomers, some with recognized toxicity (Groh et al. 2019; Hahladakis et al. 2018). Concerns over the negative effects of microplastics to ecosystems and human health has led to a growing sampling effort, revealing the presence of these particles in sediment (Abidli et al. 2018; Reed et al. 2018), soil (He et al. 2018; Watteau et al. 2018; Zhang et al. 2018), freshwater (Eriksen et al. 2013; Rodrigues et al. 2018), seawater (Frias et al. 2014; Sun et al., 2018) and air (Abbasi et al. 2019; Dris et al. 2016). However, no universal pre-treatment method has been adopted by research groups worldwide.

Proper quantification of microplastics in environmental samples is essential to understand sources and behaviour in the environment. Digestion protocols are required when environmental samples are rich in biologic material or when determining the exposure of organisms. For instance, beach sediment may contain 0.5 – 7.0% of organic matter (Crichton et al. 2017), which can be mistaken for microplastics, such is often the case of dark algae fragments (Lavers et al. 2016). Thus, organic matter removal procedures have been developed involving acid (Dehaut et al. 2016; Naidoo et al. 2017), alkali (Karami et al. 2017; Kühn et al.

2017), oxidizing (Erni-Cassola et al. 2017; Ziajahromi et al. 2017) and enzymatic (Catarino et al. 2017; Coutene-Jones et al. 2017) digestion. However, not all digestion procedures are able to remove organic matter without damaging polymers. For instance,  $\text{HNO}_3$  is reported to cause yellowing of polymers, melting of polyethylene terephthalate (PET) and high-density polyethylene (HDPE), and degradation of polyamide (PA) (Catarino et al. 2017; Dehaut et al. 2016; Karami et al. 2017). On the other hand, expenses must be taken into account when performing large scale samplings. Regardless of their efficiencies, enzymes may be too expensive to be routinely used in microplastics sampling protocols. Short digestion times are also preferred to improve sample throughput. For instance, protocols may range from 1 hour (Catarino et al. 2017) to 24 (Dehaut et al. 2016) or 48 hours (Karami et al. 2017). Higher temperatures can reduce the time needed for full digestion of biological material but may also enhance polymer damage. Munno et al. (2018) reports that temperatures above  $60^\circ\text{C}$  may lead to the destruction of microplastics in samples. Thus, there is a need for an efficient digestion protocol for microplastics sampling that can be used in large scale (Dehaut et al. 2016) and without damaging polymers.

The objective of this work was to identify efficient methods of removing organic matter from microplastics in the shortest time possible, without degrading plastics. The tested digestion solutions were chosen in order to study the effects of pH, surfactant and redox variations on the degradation of organic matter, based on their frequent use in previous works. The five selected solutions were potassium hydroxide (KOH) and nitric acid ( $\text{HNO}_3$ ) for pH, sodium dodecyl sulphate (SDS) for surfactant effect, and hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) and the U.S. National Ocean and Atmospheric Administration's (NOAA) protocol of  $\text{H}_2\text{O}_2$  with an iron catalyst ( $\text{H}_2\text{O}_2+\text{Fe}$ ; Masura et al. 2015) for redox reactions. Digestion efficiency of a pool of organic matter was determined under similar temperature and time periods to determine the best digestion conditions. Testing of individual organic matter, virgin and weathered polymers

of the best conditions followed, as well as Fourier transform infrared spectroscopy (FTIR) of polymers.

## 2. Materials and Methods

### 2.1 Digestion solutions

With the intention of studying the effects of pH, surfactant and redox, and based on frequent use in previous works, the selected digestion solutions were 30% hydrogen peroxide ( $\text{H}_2\text{O}_2$ ), 35% (v/v) nitric acid ( $\text{HNO}_3$ ), 10% (w/v) potassium hydroxide (KOH), and 10% (w/v) sodium dodecyl sulphate (SDS). KOH (Labchem, U.S.A.) and SDS (Sigma-Aldrich, Germany) were prepared in distilled water, 65%  $\text{HNO}_3$  (Chem-lab, Belgium) was diluted in distilled water and 30% (v/v)  $\text{H}_2\text{O}_2$  (Labkem, Spain) was used directly. Iron(II) (0.05 M; LabKem, Spain) was prepared following NOAA's instructions (Masura et al. 2015) and added 1:1 (v/v) to 30%  $\text{H}_2\text{O}_2$  ( $\text{H}_2\text{O}_2+\text{Fe}$ ) at the time of the experiment.

### 2.2. Natural organic matter and plastic samples

The first test evaluated the digestion efficiency of the chosen solutions on natural organic matter. Natural organic materials, comprised of *Fucus* algae, driftwood and seagull feathers, were collected in Costa Nova beach, Aveiro, Portugal, whereas fish muscle, paraffin and palm oil ( $d = 0.90 \text{ g cm}^{-3}$ ) were acquired in local markets. These samples were prepared to have similar sizes and weight in each category (**Table S-11, Supporting Information (S.I.)**), with the exception of palm oil where a drop (approximately 0.03 ml) was used as measure due to the inability to read the proper volume without losing sample.

In a second phase, nine virgin and six weathered plastics, including samples collected from the environment, were subjected to the selected digestion solutions in order to evaluate effects

on polymer integrity. Virgin plastics were obtained from pellets, such as low-density polyethylene (LDPE), polypropylene (PP) and polystyrene (PS) (Sigma-Aldrich, U.S.A.), or from consumer products, such as HDPE, PET, polyvinylchloride (PVC), expanded polystyrene (EPS), cellulose acetate (CA) and nylon (**Table S-11, S.I.**) Large pieces of weathered polymers were collected from Barra beach, Aveiro, Portugal, cut into fragments of similar sizes (**Table S-11, S.I.**), and identified by Fourier transform infrared spectroscopy attenuated total reflection (FTIR-ATR) as HDPE, PE, PP, EPS and CA (see below). This identification also allowed to evenly distribute samples according to similar degradation spectra.

In a third phase, the resistance of filters to the chosen digestion protocols was tested. The following commonly used filters (including for air sampling) were chosen: mixed cellulose esters (Gridded GN-6 White, GelmanSciences, U.S.A.), glass fibre (Prat DUMAS, France) and quartz filters (Whatman QMA, U.S.A.).

Finally, to understand the application of the selected digestion solutions to real samples, tests were performed in water, sediment and organism samples. A sample of 175 ml of water from an organic rich artificial pond in the University campus was obtained by filtration in a glass fiber filter. The digestive systems (including its contents, 7.5 g) was obtained by dissection from an Atlantic horse mackerel fish (*Trachurus trachurus*) bought in local markets (152 g, 26 cm, captured in the Portuguese coast).

## **2.3. Procedures**

### **2.3.1. Digestion efficiency of natural organic matter**

In a first phase, five solutions were tested to identify a quick and efficient method of organic matter removal. To achieve this goal, 10 ml of solutions or control (H<sub>2</sub>O) were added to pools of six organic materials in glass flasks and these were exposed to two temperature conditions,

room temperature (RT, average 25°C) and 50°C (Oasis™ Benchtop IR CO<sub>2</sub> Incubator, Caron), and two periods, 1 and 6 hours. After analysing the results of the first test, organic matter was subjected individually to 5 ml of two solutions (KOH and H<sub>2</sub>O<sub>2</sub>+Fe) and the control (H<sub>2</sub>O) under a single test condition, 50°C for 1 hour.

### 2.3.2. Resistance of polymers to digestion protocols

In a second phase, polymer resistance was tested by exposing each polymer individually to 2.5 ml of the two test solutions (KOH and H<sub>2</sub>O<sub>2</sub>+Fe) and the control (H<sub>2</sub>O) at 50°C for 1 hour. Besides weighting and photographing, samples were washed in distilled water and analysed by FTIR-ATR to evaluate the effect of digestion solution on the integrity and identification of polymers. Perkin Elmer (U.S.A.) Spectrum BX FTIR instrument at 4 cm<sup>-1</sup> resolution, 4,000-600 nm range and at 32 scans was used to obtain the infrared spectrum of each polymer. The carbonyl index for a single reading of each sample was calculated by dividing the intensity of the carbonyl peak by the intensity of the reference peak. Carbonyl peaks were read at 1715 – 1735 cm<sup>-1</sup>. Reference peaks, based on the literature, were 1471 cm<sup>-1</sup> for PE (LDPE, HDPE, PE), 1458 cm<sup>-1</sup> for PP, 1452 cm<sup>-1</sup> for PS and EPS (Rodrigues et al. 2018), 1504 cm<sup>-1</sup> for PET (Pires et al. 2015), 1328 cm<sup>-1</sup> for PVC (Balakit et al. 2015), 1030 cm<sup>-1</sup> for CA (Sakai et al. 1996), and 1464 cm<sup>-1</sup> for nylon (Cai et al. 2017) (**Table S-1 and S-2, S.I.**). The spectra of some organic matter samples were also recorded for future reference (**Figure S-1, S.I.**). To evaluate the deposition of materials in the polymer surface, a low number of samples were also analyzed by Energy X-Ray Dispersive Spectroscopy, performed by preparing the samples in carbon tape and coated by carbon evaporation (Emitech K950X, France) and analyzing using a Field Emission Gun (FEG) – SEM Hitachi S4100 microscope (Japan) operated at 15 keV.

### 2.3.3. Resistance of filters to digestion protocols



To evaluate the effects of the digestion protocols on filter integrity, mixed cellulose esters, glass fibre and quartz filters were tested for weight loss by weighting before and after exposure to 2.5 ml of the tested digestion solutions (KOH, H<sub>2</sub>O<sub>2</sub>+Fe) and a control (H<sub>2</sub>O), at 50°C for 1 hour.

#### **2.3.4. Testing protocols on environmental samples**

To test the application of the developed digestion protocols to real environmental samples, a filter from an organic rich water filtration was subjected to 20 ml of H<sub>2</sub>O<sub>2</sub>+Fe (50°C 1h) and the digestive system of the fish (including its contents, 7.5 g) was subjected to 50 ml of 10% KOH (50°C, 1h; 15% tissue weight/KOH volume) in a glass beaker.

#### **2.4. Common procedures and sample analysis**

All tests were conducted in glass flasks. Distilled water (H<sub>2</sub>O) was used as a control solution. At the end of each test, distilled water was added to the flask followed by filtration (cellulose filters, 0.45 µm, GN-6 Grid GelmanSciences, U.S.A.) and several washings with distilled water of the flask to retrieve residues, and the filter to prevent degradation. Filters were dried in Petri dishes over the counter for three days, followed by one hour at 35°C, to avoid paraffin melting (above 37°C). Each sample was weighted to the nearest 0.0001 g (Sartorius, Entris) before and after the digestion step to calculate digestion efficiency (%), as proposed by Karami et al. (2017), and photographed after digestion (Canon 550D). All treatments were conducted in 4 replicates.

#### **2.5. Statistical analysis of digestion efficiency and weight loss**

Excel 2016 was used for calculations of statistics. Digestion efficiency (%) and weight loss (%) were assessed as  $100 \times (\text{initial weight of sample} - \text{final weight of sample}) / \text{initial weight of sample}$ , as proposed by Karami et al. (2017). Due to the non-normal distribution, data was expressed as medians and box plots (except when mentioned otherwise), while averages can be found on **Section S-2. of S.I.**

### 3. Results and Discussion

#### 3.1. Digestion efficiency of a pool of natural organic matter

An exploratory study was conducted to understand the best combination of digestion solution, time and temperature on selected samples of natural organic matter. Previous works have tested the effects of sample pre-treatment on algae, feathers, squid and polychaete beaks, sheep wool, seal whiskers, fish otoliths, manila rope, fish muscle and alimentary tract, and mussels (Dehaut et al. 2016; Karami et al. 2017; Kühn et al. 2016). Six organic materials were chosen in order to represent large groups likely present in water and sediment samples: vegetable matter (algae, wood), animal matter (fish muscle, feather), a fat (palm oil) and a wax (paraffin).

A trend of increasing digestion efficiency with temperature was observed (**Figure 1**). The digestion efficiency (medians 34.2 – 40.4%) in control (distilled water) was attributed to inability to retrieve organic materials from the flask, especially palm oil, or to the loss of soluble fractions (i.e. in fish tissue). On the contrary, SDS seemed to have slightly lower digestion efficiencies than the control, possibly by facilitating the retrieval of organic matter or reducing solubility. Digestion efficiency of H<sub>2</sub>O<sub>2</sub> was similar to the control (**Figure 1**), which could be explained by the inability of H<sub>2</sub>O<sub>2</sub> in removing organic matter under the tested conditions (**Figure S-7, S.I.**), especially during the short incubation period (<6h) compared to the literature (e.g. 96 h in Karami et al. 2017). On the other hand, the cap on the highest

digestion efficiency seems to be attributed to the inability to fully digest driftwood and paraffin.

[Fig-1]

**Figure 1.** Digestion efficiency (%) of a pool of organic matter exposed to a control ( $\text{H}_2\text{O}$ ) and five solutions ( $\text{H}_2\text{O}_2$ ,  $\text{H}_2\text{O}_2+\text{Fe}$ ,  $\text{KOH}$ ,  $\text{HNO}_3$  and  $\text{SDS}$ ) under conditions of two temperatures, room temperature (RT) and  $50^\circ\text{C}$ , and two periods, 1 and 6 hours.

From the digestion solutions,  $\text{H}_2\text{O}_2+\text{Fe}$  and  $\text{KOH}$  presented the highest digestion efficiencies at  $50^\circ\text{C}$  for 1 hour, 65.9% and 58.3% respectively (**Table S-3, S.I.**). However, increasing time from 1 to 6 hours had little improvement on the digestion efficiency of these solutions, except for  $\text{HNO}_3$ . The digestion capacity of these solutions varied with organic matter, with  $\text{H}_2\text{O}_2+\text{Fe}$  efficiently removing algae, fish tissue and melting paraffin, and  $\text{KOH}$  removing bird feathers and fish tissue.  $\text{HNO}_3$  had a greater digestion efficiency (59.7%) at  $50^\circ\text{C}$  for 6 hours, but with no advantage over the previously mentioned solutions as it requires a longer incubation period and has been reported to damage plastics under certain conditions (Dehaut et al. 2016). Regarding oxidative digestion, the use of iron catalyst is recommended as it led to an increase in digestion efficiency from 38.7% for  $\text{H}_2\text{O}_2$  to 65.9% in  $\text{H}_2\text{O}_2+\text{Fe}$ , at  $50^\circ\text{C}$  for 1 hour. This is a result of the rapid formation of free radicals by the Fenton's reagent ( $\text{H}_2\text{O}_2+\text{Fe}$ ). Outliers (**Figure 1**) may be a result of accidental loss of organic matter during sample manipulation.

Based on these results, the solutions  $\text{H}_2\text{O}_2+\text{Fe}$  and  $\text{KOH}$  and the test condition  $50^\circ\text{C}$  for 1 hour were chosen as the best suited digestion protocol to be applied in the following procedures. Thus, our results show that strong redox reactions and high pH (alkali) seem to be the best suited for the degradation of organic matter in microplastic samples.

### **3.2. Digestion efficiency of natural organic matter exposed to selected digestion solutions ( $\text{H}_2\text{O}_2+\text{Fe}$ and $\text{KOH}$ )**

To better understand the digestion efficiency of each treatment, organic matter samples used in the previous test were individually exposed to the selected solutions ( $\text{H}_2\text{O}_2+\text{Fe}$ , KOH and control  $\text{H}_2\text{O}$ ) under the test conditions of  $50^\circ\text{C}$  for 1 hour (**Figure 2**). As previously observed, digestion efficiency of solutions varied with the type of organic matter.  $\text{H}_2\text{O}_2+\text{Fe}$  was the best treatment to remove algae (100.0%), driftwood (26.3%) and paraffin (17.5%). Even though in this test  $\text{H}_2\text{O}_2+\text{Fe}$  revealed to be more efficient in the removal of plant tissues, it is was also capable of removing animal tissues, leading to a weight loss of 72.6% of fish tissue. Soft tissues of bivalves have also been efficiently removed by 30%  $\text{H}_2\text{O}_2$  (without catalyst, RT, 24-48h; Li et al. 2015). Thus, this solution can also be used in the removal of animal tissues, as long as the ratio of sample weight to volume of  $\text{H}_2\text{O}_2$  does not exceed 0.025 (w/v) (Li et al. 2015). Even though the use of an iron catalyst should decrease the volume of  $\text{H}_2\text{O}_2$  required by increasing its oxidizing potential, this proportion was never exceeded in the treatment of organic material in the present study (<0.016 w/v).

KOH presented higher digestion efficiencies for fish (104.9%), feathers (72.1%) and palm fat (36.6%). Good digestion of animal tissues (crab, mussels and fish) exposed to KOH at  $60^\circ\text{C}$  for 24 hours is also reported by Dehaut et al. (2016). Furthermore, Karami et al. (2017) reports the success of 10% KOH ( $40^\circ\text{C}$ , 48-72h) in removing fish soft tissues, followed by NaI extraction to separate microplastics from the remaining hard tissues. Kühn et al. (2017) also reports the efficiency of KOH (1 M, RT, 48h) in removing most natural organic matter, except otoliths and squid beaks. Indeed, no hard tissues (e.g. bones, squid beaks) have been tested, and thus may not be removed by KOH, requiring density separation.

[Fig-2]

**Figure 2.** Digestion efficiency (%) of individual organic matter exposed to  $\text{H}_2\text{O}$  (control),  $\text{H}_2\text{O}_2+\text{Fe}$  and KOH at  $50^\circ\text{C}$  for 1 hour.

Digestion efficiencies above 100% could be justified by weight loss of the cellulose filter. However, we only observed an average weight loss during filtration of 1.3%, 0.8% and 0.7% in

H<sub>2</sub>O, H<sub>2</sub>O<sub>2</sub>+Fe, KOH respectively (**Table S-6, S.I.**). H<sub>2</sub>O is also responsible for the weight loss in certain samples, such as algae (30.3%) and fish (73.8%), possibly due to the loss of soluble fractions. From the sampled organic matter, driftwood, paraffin and palm fat were the most difficult to remove, even in treatments with the highest digestion efficiency for these samples. However, they may also be the less likely to be confounded with microplastics, except for paraffin which shares similar visual characteristics with polymers and passes the hot needle test.

Our results show that both treatments are suitable for the degradation of organic matter: H<sub>2</sub>O<sub>2</sub>+Fe (redox) is recommended when plant material is abundant, such as in sediment samples where algae can be mistaken for microplastics (Lavers et al. 2016); and KOH (pH) is recommended for removing animal tissues, such as in the determination of microplastics in the bodies of organisms. Reasonable digestion efficiencies can be achieved under 1 hour by heating the solutions to 50°C.

### **3.3. Weight loss (%) of virgin and weathered synthetic polymers exposed to selected digestion solutions (H<sub>2</sub>O<sub>2</sub>+Fe and KOH)**

Digestion protocols should be able to remove organic matter without damaging microplastics. Thus, after assessing organic matter removal, we have evaluated the effects of these conditions on nine virgin and six weathered polymers. Virgin plastics were chosen based on their demand (PlasticsEurope 2018) and previous use in similar protocols (Dehaut et al. 2016; Karami et al. 2017; Kühn et al. 2016). Weather plastics followed the same logic, after identification, limited by polymer types presence in the beach of collection. In most samples, digestion protocols involving H<sub>2</sub>O<sub>2</sub>+Fe and KOH at 50°C for 1 hour does not lead to polymer weight loss. Most medians of polymer weight loss fluctuate around 0%, as observed in **Figure**

**3.**

[Fig-3]

**Figure 3.** Weight loss (%) of virgin and weathered polymers exposed to H<sub>2</sub>O (control), H<sub>2</sub>O<sub>2</sub>+Fe and KOH at 50°C for 1 hour. CA: cellulose acetate, EPS: expanded polystyrene, HDPE: high-density polyethylene, LDPE: low-density polyethylene, PE: polyethylene, PEf: polyethylene fibres, PET: polyethylene terephthalate, PP: polypropylene, PS: polystyrene, PVC: polyvinyl chloride.

On the other hand, cellulose acetate (CA) is prone to weight loss in all solutions, but especially in KOH. Virgin CA has median weight losses of 8.6%, 12.6% and 21.8% in H<sub>2</sub>O, H<sub>2</sub>O<sub>2</sub>+Fe and KOH respectively. Weathered CA has slightly lower medians, of 4.7%, 11.9% and 15.2% in H<sub>2</sub>O, H<sub>2</sub>O<sub>2</sub>+Fe and KOH respectively, but with higher variation in results, attributed possibly to their heterogeneity (not corroborated by the pre-evaluation by FTIR), the previous loss of components under environmental conditions or differences in composition. The degradation of CA has also been reported for KOH (10%) at 60°C for 24 hours, with a reduction in weight close to 50% (Dehaut et al. 2016), and for KOH (1 M) at room temperature for 48 hours, with a decrease in 31% (Kühn et al. 2017). In our study, a lower weight loss of CA may be attributed to the limited exposure time (1 hour).

A loss in mass in LDPE (7%) was also reported for KOH (1 M) at room temperature for 48 hours (Kühn et al. 2017). This was not observed in the present study, where virgin LDPE presents loss in mass of 0.2% in KOH, probably also due to limited exposure time. Weathered PE rope fibres also present higher degradation and variation possibly due the accidental loss of fibres during filtrations. Variation, represented by interquartile range in **Figure 3**, is generally greater for weathered than virgin polymers, possible due to surface heterogeneity.

On the other hand, some polymers have gained weight after exposure to the digestion solutions, conceivably due to the adsorption of substances on their surface (e.g. iron, salts). This is the case of weathered PP in KOH (-0.9%) and virgin LDPE in H<sub>2</sub>O<sub>2</sub>+Fe (-1.3%). These samples have been analysed by Energy X-Ray Dispersive Spectroscopy, revealing the presence of only carbon and oxygen, contradicting the hypothesis of iron or salt adsorption, and

showing no significant changes in their surface after this treatment (**Figure S-4, S.I.**). Kühn et al. (2017) attribute the weight gain in synthetic materials exposed to KOH to the presence of moisture. In the present work, longer drying periods of these samples did not result in weight loss, rejecting this possibility. Dehaut et al. (2016) also reports an incoherent and unexplained weight variation in some replicates, including HDPE and PP, without changes in their microscopic morphology.

No changes have been visually observed in polymer samples (**Table S-7 to S-9, S.I.**), with the obvious exception of virgin and weathered CA. Thus, treatment with  $\text{H}_2\text{O}_2+\text{Fe}$  and KOH at  $50^\circ\text{C}$  for 1 hour does not lead to weight loss (%) and visual changes in synthetic polymers, except for CA.

#### **3.4. FTIR spectra and carboxyl index of virgin and weathered synthetic polymers exposed to selected digestion solutions ( $\text{H}_2\text{O}_2+\text{Fe}$ and KOH)**

Even though lack of weight loss (%) is important to guarantee correct weighting and visual counting of microplastics, it is not the only factor which should be considered. Infrared spectra obtained by FTIR-ATR has been used as a common method of polymer identification (Prata et al. 2019). However, polymer spectra may change due to degradation events (Da Costa et al. 2018), including during digestion procedures, hindering the identification process.

Thus, we have analysed polymer samples that undergone the previously described digestion protocols by FTIR-ATR to evaluate their effects on the spectra. As observed in **Figure S-2 (S.I.)**, most polymers retain their characteristic peaks even after the digestion protocol. The obvious exception is CA, that suffered observable degradation and lost peak definition. On the other hand, difficulty to fully cover the crystal may justify the weak signals of virgin PVC and PS (due to its shape and hardness) and the fibres of weathered PE. Decrease in peaks has also been observed in virgin PET, PS, EPS and CA for both  $\text{H}_2\text{O}_2+\text{Fe}$  and KOH. However, except for CA,

these smaller peaks would still allow determination of polymer type. A decrease in spectra intensity has also been reported by Dehaut et al. (2016) for Raman micro-spectroscopy, attributed to the location of the impact point of the laser on the surface of the polymer or to molecular changes due to digestion with KOH. Furthermore, Karami et al. (2017) report an effect of H<sub>2</sub>O<sub>2</sub> and KOH in Raman spectroscopy results of nylon and PVC. However, longer exposure (>24h) to these solutions may justify a more remarkable effect compared to our results.

To better determine the possible degradation of polymers under these protocols, the carbonyl index of a single sample was calculated (**Figure 4**). Considering the error associated with a single sample, virgin LDPE, PP, PVC and nylon, and weathered HDPE, PE and PP, do not present changes in their carbonyl index between treatments, in agreement with what is observed in the spectra (**Figure S-2, S.I.**).

[Fig-4]

**Figure 4.** Changes in carbonyl index sample of virgin (-V) and weathered (-W) polymers exposed to H<sub>2</sub>O, H<sub>2</sub>O<sub>2</sub>+Fe and KOH solutions at 50°C for 1 hour. CA: cellulose acetate, EPS: expanded polystyrene, HDPE: high-density polyethylene, LDPE: low-density polyethylene, PE: polyethylene, Pef: polyethylene fibres, PET: polyethylene terephthalate, PP: polypropylene, PS: polystyrene, PVC: polyvinyl chloride.

On the other hand, virgin HDPE presents a high index when exposed to KOH, which was not observed in weathered HDPE, possible due to differences in surface characteristics. PE fibres presented a higher index for H<sub>2</sub>O<sub>2</sub>+Fe (not observed in LDPE and HDPE), possible due to the fibres larger surface area and agreeing with a lower intensity in Pef spectra.

Virgin PS and EPS presented increasing carbonyl index with treatments H<sub>2</sub>O < H<sub>2</sub>O<sub>2</sub>+Fe < KOH, apart from weathered EPS that presented a low index in KOH. This is in agreement to what is observed in **Figure S-2 (S.I.)**, with lower intensities in virgin PS and EPS in H<sub>2</sub>O<sub>2</sub>+Fe and KOH and lower intensity in weathered EPS only in H<sub>2</sub>O<sub>2</sub>+Fe. PET presents a decrease in the carbonyl index of KOH which is related to a decrease in all the peaks (**Figure S-2, S.I.**).



Regarding CA, a large carbonyl index would be expected for  $\text{H}_2\text{O}_2+\text{Fe}$  and especially for KOH due to the large weight loss (**Figure 3**) and observable degradation (**Table S-9 and S-10, S.I.**). However, in both virgin and weathered CA, the carbonyl index of KOH is lower, while  $\text{H}_2\text{O}$  and  $\text{H}_2\text{O}_2+\text{Fe}$  present similar results. This is attributed to the fact that the reference peak, that should be stable even under degradation, decreased as well and thus gave rise to a misleading carbonyl index. Based on our results, we have attempted to find a new reference peak for CA, but with little success.

In summary, some polymers suffered an increase in carbonyl index and a decrease in peak intensity under  $\text{H}_2\text{O}_2+\text{Fe}$  and KOH treatments (e.g. PS, EPS), undetectable by weight loss (%) and visual observation. However, the spectra obtained from these particles could still be used in polymer identification, except for CA which suffered considerable degradation. Thus, treatment with  $\text{H}_2\text{O}_2+\text{Fe}$  and KOH at  $50^\circ\text{C}$  for 1 hour can be used for removal of organic matter in microplastic samples without compromising polymer identification.

#### **3.4. Choosing an appropriate filter to undergo natural organic matter removal**

In some cases, digestion solutions can be applied to filters containing filtered samples, allowing to reduce the volume of solution used, as well as the space needed. However, it is worth noting that mineral residues may also be present and not removed from these samples, interfering in the weighting process.

Filters of mixed cellulose esters, glass fibre and quartz were tested in the same conditions as previous samples: exposed to two digestion solutions (KOH,  $\text{H}_2\text{O}_2+\text{Fe}$ ) at  $50^\circ\text{C}$  for 1 hour. As highlighted in **Figure S-3 (S.I.)**, most treatments led to limited weight losses of filters. Glass fibre and quartz filters lost <10% of weight during treatments, which may still be relevant in weighting processes. On the other hand, cellulose filters were almost completely degraded when exposed to KOH at  $50^\circ\text{C}$  for 1 hour, with other treatments <5% of weight loss.

Furthermore, cellulose acetate filters should not be used in microplastics samples because of the potential for cross-contamination, hindering the identification of environmental cellulose acetate fibres (Foekema et al. 2013).

We conclude that glass fibre and quartz filters may be more appropriate for organic matter removal, as they are resistant to oxidative and alkali digestion. On the other hand, cellulose acetate filters may be used when conducting oxidative digestion, or in alkali digestion when the intention is to fully digest the filter, with the loss of environmental cellulose acetate fibres.

### 3.5. Testing the solutions on environmental samples

To test the applicability of the developed protocols in real environmental samples,  $\text{H}_2\text{O}_2+\text{Fe}$  and KOH were tested in appropriate matrices (**Figure 5**). Water samples collected from an artificial pond in the University campus were rich in organic matter, and thus perfect to visually assess the removal by  $\text{H}_2\text{O}_2+\text{Fe}$ , which is more appropriate for the removal of plant matter. However, the concentration of 30%  $\text{H}_2\text{O}_2$  revealed to be too strong for the glass fibre filter in the presence of organic matter, even though this filter was resistant to the solution when tested alone. Thus, a second test using 15% of  $\text{H}_2\text{O}_2$  was conducted showing adequate results regarding the removal of organic matter, namely of 1.7 mg (61% of the sample), and the preservation of the filter structure (**Figure 5; Figure S-5, S.I.**). Similar effects were observed in sediment samples (**Figure S-6, S.I.**). Nonetheless, 30%  $\text{H}_2\text{O}_2$  could still be used when treating directly plant rich samples, such as those collected by a manta trawl.

Animal tissues are more efficiently removed by KOH. Even though fish muscle was used during digestion efficiency testing due to its likely presence in water and sediment samples, the digestive system is more often used for quantification of microplastics in fish. In order to test the application of this protocol also to these tissues, a digestive system (with its contents) of

an Atlantic horse mackerel (*Trachurus trachurus*) was obtained by dissection and exposed to KOH at 50°C for 1 hour. After this period, the supernatant was filtered through a glass fibre filter and the larger residues kept in a petri dish.

The results of 10% KOH at 50°C for 1 hour support the use of this procedure for these tissues as well (**Figure 5**), especially considering it did not involve any form of physical disintegration and was conducted in a shorter period than previous works, namely of 48 to 72h at 40°C (Karami et al. 2017) or 24 h at 60°C (Dehaut et al. 2016). Thus, as demonstrated herein, the use of H<sub>2</sub>O<sub>2</sub>+Fe and KOH is appropriate for environmental samples.

[Fig-5]

**Figure 5.** Filtrated water samples from an artificial pond (A) and subjected to 20 ml of H<sub>2</sub>O<sub>2</sub>+Fe at 50°C for 1 hour (B and C), at H<sub>2</sub>O<sub>2</sub> concentrations of 30% (B) and 15% (C). Digestive system of *Trachurus trachurus* before (D) and after (E) treatment with 50 ml of KOH at 50°C for 1 hour.

#### 4. Conclusion

With the objective of finding a shorter, but efficient, digestion protocol, we have tested common digestion solutions in organic matter and synthetic polymers. A pool of organic matter was exposed to five solutions representing redox (H<sub>2</sub>O<sub>2</sub>, H<sub>2</sub>O<sub>2</sub>+Fe), pH (KOH, HNO<sub>3</sub>) and a surfactant (SDS), under two temperatures (room temperature – 25°C, and 50°C) and two reaction periods (1 and 6 hours). The strong redox (H<sub>2</sub>O<sub>2</sub>+Fe) and high pH (KOH) treatments presented the highest digestion efficiencies under 1 hour at 50°C, of 65.9% and 58.3% respectively. Thus, these treatments were chosen to undergo further testing. Individually testing organic matter in these conditions revealed that H<sub>2</sub>O<sub>2</sub>+Fe was more appropriate to remove plant material, whereas KOH was most efficient with animal tissues. To assess polymer degradation under the tested conditions, several virgin and weathered polymers were tested

and evaluated through weight loss and Fourier-Transform infrared spectroscopy (FTIR). Plastics (except cellulose acetate) did not present significant weight loss, whereas infrared spectra and carbonyl index presented small changes that did not compromise polymer identification. Thus, organic matter removal protocols using  $H_2O_2+Fe$  and  $KOH$  at  $50^\circ C$  for 1 hour are acceptable and can be used in microplastic samples or in filters of glass fibre or quartz. Even though this work does not aim at providing information on the recovery of microplastics in organic rich environmental samples after digestion procedures, it produced the necessary knowledge required to conduct this research in future works.

### **Acknowledgement**

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### **Supporting Information**

Supporting Information contains additional information about Fourier Transform Infrared Spectroscopy (FTIR), averages on digestion efficiencies or weight losses, Energy X-Ray Dispersive Spectroscopy and photographs of the samples.

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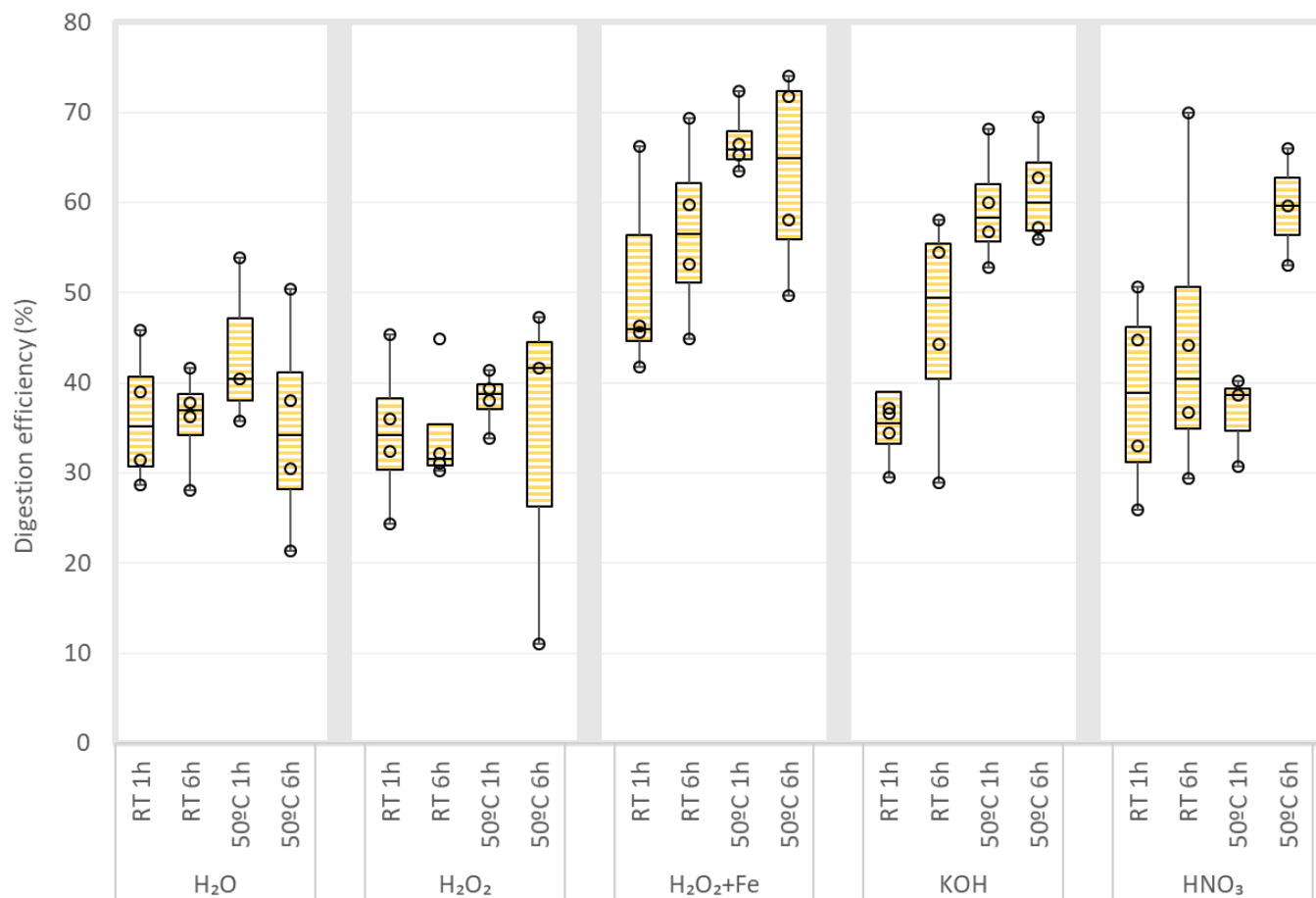
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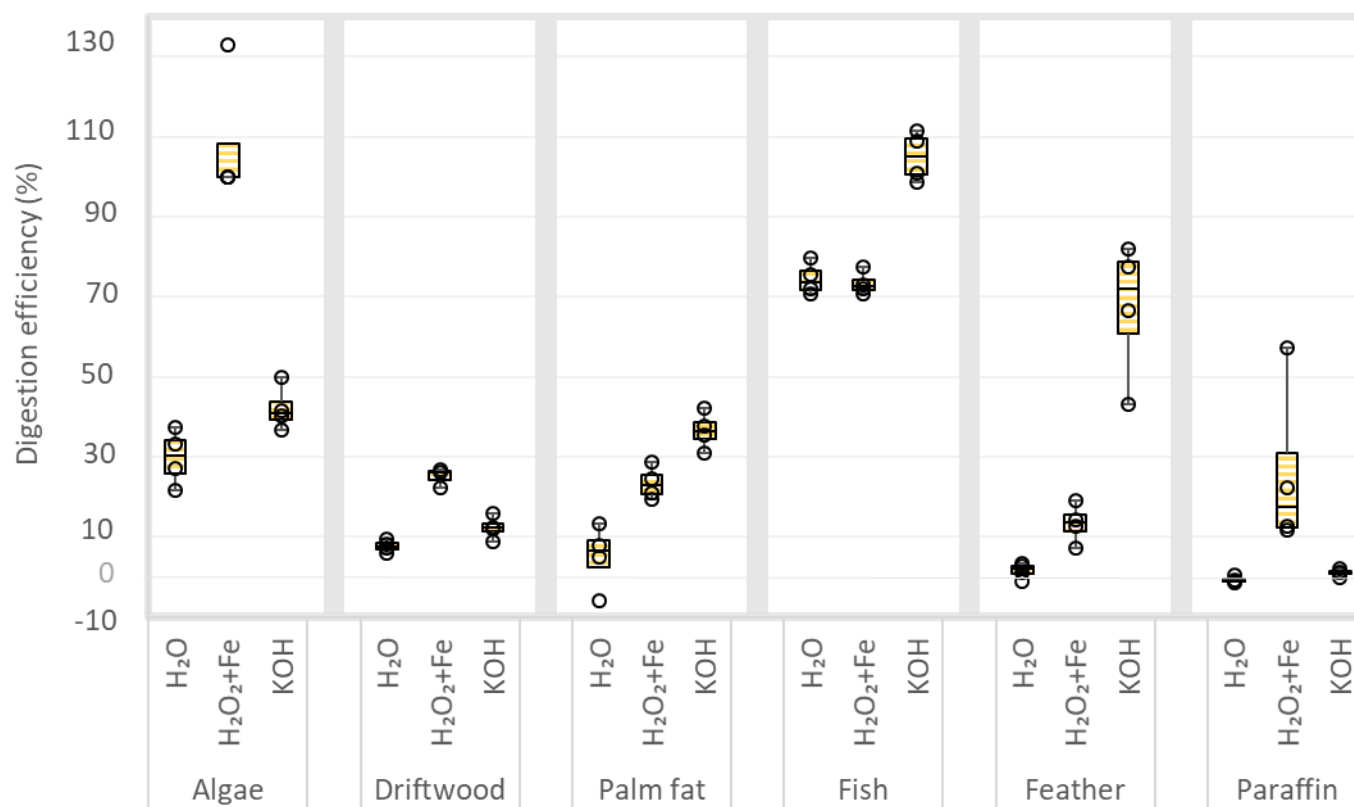
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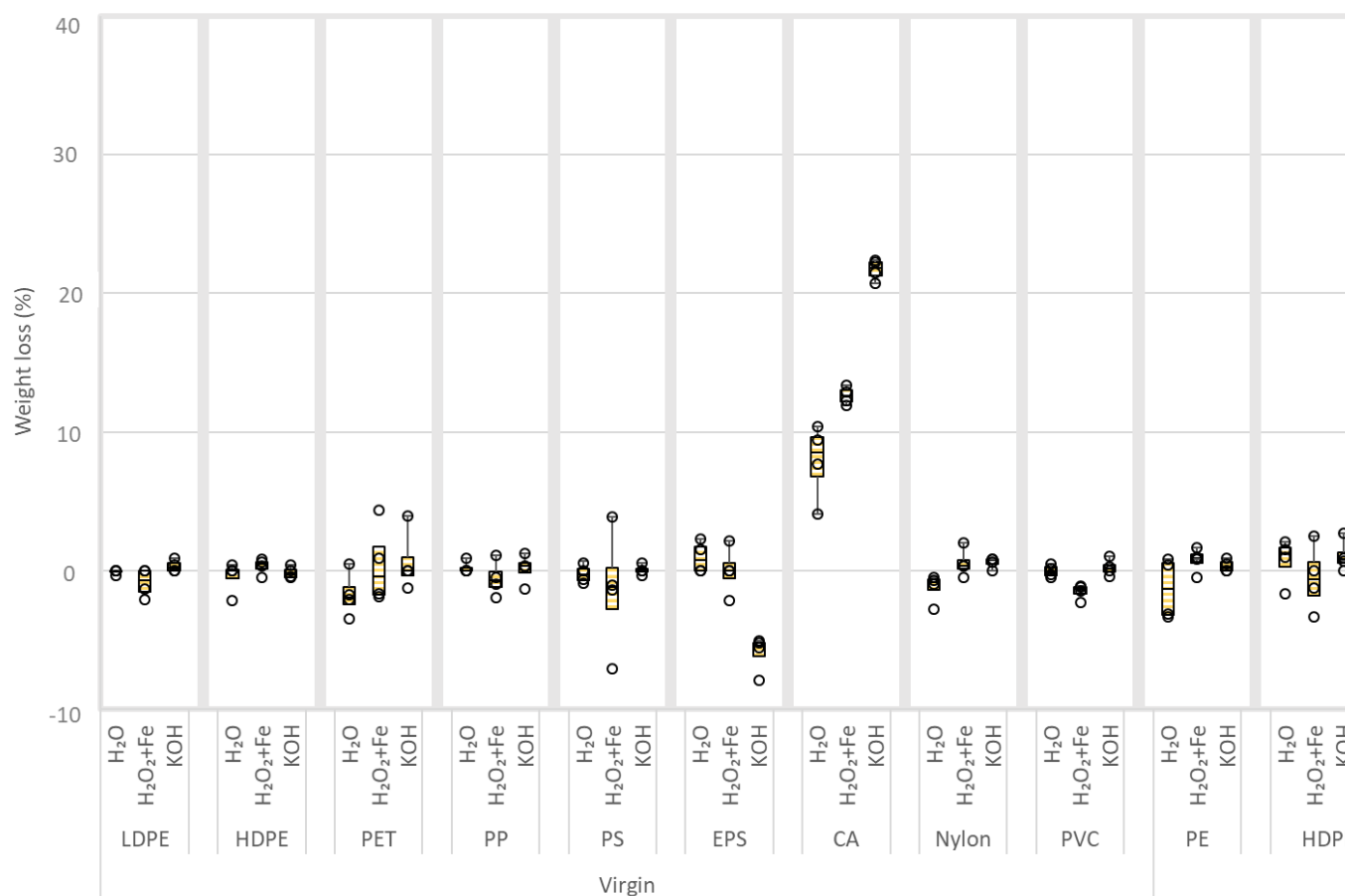
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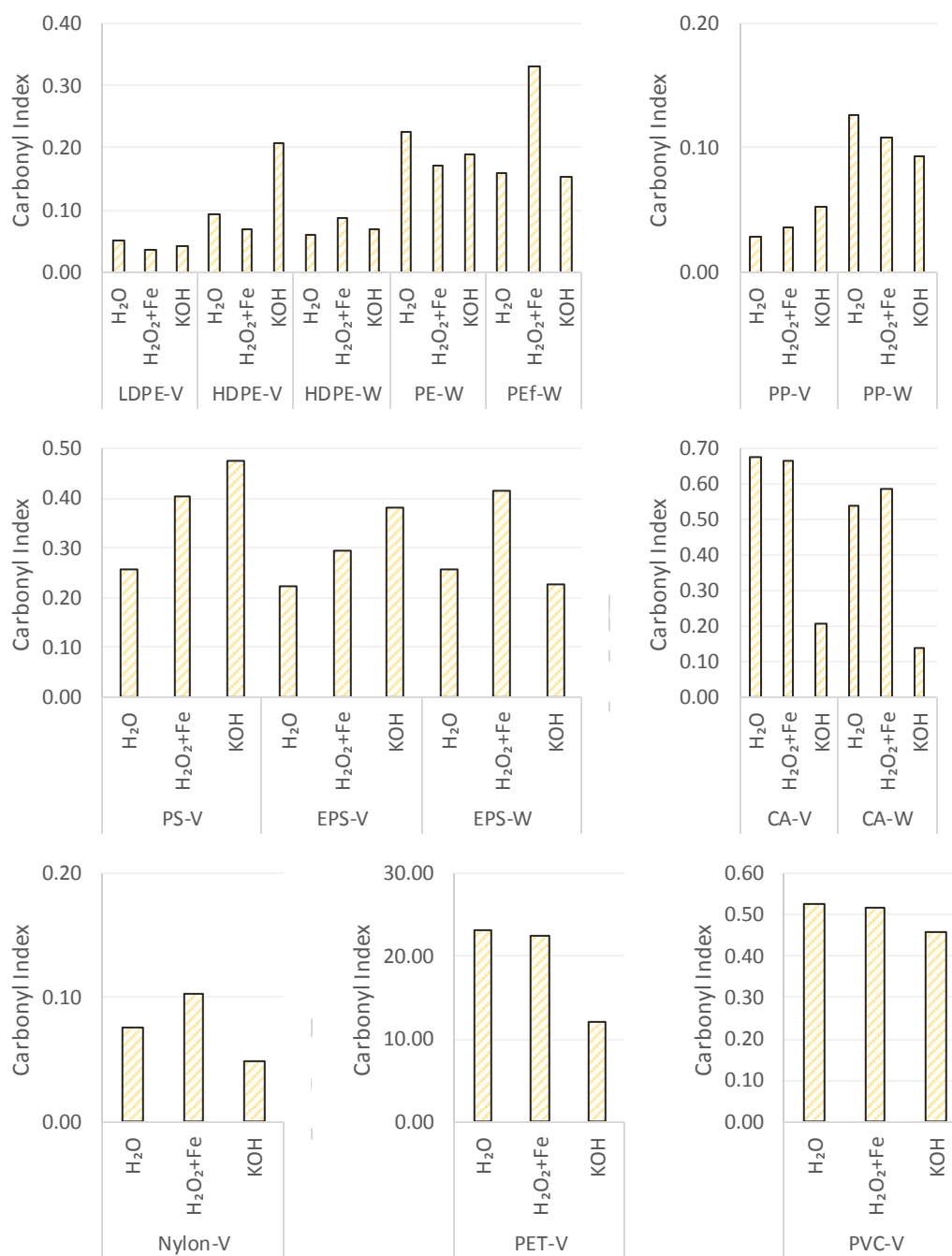
**Figure 1.** Digestion efficiency (%) of a pool of organic matter exposed to a control (H<sub>2</sub>O) and five solutions (H<sub>2</sub>O<sub>2</sub>, H<sub>2</sub>O<sub>2</sub>+Fe, KOH, HNO<sub>3</sub> and SDS) under conditions of two temperatures, room temperature (RT) and 50°C, and two periods, 1 and 6 hours.



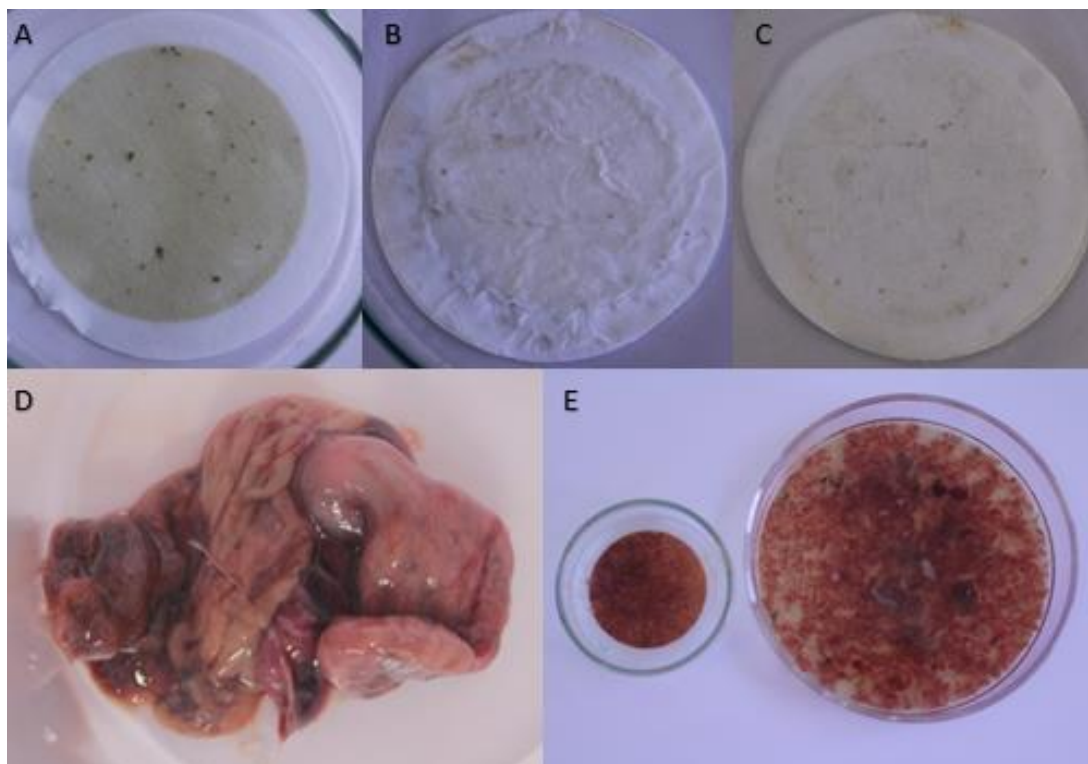
**Figure 2.** Digestion efficiency (%) of individual organic matter exposed to H<sub>2</sub>O (control), H<sub>2</sub>O<sub>2</sub>+Fe and KOH at 50°C for 1 hour.



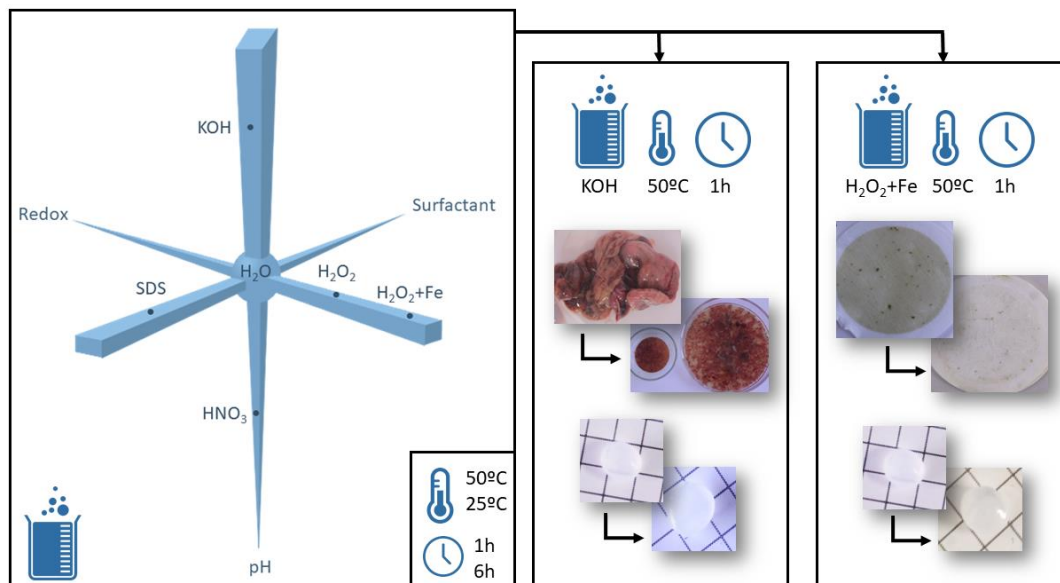
**Figure 3.** Weight loss (%) of virgin and weathered polymers exposed to H<sub>2</sub>O (control), H<sub>2</sub>O<sub>2</sub>+Fe and KOH at 50°C for 1 hour. CA: cellulose acetate, EPS: expanded polystyrene, HDPE: high-density polyethylene, LDPE: low-density polyethylene, PE: polyethylene, PEF: polyethylene fibres, PET: polyethylene terephthalate, PP: polypropylene, PS: polystyrene, PVC: polyvinyl chloride.



**Figure 4.** Changes in carbonyl index sample of virgin (-V) and weathered (-W) polymers exposed to H<sub>2</sub>O, H<sub>2</sub>O<sub>2</sub>+Fe and KOH solutions at 50°C for 1 hour for polyethylene (PE, low-density polyethylene LDPE, high-density polyethylene HDPE, polyethylene fibres PEF), polypropylene (PP), polystyrene (PS, expanded polystyrene EPS), cellulose acetate (CA), nylon, polyethylene terephthalate (PET), and polyvinyl chloride (PVC).



**Figure 5.** Filtrated water samples from an artificial pond (A) and subjected to 20 mL of  $\text{H}_2\text{O}_2$ +Fe at  $50^\circ\text{C}$  for 1 hour (B and C), at  $\text{H}_2\text{O}_2$  concentrations of 30% (B) and 15% (C). Digestive system of *Trachurus trachurus* before (D) and after (E) treatment with 50 mL of KOH at  $50^\circ\text{C}$  for 1 hour.



Graphical abstract



**Highlights**

- Highest digestion efficiencies were found for  $\text{H}_2\text{O}_2+\text{Fe}$  and  $\text{KOH}$  at  $50^\circ\text{C}$  for 1 hour;
- $\text{H}_2\text{O}_2+\text{Fe}$  is appropriate for removal of plant material and  $\text{KOH}$  for animal tissues;
- Virgin and weathered plastics were not damaged, except for cellulose acetate;
- Carbonyl index were slightly altered, but infrared spectra allowed identification.

ACCEPTED MANUSCRIPT

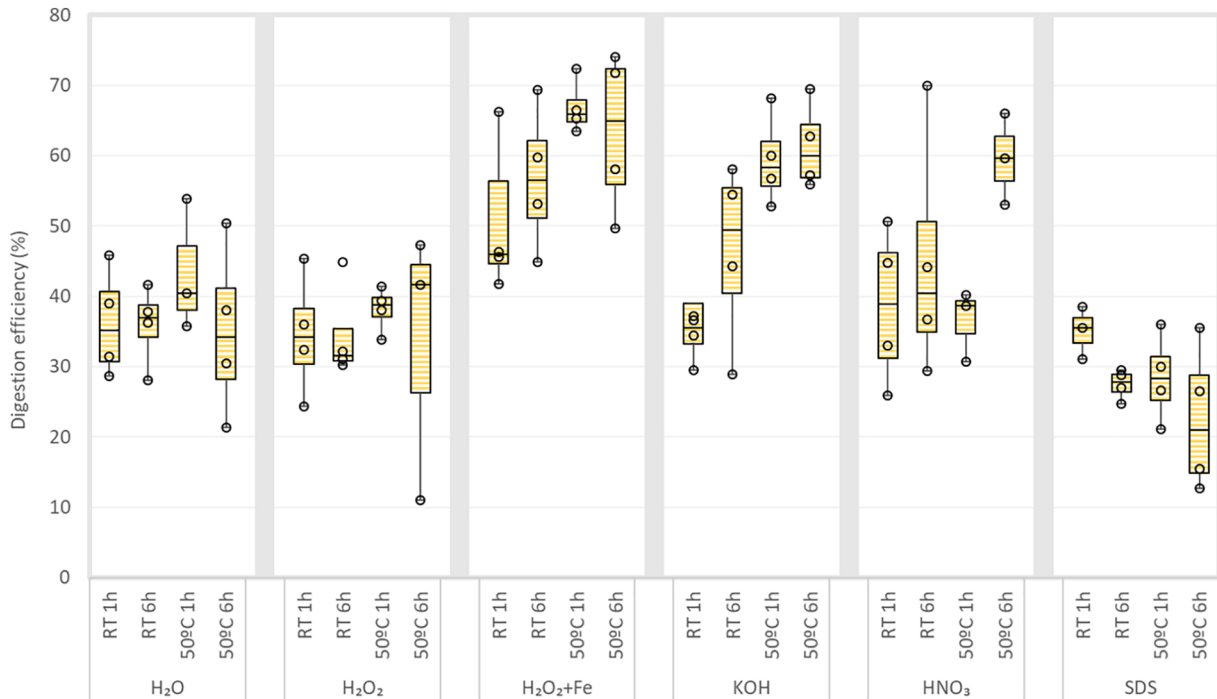


Figure 1

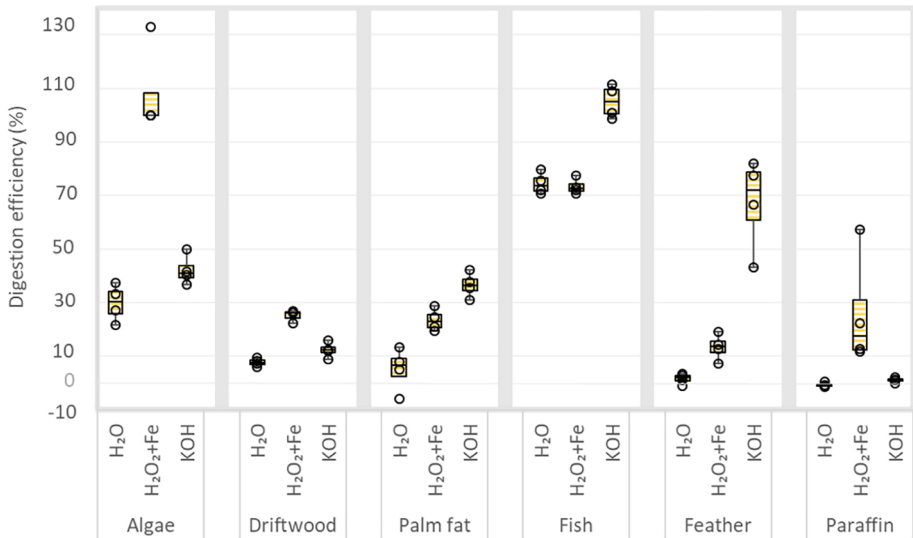


Figure 2

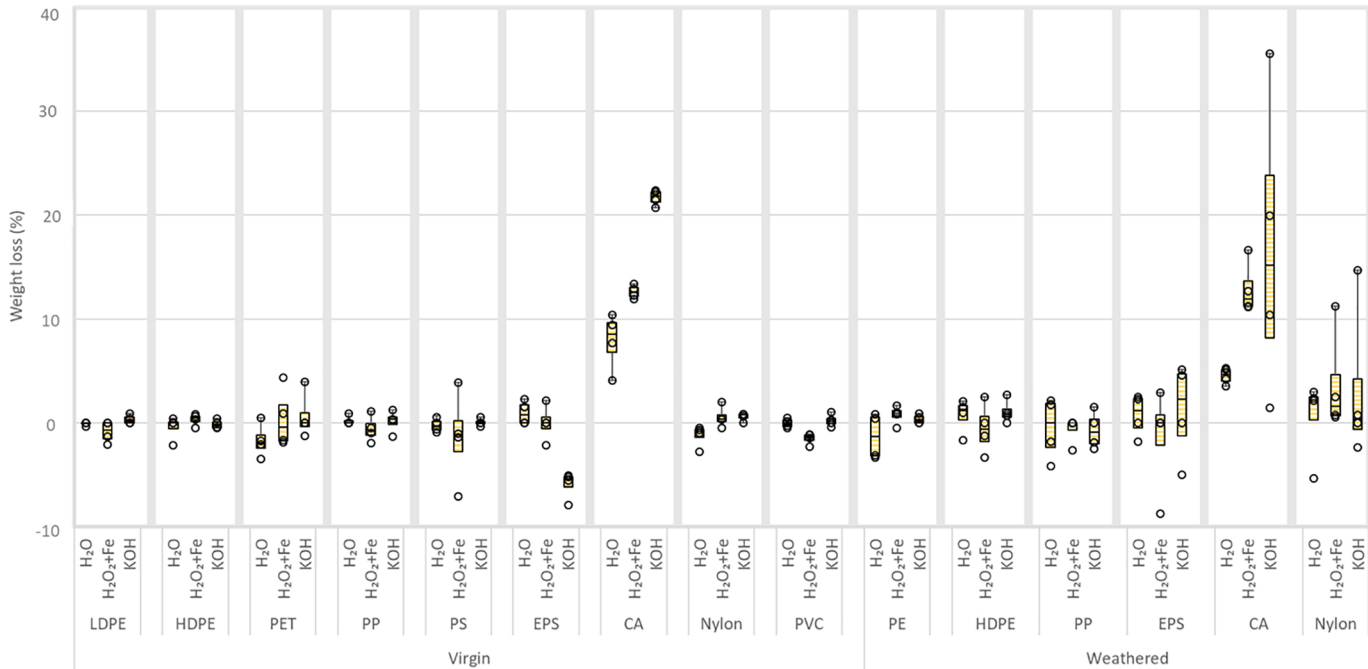


Figure 3

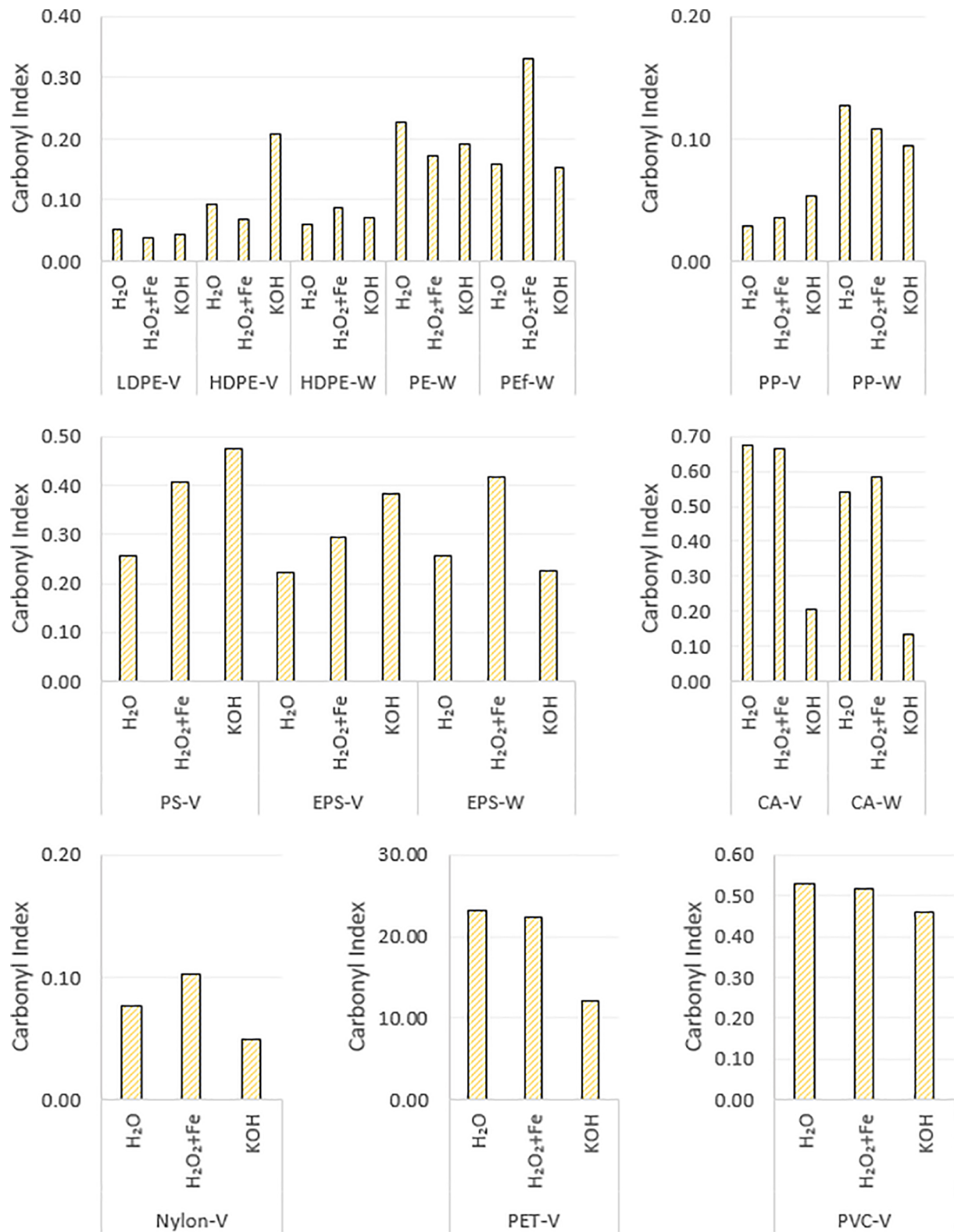


Figure 4

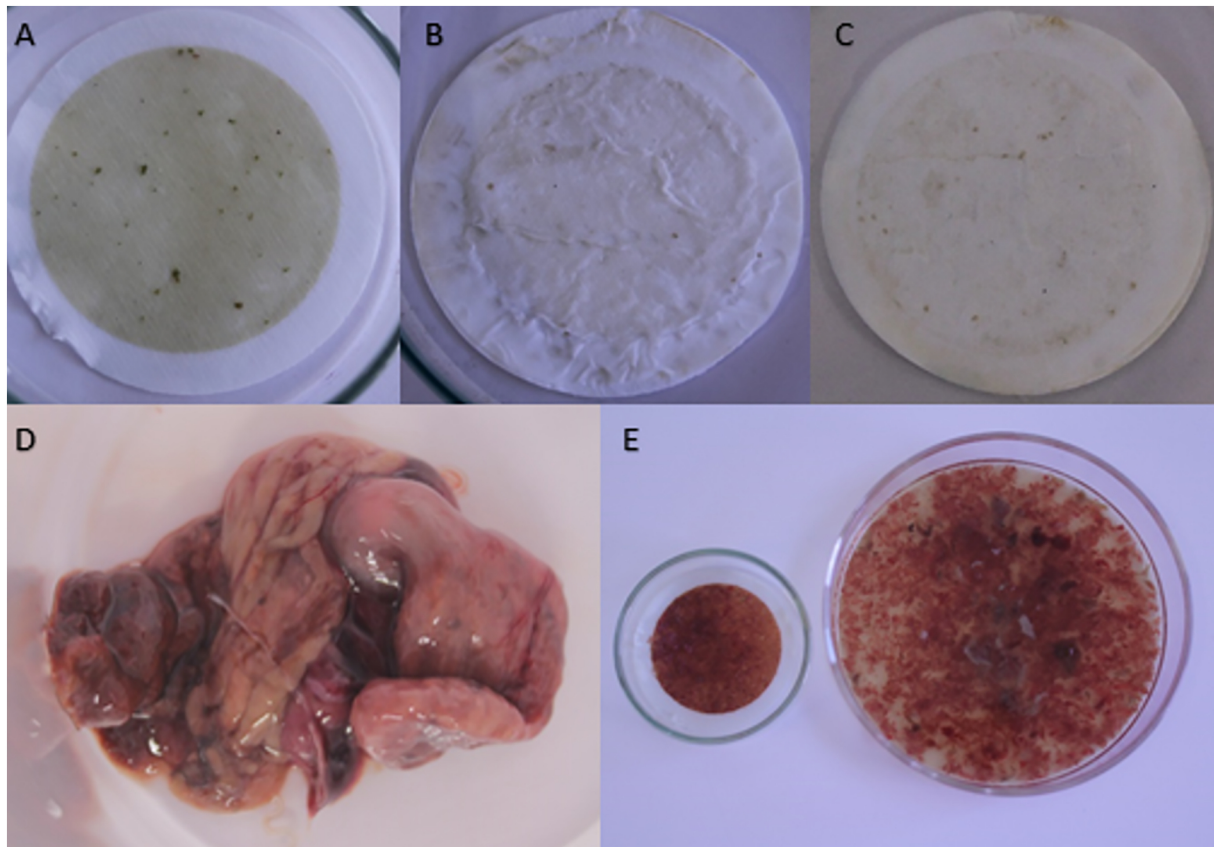


Figure 5