Don't be corrosive

A novel image analysis method for the validation of microplastic extraction procedures

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Introduction

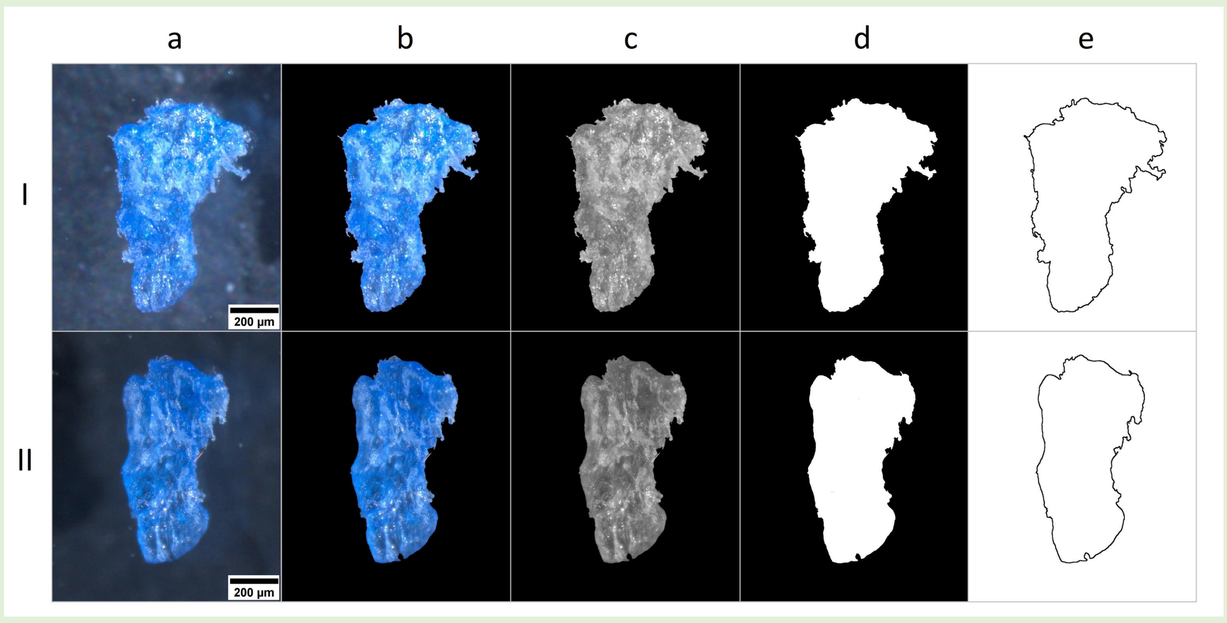
Most applied procedures to extract microplastics from complex matrices – such as soils, sediments, or biological samples – imply a digestion step to remove the biogenic component. Since the digestion efficiency of alkaline and oxidant agents can vary according to the composition of the examined matrix[1], possibly new digestion protocols will be developed in the future. The development of digestion protocols must consider that different chemicals at different incubation temperatures can attack different polymeric structures, potentially reducing the recovery of different microplastic types. Therefore, corrosiveness tests represent a crucial key step in validating new microplastic extraction procedures[2].

In this study we propose a methodological approach based on image analysis to detect the corrosive effect of digestive solutions. To reach this goal, we performed an experiment to verify the detection of expected shape variations in different microplastic types (tested polymers: nylon [**NY**], polyethylene [**PE**], polyethylene terephthalate [**PET**], polypropylene [**PP**], polystyrene [**PS**], and polyvinylchloride [**PVC**]) treated with two of the most commonly used solutions, namely **10% KOH at 60 °C** or **30% H_2O_2 at 50 °C** (incubation time: **12 h**)[1,3-4]. A treatment with **Milli-Q ultrapure water at room temperature** was used as control treatment (**CTRL**).

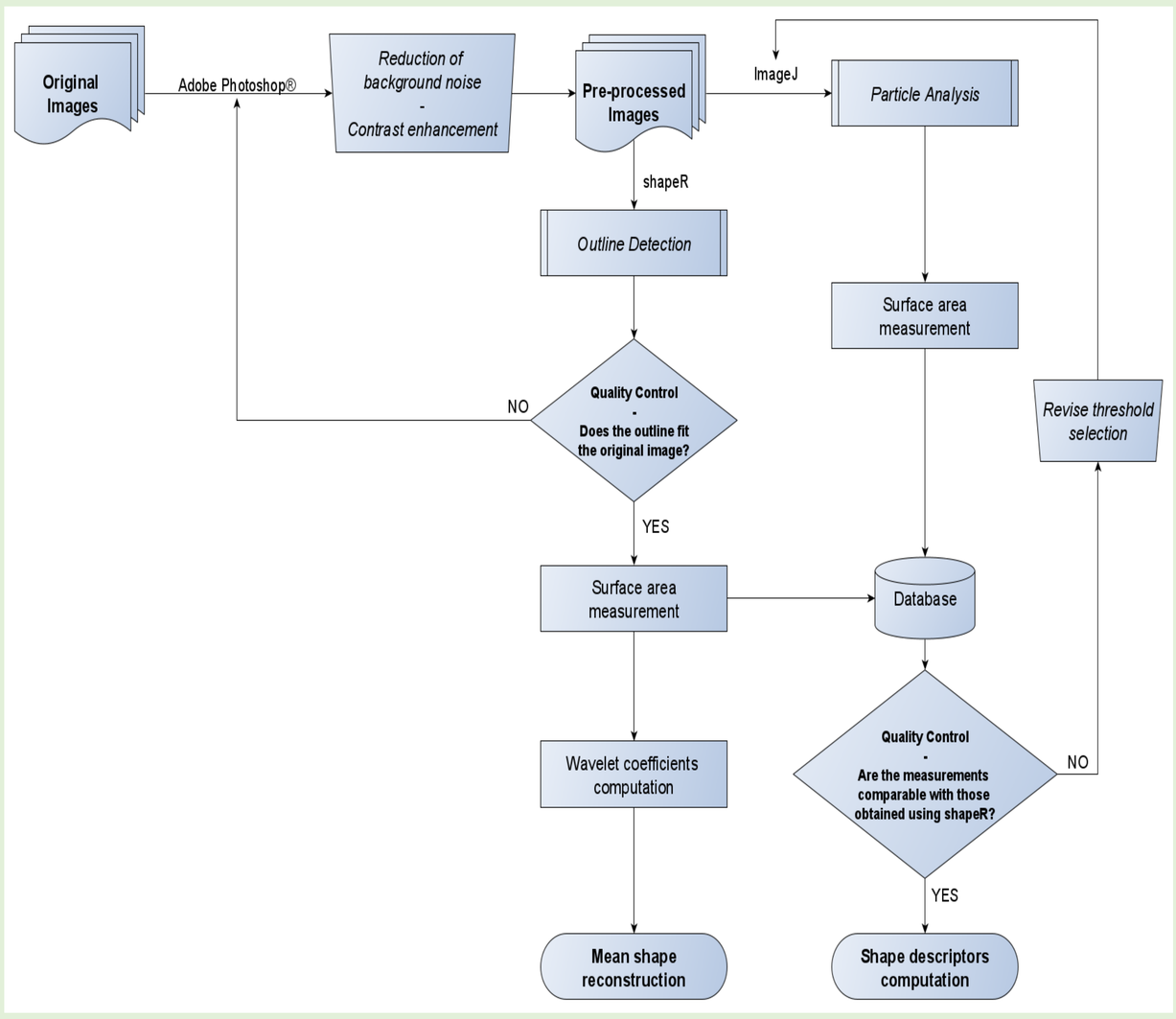
Materials and methods

Microplastics for the analysis (size range: 0.170 – 1.534 mm^2) were obtained by fragmenting daily-use plastic products or laboratory materials. The polymer composition of all materials was verified using a Nicolet iS10 Fourier Transform Infrared Spectroscopy with Attenuated Total Reflection (ATR) FT-IR (Thermo Fisher Scientific, Madison, WI, USA).

Pictures of 540 microplastics (30 particles x 6 polymers x 3 treatments) were taken before and after their treatment using a camera-equipped dissecting microscope (ZEISS SteREO Discovery.V20; AxioCam ERc5s camera). The 1080 images (2560 x 1920 pixel, 1143 pixel x mm^{-1}) were processed using the open-source software ImageJ (https://imagej.nih.gov/ij/) and the shapeR package for R[5] to obtain data on pre-post shape variations. **Figure 1** and the workflow diagram in **Figure 2** explain the image elaboration process in detail[6].

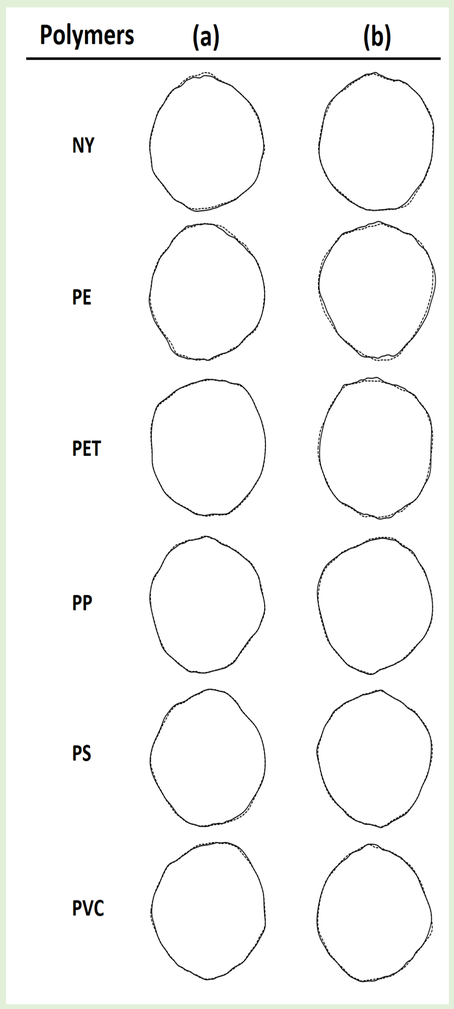


↑**Figure 1.** ♦ *Image processing.* Sample images: PET particle before (I) and after (II) a treatment with 10% KOH at 60 °C. **a)** original images stored in full color; **b)** pre-processing to reduce background noise and enhance the contrast; **c)** conversion to 8-bit images; **d)** threshold selection for extracting the particle from the background; **e)** outline detection.



→**Figure 2.** ♦ *Workflow and outputs.* The shapeR package was used to automatically extract the contour outline of each microplastic. The considered outputs were surface area of each microplastic and their 64 Wavelet coefficients[5]. The automated use of ImageJ with Macros and Batch Processing was employed to get validated surface area measurements, and also other shape descriptors not considered in this study (*e.g.*, compactness, solidity, and convexity). For more information see **Valente *et al.*** [6]

Results and discussion



←**Figure 3.** ♦ *Mean shape reconstruction.* Graphical assessment of the average shape variation of microplastics treated with **(a)** 30% H_2O_2 at 50 °C, and **(b)** 10% KOH at 60 °C. Drawings are based on wavelet reconstruction: the solid lines represent the mean shape before the treatment; the dashed lines highlight the deviations detected after the treatment.

→**Table 1.** ♦ *Surface area variations [mm^2].* ANOVA and Tukey's HSD tests were used to highlight significant differences among treatments applied to each polymer. Values with "*" indicate significantly higher surface area variations ($\alpha = 0.05$).

Polymer	CTRL	30% H_2O_2 (50 °C)	10% KOH (60 °C)
NY	-0.010±0.003	0.018 ± 0.003*	0.000 ± 0.003
PE	0.008±0.004	0.003±0.003	0.013±0.003
PET	0.013±0.002	0.015±0.002	0.061±0.003*
PP	0.003±0.003	0.005±0.002	0.010±0.002
PS	0.012±0.003	0.023±0.002*	0.014±0.003
PVC	0.005±0.002	0.017±0.002*	0.009±0.002

Despite the lack of strong shape differences (**Figure 3**), the analysis of surface area variations (**Table 1**) shows that NY, PS, and PVC are the most susceptible polymers to the 30% H_2O_2 treatment, likely due to possible changes of the polymeric structure (as previously evidenced by Karami et al.[2]). The largest decrease in surface area was recorded for PET particles treated with KOH at 60 °C. In this case, degradation may be led by the high incubation temperature, which is close to the softening point of PET (74 – 85 °C)[2,7]. PE and PP are confirmed as the most resistant polymers to the applied treatments[2]. Since our results are in line with what was expected, the proposed image analysis approach could represent a replicable method for quantifying the corrosiveness of digestive solutions on microplastics with different composition.

♦ From: Valente et al. (2022)[6]

Main References

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