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Classification Study of Ingested Plastic Particles in Marine Organisms using Electron Microscope: A Case Study of Cameroon Beaches

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ABSTRACT

Plastic wastes are non-biodegradable whose fragmentation often leads to the formation of micro plastics. These are likely to be ingested by marine organisms with grave consequences to human health. A plastic waste classification study was carried out using (Pseudotolithus sp and E. fimbriata) to determine the quantity and quality of ingested plastic particles. It is on this basis that a laboratory analysis was carried out using an electron microscope. From a qualitative and quantitative point of view, plastic debris ingested by organisms (Pseudotolithus sp and E. fimbriata) was classified into five groups: (a) fishing lines (47%), (b) plastic particles (9%), (c) cord filaments (23%) thongs (13 %), and other particles (8%). The results from this study will certainly enhance policy development where protein from fish is considered the ultimate source for protein for humankind, not forgetting fish pollution which is a common cry to communities that depend on fish for their livelihoods and as an ideal source of protein.

Keywords: *Plastic Waste Classification, Marine Pollution, Marine Environment, Human Health.*

1. INTRODUCTION

In the environment, plastic pollution can have several consequences. Aside from the visual pollution they cause, plastics affect marine organisms directly or indirectly at different levels of the food chain [1]. Chemically, plastics are made up of chains of identical sequences (or polymers) of carbon molecules, mainly hydrocarbons, organic molecules toxic to many organisms, liable to accumulate along food chains. In areas of accumulation, the concentration of microplastics observed (0.5 to 5mm in size) is comparable to that of zooplankton (between 0.005 mm and more than 50 mm).The Mediterranean, for example, has microplastic / zooplankton ratios between 1/10 to ½ [2].

The risk for zooplankton predators (i.e. fish) to ingest microplastic is therefore considerable. The residence time of plastic in small pelagic fish is estimated to be between 1 day and 1 year [3]. The fragments of ingested microplastics are found in animal droppings, they can sink with corpses or be transferred to predators and thus reach the upper echelons of the food chain [4]. Plastics are also vectors of dispersal of toxic compounds which can also accumulate in food chains. These compounds can be directly present in the composition of plastics, or else adsorb on their surface. In the first case, they are additives (phthalates, biphenyls) incorporated into certain plastics to increase their resistance. Various studies have shown that these compounds can be toxic to certain animals and humans [5].

Other toxic compounds (hydrocarbons, pesticides, DDT, PCB) can be adsorbed to plastics, which is likely to increase their dispersion, persistence at sea and accumulation in the highest trophic levels [6]. If plastic debris in the environment arouses so much interest, it is mainly because there are many known or suspected impacts.The most obvious [7] are the visual impacts, negative for tourism for example, boating accidents involving large plastic objects (buoys or drift nets) are also more and more numerous. The physical impacts on fauna have been observed for many years, for example in the albatross [8]. Many other species are concerned: [9] list 250 species in which strangulations or ingestions of plastic causing suffocation or obstruction of the digestive tracts have been observed, ranging from penguins to whales, including fish, crustaceans and various birds.

The disastrous effects of ingesting plastic debris mistaken for prey are also well documented, with consequences for the digestive systems of animals such as fish, birds, sea turtles and marine mammals, which can lead to their death [10]. This debris is also considered as a vector for dispersing toxic algae and pathogenic microorganisms [11].

2 MATERIAL AND METHODS

2.1 Presentation of the Study area

The study took place from July 13 to December 5, 2016 at the Specialized Center for Research on Marine Ecosystems (CERECOMA-IRAD of Kribi), located in the Southern Region of Cameroon.

The Kribi continental shelf is approximately 10,600 km^2. In the east of the region, the highest relief reaches 300m. There is an alternation of sandy beaches and metamorphic rocky outcrops, frequently opened by silted estuaries. The relief of the continental shelf is rugged due to rocky banks and sand mounds [12]. Typically, soils in Kribi have an acidic pH, in the order of 4.1. The permeability is high at the surface and decreases with depth, and the organic matter content is of the order of 2%. The variations in the physiognomy of the profiles, in particular with regard to the presence of coarse elements (gravel, ferralitic concretions, blocks of rock) suggests the existence of two types of soil: ocher soils (without coarse elements) and gravelly soils [13].

The climate encountered in the southern region is equatorial, subject to marine influence and the dynamics of the inter tropical fronts receives an average of 2900mm of rain in 204 days. There are four seasons: a large rainy season from mid-August to November, a small rainy season from March to June, a large dry season from December to mid-March and a small dry season from June to mid-August [14]. The average temperature in the South is around 25 ° C; this value can rise to 28 ° C in the dry season with a peak at 31 ° C in March. Winds are weak (0.5 to 2m / s).The hydrographic network is dense, with catchments of small rivers (Table I), rocky creeks and rapids and small waterfalls.

Table I: Characteristics of the main rivers of the coastal region of Kribi (Adapted from) [15].

River	Lenght (Km)	Watershed (Km²)	Average flow (m²/s)	Average flow (m^3/s)
Nyong	800	14000	$2,8*10^9$	376
Lokoundjé	216	1150	28,2	118
Kienké	130	1435	49,2	177
Lobe	130	2305	102	390

Source:(modified)

Several types of vegetation are identified on the south coast; they alone are home to more than 1,500 plant species divided into 640 genera and 141 families. The flora is influenced by the climate and we observe a grouping in herbaceous layer, shrub layer and littoral forest. In addition to the six mangrove species present in the Kribi-Campo area, there are also coastal border forests or forests of Avicenniacae, Caesal pinioidae rich in Socoglotis gabonensis, Hibiscus escalentus (Gombo), Dalbergia acastaphyllum, Drepanocarpus lunatus and Arécacae sp (palm trees, coconut trees) [16].

The fauna is very rich and varied depending on the space. This is how we meet terrestrial, aerial and aquatic fauna. The terrestrial fauna consists of mammals, hedgehogs and reptiles; the avian fauna is composed of birds, insects especially bees and the aquatic fauna is mainly composed of molluscs, shrimps, crabs and fish and occasionally sea turtles [12].

2.2 Marine organisms

Ingestion of micro plastics by various marine vertebrates and invertebrates in laboratory and field conditions has been reported in the literature [17]. Sampling strategies are numerous and highly dependent on the target organisms, they only give an overview of the control organizations for the ingestion of micro plastics with a focus on the field sampling [1; 18]. Laboratories studying the ingestion of micro plastics by organisms frequently use microscopic plastic beads of known polymer origin, which can be easily identified and counted under the microscope in the contents and excretions of the gut or in the case of the organisms transparent planktonic cells or in the organism itself [19;20]. After dissection, the stomach contents or the entire digestive tract was stored or frozen for later analysis.

2.3 Sediments and beaches

Detected worldwide on beaches and in subtidal sediments (Table 2), the extraction method used by the majority of authors was developed [21]. This technique, which is currently the most widely used, relies on the density of a concentrated solution of NaCl (1.2 kg L-1) to separate the sediment from the particles of micro plastics. This is because when this salt solution is added to the sediment sample, the low density micro particles float to the surface. However, this method is only effective for polymers with a

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density lower than that of the saturated salt concentration, i.e. 1.2 g cm^3, and not suitable for the extraction of high density polymers. Plastics such as polyvinyl chloride (density 1.14–1.56 g cm3) or polyethylene terephthalate (density 1.32–1.41 g cm^3) will not float in the concentrated NaCl solution. These two polymers, however, account for 18% of European plastic demand [22] representing a significant proportion of current micro plastics in the marine environment. Particularly in marine sediments, the proportion of these high density plastics may be higher: due to their high density, these types of plastics will tend to sink more easily than lighter plastics.

Table2: Maximum concentration of micro plastics found in sediment [27]

Country	Location	Max concentration	Unit	Reference
India	Ship-breaking yard	89	mg kg[1]	[23]
UK	Beach[a]	9	mg kg[1b]	[21]
UK	Estuarine[a]	35	mg kg[1b]	[21]
UK	Subtidal[a]	86	mg kg[1]	[21]
Singapore	Beach	16	mg kg[1b]	[24]
UK	Sewage disposal site	15	mg kg[1]	[25]
Belgium	Harbour	391	mg kg[1]	[26]
Belgium	Continental shelf	116	mg kg[1]	[26]
Belgium	beach	156	mg kg[1]	[26]

a Only fiber concentrations were reported.
b Original unit (number of fiber 50 mL1 sediment) converted using an average sediment density of 1600 kg m^3 [28] and 1.25 as average wet sediment/dry sediment ratio.

2.4 Water surface

Due to their relatively low concentrations in the sample, sampling of micro plastic particles generally requires large volumes of control water. Thus, samples from open water are usually taken with plankton nets of different mesh sizes. The sea surface is sampled for micro plastics floating by the Manta trawl net [29] or neuston fillets [30]. While neuston catamarans (fig. 2a) can be operated even in higher waves, a manta trawl (fig. 2b) is used in calm waters. The volume filtered by a net is usually recorded by a flowmeter mounted at the opening, allowing the normalization of the filtered volume and thus a calculation of the concentrations of microplastics (particles / g) per unit volume of water. The relation of concentrations to the area sampled is also possible by multiplying the trawl distance by the horizontal width of the opening. Trawl speed depends on weather conditions and currents, but usually ranges between 1 and 5 knots. The plankton sample is concentrated in the snare at the end of the net, the latter must be thoroughly rinsed from the outside to ensure that all plankton and debris are washed and concentrated in the snare [29]. The contents of the collar are finally transferred to a control container and fixed with plastic friendly fixatives (e.g. formalin) or stored frozen. If the particles are taken out directly, they are dried and kept in the dark before analysis [31].

The size of the particles maintained and also the filterable volume is a direct consequence of the mesh used. The meshes used for sampling in previous studies have changed between 50 µm and 3000 µm [31]. Another factor influencing the filtered volume is the size of the net, i.e. the sector, which acts as a filter. Seasons with tides or red flowers of plankton and jellyfish are generally unfavorable for the withdrawal of large volumes of water. Nets are usually 3–4.5 m long and a mesh size of around 300 µm is most commonly used. These nets do not collect micro plastic particles <300 µm quantitatively but take into account the withdrawal of larger volumes of water. The non-standardized use of different nets and meshes seriously impedes the comparability of data on pelagic micro plastic concentrations [32]. Besides common net sampling, other techniques are occasionally used to assess microplastic concentrations in the water column: bulk sampling with sequenced filtration [24; 33]. Continuous Plankton Recorder (CPR) screening [21], or using direct in situ filtration [34].

A highly promising technique, currently under development, is the use of the direct fractional pressure filter of large (> 1 m^3) water volumes by a filter cascade (developed by Technologie GmbH of -4h-jena). This approach theoretically takes into account the simultaneous removal of different size fractions of microplastics downwards and thus allows a more complete resolution of the size spectrum.

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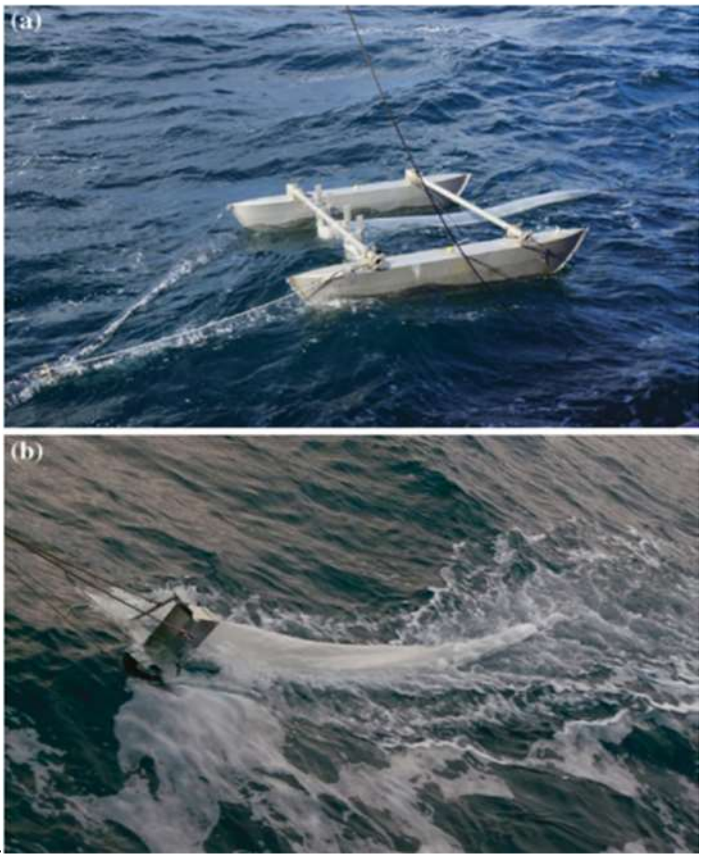

Figure 2: Catamaran neuston (a) Manta (b) [20].

2.5 Quantity of micro plastics in marine biota

Marine organisms are known to ingest micro plastic particles. A good part of the commercialized marine catches (fish, bivalves, crustaceans, etc.) are known to contain particles of micro plastics, with several possible routes via the mouth for example and therefore the digestive system or via the gills [35].

2.5.1 Bivalves

Bivalves such as mussels are suspension feeders that can filter about 2 liters of seawater per hour [36] and therefore, it is not surprising that they contain particles of micro plastics. Cultured mussels showed lower proportions of micro plastics than wild mussels when collected, and a large part of these contaminants consist of plastic cord [37].

Another study of blue mussels cultivated in Germany (the North Sea) and oysters cultivated from Brittany, France (North Atlantic), showed that both species contained micro plastics, i.e. 0.36 ± 0.07 particles / g-1 (wet weight) and 0.47 ± 0.16 particles / g-1 (wet weight) respectively [38]. The significance of micro plastic pollution on seafood safety is not known, although it is important to note that the concentrations determined in mussels and cultivated oysters are relatively low [38].

2.5.2 Crustaceans

Barnacles (crustaceans) which are sessile marine organisms also living in the intertidal zone. are like mussels, they are suspensivores and therefore it is not surprising that 33.5% of North Pacific barnacles contain particles of micro plastics. The average number of microplastic particles is typically between 1 and 30 [39]. In Clyde Bay (west coast of Scotland) 83% of Nephrops norvegicus found were contaminated with micro plastic particles and 62% of these particles were present as tightly entangled beads (Figure 3) [40].

Langoustines are omnivorous and therefore consume a lot of different benthic fauna such as crustaceans, polychaetes, bivalves and the ingestion of the plastic particles is likely to be due via the food or passively from the sediment when 'they feed [40].

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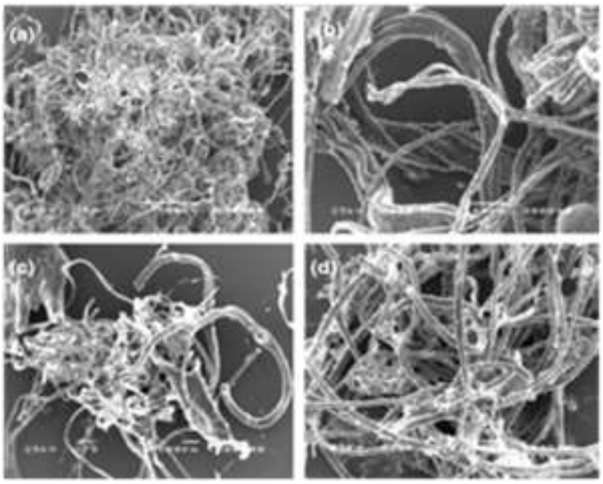

Figure 3: Image of micro plastics in N norvegicus by scanning electron microscopy [40].

2.5.3 Fish

While the first ingestion of plastic by fish was reported by [41], research on this topic is recent. According to several studies, the types of plastics ingested differ according to species and regions [42]. Fish from the English Channel appear to be exposed, but this has only been confirmed by three studies focused on seas in northern Europe [43; 17; 44]. In the North Sea, [43] reported that 2.6% of the fish collected contained plastic in their stomach.

In another report, [17] highlighted that the majority of polymer particles were fibers. Of the 351 pieces of polymer found, 58% were radiated. Another study indicating plastic ingestion by fish in northern Europe found 21 plastics in 290 fish [44], Polyethylene was the most registered polymer.

In the Mediterranean Sea, elasmobranchs appeared to be more exposed than the teleost species [45]. 3% of elasmobranchs ingested plastic debris, which accounts for 86.5% of all ingested debris. Other studies in the Mediterranean Sea reporedt a higher percentage of ingestion [45; 46; 47; 48; 46]. All three species ingested micro and macro-plastics with a similar color tendency: transparent, white, yellowish and blue particles being preferentially ingested.

In another study, [47] reported an ingestion frequency of 24% in Trachinotus ovatus and the same color trend was found. The highest frequency of ingestion occurred in Boops boops (58%) but no dominant color was reported [48].

2.6 Methods of isolating micro plastics

Majority of the methods have been examined and validated on mussel tissues or stomach contents of fish (Table 3). However, few studies employ chemical methods to isolate micro plastic particles. The most common isolation method is the digestion of organic matter with a 10% solution of potassium hydroxide (KOH) first described by [43].

Table 3: Summary of methods for isolating plastics from biological samples

Samples	Chemical	Advantages	Pitfalls	References
Mussel soft tissues	HNO_3	Cheap and quick	Polyamide fibers not recovered, tested by [51].with low recovery	[27].
Mussel soft tissues	HNO_3:$HCLO_4$ (4 :1 v :v)	Cheap and quick	No information available on the resistance of plastic polymers	[49].
Plankton	Enzyme (Proteinase-K)	Cheap and quick	Expensive	[50].
Stomach content fish	Rose bengal stain	Cheap	Based on visual sorting	[3].
Stomach content fish	KOH	Cheap	Time consuming, polycarbonate and polyamide not resistant	[43].
Stomach content fish	NaCl (density gradient separation) and H_2O_2	Cheap and quick	Validated on 2 polymers	[51].

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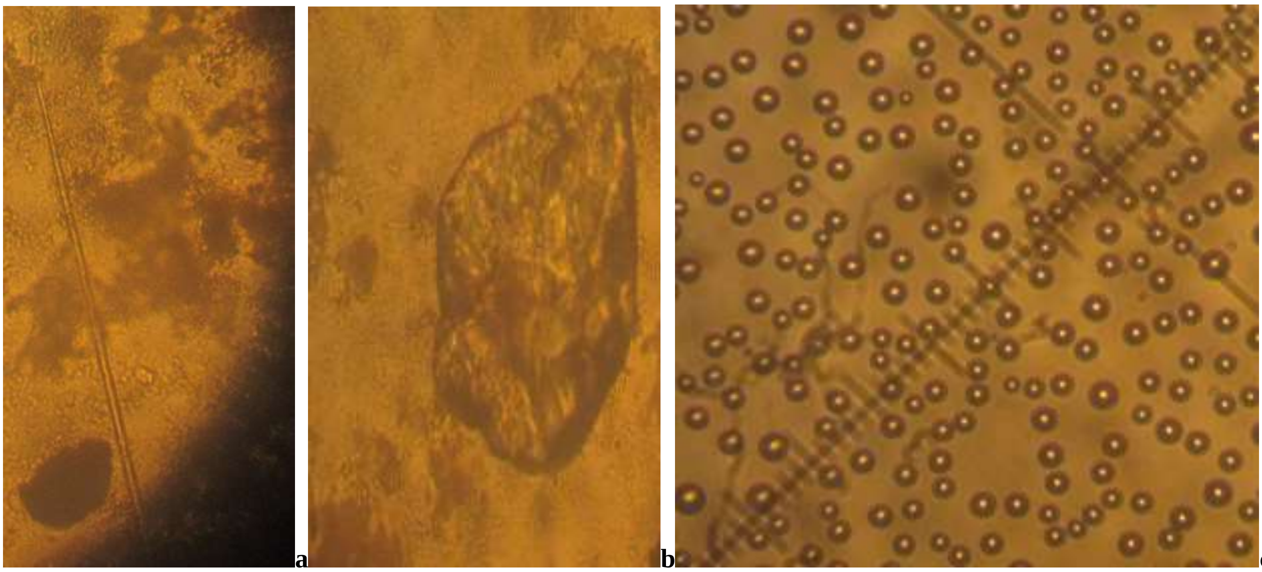
3. RESULTS AND DISCUSSION

From a qualitative and quantitative point of view, and using a scanning electron microscope, plastic debris ingested by organisms (Pseudotolithus sp and E. fimbriata) were classified into five groups: (a) fishing lines (47%), (b) plastic particles (9%), (c) cord filaments (23%) (d) thongs (13 %), and (e) other particles (8%) (Figure 4).The result is in line with [52], who used two species of coral trout as one single group for data analyses, given their similarities in life history, diet and feeding strategies.



a b c

Figure 4 :Micro plastics found in the samples (a) fishing line, (b) plastic particle, (c) cord filaments

4. CONCLUSION

The present Classification study of Ingested Plastic Particles using electron microscopy in a laboratory data analysis allowed us to obtain information on the quality and quantity of ingested micro plastic particles by two marine species Pseudotolithus sp and E. fimbriata). The results from this study will certainly enhance policy development where protein from fish is considered the ultimate source for protein for humankind, not forgetting fish pollution which is a common cry to communities that depend on fish for their income and livelihoods.

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