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Life on bottles: Colonisation of macroplastics by freshwater biota

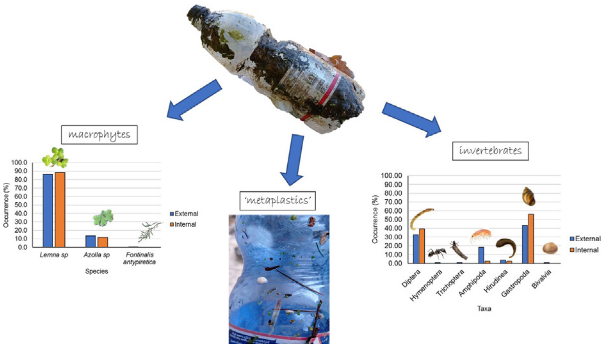
L. Gallitelli [a], G. Cesarini [a,*], A. Sodo [a], A. Cera [b], M. Scalici [a]

[a] Department of Sciences, University of Roma Tre, Viale G. Marconi 446, 00146 Rome, Italy
[b] Institute of Freshwater Biology, Nagano University, 1088 Komaki, Ueda, Nagano 386-0031, Japan

HIGHLIGHTS

- Colonisation and degradation of riverine plastic bottles by a novelty approach
- Bottles were covered by macrophytes, while internally entrapped invertebrates.
- Correlation between the colonising taxa abundance and the bottle degree degradation
- Introduction of metaplastic concept: plastic particles carried by other plastics

GRAPHICAL ABSTRACT



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ABSTRACT

While rivers are known to be the main vectors of plastics to the sea, it seems surprising that studies on interactions (e.g. colonisation/entrapment and drift) between macroplastics and biota continue to remain largely neglected, notwithstanding they represent unexpected threats to freshwater biota and riverine habitats. To fill these gaps, here we focused on the colonisation of plastic bottles by freshwater biota. To do so, we collected 100 plastic bottles from the River Tiber in summer 2021. Overall, 95 bottles were colonised externally and 23 internally. Specifically, biota mainly occurred within and outside the bottles rather than plastic pieces and organic debris. Moreover, while bottles were externally covered mainly by vegetal organisms (i.e. macrophytes), they internally entrapped more animal organisms (i.e. invertebrates). The taxa most occurring within and outside the bottles belonged to pool and low water quality-associated taxa (e.g. *Lemna* sp., Gastropoda, and Diptera). In addition to biota and organic debris, plastic particles also occurred on bottles reporting the first observation of 'metaplastics' (i.e. plastics encrusted on bottles). Furthermore, we observed a significant positive correlation between the colonising taxa abundance and the bottle degree degradation. In this regard, we discussed how bottle buoyancy may change due to the organic matter on the bottle, affecting bottle sinking and transport along rivers. Our findings might be crucial for understanding the underrepresented topic of riverine plastics and their colonisation by biota, given that these plastics may act as vectors and cause biogeographical, environmental, and conservation issues to freshwater habitats.

1. Introduction

Plastics are an emerging contaminant that is widely distributed across aquatic habitats (Morales-Caselles et al., 2021; Veiga et al., 2022; Cera et al., 2023; Nyberg et al., 2023; Rakib et al., 2023; Tang et al., 2023). In particular, plastic pollution has been well studied in marine ecosystems, while freshwaters have been understudied (Blettler et al., 2018; Cera et al., 2020, 2022a; Gallitelli et al., 2022). However, among freshwaters, rivers have the pivotal role of acting as a vector of plastics from the land to the sea (Gallitelli et al., 2020; Meijer et al., 2021). Several inland-based sources of waste contribute to the amount of discharged litter that is

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* Corresponding author.
E-mail address: giulia.cesarini@uniroma3.it (G. Cesarini).

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transported downstream by watercourses (Schirinzi et al., 2020; Gallitelli et al., 2022; Cesarini et al., 2023). The riverine transport is affected by different environmental factors, such as current, water level and discharge (van Emmerik et al., 2019; Castro-Jiménez et al., 2019). During the transport, plastic items can sink (van Emmerik and Schwarz, 2020), accumulate in riverbanks (Liro et al., 2020), and become entrapped by vegetation (Cesarini and Scalici, 2022; Gallitelli et al., 2022). However, floods and high-water levels can remobilize plastics, transporting them downstream to the river mouth as floating macroplastic litter (Liro et al., 2020). Among the floating litter, single-use plastic items are among the most widespread debris in rivers and plastic bottles represent one of the most occurring litter items (Crosti et al., 2018; Castro-Jiménez et al., 2019; González-Fernández et al., 2021; Cesarini et al., 2023).

Specifically, plastic bottles are objects with a high buoyancy and therefore they can float for long periods and be transported over long distance (Fazey and Ryan, 2016; Maclean et al., 2021). However, the sources and sinks of macroplastics are not well studied or identified (Lechthaler et al., 2020). In particular, literature studies focused on river storage as temporary sinks for macroplastics (van Emmerik and Schwarz, 2020; Liro et al., 2020; van Emmerik et al., 2022) and on floating plastics giving emphasis on the origin and the final fate of plastics (González-Fernández et al., 2021). Additionally to plastic sink and sources, plastic bottles can survive currents, wind and other abiotic factors affecting their durability (Pasternak et al., 2018; Winkler et al., 2019). However, after several years, macroplastic (MA, > 25 mm) bottles occurring along watercourses may undergo fragmentation and degrade into mesoplastics (5–25 mm) and microplastics (0.001–5 mm) due to abiotic and biotic factors (Windsor et al., 2019; van Emmerik and Schwarz, 2020; Gallitelli et al., 2021). All the different sizes of plastic items may have detrimental impacts on freshwater biota and riverine habitats (Gallitelli et al., 2020; Azevedo-Santos et al., 2021). While, for several marine species, many studies reported the use of plastics as substrate, nesting material, dispersal vector, refuges and shelters (Winston et al., 1997; Bergmann, 2015; de Carvalho-Souza et al., 2018; Rech et al., 2018; Battisti et al., 2019; Crocetta et al., 2020; Lavers et al., 2020; Póvoa et al., 2021; Cesarini et al., 2022), research on freshwaters remains largely lacking these interactions (Blettler and Wantzen, 2019; Blettler and Mitchell, 2021). Among these interactions, plastics may be colonised by a complex microbial community (e.g. microorganisms, bacteria, diatoms) that is called plastisphere (Zettler et al., 2013).

Plastisphere is composed by various organisms, with a biofilm setting soon and then bacteria, algae and fungi occurring and so composing a well-structured community (Zettler et al., 2013; Taurozzi et al., 2023). Moreover, plastic items may act as a sponge transporting contaminants (Joo et al., 2021), heavy metals (Liu et al., 2021), pathogens (Naik et al., 2019), antibiotics (Naik et al., 2019), and alien species (Zettler et al., 2013). Particularly, given that plastics are hydrophobic, the biofouling can be enhanced relatively soon, causing the following colonisation by micro- and macro-organisms (see Amaral-Zettler et al., 2021). This colonisation by the plastisphere community modifies the buoyancy and consequent fragmentation of the plastic items that have been colonised (Reisser et al., 2014).

Little is known about plastics in freshwater and the biotic colonisation of MA along rivers, although few studies highlighted that MA may be harmful to biota, the effect and interaction of MA on biota are understudied (Blettler and Mitchell, 2021; Blettler et al., 2019), except for microbes and bacteria (see Zettler et al., 2013; Oberbeckmann et al., 2018; Wang et al., 2021). Particularly, MA biofouling is not well studied in freshwaters (Maclean et al., 2021), while studies focused on fouling in marine ecosystems describing how biofouling can affect plastic buoyancy and sinking (Fazey and Ryan, 2016; Amaral-Zettler et al., 2021; Maclean et al., 2021).

To fill these gaps, we assessed the biota colonisation of plastic bottles by investigating the occurrence, abundance, and composition of taxa to evaluate the plastic transport of biota downstream and discussing the possible correlated effects on the plastic transport. Indeed, we considered plastic bottles as an object that transports biota downstream increasing their drift downstream along watercourses. Our main hypothesis is that plastic bottles might be used as a new substrate by biota. More specifically, we hypothesized that more the plastic bottles are degraded more they resulted colonised by freshwater biota. Thus, with this research, we aimed at investigating three main hypotheses: (i) plastic bottles transport biota; (ii) bottles act as a substrate for biota; and (iii) more degradation of plastic bottles leads to more colonisation by biota.

2. Methods

2.1. Field sampling

The Tiber River is a major river in Central Italy, its source is located on Mount Fumaiolo in Central Apennines and its mouth is in the Tyrrhenian Sea. Tiber River lower course belongs to the Mediterranean hydroecoregion (Traversetti et al., 2013) and is mainly characterized by large width (80–85 m) and lower flow rate (8–9 m^3/s, di Lascio et al., 2013), with calcareous lithotype and sandy-muddy substrate (Ceschin et al., 2010). Along its lower course, Tiber River flows through the city of Rome (Central Italy), a highly populated urban area (about 2 million inhabitants). We selected this urban part of the river as we observed that it is a plastic storage zone (i.e., plastic hotspot area) compared to upstream and downstream the city (Cesarini and Scalici, 2022; Gallitelli et al., 2022). We sampled plastic bottles along a 1 km transect along a straight section of the Tiber River in the centre of Rome (see Fig. 1), characterized by concrete river banks. Specifically, we sampled 100 bottles as considered a representative statistical sample size. All the floating plastic bottles found were collected using a handmade net (made by wooden handle and aluminium mesh) from the riverbank and analysed in situ during the summer of 2021. All the bottles were identified as polyethylene terephthalate (PET) bottles considering the recycling code (or resin identification code) reported on the bottle (i.e., PET, 01). Then, bottles were checked for the identification of freshwater biota attached to the external and internal surfaces. Biota occurring on the external surface of the bottles allows us to assess the colonisation of plastic items, whereas the internal surface investigates the detrimental effects (i.e., the entrapment) on freshwater biota. All the found items were stored into alcohol 70 % to then be analysed in laboratory. All the organisms were counted and identified to the lowest level possible by using a stereomicroscope (Nikon C-LEDS, China), and their occurrence and abundance were recorded. Specifically, for the plastic items attached to the bottles (i.e., metaplastics), we followed the procedure protocol by Gallitelli et al. (2020) for counting and identifying them under stereomicroscope (Nikon C-LEDS, China). More information on the type, size and colour of metaplastics was noted. Also, we characterized the polymers of metaplastics and of plastics occurring in the bottles by Raman spectroscopy. We acquired Raman spectra by an InVia Renishaw micro-Raman spectrometer (785 nm laser source) and Wire software. The interpretation of spectra was carried out using the libraries SLoPP and SLoPP-E (Munno et al., 2020). For more detail, we adapted the procedure by Cera et al., 2022b, and more information is provided on methodology is provided in Supplementary Materials.

2.2. Data analysis

Regarding the analysis on biota, we considered abundance (i.e. number of individuals) for animal taxa, while coverage for vegetal taxa. To investigate a possible relationship between taxa abundance and bottle degradation degree, we performed a Spearman correlation test as data were not normally distributed. In this case, for conducting the correlation, we transformed data on vegetation coverage in abundance data to allow performing the test. For achieving it, we obtained the ordinal quantitative data belonging to macrophyte coverage transforming raw data (i.e. macrophyte coverage, expressed in %) into a scale ratio ranging between 0 and 5. Therefore, in the case of macrophyte absence (0 %) the abundance corresponded to 0, while 1 ranged between 1 and 20 %, 2 between 21 and 40 %, 3 was 41 and 60 %, 4 between 61 and 80 %, and 5 between 81 and 100 %. The new index called ‘bottle degradation degree’ (hereafter, BDD) was estimated on a scale

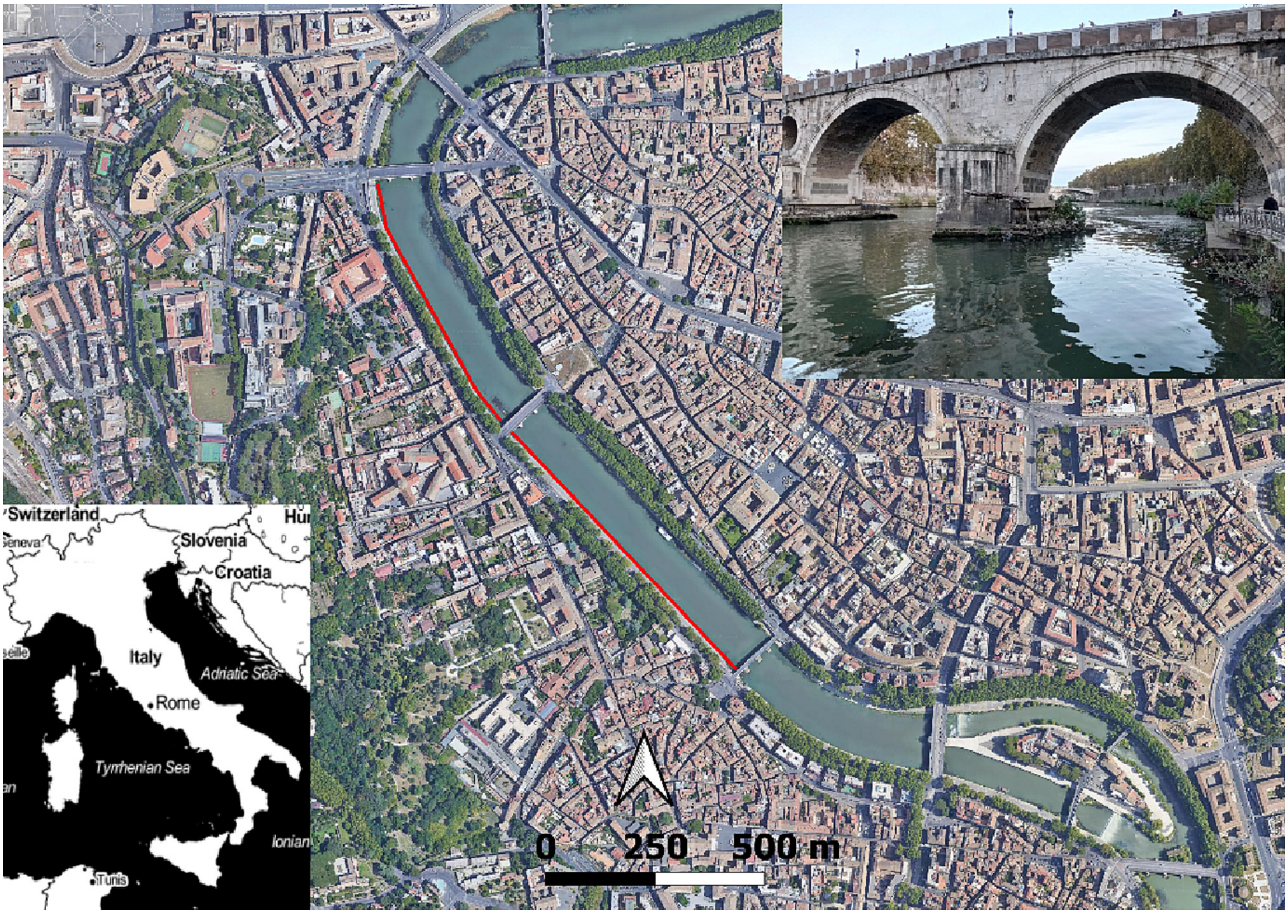


Fig. 1. Study area along the Tiber River located in the centre of Rome. The red line indicates the transect (1 km) surveyed. Image obtained by Google Earth and processed with QGIS version 3.10.

ranging between 0 and 5, relying on the damage caused to the bottles. We assigned 0 to freshly discarded bottles, 1 to not very evident sign of degradation (e.g. scratched bottle), 2 for scratched and slightly deformed/dented, 3 to scratched and deformed dented, slightly worn cap, 4 for scratched, deformed dented, degraded cork, bottle with small cuts, and 5 to bottle turned into fragments (meso- and microplastics formation) (see Fig. 2).

Furthermore, to investigate if there was a relationship between the size of bottles and items (i.e. biota, plastic particles, and organic debris) occurrence, we transformed data into absence/presence data and then we summed all the occurrences. After that, we investigated the relationship between the total occurrence of items occurring on bottles with the specific plastic bottle sizes. Moreover, to investigate if the abundance of biota normalized for the surface area of the bottles was different between bottle sizes, we carried out a Wilcoxon matched-pairs signed rank test. To obtain the bottle area, we approximated the bottle to a cylinder, composed by lateral area plus two base areas (i.e. bottle top and bottom). Thus, for the lateral area, we multiply π (3.14) with the circumference (using the bottle diameter) with the bottle height. Then, we added the base areas multiplying π (3.14) with the square radius (r^2). After that, we divided the

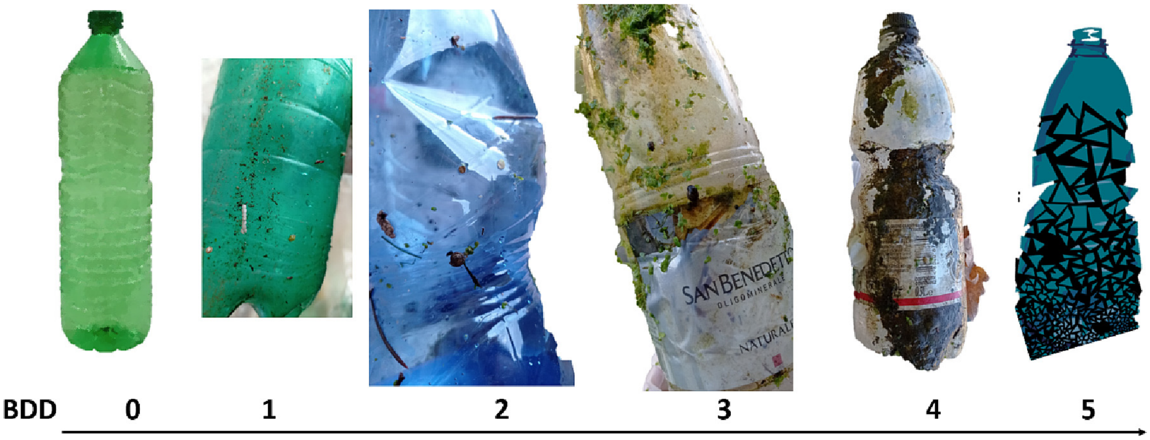


Fig. 2. Bottle degradation degree (BDD) estimated by using a reference scale ranging 0 (not degraded) – 5 (highly degraded).

abundance of biota by this total cylinder area. To carry out the analysis, we transformed the bottle size into rank class: 1 to 10–20 cm bottles, 2 to 20–30 cm, and 3 to the 30–50 cm.

To understand if most occurring taxa were attracted by a specific colour of bottles, we performed a Kruskal Wallis test, followed by a Dunn's post hoc test to reveal significative difference among groups.

All statistical analyses were performed using GraphPad Prism 8.4.2 and was considered significant if *p*-value levels were < 0.05.

To investigate whether the encrusting items were distributed on the bottles following a certain pattern, a co-occurrence analysis was carried out using the software EcoSim (Gotelli and Entsminger, 1999). Co-occurrence was tested using a C-score index and the V-ratio. For this purpose, a C-score index was conducted following the best combination of randomization with 5000 iterations and the common pattern fixed-fixed (ff, row sums fixed, column sums fixed) (Gotelli, 2000). Moreover, the V-ratio is an immediate test for detecting covariation patterns and uncommon species combinations (Schluter, 1984). Aggregation of taxa occurs when the C-score ratio values were smaller than expected by chance while the V-ratio larger than expected by chance (Gotelli, 2002). Taxa segregated if the C-score was larger and V-ratio smaller than expected by chance (Gotelli and Rohde, 2002). Significant differences were assumed setting always $\alpha = 0.05$ (following Gotelli and Graves, 1996). In addition, for V-ratio, we used the fixed-equiprobable (fe) pattern without constraining the number of species. This is because the ff algorithm maintains the differences and cannot be used for V-ratio because the latter is determined by marginal totals of the matrix rather than by the species co-occurrence pattern (Gotelli, 2000).

3. Results

Overall, a total of 100 plastic bottles were sampled. Out of 100 bottles, 95 were colonised externally and 23 internally (Table 1; Tables S1 and S2 in Supplementary Materials). Overall, 22 bottles were colonised both internally and externally. Moreover, bottles were mainly colonised by aquatic taxa, however terrestrial taxa were also found, i.e. one terrestrial gastropod and one individual of Hymenoptera (a terrestrial ant).

3.1. Colonisation of plastic bottles

3.1.1. External and internal colonisation

Concerning the external colonisation, bottles were covered mainly by biota (60.9 %), followed by plastic particles (28.8 %), and organic debris (10.3 %, e.g. leaves and small vegetal rest). Among biota, plant (i.e., macrophyte) organisms were more abundant (60.5 %) than animals (i.e., invertebrates) (39.5 %) (Fig. 3a). The most represented orders colonising bottles externally (Fig. 4a) were Gastropoda (39.7 %), Diptera (32.6 %), and Amphipoda (18.4 %) for invertebrates, while Arales (*Lemna* sp., 86.3 %), Salviniales (*Azolla* sp., 13.6 %), Hypnales (*Fontinalis antipyretica*, 0.10 %) for macrophytes (Fig. 4a). Specifically, among animals, the most occurring taxa belonged to Chironomidae (18.4 %) and Gammaridae (18.4 %), Gastropoda eggs (12.1 %), Physidae (11.3 %), Lymneaidae (10.6 %), and Stratiomyidae (7.80 %) (Fig. 4a; Fig. 5a; Fig. S1).

Regarding internal colonisation (bottles as traps), plastic bottles entrapped mainly biota (79.2 %), followed by plastic particles and organic debris (10.4 %). In this case, invertebrate organisms (57.6 %) were more abundant than macrophytes (42.4 %) (Fig. 3b). The internal entrapped taxa (Fig. 4b) were mainly represented by the invertebrate Gammaridae (21.4 %), *Lymnaea* sp. (16.7 %), and *Bithynia* sp. (16.7 %) and the macrophytes *Lemna* sp. (87.1 %) and *Azolla* sp. (12.9 %) (Fig. 4b; Fig. 5b).

Table 1
The number of bottles shows external and internal colonisation.

Type of colonisation	Description of bottles	Number of bottles
External colonisation	Colonised	95
	Not colonised	5
Internal colonisation	Open	23
	Closed	77

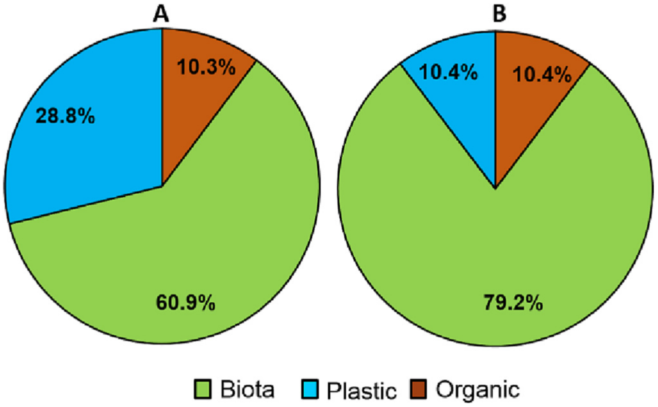


Fig. 3. Items recorded on the outside surface (A) and the inside bottle (B): biota (green), plastic particles (blue), and organic debris (brown).

3.1.2. Metaplastics

In addition to biota, we observed that plastic bottles carried downstream also other plastics (i.e., metaplastics). Externally, 45 plastic particles were found encrusted on bottles with the majority being polystyrene pieces (91.1 %), while inside the bottles 21 plastic particles were found (polystyrene pieces accounted for 85.7 %) (Fig. 5c). More information on the polymers spectral interpretation is reported in Supplementary Materials (see Figs. S1 and S2).

The analyses of the co-occurrence among colonising external items showed that biota (i.e., invertebrates and macrophytes), organic, and plastic particles segregated on plastic bottles (C-score, ff: obs > exp., $p < 0.05$; fe: obs < exp., $p < 0.05$; V-ratio: obs > exp., $p < 0.05$). Among the most occurring taxa on bottles, Gastropoda (*Lymnaea* sp., *Physa* sp., and *Bithynia* sp.) co-occurred mainly with *Lemna* sp. and *Azolla* sp. Gammaridae showed a high co-occurrence (i.e., iterations) with *Lymnaea* sp. (70), *Physa* sp. (120), *Lemna* sp. (476) and *Azolla* sp. (312), while Chironomidae co-occurred with Gammaridae (144), *Physa* sp. (98), *Lemna* sp. (710) and *Azolla* sp. (390) (C-score, ff/fe: obs > exp., $p < 0.05$; V-ratio: obs < exp., $p = 0.36$).

The internal entrapped taxa were randomly segregated (C-score, ff: obs > exp., $p = 0.29$; fe: obs < exp., $p < 0.05$; V-ratio: obs > exp., $p < 0.05$). Among the biota taxa, Gammaridae co-occurred with *Lymnaea* sp. (16), *Lemna* sp. (70), *Azolla* sp. (15), while Chironomidae co-occurred with *Lymnaea* sp. (10) and *Lemna* sp. (28), and Psychodidae with Gammaridae (20) and *Lymnaea* sp. (20) (C-score, ff: obs > exp., $p = 0.26$; fe: obs < exp., $p < 0.05$; V-ratio: obs > exp., $p < 0.05$).

3.1.3. Relationship between colonisation and physical characteristics of bottles

As regards the physical characteristics of found bottles, the main occurring plastic bottle size was 30–50 cm (56.0 %), followed by 20–30 cm (38.0 %) and 10–20 cm (6.0 %). Concerning the colours, transparent bottles (57.0 %) occurred more, followed by green (27.0 %) and blue (11.0 %) (Table 2).

Furthermore, for both external and internal items, the low-medium value of item occurrence (i.e., biota, plastic particles, organic debris) and taxa occurrence (i.e., 0, 1, or 2) was linked to the plastic bottle size with most of the items and the taxa transported by medium and large bottles (20–30 cm, 30–50 cm, Fig. 6). In this regard, we found that the abundance of biota normalized for the surface area of the bottles was different between bottle sizes ($W = 5050$, $p < 0.05$).

Among colours, transparent bottles (57.0 %) occurred more than the green (27.0 %) and blue (11.0 %) ones. For the most occurring taxa, *Lemna* sp., *Azolla* sp., and Chironomidae were occurring likewise on the green, transparent, and blue bottles. On the contrary, Gammaridae was

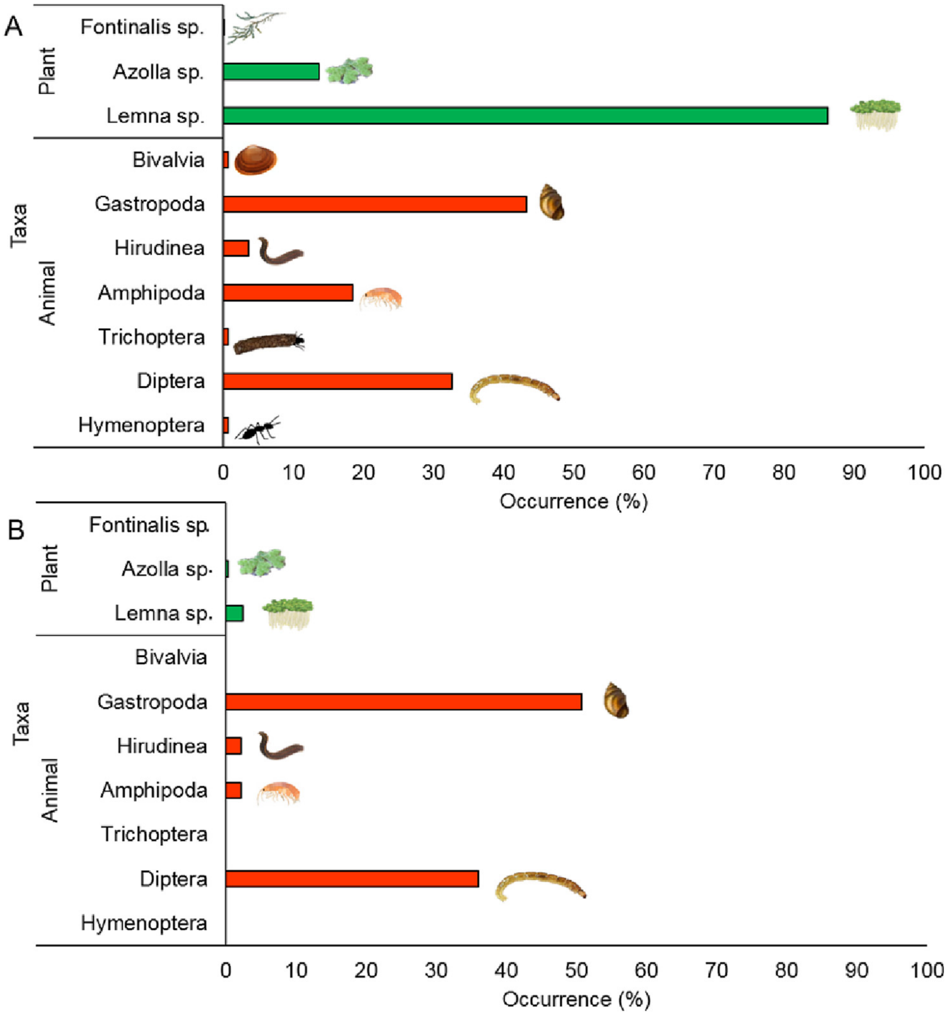


Fig. 4. Plant (i.e. macrophytes) and animal (i.e. invertebrate) taxa colonising plastic bottles externally (A) and internally (B) plastic bottles with relative percentage of taxa occurrence.

found more on the rest of the bottles (i.e., red, brown, orange) than on green, transparent, and blue (40.0 % vs 21.1 %) (H′ = 13.08, $p < 0.05$). Dunn's post hoc revealed that transparent and “rest” bottles showed a significant difference with Gammaridae occurring mostly on transparent and orange bottles (Dunn's post hoc, $p < 0.05$).

3.2. Degradation of plastic bottles

Concerning the bottle degradation degree, bottles showed evident signs of degradation (degree 2, 42.0 %) or small sign (degree 1, 26.0 %), or more evident (degree 3, 17.0 %) (Fig. 2). There was a correlation between most colonised bottles (i.e., high taxa abundance) and bottle degree degradation (total taxa abundance: $\rho = 0.47$, $p < 0.05$, Fig. 7). Thus, the most degraded bottles were the most colonised (by invertebrates: $\rho = 0.38$, $p < 0.05$; by macrophytes: $\rho = 0.05$, $p = 0.62$).

Moreover, there was a positive significant regression between the most colonised bottles and the bottle degree degradation ($R = 0.15$, $p < 0.05$; $y = 0.1167*x + 1.589$). Also, the correlation and regression results remained significant ($\rho = 0.45$, $p < 0.001$; and $R = 0.16$, $p < 0.0001$; $y = 0.2269*x + 1.367$, see Fig. S3 in Supplementary Materials), after eliminating the single outlier (see Fig. 7).

4. Discussion

In our study, we focused on the understudied and cuttle-edge aspects of plastic bottle colonisation by freshwater biota along a small stretch of an urbanised river. This is the first time that a thorough investigation observed the colonisation of plastic bottles by freshwater biota in a riverine ecosystem.

4.1. External colonisation of plastic bottles

Most bottles were colonised externally and mainly by biota, but also encrusted by plastic particles and organic debris. Among the biota, Gastropoda (i.e., freshwater snails), Diptera and Gammaridae shrimps were the most abundant invertebrates occurring on the external surface of the bottles. While Gammaridae were found able to attach on bottles as they are occurring where organic matter is higher, Gastropoda and Diptera larvae and pupae may remain attached on bottles in a long-term period due to their adhesive organs (e.g., hairy and smooth pads, see Walker, 1993; Armitage et al., 2012; Dirks, 2015). In literature, other studies highlighted that macroinvertebrates can rapidly colonise plastic substrates in freshwater habitats (Booth et al., 2013; Wilson et al., 2021). In particular, Booth et al. (2013) observed that plastic items were less colonised by taxa than brick and gravel substrates. Additionally, colonisation rates and macroinvertebrate composition largely varied on these different substrates, also having a possible influence on predator-prey relationships (Booth et al., 2013). Our results corroborated these studies. In fact, Booth et al. (2013) mostly found Chironomidae (1330 individuals), Coenagrionidae (505 individuals) and Libellulidae (102 individuals) on plastic substrates, while Wilson et al. (2021) observed mainly Muscidae dipters and Planorbidae gastropods. Thus, the comparison with these studies pointed out that in

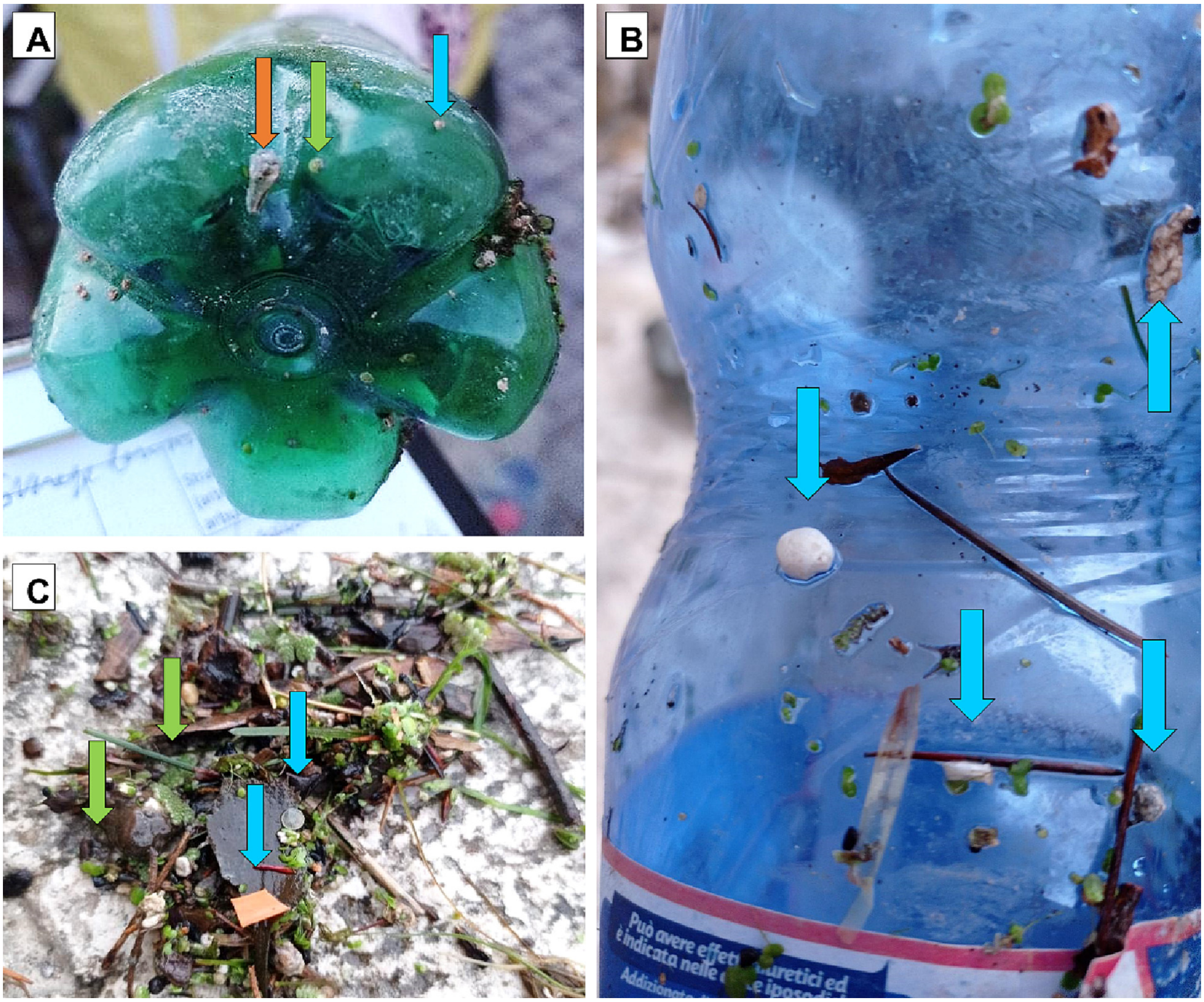


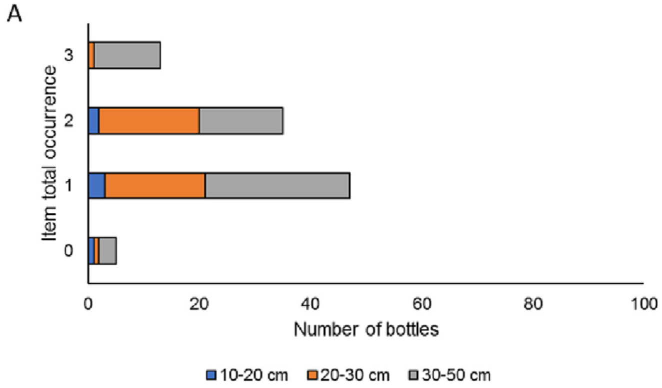
Fig. 5. (A) Diptera pupae, *Lemna* spp., and polystyrene piece on a plastic bottle (i.e. outside colonisation); (B) *Lemna* sp., *Azolla* sp., plastic particles and microbeads trapped in a plastic bottle (i.e. inside colonisation). The orange arrows indicate invertebrates, while green indicate macrophytes, and the blue indicate plastic pieces. (C) Example of plastic particles (i.e. polystyrene pieces, indicated by blue arrows) found encrusted on plastic bottles.

the Tiber River we observed more Gastropoda and Diptera on bottles as reported also by Wilson et al. (2021). However, no Odonata individuals (i.e. Coenagrionidae and Libellulidae dragonflies, see Booth et al., 2013) were observed as our study focussed on a lotic environment where river current occurs while Booth et al. (2013) worked in a reservoir lentic habitat. In addition, we should also consider that taxa distribution and local water quality influence variation in taxa occurring on plastic bottles.

Additionally, biota can be transported further downstream as they remain attached to the bottles and this could be a threat to local populations enhancing their drift to the river mouth. Furthermore, given that biota (e.g., invertebrates and macrophytes) are encrusted on plastics and bottles are transported downstream by river current (Crosti et al., 2018; Schirinzi et al., 2020), this local biota population may be flushed away from their local optimal habitat. We hypothesized that these organisms are most prone to colonise the bottles when they are in the river pools. Then, when the bottle is carried away by the current only gastropod eggs, gastropods and polystyrene particles might remain attached, while all other organisms are washed away by the current force. We noticed that the most degraded bottles were significantly the most colonised by invertebrates rather than by macrophytes, highlighting how animals need to anchor to find a favourable substrate for feeding and protecting from currents and predators, while plants are less linked to a substrate and are more prone to be transported away by currents. This process can cause a possible *substratum* fragmentation for the population of different species, being seen as an index of increased connectivity, due to the drift effect enhancement, also probably alterating the genic flow as well. Moreover, the arrival of plastics could enhance the diversity of taxa in some cases or diminish it in others, transporting the taxa on the plastic items (Zettler et al., 2013). In this regard, although we have not tested the connectivity and the drift topics, the enhance of taxa diversity is based on our result that the more degraded bottles are more colonised by freshwater biota. However, it seems certain that this topic should be further still investigated. In this regard, plastic fragmentation might alter ecological connectivity in riverine organism populations. Although it can be possible that some species are well adapted to the environment to which they are transported, in other cases it might also be true that many species may be able to survive at different levels of

Table 2
Number of bottles occurring for each colour.

Colour	Number of bottles
Transparent	57
Green	27
Blue	11
Brown	2
Orange	2
Red	1



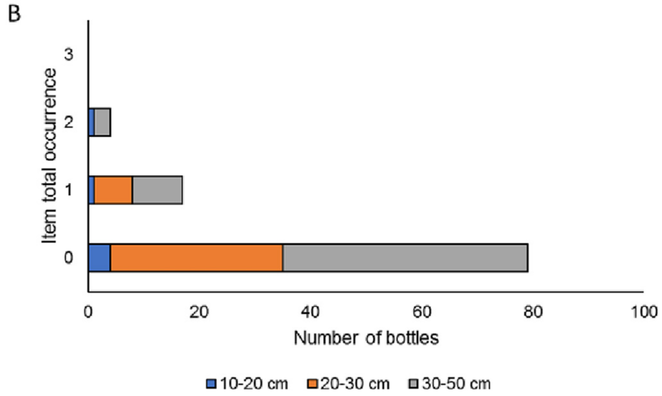


Fig. 6. Relationship between the size of the bottles (cm) and items (i.e. biota, plastic particles, and organic debris) total occurrence on the outside (A) and the inside (B) bottles.

environmental factors and this is a problem in the case of alien species. Some invasive species could benefit from the encounters to the detriment of environmental health, using plastics as settlement *substrata*, such as *Dreissena polymorpha* found in the Tiber River on natural *substrata* (Cianfanelli et al., 2007; Grano et al., 2020). In particular, it is of great importance to highlight that *D. polymorpha* species is one of the most harmful invasive species, also listed in the 100 of the World's Worst Invasive Alien Species (Lowe et al., 2000). Also, we should consider all the microscopic species that may occur on this *substratum* (e.g., alien diatoms such as the marine ones found on Plastisphere by Zettler et al., 2013).

Our findings might support understanding the underrepresented topic of "flowing" and sink plastics (i.e. plastics within the water column and on the bottom of rivers). Although several researchers observed encrusted bottles in marine ecosystems (Winston et al., 1997; Gregory, 2009), in freshwaters the topic is completely understudied but with high importance. Indeed, little research discussed the possible process of sinking plastics (see Liro et al., 2020), however only Maclean et al. (2021) preliminary observed that the organic debris and the biota colonisation on the bottle can change the buoyancy of the bottle which can then sink remaining entrapped into the riverbed substrate. Floods and high river currents may remobilize the colonised bottles that were stuck in the river sediment and in the riverbank, making them more easily to be carried downstream by the current towards the sea.

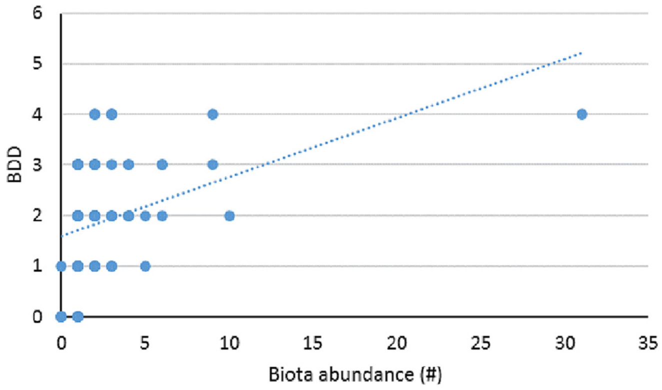


Fig. 7. Correlation between biota abundance (i.e. number of individuals, #) and bottle degradation degree (BDD) score obtained through Spearman test.

4.2. Internal colonisation of plastic bottles

Concerning the bottle acting as a trap for biota, we found a few open bottles in our study area. Open bottles may act as a temporary trap transporting species downstream. Recently, few studies highlighted the threat posed by open bottles revealing a global concern of abandoned containers as deadly traps for organisms (Blettler and Mitchell, 2021; Kolenda et al., 2021). In the sampled bottles we found both dead and alive organisms, making bottles as a trap but also a vector for biota. We found that, among taxa, Gammaridae was found more on the rest bottles and thus this taxon might be attracted by several colours (i.e. red, orange, brown). These results highlighted that plastic bottle colour might have a key role in attracting aquatic invertebrates, so they may have colonised specific bottles due to the colours and the polarised light (see Gallitelli et al., 2021). Furthermore, Blettler and Mitchell (2021) observed that birds represented the taxon most occurring in interaction with MA. However, the few investigated interactions between MA and biota focus on vertebrates (Blettler and Mitchell, 2021), only Kolenda et al. (2021) reported invertebrate biota interaction with MA. In particular, they reported that MA containers act as a trap for invertebrates, finding 1050 individuals dead in containers (Kolenda et al., 2021). It could also be that animals may have chosen bottles as a new more repaired habitat to live. However, given the importance of freshwater invertebrates, as they stand at the base of the food web, representing an important part of freshwater ecosystems, studies in this regard are mandatory (Vannote et al., 1980).

4.3. The concept of metaplastics

We also recorded plastic particles attached to the bottles, thus we introduced for the first time the concept of metaplastic, i.e., plastics carried by other plastics. In this case, MA might carry smaller plastic items. However, although microcosms have been observed on bottles (Mieczan, 2020), no plastics have been reported to date. Moreover, plastic particles in addition to transporting alien and pathogen species (i.e., plastisphere, see Zettler et al., 2013), can be harmful, representing a vector of environmental contaminants (Hartmann et al., 2017). Particularly, these contaminated plastics can be ingested by animals (i.e., birds and fish), used as nesting material (i.e., by birds) or cause entanglement (i.e., fishing gear entrapping fish) (Jabeen et al., 2017; Blettler et al., 2020; Blettler and Mitchell, 2021). Moreover, plastic ingestion can cause suffocation and mechanical blockage with a consequent accumulation of plastics within the gastrointestinal tract (Lusher et al., 2015; Jâms et al., 2020) and also cause several ecotoxicological effects on freshwater biota (Anbumani and Kakkar, 2018; de Sá et al., 2018; Binelli et al., 2022). The entanglement by MA occurs when plastics entrap animals, causing a loss of mobility and also strangulation and suffocation (Allen et al., 2012; Blettler and Mitchell, 2021; Andrades et al., 2021).

4.4. Relationship between degradation and colonisation of plastic bottles

Regarding the relationship between bottles and biota, we pointed out that there is a correlation between the size of bottles and the abundance of biota, and then we investigated a co-occurrence between the BDD and taxa abundance. In particular, our results highlighted that most colonised bottles (i.e. high taxa abundance) were correlated with BDD so the most degraded bottles were the most colonised. Moreover, the higher density (biota abundance on bottle area) is related to the bottle size. This means that a higher number of biota might live on small area bottles, thus there could be a taxa aggregation due to taxa ecological traits and life-history strategy. However, these are the first results to show this topic in freshwater, so future studies should focus on the community ecology of biota on bottles. In literature, research conducted in marine ecosystems highlighted that a higher area of plastic litter increased the number of fouling taxa (Shabani et al., 2019) enhancing plastic colonisation. Moreover, the abundance of fouling taxa is due to the residence time of the bottles occurring in water. In fact, bottles with a higher residence time might be most prone to be colonised by biota, while bottles that stay in water for less time should be less colonised (L. Gallitelli, personal communication). In detail, bottles, similarly to other plastic litter, undergo a process of colonisation over time. Temporal colonisation of plastics is complex (e.g. Richardson, 1992; Hofer and Richardson, 2007). Firstly, plastic items (i.e. bottles) might be colonised by microbes and bacteria (Zettler et al., 2013; Oberbeckmann et al., 2018; Wang et al., 2021), which allow colonisation of plants according to the ecological theory (Clements, 1916; Gleason, 1926). Indeed, biofilm development may allow plastic colonisation by plants and animals (Arsuffi and Suberkropp, 1985). This colonisation is following the ecology of succession whereby habitat is colonised firstly by primary producers (i.e. fungi, bacteria) and then by more complex organisms (i.e. moss and macrophytes) and apex predator species (i.e. macroinvertebrates and fish). After that, habitats (in this case bottles) should reach the climax community (following Whittaker, 1974).

5. Conclusions

Although riverine MA distribution and accumulation is under-studied, MA (i.e., plastic bottles) hosts several encrusters and biota attached to them. Given that the fouling and colonisation activities may affect plastic buoyancy, our research might have large impact on understanding the riverine plastic transport, as also highlighted by Maclean et al. (2021). Moreover, further detailed research on the interaction between MA and biota should focus on investigating further whether colours could affect specific plastic colonisation by biota. In this regard, cutting-edge technologies (i.e. metagenomic and environmental DNA, eDNA) might be used to assess biodiversity on plastics in rivers, evaluating the composition of biofilm microorganisms on plastic bottles as well as the colonisation of macrophytes and macroinvertebrates. In addition, colonisation and, consequently, the possible plastic degradation by biota can be explored from a spatial and seasonal point of view to further explore the dynamics behind these processes. Concerning several interactions among MA and biota, floating plastics may be drivers for alien or threatened species, transporting downstream part of the local population. This topic should be developed further with future studies as it may pose a risk to biota surviving and consequently problems on fragmenting populations. In this view, pelagic plastics may cause biogeographical, environmental, and conservation issues to freshwater habitats.

CRediT authorship contribution statement

L. Gallitelli: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Writing – original draft, Writing – review & editing. **G. Cesarini:** Methodology, Investigation, Formal analysis, Writing – review & editing. **A. Sodo:** Investigation, Writing – review & editing. **A. Cera:** Investigation, Writing – review & editing. **M. Scalici:** Conceptualization, Writing – review & editing, Funding acquisition, Project administration.

Data availability

Data will be made available on request.

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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Appendix A. Supplementary data

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