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Method Article

Patent method for the extraction and determination of micro- and nano- plastics in organic and inorganic matrix samples: An application on vegetals

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A B S T R A C T

The uncontrolled introduction into the environment of plastic polymers have caused the dispersion of plastic debries, known as microplastics (MPs), that represent an important topic for environmental and human health threats. So far, the absence of effective and efficient extraction methods of MPs (especially for plastic particles with diameters inferior than 10 µm) from complex matrices (water, food, etc.) did not allow to perform the risk estimation and, the consequent assessment of the health impact associated with the exposure to these emergent contaminants.

In this paper, a new patented method for the extraction and determination of micro- and nano-plastics in organic and inorganic matrix samples is reported. The method applied in the study has been nationally and internationally protected. The code of the submitted request of international patent's extension in several country of world is PCT/IB2019/051,838 of 7 March 2019, coupled with the accepted Italian patent n. 102,018,000,003,337 of March 7 of 2018 entitled "Method for extraction and determination of microplastics in samples with organic and inorganic matrices". The method applied to our study is based on sedimentation of the particles with density higher than 1 g/cm^3. The method can be applied to organic and inorganic samples as water, food, soil, waste, air, biological sample (blood, urine, tissues, etc.).

After acid digestion of sample matrix, MPs are recovered by sedimentation in dichloromethane and then they are dispersed in metallic stub. Analysis is performed by SEM-EDX.

- New method for the extraction and determination of total microplastics <10 µm.
- The method is based on the sedimentation of particles with density higher than 1 g/cm^3.
- The method can be applied to organic (food, soil, biological sample, etc..) and inorganic samples.
- Total micro- and nano-plastics quantification is performed by SEM-EDX.

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Specifications Table

Subject Area	Environmental Science
More specific subject area	*Environment, food and human risk assessment*
Method name	*Method for extraction and determination of microplastics in samples with organic and inorganic matrices*
Name and reference of original method	*Method for extraction and determination of microplastics in samples with organic and inorganic matrices* *The request of international patent's extension in several country of world is PCT/IB2019/051,838 of 7 March 2019, coupled with the accepted Italian patent n. 102,018,000,003,337 of 7 March 2018*
Resource availability	WIPO/PCT, WO 19/171,312 A1

Method details

The method has been nationally and internationally protected. The code of the submitted request of international patent's extension in several country of world is PCT/IB2019/051,838 of 7 March 2019, coupled with the accepted Italian patent n. 102,018,000,003,337 of March 7 of 2018 entitled "Method for extraction and determination of microplastics in samples with organic and inorganic matrices". Based on the sedimentation of the particles with density higher than 1 g/cm^3, it is possible to count the number and the diameter of the plastic particles both in organic and inorganic samples as water [1,2], food [3], soil, waste, air, biological sample (blood, urine, etc.).

To avoid sample contamination, some precautions have been taken. Nitrile gloves and laminar flow hoods in order to minimize the contamination of the sample by airborne dust in the environment have to use. In all operations, from the acquisition of the samples, to the pre-treatment, extraction and analysis phases, only glass equipments and containers were used, any plastic material and any product whose chemical structure was made up of inorganic carbon (containers, caps, pipettes, filters, holders, etc.) have been carefully avoided. All containers and equipments that have come into contact with the sample were first washed with UPLC-MS Grade water (Merk, Darmstadt, Germany) and subsequently with acetone (Merk, Darmstadt, Germany).

Dichloromethane and acetonitrile, both with LC-MS hypergrade certified, and Suprapur® nitric acid 65% v/v were provided by Merck (Merk, Darmstadt, Germany). Ultra-pure water (filtered 0.22 µm) was purchased by Merck Millipore (Bedford, MA, USA).

In this paper, the method is referred to extraction of aliquots of 1 ml or 1 g of sample. The quantity can be modified based on expected microparticles concentration.

Sample extraction

- After homogenization of vegetal samples by vortex, 1 g (or 1 ml) of each one is transferred to 25 ml transparent glass test tubes (24 × 100 mm), with a conical bottom.
- Afterwards, 3 ml of nitric acid 65% is added and mineralization of the samples is performed in an open vessel at 60 °C for 24 h.
- After this, 3 ml ultrapure water and 9 ml of dichloromethane are added in each sample.
- Subsequently to vortex, samples are centrifuged at 4000 rpm for 15 min.
- The solvent is transferred into a glass tube and, after, it is evaporated.

- Each sample is extracted with other 9 ml of dichloromethane.
- Dried extracts are re-suspended with 200 µl of acetonitrile and are disperse on an aluminum and copper alloy stub with a diameter of 25 mm through nebulization by a diaphragmizer.
- After stubs are coated with gold, samples are ready to SEM-EDX analysis.

SEM-EDX sample analysis

The analyses of the samples were carried out by means of Scanning Electron Microscopy coupled with an Energy Dispersion Detector (SEM-EDX).

- Calibrate the EDX with cobalt standard provided by Oxford Inca Xstream.
- Calibrate the SEM for size measurement using the traceable magnification standard MRS-3XYZs/n R18–198 of Agar Scientific.
- SEM observations are performed with an accelerating voltage of 20 kV.
- The analysis is based on two contemporary procedures: micro-analytical acquisition (reading and recognition of the particles) and measurement and counting (determination of particle size and counting). The reading aims to verify that the particle chemical structure consists mainly of carbon. The identification of particles by SEM-EDX is according to the following inclusion criteria of:
  - high percentages of carbon;
  - the sum of the percentages of any present cations must not exceed 20% than *C*;
  - there must not be Si, N, S, P, Bi, Pb.
- The counting method is applied to an overall reading area within the stub of 1.0 mm^2, corresponding to a total of 228 fields at magnification of 1500x (the number of fields can be reduced in case of abundance of particles).
- Count and measure the diameters of each individual particle (length and width) for all plastic particles of any size, and which lie in the field of observation without position discrimination.

Expression of results

Concentration of microplastics, expressed as number of particles p on sample quantity Q (mass), is calculated as:

$$C(p/Q) = p_{tot}/Q_s,$$

where Q_s is the mass (g) of sample and p_{tot} is total number of particles calculated as:

$$p_{tot} = \left(n_p * A_{tot}\right)/\left(A_f * f\right),$$

where n_p is the number of found particles, f is the number of examined fields, A_{tot} is the total area of stub and A_f is the area of one field at magnification of 1500x.

Method sensitivity

It is possible to estimate the sensitivity of the described method (defined as the minimum amount of MPs present in the sample that can be detected by the method) starting by the hypothesis of a random distribution of the particles on the stub, the number N of the particles sampled on a given surface has a Poissonian distribution.

The minimum detectable concentration of MPs is the concentration at which the average number of MPs, on the overall checked area of stub ($n{\cdot}a$), is sufficiently high because at the level of probability fixed (usually the 95% level is adopted) the lower fiduciary limit is ≥ 1 particle (i.e. the possibility of observing at least one particle with the fixed probability level is guaranteed).

Assuming a level of 95%, the average number of MPs must be at least 4 (which corresponds to a lower trust limit of 1 and a higher trust limit of 10).

The sensitivity of the method depends on various factors: quantity of extracted sample, working conditions of the microscope, area of deposition of the sample on the stub, number of SEM-fields of reading. In any case, a sensitivity of about 48.9 p/g can be estimated when the sample consists of about 10 g of extract over an area of about 490 mm^2 (circular surface of about 25.0 mm diameter) and 228 fields are inspected at 1500 $\times$ magnification. Sensitivity will be modified depending to the quantity of the extracted sample.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

[1] P. Zuccarello, et al., Exposure to microplastics (<10μm) associated to plastic bottles mineral water consumption: the first quantitative study, Water Res 157 (2019) 365–371, doi:10.1016/j.watres.2019.03.091.
[2] P. Zuccarello, et al., Reply for comment on Exposure to microplastics (<10μm) associated to plastic bottles mineral water consumption: the first quantitative study by Zuccarello et al. [Water Research 157 (2019) 365-371], Water Res 166 (2019) 115077, doi:10.1016/j.watres.2019.115077.
[3] G.O. Conti, et al., Micro- and nano-plastics in edible fruit and vegetables. The first diet risks assessment for the general population, Environmental Research 187 (2020) 109677, doi:10.1016/j.envres.2020.109677.