



Micro- and nano-plastics in edible fruit and vegetables. The first diet risks assessment for the general population

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ABSTRACT

Microplastics (MPs) represent a current public health concern since toxicity has not yet fully investigated. They were found in several foods, but to the best of our knowledge, at this time no data was reported for the edible vegetables and fruits. We focused on diet exposure aiming to evaluate the number and the size ($< 10 \mu\text{m}$) of MPs in the most commonly consumed vegetables and fruits, in relation to their recommended daily intake too. MPs extraction and analysis were carried out using an innovative Italian methodology and SEM-EDX, respectively. Finally, we calculated the Estimated Daily Intakes (EDIs) for adults and children for each type of vegetal and fruit.

The higher median (IQR) level of MPs in fruit and vegetable samples was 223,000 (52,600–307,750) and 97,800 (72,175–130,500), respectively. In particular, apples were the most contaminated fruit samples, while carrot was the most contaminated vegetable. Conversely, the lower median (IQR) level was observed in lettuce samples 52,050 (26,375–75,425). Both vegetable and fruit samples MPs levels were characterized by wide variability. The smallest size of MPs was found in the carrot samples (1.51 μm), while the biggest ones were found in the lettuce (2.52 μm). Both vegetable and fruit samples had size of the MPs characterized by low variability. We found the highest median level of MPs in samples purchased from the "fruiter 3" (124,900 p/g) and the lowest in those purchased in "supermarket" (87,600 p/g). The median size of the MPs had overlapping dimensions in all the purchase sites, with the exception of the samples purchased at the "shop at km zero 2" which had slightly smaller size (1.81 μm).

The highest adults' (4.62 E+05) and children's (1.41 E+06) EDIs are due the ingestion of apples, instead the lowest are due to the ingestion of carrots (adults: 2.96 E+04; children: 1.15 E+05).

We hypothesized that the mechanism of uptake and translocation of MPs can be the same described and reported for carbon-nanomaterials. This may be a possible translocation route of MPs by environment to vegetables permitting, so, the translocation or uptake inside of their biological systems.

Based on the results obtained it is urgent important to perform toxicological and epidemiological studies to investigate for the possible effects of MPs on human health.

1. Introduction

Agricultural systems are the final recipients of a number of several pollutants (Razzaghi et al., 2018) and nanomaterials, including microplastics (MPs), with effects relatively unknown. The general lack of understanding regarding nanomaterial fate and effects in agricultural systems is troublesome given the potential for food chain

contamination and for an uncharacterized pathway of human exposure. MPs nanotechnology is a rapidly growing area of nanotechnology research. MPs are capable to penetrate the seed, root, culm, leaves and fruits plant cell based on their size and type (Dietz and Hertz, 2011) and this is the only study on plants; no study is available on the edible plants. The size of MPs alone is of great significance in food and health risk assessment. The effects of penetration of microplastics into the

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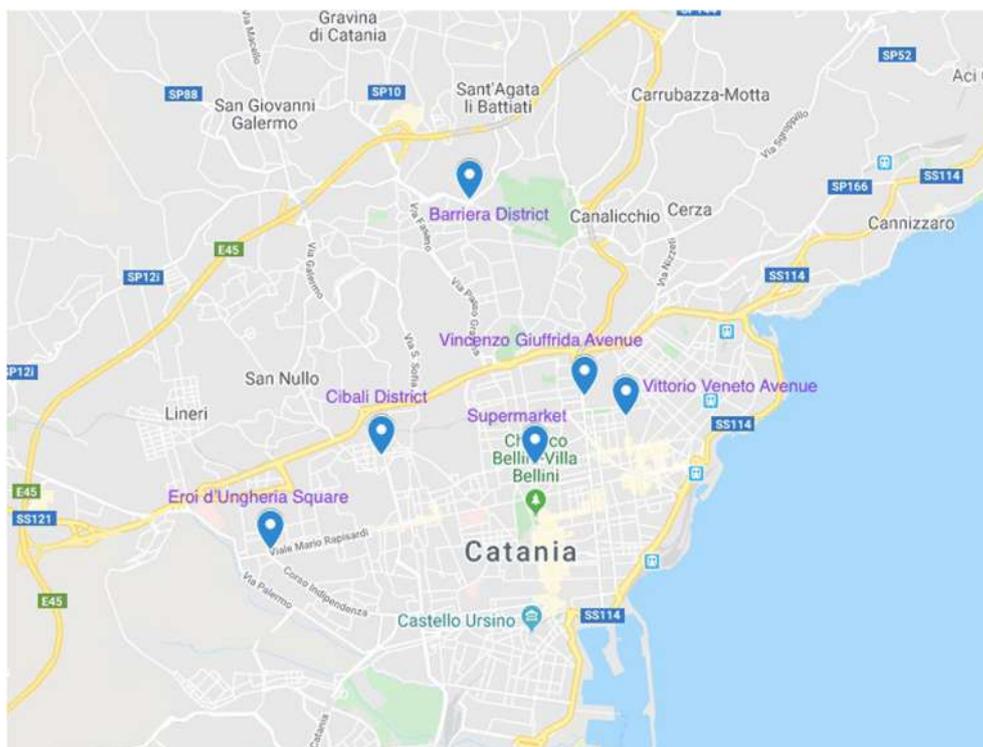


Fig. 1. Sites of the city of Catania where vegetable and fruit were acquired.

plant system can be speculated to modify the growth and also other characteristics such as taste. However, no data are available until today on this topic. The nanobiotechnology may be helpful for the advancement of plant science assessment but, for a more correct human's risk assessment too.

Thus, chemical identity needs to be considered on regard to potential effects of nano-plastics (NPs) on plants. This is of particular importance for the investigation on the food chain, which remains as one major research need.

The WHO indicates that Mediterranean diet is the best prevention tool for a healthy life (Renzella et al., 2018 Romagnolo and Selmin, 2017); against noncommunicable diseases and at least 400 g (i.e. five portions) of fruit and vegetables per day are indicated for a good health status maintaining, excluding potatoes, sweet potatoes, cassava and other starchy roots. So, it is extremely important to monitor the food quality and, in consideration of the high widespread of MPs in the environment and due to the lack of specific national and international policies and/or food standard limits for the plastic contamination control, the assessment of nano and micro plastics is particularly relevant. MPs are categorized into primary and secondary types. Primary MPs are produced and these are characterized by a size < 5 mm for several industrial applications, while secondary MPs result from the breakdown of larger plastic items. Microbeads in personal care products or cosmetics are an example of primary MPs (Kang et al., 2019; Duis and Coors, 2016; GESAMP, 2016).

In particular, plastic particles with smallest sizes < 100 μm represent an emerging issue and, also, these were difficult to be detect. Recently, MPs < 10 μm have been recognized as the most abundant particles e.g. in PET bottled waters (Zuccarello et al., 2019a, 2019b Welle and Franz, 2018).

No risks assessment is available currently also, with the exception of the last calculated Estimated Daily Intake (EDI) of MPs in bottled mineral waters reported by Zuccarello et al. (2019a, 2019b), where the smallest size of MPs was 0.1 μm , and the total exposure of MPs by food (seafood, water) and cosmetics calculated for the USA people by Cox et al. (2019) in a systematic review. The smallest size of particles

detected by Cox et al. is 6.5 μm (Cox et al., 2019 Bosker et al., 2019).

The evaluation the food/fruit and vegetables quality in term of MPs content besides the classical monitored pollutants can be a better approach in diet risk assessment and permits the assessment of the real EDI of total MPs ingested through the agrifood products. Currently, no information is available on this topic due to the absence of data about the nano- and microplastics presence in edible vegetables tissues. To fill this gap, we applied our method to evaluate the NPs and MPs < 10 μm presence and their concentration in common edible fruit and vegetables.

So, the aims of this study were:

- to investigate, for the first time, the presence of MPs (< 10 μm) in vegetables carrot, lettuce, broccoli, potato) and fruits (apple and pear)
- to identify and quantify the number of the MPs using our new patent/method
- to assess for the first time the Estimated Daily Intakes (EDIs) of MPs (< 10 μm) both for adults and children.

2. Materials and methods

2.1. Samples collection

In order to represent the vegetable/fruits that general population consumed daily (ISTAT, 2019), we have purchased vegetables and fruits from local markets (n. 3 fruiterer, n. 1 supermarket and n. 2 shops at km zero) that citizen identified for grocery shopping. We specifically purchased fruits (*Malus domestica*, *Pyrus communis*) and vegetables (*B. oleracea italic*, *Lactuca sativa*, *Daucus carota* and *Solanum tuberosum*) that are frequently consumed (almost one times/day) in order to better assess the dietary intakes of MPs and NPs.

Thirty-six samples, 6 for each vegetable and fruit (each sample is made by a pool of three samples), acquired in six different sites of the city of Catania (Cibali, viale Vincenzo Giuffrida, Viale Vittorio Veneto, Piazza Borgo, Barriera and finally piazza Eroi d 'Ungheria, see Fig. 1).

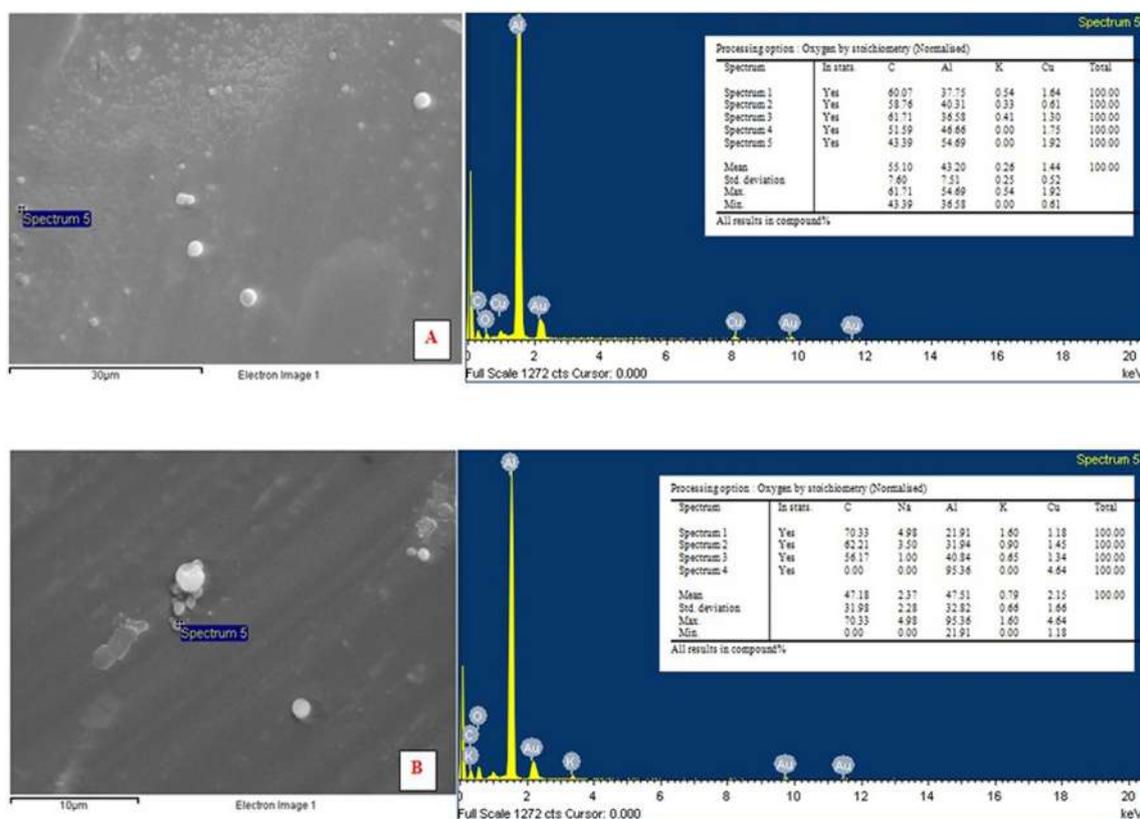


Fig. 2. SEM-EDX analysis in *M. domestica* (A) and *D. carota* (B), respectively.

2.2. Samples preparation

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/IB2019/051838 of March 7, 2019, coupled with the accepted Italian patent number 102018000003337 of March 07, 2018 and well described by Zuccarello et al. (Zuccarello et al., 2019a, 2019b).

Briefly, to avoid sample contamination, some precautions have been taken.

We used nitrile gloves and laminar flow hoods in order to minimize the contamination of the sample by airborne dust in the environment.

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 equipment 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).

Fruits and vegetables were thoroughly washed with UPLC-MS Grade water. After washing, all the samples of the same type of vegetable were peeled (with the exception of lettuce and broccoli) and blended with a blender made entirely of aluminum and stainless steel (without plastic parts), so as to obtain a representative pooled sample.

Since vegetables are high in water ranging by 79%–95%, all samples were placed on FKV stove (Orio del Serio, Italy) for 24 h at a temperature of 80 °C. To obtain the exact percentage of humidity, the samples were weighed before and after drying using a Mettler Toledo Excellence analytical balance.

From each homogenate pooled sample an aliquot 0.1 g were transferred into 16 ml transparent glass tubes with conical bottom and an emery neck with a glass stopper for a total of 6 samples.

All pooled samples were mineralized by adding 1 ml of 65% nitric

acid (Merk, Darmstadt, Germany) at 80 °C for 90 min, using a graphite digestion block system (digiPREP LS, QuantAnalytica, Italy).

At the same time, six reagent blanks (B) were examined to check the cross contamination by the analytical process.

After digestion, 3 ml of dichloromethane were added to each sample and finally the miscela was vortexed for 30s using a Fisherbrand™ vortex (Thermo-Fisher). The samples were then centrifuged at 4000 rpm for 5 min using an Eppendorf 5427 R bench centrifuge with angular rotor. Subsequently, the lower organic phase was transferred to a new test tube. The extraction was performed twice. The extracts were dispersed in aluminum stubs for scanning electron microscope "SEM Specimen Stubs" with a diameter of 25 mm, previously washed with 100% dichloromethane. The dispersion of the sample on the stubs surface was carried out through the use of a diaphanizing. As soon as the solvent evaporated, the stub was metallized with gold (metallizer Cressington Sputter Coater 108 Auto). All methodology is protected by its use through the patent.

2.3. Microplastics analysis and counting

The samples have been analyzed by Scanning Electron Microscopy (Cambridge Instruments Mod. Stereoscan 360) combined with an X Energy Dispersion Detector (SEM-EDX) (Diffractometer Rigaku Miniflex) using Inca software. The criterion of calculation was applied to an overall reading area within 1 mm² of stub, examining 228 fields at 1500 K magnification.

It allows the automated calculation of the total number of the microparticles by adjusting the results in real time at vary the number and size of the particles counted and based on the values of the other parameters noted in the appropriate boxes of insertion (quantity of sample analyzed, diameter of microplastic, etc.). From the registered diameters, the electronic sheet will calculate the average radius of each particle. The results were expressed as number of particles per gram of sample.

In Fig. 2 we reported the SEM-EDX of microplastics detection in apple and carrot samples.

The sensitivity of the described method (defined as the minimal number of MPs present in the sample that can be detected by this protocol) is based on the hypothesis of a random distribution of the MPs particles on the stub, so, 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 inspected area of stub (n -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 mm² (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.

For this study, sensitivity of the method was calculated based on the quantity of extracted sample (0.1 g) and on the detection of one single particle in the 228 SEM-fields at magnification of 1500 \times corresponding to inspection of an area of about 1.0 mm² of the whole stub where the extract was dispersed. Sensitivity was 4.89 $\times 10^3$. This value was considered appropriate since it was significantly inferior to the lowest number of MPs in samples.

2.4. Statistical analysis

Statistical analysis was performed using SPSS for Windows (Statistical Package for the Social Science, version 21.0; SPSS Inc., Chicago, IL, USA).

Continuous variables were expressed as median (interquartile range) and mean \pm standard deviation. The values of the number of particles per gram of sample were corrected with B samples.

Finally, the Estimated Daily Intakes (p/kg * day) were calculated through the equation (CAC, 2006):

$$EDI = (C \times IR)/BW$$

where: "C" is the average concentration of microplastic (w/g); "IR" is the per capita ingestion rate of the entire population for apples and pears (165.3 and 115.7 g/day for adults and children, respectively), lettuce and broccoli (53.0 and 24.2 g/day for adults and children, respectively), with potatoes (78.5 and 65.0 g/day for adults and children, respectively) and finally with carrots (20.3 and 18.0 g/day for adults and children, respectively) (Leclercq et al., 2009; Conte et al., 2015, 2016 Ferrante et al., 2018); finally, the "BW" is the average body weight of the adult population (70.0 kg) and children (16.0 kg) (CREA, 2019).

3. Results

Distribution of median levels and size of MPs (particles for gram or p/g) for each specie and EDIs are reported in Table 1. The higher median (IQR) level of microplastics in fruit and vegetable samples was 223,000 (52,600–307,750) and 97,800 (72,175–130,500), respectively. In particular, apples were the most contaminated fruit samples, while carrots were the most contaminated vegetable. Conversely, the lower median (IQR) level was observed in lettuce samples 52,050 (26,375–75,425) (Table 1). Both vegetable and fruit samples MPs levels

were characterized by wide variability (Fig. 3).

The smallest MPs' size was found in the carrot samples (1.51 μ m), while the biggest ones were found in the lettuce (2.52 μ m) (Table 1). Both vegetable and fruit samples had size of the MPs characterized by low variability (Fig. 3). The adults' (4.62 E+05) and children's (1.41 E+06) EDIs had the highest increasing following the ingestion of apples and the lowest following carrots ingestion (adults: 2.96 E+04; children: 1.15 E+05) (Table 1).

We found the highest median level of MPs in samples purchased from the "fruiter 3" (124,900 p/g) and the lowest in those purchased in "supermarket" (87,600 p/g) (Table 2).

The median size of the MPs had overlapping dimensions in all the purchase sites, with the exception of the samples purchased at the "shop at km zero 2" which had slightly smaller size (1.81 μ m) (Fig. 4). The fruits have showed the highest MPs (< 10 μ m) contamination compared to vegetables.

The highest adults' (4.62 E+05) and children's (1.41 E+06) EDIs are due to the ingestion of apples, instead the lowest are due to the ingestion of carrots (adults: 2.96 E+04; children: 1.15 E+05). The EDIs of children are highest for all studied edible vegetable and fruits than adults due to the lowest BW of children, in fact, children ingest smaller quantities of all the studied vegetables and fruits but, the exposures are greater than adults when considered in relation of BW.

4. Discussion

For first time we detected MPs in edible fruit and vegetables. Results open a new scenario both in environmental and medical sciences. No experimental data are available about uptake and translocation of microplastics in vegetal tissues.

We can suppose that microplastics may be absorbed to the similarity of carbon nanomaterials and translocated in vegetal tissues similarly to the modality of uptake of the more studied carbon-nanomaterials (Herremans et al., 2015 Dietz and Herth, 2011). For this type of nanoparticles the uptake by plant system is inversely proportional to its size and can affect the plant growth and fruits (Husen and Siddiqi, 2014 Bosker et al., 2019). So, the particles with a large size are forbidden to enter the plant cell and therefore, get adsorbed on the surface. Carbon nanomaterials may be absorbed through roots of the plants but, in seeds it may penetrate when have appropriate size and translocated to the shoot also by endocytosis, in the aerial parts through capillary action to places where the passage adequate of their size (Husen and Siddiqi, 2014) therefore, the nanomaterial accumulated in plant tissues may be transferred to the consumer through diet.

We suppose that the same mechanism of uptake and translocation may be possible for micro and nanoplastics being these also them self the carbon based particles Bosker et al. (2019). Show that exposure to plastics caused significant impacts on germination and root growth of the terrestrial vascular *Lepidium sativum*, and Hasen et al. (2014) highlighted that the accumulation of Carbon nanomaterial of appropriate size in the plant cells hampers the absorption/uptake of essential nutrients delaying the growth and flowering.

Herremans et al. (2015) carried out a study on *M. domestica* using the X-ray micro-tomography at various spatial resolutions to investigate the growth of void network and vascular system transport structures in 3D during fruit development of "Jona gold" apple. The developing fruit showed a more extensive vascular network compared to their rather small volume. Herremans et al. report that also the vascular bundles increase, both in terms of average diameter as well as length, wise with the growth progression (Herremans et al., 2015).

About that, Xylem and Phloem are the two types of transport tissue in vascular plants (Pace, 2019). The basic function of Xylem is to transport water from roots to stems and leaves, but it also transports NPs as reported by Dietz and Herth (2011) review study. The Xylem cells are long tracheary elements that transport water and nutrients with a mean diameter ranging by 200–700 μ m for the Angiosperms

Table 1
Distribution of levels and size of micro- and nano-plastics by species (p/g)* and EDIs (p/kg day) **.

Species	Micro- and nano-plastics Median (IQR)	Micro- and nano-plastics Mean ± SD	Size (µm) Median (IQR)	EDIs children	EDIs adults
<i>M. domestica</i>	223000 (52600–307750)	195500 ± 128687	2.17 (1.56–3.19)	1.41 E + 06	4.62 E + 05
<i>P. communis</i>	176500 (98325–302250)	189550 ± 105558	1.99 (1.87–2.59)	1.37 E + 06	4.48 E + 05
<i>B. oleracea italica</i>	94900 (65025–201750)	126150 ± 80715	2.10 (1.86–2.95)	1.91 E + 05	9.55 E + 04
<i>L. sativa</i>	52050 (26375–75425)	50550 ± 25011	2.52 (2.18–2.78)	7.65 E + 04	3.83 E + 04
<i>D. carota</i>	97800 (72175–130500)	101950 ± 44368	1.51 (1.36–2.00)	1.15 E + 05	2.96 E + 04

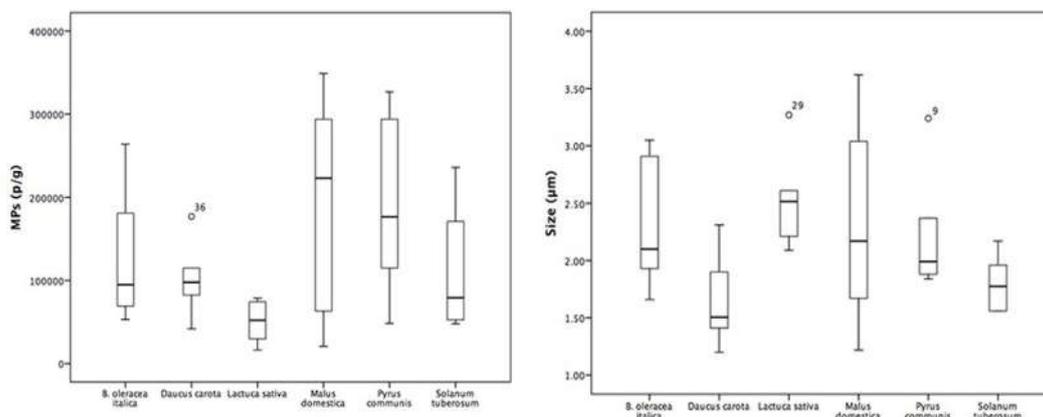


Fig. 3. Distribution of the MPs ($p = 0.071$) and their size ($p = 0.025$) by fruit and vegetable species.

Table 2
Distribution of levels and size of micro- and nano-plastics by purchase sites (p/g)*.

Purchase sites	Micro- nano- plastics Median (IQR)	Size (µm) Median (IQR)
Fruiterer 1	89250 (72975–192500)	2.02 (1.52–2.63)
Fruiterer 2	105950 (43200–173500)	1.93 (1.73–2.60)
Fruiterer 3	124900 (50275–201750)	2.10 (1.66–2.37)
Supermarket	87600 (47000–225500)	2.16 (1.91–2.46)
Shop at km zero 1	114750 (19700–271500)	2.29 (1.77–3.09)
Shop at km zero 2	87750 (62650–217500)	1.81 (1.47–2.33)

*Particles for gram.

group (Pace, 2019), in which all our species studied are included.

Phloem is the vascular tissue in charge of transport and distribution of the organic nutrients, is a pathway for the distribution of signaling

molecules and, finally, has a structural function in the plant body. The diameter (µm) of sieve pores in Phloem ranging by 4.0–100 µm in Angiosperm (Mullendore et al., 2010), generally is composed of three cell types: sieve elements, parenchyma, and sclerenchyma (Pace, 2019). Also, the void network is important pathway for the transport of gases, water and solutes in fruit (Herremans et al., 2015).

NPs are adsorbed to plant surfaces and taken up through natural nano or micrometer scale plant openings. Several pathways exist or are predicted for NP association and uptake in plants (Dietz and Hert, 2011; Vithanage et al., 2017). The vascular system is more complicated and largest compared to vegetables. So, we assume that the large quantity of microplastics detected in both fruits is justified for their vascular network complexity due to the necessity to nourish and protect the seeds.

Cellular uptake of various types of NPs has been extensively studied, revealing capability of active endocytosis involved in their internalization in vegetal tissue. This capability is verified also for

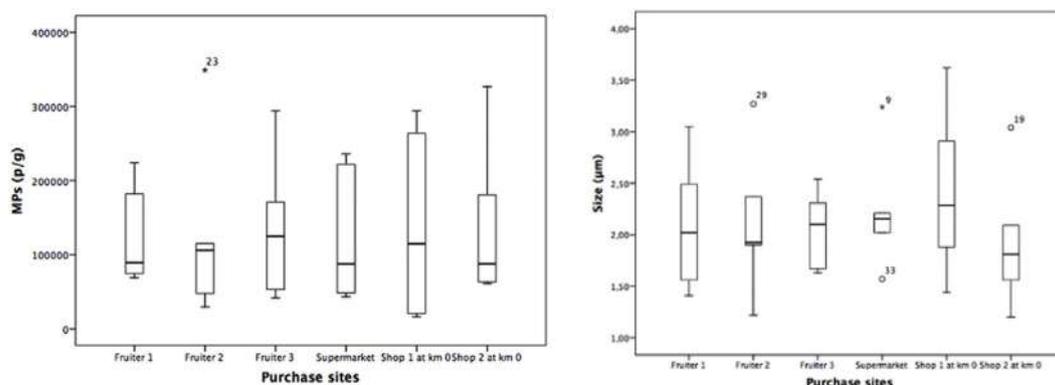


Fig. 4. Distribution of the MPs ($p = 0.998$) and their size ($p = 0.830$) by purchase sites.

NPs > 150 nm as reported by several authors in animal in vitro study (Wang et al., 2017; Zhang et al., 2009). By endocytosis, and/or using also carrier proteins or ion channels, the carbon nanoparticles (CNTs) can enter into the cytoplasm of plant cells. It was advanced hypothesis that translocation of MultiWalled Carbon Nanotubes (MWCNT) (ranging on size between 3500 and 3900 nm) occurs together to the uptake of water and nutrients at roots level transporting these at shoots. In fact Martı'nez Ballesta et al. (2016), showed that MWCNT entered the cells in adult broccoli plants with high accumulation under saline conditions (Martı'nez-Bellesta et al., 2016). Further, it was demonstrated also that MWCNT could be downward transported from leaves to roots using the network of phloem specifically when MWCNT enter into plants through leaves. About that, Chen et al. has provided scientific evidence about MWCNTs capability to be absorbed by roots of mature mustard plants and then, translocated to other parts of the same roots and carried up to the leaves too (Chen et al., 2015). However, due to the relatively larger size of MWCNT than fullerene, the internalization of CNTs into the cells is easier (Vithanage et al., 2017; Lin et al., 2009; De La Torre-Roche et al., 2013; Gogos et al., 2016). The same mechanisms can be assumed for microplastics uptake and translocation.

The EDIs to microplastics is lower from the intake of agrifood products compared to that due to the intake of mineral water in plastic bottles against 1.5 E+06 and 3.4 E+06 from mineral water, respectively in adults and children (Zuccarello et al., 2019a). Therefore, although the abundant presence of MPs in the investigated vegetables was of considerable concern, exposure to MPs through the ingestion of these foods was less than PET bottled mineral water consume.

The present work provided for first time clues about the presence of MPs in vegetables and fruits (carrots, lettuces, broccoli, potato apples and pears). Moreover, our results indicated that plastic particulates are more concentrated in fruits than in vegetables edible parts and that plastic sizes are different in the assessed plants being very small in carrots tissues.

We can hypothesize that the fruits contain more MPs not only because of the very high vascularization of the fruit pulp but also due to the greater size and complexity of the root system and age of the tree (several years) compared to the vegetables (60–75 days for the carrot). Also, the carrot has small, microscopic hairs on the outside of the epidermis of central root and these serve to increase the surface area of the root but survive for only a few days. The moisture content of carrot and apple are similar, from 86 to 89% (Sharma et al., 2012) for carrot and 84–86% for apple (Canet, 1988).

Finally, our data highlighted for the first time the worrying Estimated Daily Intakes either for adults or for children in term of plastic particles. At present, it is premature to draw conclusions about the potential effects of naturally occurring levels of microplastics on agrifood products. Future researches should specifically focus on understanding nanotoxicity in plants but also in human through the diet. An important point is to assess MPs persistence in plants depending on size and chemistry of MPs, similar to studies in animal cell systems.

Major developments were required to establish standardized procedures for collecting, fractionating, characterizing, and quantifying polymer particles. Today a new patent answers to these needs as reported and deeply discussed by Zuccarello et al. (2019a, 2019b), opening a new scenario and opportunity of human risk assessment for microplastics < 10 µm.

Author contributions

OCG designed and concept the project and, wrote the manuscript; FeM contributed to the final version of the manuscript, to the main conceptual ideas, supervised the study and the proof outline; BM contributed to the final version of the manuscript; FC, NI and CA performed the laboratory measurements, FiM performed the statistical analysis and analysis of the results, ZP worked out almost all of the technical details, performed the numerical calculations for the suggested

experiment and contributed in revision of final version of the manuscript.

Declaration of competing interest

The authors declare no competing of interests.

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