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Research Article

Paraffin waxes in the North-Western Mediterranean Sea: A comprehensive assessment in the Pelagos Sanctuary, a Specially Protected Area of Mediterranean Importance

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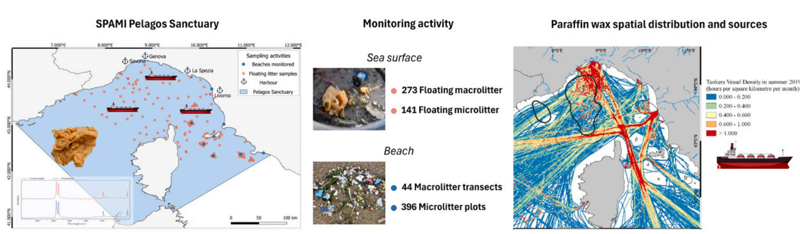
HIGHLIGHTS

- Urgently needs to standardise wax classification and assess its ecological implications.
- Paraffin waxes have been detected floating on the sea surface and stranded on beaches.
- ATR-FTIR used for chemical identification reveals the PE composition of waxes.
- Paraffin wax distribution seems to be related to tanker navigation routes in the North-Western Mediterranean Sea.
- Management strategies are essential to address and reduce the impacts of waxes.

GRAPHICAL ABSTRACT



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ABSTRACT

Paraffin waxes are widely recognized as emerging marine pollutants, even their classification by the recent monitoring programs and the knowledge of their occurrence, and sources of contamination in marine ecosystems are poorly defined and reported. Wax presence and distribution have been evaluated in different environmental compartments in the Pelagos Sanctuary (Mediterranean Sea) floating on the sea surface and stranded on beaches, focussing on their characterization, accumulation areas and pollution inputs. More than 2500 yellow paraffin residues were detected and analysed in the study area showing a prevailing dimension smaller than 5 mm. The Genoa Canyon and the waters facing Gorgona Island resulted in the more polluted areas representing two distinct hotspots of wax accumulation potentially related to the high density of tanker vessels sailing to and from the harbour of Genova and Livorno. Higher concentrations of beached particles were found along the Tuscan coast (11 items/100 m) and on Pianosa Island (110 items/m^2). This study gives valuable insights into paraffin wax pollution in the Pelagos Sanctuary, emphasizing the need for harmonized monitoring and detection methods to

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elucidate the potential impacts on marine organisms. Moreover, mitigating actions are crucial to prevent and curb the waxes pollution of marine ecosystems.

1. Introduction

Petroleum waxes (e.g., petrolatum, paraffin, and microcrystalline products) are by-products of heating or distilling crude oil consisting of saturated long-chain hydrocarbons. Usually solid at ambient temperatures, these materials show melting points between 35 °C and 95 °C [21,35]. They account for 85–90% of worldwide wax use and are by far the most significant in terms of worldwide production volume (about 4.79 million tons) and economic effect [17,42,22]. Their high adaptability and low reactivity make paraffin waxes appropriate for a wide range of industrial applications, including single-use packaging [24,27,42]. Every year, tankers and cargo vessels transport significant amounts of fully refined or unrefined petroleum wax throughout the world [42]. After unloading, certain amounts of the product frequently remain on the bottom of cargo tanks or crystallize on bulkheads and interior components and can then be handled at port receiving facilities or dumped at sea under particular conditions. The International Maritime Organization's (IMO) Annex II to the International Convention for the Prevention of Pollution from Ships (MARPOL 73/78) governs operational practices by containing provisions to control pollution from Noxious Liquid Substances (NLS) carried in bulk and establishing the standards and principles to be followed when discharging pollutants at sea. According to the version of Annex II (2007), petroleum waxes are classified as "high viscosity and solidifying substances" which fall into medium pollution category Y: "Noxious liquid substances which, if discharged into the sea during tank cleaning or ballast unloading, pose a risk to marine resources or human health or adversely affect amenities or other legitimate uses of the sea and therefore warrant a restriction on the quality and quantity of discharge into the marine environment". As a result, cargo residues can be lawfully discharged into the sea as long as they are below the waterline, moving at a minimum speed of 7 knots, at least 12 nautical miles from the nearest shore, and in water depths more than 25 m. The only exception is Antarctica, where any discharge of NLS is prohibited. To minimize the discharge at sea, this regulation was improved in 2019 [19], following the amendment adopted on the control of discharges of residues of NLS. Since 1 January 2021, ships have been required to wash out their tanks and discharge the washing water in specialised port facilities rather than at sea. In the Netherlands, the adoption of a voluntary improved prewash procedure for tanks and the delivery of contaminated waters to port reception facilities [20] has successfully led to the total elimination of paraffin residues on the Dutch coastline since the initiative came into effect.

While the new regulation is in place, discharge at sea, outside of territorial waters, and mismanagement in harbours may still occur. As an example, and without consideration of accidents and spills, paraffin wax may average, by number, 10.8% of the micro and meso particles found on beaches from the Baltic Sea, and may reach 41% in Lithuania and Russia, Kaliningrad, most probably related to uncontrolled discharges from the harbours of Klaipeda and Baltiysk where tanks are washed, with prevailing winds transport toward the shores (Haseler et al., 2020).

Actually, industrial wax, widely described as a solid form of petroleum-based pollutants, is not usually considered plastic or polymer due to its relatively low molecular weight, despite its thermoplastic origin. Macro residues are generally considered chemical pollution rather than litter by the recent Joint (codes J216 - J218) and Master List of Litter items (code G213) from Task Group 10 of Marine Strategy Framework Directive (MSFD) [10,13,29], as well as by OSPAR (codes 109, 110 and 181) [4] and UNEP/MED WG.555/5 [38] beach litter monitoring protocols (code OT01), while wax particles smaller than 2.5 cm are not mentioned in any guideline or protocol adopted by the scientific community and they are generally identified as other pollutants category.

Lumps and pieces of wax are commonly found during beach litter surveys, despite the chemical identification of these materials is rarely provided by authors. There are no reliable estimates on the amount of petroleum waxes being discharged at sea every year, but massive pollution events have already been reported since the early 1990 s [35]. Kimo (2017) reported a minimum of 91 incidents in 5 years (2012–2016) in 5 Northern EU countries (Denmark, Germany, The Netherlands, Sweden, and France), costing over 1.4 million euros to clean up. In the Mediterranean Sea, the presence of paraffin waxes is rarely explicitly reported in the scientific literature, despite some fragmented data [35,36,40,43], and their actual occurrence in the marine environment is largely unknown.

In this basin, heavily influenced by vessel traffic, the uncontrolled discharge of wax residues could represent a threatening issue for marine organisms, especially in protected areas such as the Specially Protected Area of Mediterranean Importance (SPAMI) Pelagos Sanctuary (North-western Mediterranean Sea) which has also recently been included in North Western Mediterranean Sea Particularly Sensitive Sea Area (PSSA), designated and adopted by IMO, to protect marine organisms from collisions. The Pelagos Sanctuary is characterized by 3,757,587 km of vessel traffic originating from 82,831 transits by 4205 distinct vessels (navigating under the flag of 90 different states) [5]. The spatial and temporal distribution of marine traffic in this area varied according to the vessel type, identifying the passenger vessel as predominant, with 26,264 transits totalling 1,385,361 km, followed by cargo (21,753 transits totalling 1,427,681 km) and tanker (10,352 transits totalling 369,026 km) [5].

To gain information on the potential ecotoxicological risk that waxes represented in the Nort-Western Mediterranean Sea, their presence and distribution have been evaluated in different environmental compartments, floating on the sea surface and stranded on beaches in the SPAMI Pelagos Sanctuary with a particular focus on the Tuscan Archipelago National Park. The physical characteristics and chemical composition of this material were investigated to confirm its anthropogenic origin. Moreover, the relationship between paraffin wax accumulation areas and both the main tanker navigation routes and commercial harbours have been explored to highlight potential sources of pollution in a relevant ecological and high biodiversity area.

2. Materials and methods

Sampling activities were planned and carried out within the Plastic Busters MPAs Interreg Mediterranean Project campaigns during 2019–2020. Special attention has been paid to the presence evaluation of paraffin waxes in the monitoring of macro and microlitter floating at the sea surface and stranded on beaches. Wax objects found have been respectively classified according to the categories J217 and G213 of the Joint and Master List of Litter items proposed by Task Group 10 of the Marine Strategy Framework Directive [29].

Floating macro and microlitter simultaneous assessment has been performed in pelagic and neritic areas of the SPAMI Pelagos Sanctuary from May to September 2019, focusing on the northern and central-western sectors of the Ligurian Sea, the coastal waters off the 7 main islands of the Tuscan Archipelago National Park and the northeast and west coast of the island of Corsica (Fig. 1). Beach macro and microlitter monitoring activities were carried out between 2019 and 2020 at three beach sites along the Tuscan coast and on 5 islands (Capraia, Elba, Pianosa, Montecristo, and Giglio) of the Tuscan Archipelago National Park for a total of 8 beaches (Fig. 1A). The total number of transects performed and samples collected are reported in Fig. 1B.

2.1. Floating litter monitoring activities and characterization

Floating waxes were simultaneously identified during the macrolitter monitoring transects and isolated from the neustonic samples performed with a manta net. As deeply described in [14] the distribution, abundance, and composition of floating macroparticles (>2.5 cm) were assessed using the fixed-width strip transect method. A total of 273 transects were performed with the naked eye from the bow of the ship (3 m above sea level) at a speed of 4 knots for 30 min. Due to the characteristics of our observation set-up, a strip of 7 m was monitored, following the recommendation of Galgani et al. [13]; Galgani et al. [12]. Each item sighted floating at the sea surface, was characterised according to the size class (B. 2.5–5 cm, C. 5–10 cm, D.10–20 cm, E. 20–30 cm, F. 30–50 cm, G. > 50 cm), category and colour. Finally, counts of scattered objects were converted to density values (Di) by dividing the total number of objects sighted by the effective area sampled in each transect:

$$Di = n / (Li \times W)$$

Where n is the number of items seen on the transect, L is the length of the transect, and W (7 m) is the fixed width of the strip observed and expressed as items/km^2.

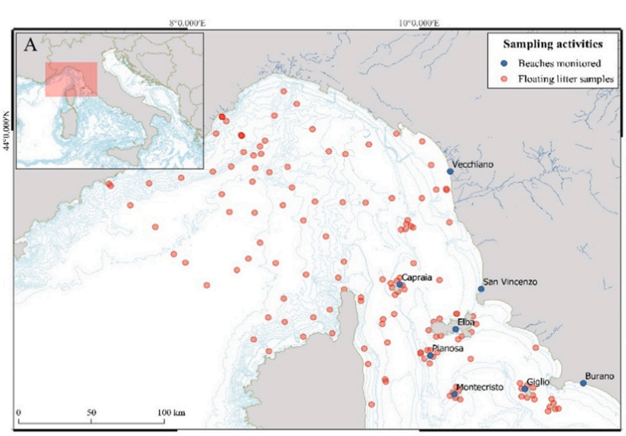
A total of 141 floating samples were collected using a manta trawl (330 µm mesh size, 16 ×60 cm mouth opening) towed at 2–3 knots on the water surface for 30 min, held to the side of the boat to avoid the turbulence caused by the wake of the vessel. Samples were filtered through a 300 µm metal sieve and stored in a 70% ethanol solution for synthetic particle analysis. Samples were then observed under an NBS stereo zoom microscope (Mod. NBS-STMDLX -T) equipped with an LED light and a micrometered eyepiece. The microparticles were manually isolated in a glass Petri dish and allowed to dry overnight at room temperature. Each Petri dish was then photographed and analysed for particle size measurement (expressed in mm) using ImageJ software (Fiji Distribution) [14]. The isolated particles were characterised according to different size classes: 0.3 - 1 mm, 1−2.5 mm, 2.5 - 5 mm, 5 - 25 mm, and > 25 mm, and colour. The data obtained, expressed as items/km^2 were normalised, if necessary, by applying the correction factor proposed by Kukulka et al. [25].

2.2. Beach litter monitoring activities and characterization

Following the beach litter sampling protocols proposed by Vlachogianni [41] and the Plastic Busters MPAs projects, the presence and abundance of beached paraffin wax items (>2.5 cm) at the selected eight sites were assessed by performing two 100 m transects separated by a 50 m stretch for each site along the Tuscan coast and only one transect in the Tuscan Archipelago islands since the total length of the beaches was shorter than 100 m. The monitored area covered the entire transect from the beach line to the back of the beach where natural vegetation or dunes begin. Data obtained by the two consecutive transects performed in the same beaches have been averaged and the concentrations of the items found expressed as items/100 m. In the same 100 m transects conducted for macrolitter distribution evaluation, beached wax microparticles were monitored in the eight sites. For each transect, nine plots of 1 m^2 were carried out in correspondence to three different zones located respectively at the end of the swash zone (where waves run up the beach), below the dunes and in an intermediate area due to the different accumulation zones which may occur in the width of the beach. The top 3–5 cm of sediment were collected using a metal shovel. The sand was weighed (kg) and sieved through two metal sieves with mesh sizes of 5 mm and 1 mm, respectively. Samples were oven-dried (50 °C overnight) and wax meso and microparticles were carefully isolated in a glass Petri dish and analysed as described above for floating microparticles. Isolated particles were characterised according to two size classes ranging from 1 to 5 mm and from 5 to 25 mm, and colour. The data obtained were averaged if obtained by two consecutive transects and expressed in terms of concentrations as items/m^2. All the sampling monitoring, including macro and microlitter evaluation, was repeated in each beach once per season during the autumn and winter of 2019 and spring and summer of 2020. The corresponding total number of transects performed and samples collected are reported in Fig. 1B.

2.3. Chemical analysis

The chemical composition of the isolated particles both from neustonic and beach samples was evaluated using Fourier infrared spectroscopy (FTIR). Spectra of all wax particles were analysed using an Agilent Cary 630 Spectrophotometer, recorded in transmission mode in the spectral range of 4000 to 650 cm^{-1} with a collection time of 3 s and 16 co-scans for each measurement. The spectral resolution was set to 4 cm $^{-1}$ and the Agilent Micro Lab FTIR software was used for the output spectra identification and elaboration. After each polymer identification, the instrument was settled to perform an autocalibration and autozeroing of the internal setting and each residue eventually remained on the crystal holder where particles were placed down were eliminated and cleaned using a mixture of hexane-dichloromethane 1:1 v/v. Reference spectra (Fig. 2 F) to accurately determine and confirm the chemical composition of smaller residues isolated both from neustonic



B

Monitoring activity	Number of samples/transects
Floating macrolitter	273 transects
Floating microlitter	141 manta trawls
Beach macrolitter	44 transects of 100m
Beach microlitter	396 plot of 1m^2

Fig. 1. Study area monitored for the presence of paraffin wax particles including the SPAMI Pelagos Sanctuary and the Tuscan Archipelago National Park including the islands of Capraia, Elba, Pianosa, Montecristo and Giglio (A); summary of the total samples collected and transects performed (B). Red dots represent the sampled sites for the evaluation of wax residues floating at the sea surface and blue dots the monitored beaches.

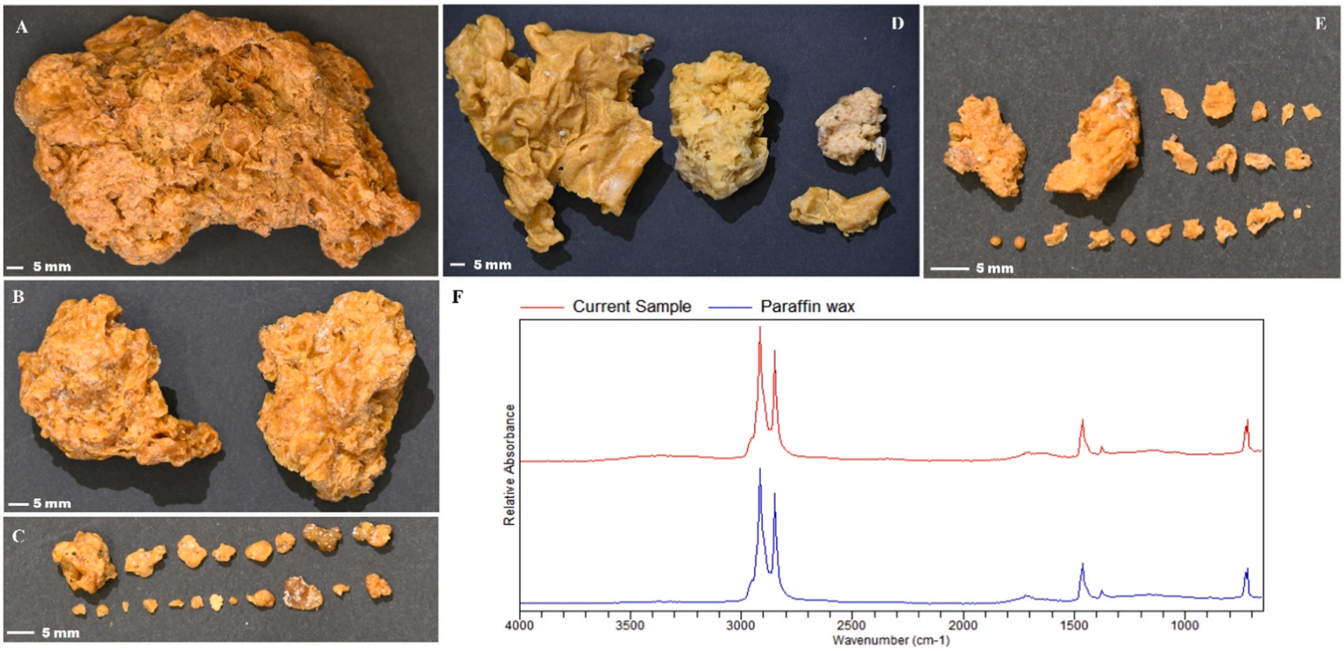


Fig. 2. Examples of paraffin wax macro and microparticles isolated from sand (A-C) and neustonic samples (D-E). Reference spectra (F) obtained by the analysis of larger items found floating at the sea surface and stranded on beaches matched with spectra obtained by the Agilent library database.

and sand samples, were obtained by performing several analyses of the external and internal sections of larger wax residues collected floating at the sea surface and beached during the sampling campaigns (Fig. 2 A-E). The obtained spectra were compared to different spectral library databases provided by Agilent and personally created, confirming the paraffin-polyethylene origin of the analysed material. Thus, for the chemical composition evaluation of collected particles, a similarity index based on the matching percentage with previously acquired reference spectra was calculated followed by a visual analysis comparison of characteristic bands in the reference spectrum. Components with match $\geq$ 80% are considered acceptable [3].

2.4. Statistical and spatial analysis

Wax particle concentration data were imported and processed using the Quantum GIS platform (version 3.10.1 A Coruna), and Rstudio (version 1.1.4.1106) to perform spatial and statistical analysis, respectively. Descriptive statistics and normality tests (Shapiro-Wilk normality test) were performed to examine the parametric or non-parametric statistical analyses to be used. A Spearman's rank correlation test was performed between macro and micro wax particle concentrations to evaluate a statistically common distribution pattern. Concentration areas have been identified by Kernel analysis, where H50 and H95 density contours, referring respectively to the 50% and 95% of overall wax items densities, have been identified as the core (H50) and range (H95) distribution. Hotspots, as well as coldspots, were characterised by the Getis-Ord Gi*analysis [16], allowing the identification of areas where particles of paraffin wax were clustered, compared to the overall sampled area.

3. Results and discussions

3.1. ATR-FTIR chemical composition determination

The chemical composition of 2400 particles was evaluated to confirm their paraffinic-polyethylene origin using the ATR Fourier transform spectrometry technique. All the particles collected on beaches (n = 219; Fig. 2 A-C) and isolated from neustonic samples (n = 2181; Fig. 2 D-E) were characterized as paraffinic polyethylene waxes, confirming the previous results presented by Suaria et al. [35] in the same area and providing contextual elements to the present study. No differences were found between the spectra obtained from floated and beached particles (Fig, 2 F). Comparing these spectra with those chemically described in detail by [35] and [26], appears clear how the paraffin waxes detected in the Mediterranean Sea are very similar to those found in the North Sea region (category 2 in [26]), often found after their uncontrolled discharge at sea by cargo and tank vessels. The top bands around 2900 cm^{-1} and 2840 cm^{-1} are shown as well as the secondary bands around 1460 cm^{-1} and 700 cm^{-1}.

3.2. Floating paraffin wax presence and spatial distribution

During the sampling campaigns carried out in the Pelagos Sanctuary in 2019, different concentrations of yellow-coloured wax residues were found floating in the surface water and stranded on the Tuscan coast. Their presence was detected in 22% of the macrolitter monitoring transects performed with a total of 187 macro residues floating in the Ligurian Sea and corresponding to an average total concentration of 29.8 $\pm$ 87.1 items/km^2. In the Tuscan Archipelago National Park, the highest concentration was found floating in the facing waters of the island of Gorgona with a mean of 17 $\pm$ 31 items/km^2 (Fig. 3A). Approximately 80% of the residues isolated had a size between 2.5 cm and 5 cm, and any items larger than 30 cm were found.

Focussing on wax microparticle abundance, their presence was detected in 62% of neustonic samples collected with the manta net accounting for a total of 2181 wax items (<25 mm). No difference in terms of the average concentration of these particles was found between the Tuscan Archipelago National Park (12,911 $\pm$ 45,171 items/km^2) and the SPAMI Pelagos Sanctuary waters (12,363 $\pm$ 20,602 items/km^2) (Fig. 3B). The majority of particles isolated (90%) belonged to the microlitter category (<5 mm), while mesolitter (5 – 25 mm) represented only 10% of items described. The highest concentration was found in the size class ranging between 1 and 2.5 mm (54%) confirming how in the Mediterranean Sea [15,36,6] and in the Pelagos Sanctuary [11,14,3] this dimensional range constitutes the mostly found and potentially the most available for marine organisms being in the same dimension of

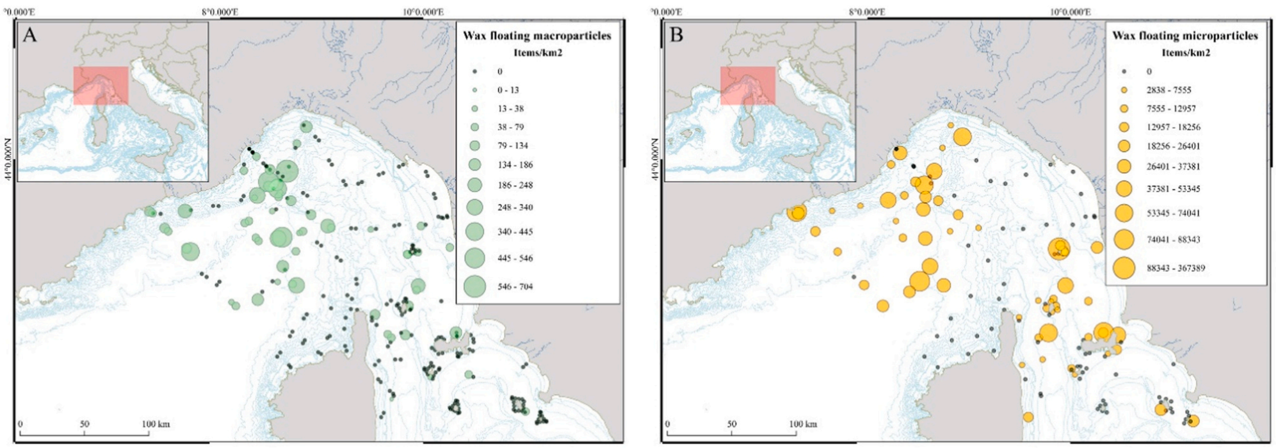


Fig. 3. The concentration of paraffin wax macroparticles (A) and microparticles (B) detected on the sea surface within the SPAMI Pelagos Sanctuary and the Tuscan Archipelago National Park.

several zooplankton preys [31]. The lowest abundance (6%; n. 122 items) was represented by particles between 300 µm and 1 mm.

In the Mediterranean Sea, the presence of wax is rarely reported and information on the physical and chemical characterization of this material is fragmented. Its presence was first detected in the Adriatic Sea in 2013 [36] and in 2018 [40,43]. During the summer of 2017, a massive accumulation event was described in the SPAMI Pelagos Sanctuary (Ligurian Sea), with more than 350 kg of yellow wax recovered in the surface waters off the northern side of Elba Island [35]. Information on the presence of waxes floating at the sea surface was reported also in the North Sea by the German Federal Maritime and Hydrographic Agency isolating particles in 24 out of 33 trawls carried out and by the study of Lorenz et al. [26] in the southern sector of this basin reporting a concentration ranging from 0.82 to 3.66 items/m^3.

A significant strong correlation (p-value $<$5.9e-9, r = 0.47) (Fig. S1) was found between the spatial concentrations of macro and micro wax particles, confirming the effectiveness of the simultaneous sampling performed and highlighting the common behaviour of litter objects to accumulate in the same areas as already reported by [14]. These data were confirmed also by the spatial analysis evidencing a common general patterns of distribution with some differences in the hotspot accumulation areas according to wax dimension (macroparticles *vs.* microparticles) (Fig. 4A and B). For the items larger than 2.5 cm, the core distribution area (H50) was located in the Genoa Canyon area, including the Janua seamount (44 ± 106 items/km^2) (Fig. 4A). A smaller but distinct core area was found also in the canyon off Nice

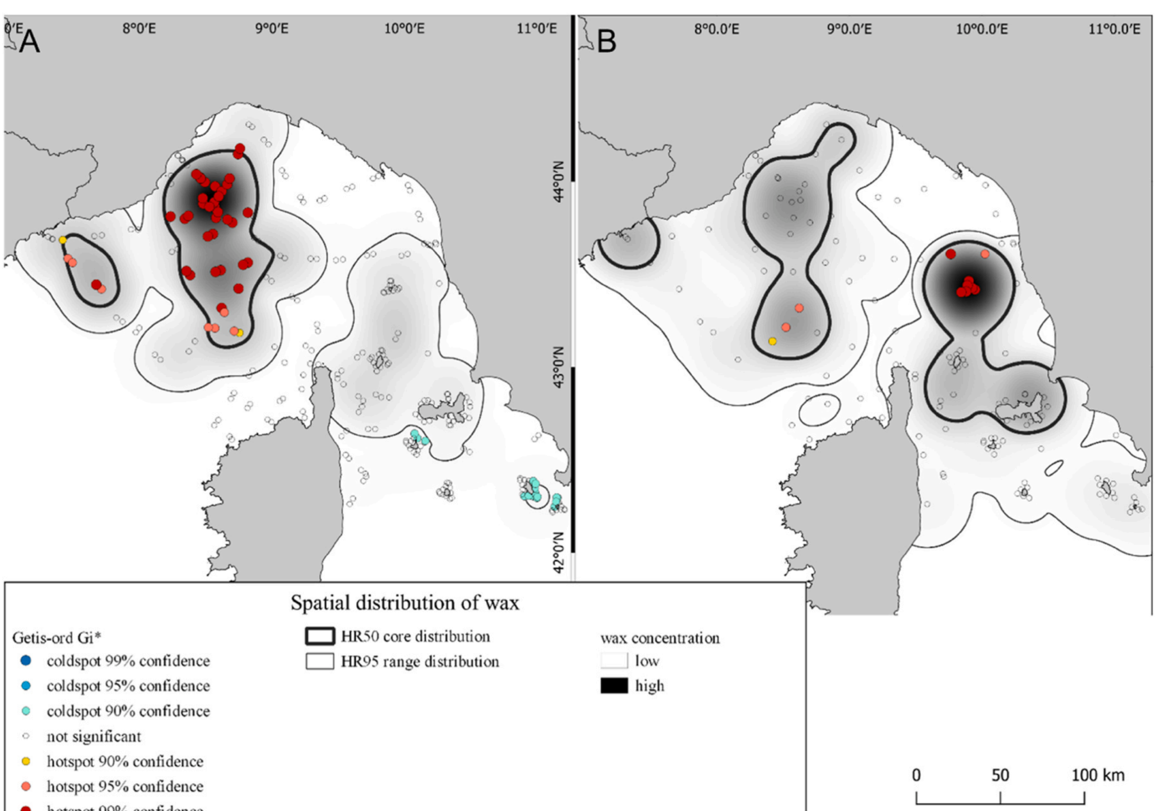


Fig. 4. Spatial analysis of wax particle distribution: macroparticles (A) and microparticles (B), and identification of hotspots.

(France). Both areas were confirmed as accumulation hotspots, while no statistically significant coldspots were evidenced by the Getis-Ord Gi* analysis. Regarding wax microparticles, their range distribution includes almost the entire study area, confirming the high dispersion level of smaller items in the marine environment. The Genoa Canyon and Nice area were confirmed again as core distribution areas (Fig. 4B), with the first one being enlarged southern reaching the deepest portion of the basin and the northern Corsica. An additional core area is evidenced in the Tuscan Archipelago National Park, specifically including Elba, Capraia, and Gorgona Islands (Fig. 4B).

Hotspot analysis confirmed the presence of an accumulation area in the southern part of the Genova canyon system as well as a distinct hotspot in this second core area, specifically around Gorgona Island. The wax average concentration here measured was five times higher (68,676 items/km^2 vs. 12,586 items/km^2) than the average concentration measured in the whole study area. Already highlighted as a site of transient accumulation of MPs, this area of the Ligurian Sea was also identified by Suaria et al. [35] as particularly affected by the presence of wax and also confirmed by the data of floating litter shown in Galli et al. [14].

3.3. Beached paraffin wax presence and spatial distribution

Residues of these petroleum materials (n = 219 in total) were also found stranded along the Tuscan coast and on four out of the five islands of the Tuscan Archipelago monitored (Elba, Pianosa, Montecristo and Giglio). The highest concentration of wax residues larger than 25 mm (58 in total) was found in the northernmost and southernmost beaches monitored along the Tuscan coast (Vecchiano and Burano), accounting for 11 items/100 m (Fig. 5A). Their presence was exclusively found on most pelagic islands of the Tuscan Archipelago National Park, Pianosa and Montecristo, ranging from 4 - 5 items/100 m, respectively. The presence of wax residues in these areas seems connected exclusively with the influence of currents and other hydrodynamic features determining their transport, distribution and accumulation after their discharge at sea. This behaviour appears clearer focusing on smaller items (n = 161 in total), ranging mainly from 1 and 5 mm (68%) in size. The highest abundance of these particles was found exactly on the beach of Cala Giovanna on the island of Pianosa (110 items/m^2) (Fig. 5B). This area has been already described as particularly affected by massive strandings of floating microplastic particles [14]. The total absence of paraffin waxes on the island of Capraia, despite the core area of distribution evidenced by the spatial analysis, may be due to superficial current systems facilitating their accumulation in the foreshore waters rather than the strandings and also be connected with the physical characteristics of its rocky cliffed coasts and the presence of fewer pebbles beaches that may hamper the litter strandings and influence the sampling of this material.

Regarding potential differences in the wax abundance according to seasons, no clear pattern of accumulation was found among the sites monitored. The highest number of beached wax macro particles were recorded during the autumn and winter seasons in 2019 (3 ± 5 items/100 m) (Fig. S2), while the higher concentrations of smaller particles were detected during the sampling activities performed in the winter and spring seasons (4 ± 6 items/m^2) (Fig. S3). Exceptionally high abundances were found in the summer of 2020 on Pianosa Island (80 items/m^2).

The presence of paraffin waxes stranded on beaches was widely reported in the North Sea region, affecting several countries such as Denmark, Sweden, France, Germany, Belgium and the Netherlands. Data from the OSPAR beach litter database between 2001 and 2016 confirm the presence of paraffin pieces in 371 out of 2824 litter surveys performed on 151 different beaches, with a mean estimated frequency of 14.6 items/m of beach line (maximum 738 items/m). The presence of this material was also detected on Lithuanian beaches, accounting for 63% of all isolated litter [18], and in the Russian Baltic Sea as aggregates with microplastics showing an average concentration of 31.1 ± 18.8 particles per sample or 11,479 ± 10,785 items per kg of wax [9].

In the Mediterranean Sea, the only data reporting the stranding of paraffin waxes focused in the Ligurian Sea, and in the SPAMI Pelagos Sanctuary. An exceptional wax strandings, along a 200 km stretch of Tuscan coastline, in an area well described to be affected by litter accumulation [28,33], was reported in 2018 by Suaria and coworkers, with average densities of 15 kg/m^2 and 16,400 fragments/m^2, mainly with diameters between 5 and 30 mm. These results highlighted the urgent need to deeply assess the ecological risk connected with this underestimated threat, especially in marine protected areas.

3.4. Paraffin wax potential sources of pollution and relative ecological implications

The spatial distribution of paraffin waxes described and their prolonged residence time in the marine environment (5 months after the stranding event described by [35], many wax particles are still present) may influence the direct ingestion by marine organisms and the potential desorption of additives, dyes and other compounds (volatile organic and inorganics contaminants). It is widely known that different PAHs, classified as carcinogenic by the European Union, have been detected in wax residues [7,37] as well as some chlorinated cleaning solvents used in the shipping industry such as perchloroethylene or trichloroethylene [34]. The ingestion of wax particles has been reported in the stomach contents of northern fulmars (*Fulmarus glacialis*) [2,39],

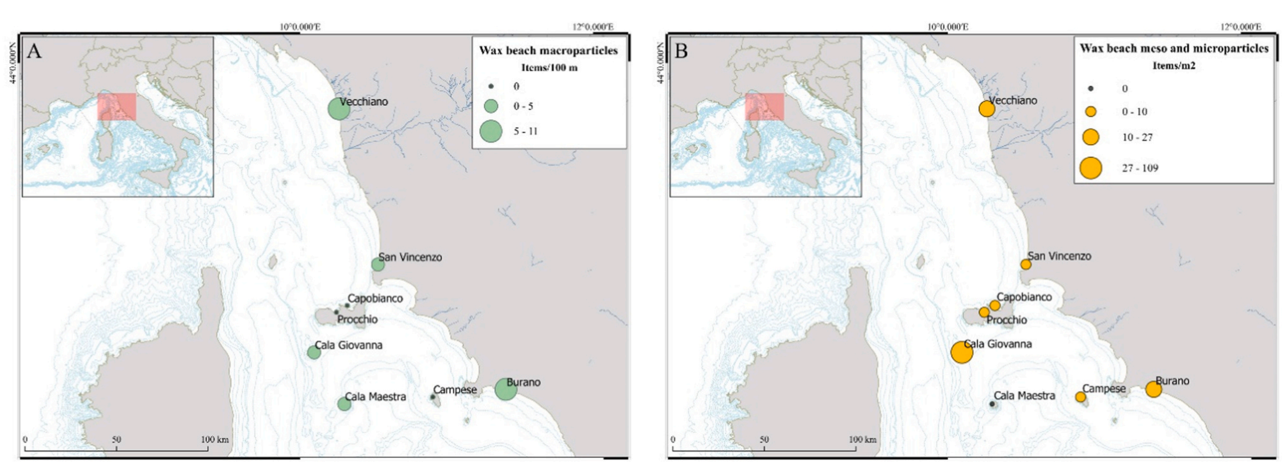


Fig. 5. The total mean concentration of paraffin waxes macroparticles (A) and microparticles (B) isolated from beach sediments within the SPAMI Pelagos Sanctuary and the Tuscan Archipelago National Park.

in regurgitates from Black Legged Kittiwakes (*Rissa tridactyla*) and Great Cormorants (*Phalacrocorax carbo*) in Ireland [1] and in a post-hatchling loggerhead turtle *(Caretta caretta*) on a South African beach [32]. However, many aspects of the ecotoxicity connected with this material are still unknown, and the knowledge on this issue is limited only to preliminary theoretical considerations [23]. The most likely route of exposure to paraffin waxes and the associated release of chemicals is the direct contact with respiratory surfaces and absorptive gut epithelium once ingested. To date, only the study of Nunes et al. [30], attempts to evaluate the ecotoxicity of paraffin microbeads exposing the filter-feeding organism *Mytilus galloprovincialis*. Although ingestion was confirmed, biochemical effects were not as toxicologically significant for the biomarkers considered involving the measurements of the activities of four enzymes regulating important cellular processes (e.g., antioxidant defence, glutathione reductase and peroxidase, and phase II metabolism). Nevertheless, the potential ecological risk connected with this material itself and acting as a combined stressor with the simultaneous presence of floating plastic, exacerbating their physical and chemical impacts on marine organisms, urgently requires the acquisition of information on possible sources of contamination to define effective mitigation actions.

According to that, the high number of vessels moving towards the port of Genova and Livorno, exceeding respectively the 1000 and 600 units from 2018–2020 [8]; https://www.emodnet.eu/), may represent a potential source of pollution of this material (Fig. 6).

The presence of floating waxes sighted during the macrolitter monitoring transects seems to be linked with areas where tankers are mainly stationary, as shown in Fig. 4. Core distribution areas coinciding with the Genoa Canyons system overlap areas where tanker density is higher (>1000 h per square kilometre per season) (EMODnet Human activities portal; https://www.emodnet.eu/) and above all not linked

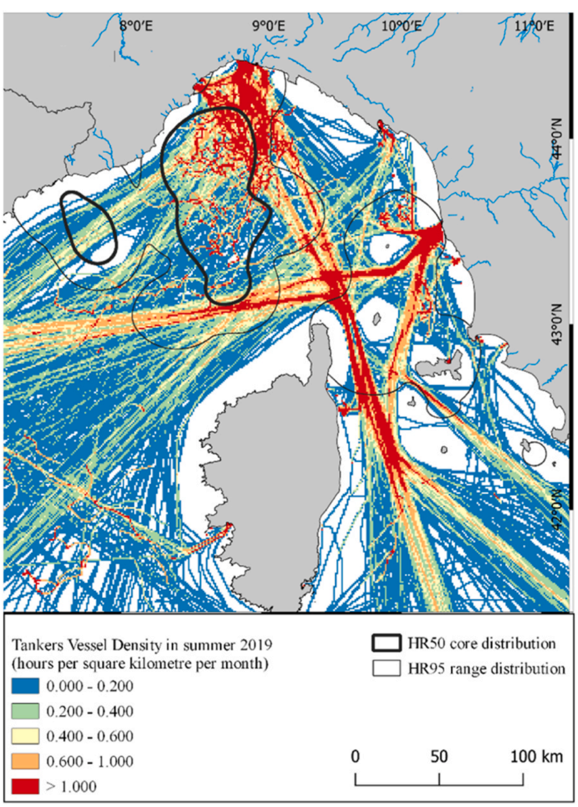


Fig. 6. Overlap of core (H50) and general (H95) distribution of wax sighted during macrolitter transects with tanker vessel density (hours per square kilometre per month, cumulated for summer 2019). Data
Source: EMODnet Human activities portal; https://www.emodnet.eu/.

with specific corridors. The lack of linear patterns can be an index of the stationary presence of tankers and may indicate a potential discharge into the sea during tank cleaning or ballast unloading. On the other hand, in areas where tanker presence is not allowed, such as in the proximity of islands of the Tuscany Archipelago, wax macroparticles were mainly absent evidencing also some coldspots (Fig. 6). Despite the more dispersed distribution of wax microparticles, the accumulation hotspot found in the facing waters of the Gorgona Island, about 18 nautical miles westward from the Livorno harbour, may be related to the higher tanker density that characterised this area (>1000 h per square kilometre per season) (EMODnet Human activities portal; https://www.emodnet.eu/) and significantly affected by the transport of litter by the superficial currents affecting it during the summer months, already described as a transient zone of plastic accumulation [11,14,36].

4. Conclusions

Despite the recent amendments to the regulation made by IMO in 2019 that limited the discharge of paraffin wax at sea, the paucity of data available in the literature and the lack of clear definition, identification and characterization of this pollutant highlight the need to improve and standardise monitoring protocols addressing its ecological impacts. Even if the ecotoxicity of paraffin waxes is mainly still unknown, their chemical-physical characteristic, routes of exposure related to direct ingestion by organisms and/or indirect chemicals leaching and the potential combined impacts with plastic and microplastics make clear the potential ecological risk connected with this material. Within MSFD, solid paraffin and wax have now been included in both the updated Joint list concerning the litter items to be considered for monitoring beaches and the list regulating the ingested litter by biota, but these residues are not considered by protocols and monitoring guidelines for floating items, however. As a consequence, the monitoring of changes within time and for assessing the efficiency of measures may be limited to these components of the marine environment. In the Mediterranean region, especially in protected areas such as the Pelagos Sanctuary where discharge restrictions are not regulated, an impact of both the IMO regulation and MSFD is expected, noting that UNEP MAP has included solid paraffin in its list of items for riverine inputs and surface waters. The results achieved by this study provide a basis for future assessments of floating marine litter made of wax and paraffin, in a complex and highly ecologically valuable area. The information on the relationship between main tanker density routes and wax distribution reported, suggests a potential discharge of these residues by vessels in the offshore waters facing the Genova and Livorno harbours posing a threatening risk for marine organisms inhabiting these areas. Finally, the distribution patterns of the macro and microparticles of waxes detected, and above all the more widespread presence in the environment of the microlitter component, suggest the need to identify possible sources where to address specific policies strengthen the limitation to their uncontrolled discharge facilitating their distribution trough the sea currents and reducing the associated risks deriving from the presence of paraffin wax in the marine realms.

Environmental Implication

This study provides a comprehensive assessment of the quantity and composition of paraffin wax particles in areas of high ecological value in the Mediterranean Sea. The relationship between wax distribution and tanker density routes suggests the potential discharge of polyolefin residues from ships in offshore waters. The information obtained provides an affordable basis for the implementation of effective measures for the prevention and reduction of paraffin wax pollution. The relevant information obtained represents an affordable basis for future toxicological studies aimed at assessing the impact on organisms living in Mediterranean protected areas, which represents an essential gap to be filled.

CRediT authorship contribution statement

M. Galli: Formal analysis, Investigation, Visualization, Writing – original draft, Writing – review & editing, Data curation. **M. Baini:** Conceptualization, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **C. Panti:** Conceptualization, Investigation, Methodology, Resources, Writing – original draft, Writing – review & editing. **P. Tepsich:** Formal analysis, Investigation, Visualization, Writing – original draft, Writing – review & editing. **M. Rosso:** Investigation, Visualization. **F. Giannini:** Investigation, Visualization. **F. Galgani:** Investigation, Writing – original draft, Writing – review & editing. **M.C. Fossi:** Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – original draft, Writing – review & editing.

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.

Data Availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2024.133677.

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