Bioaccumulation of microplastics in *Echinolittorina* sp. along intertidal areas of Barangay Buru-un, Iligan City, Philippines

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Abstract. Microplastics are of major environmental concern because they are not biodegradable and can persist in the environment for hundreds of years. These microparticles have now found its way inside the gastrointestinal system of many vertebrate and invertebrate organisms. This study focuses on detection and identification of microplastic ingestion by *Echinolittorina* snails. The two stations established in Barangay Buru-un, Iligan City, Philippines were observed to be littered with plastics and other materials which may also indicate microplastic pollution. The processes involve visual identification for isolation and utilization of Fourier Transform Infrared (FT-IR) analysis for polymer identification. Two isolated materials from the samples were subjected to FTIR testing. The results were compared to the database's accessible spectra and it was found out that only the suspected microfiber sample was identified as a microplastic, where it exhibits a strong spectral similarity to polyethylene. The findings support that microplastics were ingested by the *Echinolittorina* snails. This would imply that microplastics are indeed present in the area and may cause of stress to the marine life and its environment. It is strongly recommended that additional studies be conducted in terms of microplastic abundance in the area that would become supplementary data for establishing proper waste management.
Key Words: FT-IR analysis, gastropod, mollusc, pollution, snails.

Introduction. Plastic pollution is undeniably one of the greatest environmental concerns we have today. Despite the problems these plastics pose, plastic production is still substantially increasing due to its demand in society, especially in various industries (D'Ambrières 2019). Microplastics, by definition, is simply a plastic of a very small size (1 to 5000 µm particles). They are found in diverse forms, including spheres, fragments, and fibers. Due to its small size, microplastic can be unintentionally ingested by organisms, which can lead to ecological impacts on organisms. The irresponsible disposal of these materials may present a huge risk to marine life (Jambeck et al 2015; Rochman et al 2016).

Gastropods are recognized to be one of the most diverse groups of animals, being the largest group of mollusks comprising 80% of extant species (Malaquias & Reid 2008). Gastropods are one of the molluscan classes able to colonize the majority of habitats: marine, freshwater, and terrestrial environments. These animals are well-known, as they have been associated with humans for various uses such as food, tools, ornaments, and much more. To add, they are an important component in aquatic ecosystems worldwide since they are an essential food source for predatory animals and therefore may have an extensive impact on algal primary productivity (Baker et al 2014). Thus, the role of gastropods cannot be ignored especially in the rise of plastic pollution.

The family Littorinidae, commonly known as periwinkle, comprises marine snails that are intertidal or inhabit the littoral region's rocky shore. These snails are small in size and have the habit of grazing algae along the shores. In several locations, periwinkles are used as food (Reid 1989; Duncan 2003).

Dispersive particles such as microplastics can be damaging to an organism's feeding as well as other life processes. This problem of microplastics has caught the awareness of the scientific community and the public (Hale et al 2020; Horton & Barnes 2020). Recent studies mainly involve the effects of microplastics on fishes and small crustaceans (de Sá et al 2018), leaving invertebrates such as gastropods underrepresented. To add, there is insufficient data regarding bioaccumulation of microplastics in marine life published in the Philippines (Abreo 2018). Gastropods such as snails, which are easy prey for larger organisms, are potentially transferred in the food webs, which may bring implications in food safety (Rochman et al 2016). Therefore, there is a need for more studies focusing on the said matter. This study involves collection of intertidal snails on selected areas in Barangay Buru-un, Iligan City, Philippines. Specifically, this study aims to: (1) investigate the type of microplastics detected in the species based on its form by using standard microscopy; and (2), verify whether the isolated materials from the snail are microplastics by utilizing Fourier Transform Infrared Spectroscopy (FT-IR).

The information obtained from this research will raise awareness on the accumulation of microplastics ingested by *Echinolittorina* spp. snails. Additionally, the data in this study will inform locals that the presence of microplastics in this organism may be a potential risk in food safety. Furthermore, one of the significant goals of this study is to open up communication between local stakeholders and other researchers on developing solutions to the problem posed by microplastics in Iligan Bay.

Material and Method

Study area. The research was conducted from February to May 2022 in Barangay Buru-un, Iligan City, Philippines, as the area is recognized by the Bureau of Fisheries and Aquatic Resources (BFAR) to be one of the major fishing grounds in the area known for its abundant marine resources. Buru-un (Figure 1) is one of the coastal barangay of Iligan and is situated along the National Highway.

Figure 1. Map of the study site (A) Iligan City (B) Barangay Buru-un (C) the two established study sites.

The barangay represents 4.64% of the total population of Iligan City as reported by the census back in 2020, and has depended on its rich water source, which resulted in the rise of different livelihoods such as laundry and car wash services (Bersales 2015). Buru-un is situated at approximately 8.1872° latitude, 124.1688° longitude, in the island of Mindanao. Two stations along the coasts were identified for collection of samples. The stations were 1.5 km away from each other, both adjacent to a residential area. Table 1 shows a brief description of the two sites established.

Table 1

Location and description of sampling station

Sampling site	*Station*	*Location (coordinates)*	*Station description*	*Common litter found*
Buru-un	S1	8.187939° latitude, 124.169021° longitude	Adjacent to some commercial establishments and densely populated residential site	Food packaging, clothes, toys.
	S2	8.193964° latitude, 124.183295° longitude	Adjacent to a residential site	Cellophanes, sachets, food packaging.

The first sampling station (Site 1, Figure 2A) is at a shore near the center of barangay Buru-un. The area adjacent to it is covered with densely packed houses and commercial establishments. Few people were observed to be visiting the shore. The second sampling station (Site 2, Figure 2B), though far from the barangay's center, is also adjacent to a residential area. Less people were observed compared to the first station.

Figure 2. The two sample stations; (A) Site 1: shore near Buru-un's Barangay Hall; (B) Site 2: a residential area away from the center of the barangay.

Collection of samples. The needed samples were collected in the study area at around 05:00 in the morning during low tide. Purposive random sampling method was employed in collecting the samples. Thirty (30) snails were collected from each sampling site. The collected samples were preserved in a container filled with fresh water. The samples were identified through morphological characteristics of its shell based on Poppe (2008).

Laboratory analysis of the samples and identification of microplastics. The analysis of microplastics in *Echinolittorina* sp. was performed for each sampling site. Methods used in the study of Fathonia & Patria (2021) were utilized with modifications for the analysis. The process began by subjecting the samples to a container filled with 70% alcohol for further processing. The sea snail's body tissue was removed from its shell using small instruments like tweezers and transferred into two separate beakers intended for each site. Samples were digested using a solution of 10% potassium hydroxide (ratio of snail to KOH 1:5) in a glass beaker, covered with aluminum foil, and was incubated in an oven under 60°C for 24 h, until the sample has completely disintegrated. Immersion in KOH aims to dissolve the soft tissue of the shells, but not dissolve the microplastic present. NaCl was added to the immersed solution until it was saturated. The solution was left out until sediments and other heavy particles settled. The supernatant containing microplastics was collected and filtered using a Whatman glass microfiber filter (47 mm). The filters were left to dry in clean, sealed containers to avoid contaminations, and remaining solutions were removed by subjecting it to heat between 40-70°C.

Following the process, the quantity and types of microplastics should be ascertained. Of the methods utilized, visual identification, can be employed to identify the type of microplastics. Visual identification can be conducted using a stereomicroscope with a 40x magnification. The filter was examined under a microscope to identify and isolate possible microplastics. The suspected microplastics were placed into four groups, namely pellets, fibers, films, and fragments. The suspected microplastics of each type observed were subjected to spectral analysis by using the Fourier Transform Infrared Spectroscopy. This method was used to confirm whether the isolated material from the snail samples is a microplastic. Table 2 shows the types of microplastics classified according to morphology (Lusher et al 2017).

Table 2

Classification of microplastic according to shape

Shape classification	*Other terms used*
Beads	Grains, spherical microbeads, microspheres
Fibers	Filaments, microfibers, strands, threads
Film	Thin, film-like structure
Fragments	Irregular shaped particles, crystals, fluff, powder, granules, shavings
Foams	Polystyrene, Expanded Polystyrene
Pellets	Resin pellets, nurdles, pre-production pellets, nibs

Data analysis. The raw data obtained were analyzed to help identify the type of material isolated from the gastropods. This was done by comparing the procured data from existing data spectra of materials available in Center for Sustainable Polymers in the College of Engineering Technology Building.

Results and Discussion

Microscopic analysis of microplastic. After observation, only two of types possible microplastics were identified. These were the fibers (Figure 3) and fragments (Figure 4). Microfibers comprise most of the suspected microplastic materials.

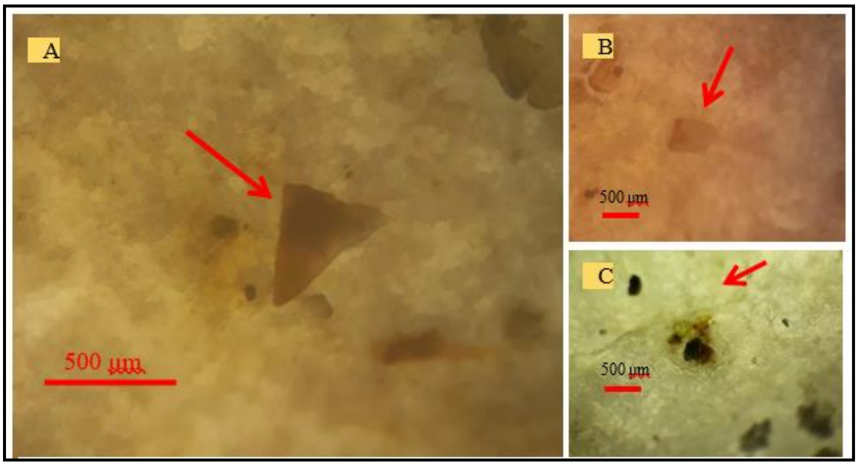

Figure 3. Suspected microplastic fragments under 40x total magnification (A and B) from site 1 and (C) from site 2.

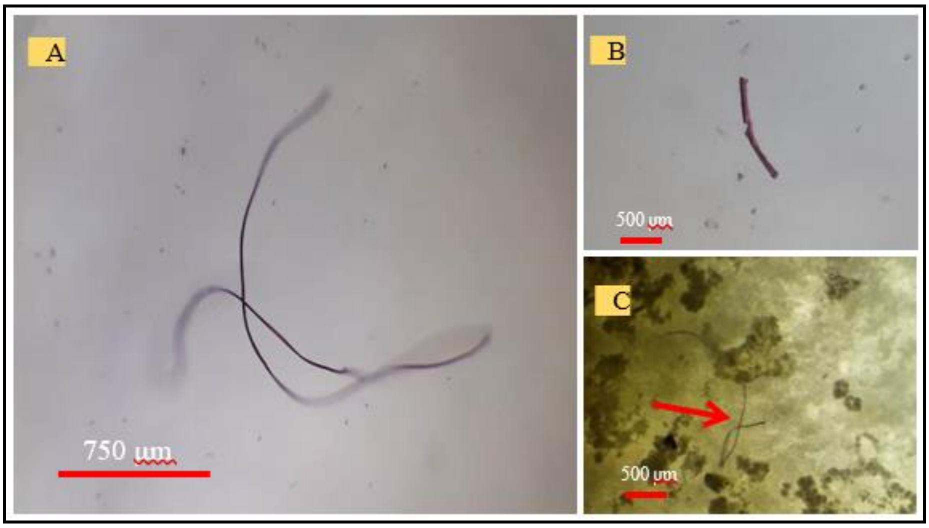

Figure 4. Suspected microplastic fibers under 40x total magnification (A and C) from site 1 and (B) from site 2.

FT-IR verification. Identification of the functional groups was based by frequency regions (Table 3) (Infrared Spectroscopy Absorption Table 2014).

Table 3

A section of the infrared spectroscopy absorptions

Frequency region	*Intensity of the peak*	*Functional group*	*Type of bond*
3000-2840	Medium	C-H	Alkane
2830-2695	Medium	C-H	Aldehyde
2600-2550	Weak	S-H	Thiol
2349	Strong	O=C=O	Carbon dioxide
2275-2250	Strong	N=C=O	Isocyanate
2260-2222	Weak	C≡N	Nitrile

Figure 5 displays the result of the FT-IR spectroscopy of a sample suspected as microfiber, while Figure 6 shows the FT-IR spectroscopy result of a sample suspected as microplastic fragment material.

Upon thorough inspection of the sampling sites, it becomes evident that Barangay Buru-un, while relatively lacking in establishments compared to other barangays in the city, thrives with fishing activities and boasts a variety of attractions, including beaches and swimming pools. This scenario raises concerns about the potential presence of microplastics in the area, as highlighted in a study conducted by Kaviarasan et al (2022), who underscore that terrestrial anthropogenic activities, alongside fisheries activities, significantly contribute to the escalating abundance of plastic pollution in the vicinity. Both sampling sites surveyed in Barangay Buru-un, Iligan City, Philippines are littered with varying plastic materials, including sachets, styrofoam cups, diapers, food packaging. Other litters such as organic materials, clothes, glass, rubber, and aluminum cans are also be observed in the area. This confirms the findings of Paler et al (2019), that plastics such as sachets are common litters found in local marine ecosystems. This is supported by the findings of Zhang et al (2019), where it was shown that concentration of microplastics ingested by marine organisms are directly related to the available plastic litter in the environment.

Figure 5. FT-IR spectra of the suspected microfiber.

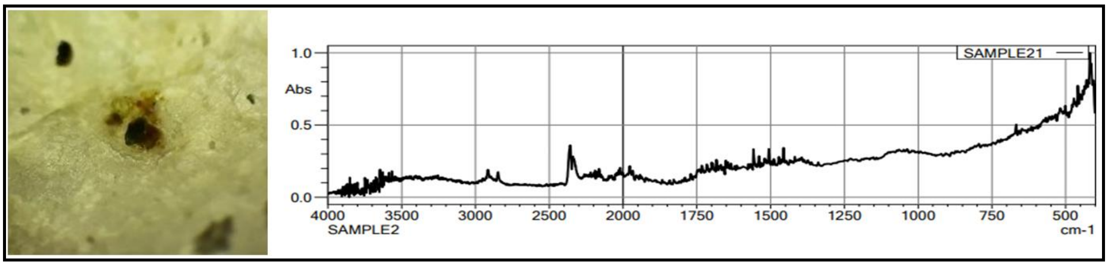

Figure 6. FT-IR spectra of the suspected microplastic fragments.

Microplastic fragments may vary in shape, but are easily differentiated from organic matter based on their straight edges. These may be irregular shaped particles, crystals, granules, shavings. The number of suspected microplastic fragments were manually counted under the light microscope. A total of five fragments where identified, two of which came from site 1 and three from site 2. In a similar study conducted in Eastern Visayas, Philippines, fragments were also isolated from the guts of fishes *Valamugil speigleri* and *Siganus canaliculatus* (Cabansag et al 2021).

Microfibers are easily identified under the microscope. They look like thin threads and are often colored. 13 suspected microplastic fibers were counted. Eight possible fibers were identified from site 1 and five more fibers were observed in site 2 samples. Similar findings were also observed in a study of microplastic ingestions by fishes collected from Eastern Visayas, Philippines. The findings exhibited a dominance of fibers over fragments in the gut of the sample fishes examined. A related study led by Hasbudin et al (2022) also reviewed bivalves *Perna virdis* obtained in Bacoor Bay and Tayabas bay in the Philippines. From the different locations established around Southeast Asia including the Philippines, microplastic fiber was the most common type of microplastic found in marine invertebrates. Smaller plastics such as fibers are easily distributed and transported (Reisser et al 2015), therefore are more prone to be ingested by marine organisms.

FT-IR was used to confirm the identity of the material isolated from the samples. FT-IR determines the structure of molecules through analysis of their absorption spectra. Figure 7 shows the amount of energy that was absorbed by the sample at different wavelengths of energy. The results are presented by the intensity of light absorbed on the y-axis and wave number on the x-axis (cm^{-1}).

The peak on wavenumber 2900 is indicative that an alkane is present in the material. Alkanes are key chemical industry raw materials, and the main ingredient in lubricants and gasoline. Additionally, their derivatives are used to synthesize a variety of products such as plastics, paints, detergents, and many more (http://hyperphysics.phy-astr.gsu.edu/hbase/Organic/alkane.html). The peak on 1400-1500 shows that the nitro-compound group is also present. These are organic compounds that contain the nitro functional group (−NO2). Because of its great chemical resistance, this type of rubber is utilized to create seals, hoses, and protective gloves (Dunn 2001). The fingerprint region is unique to a chemical, and is typically used to compare the result to known listed substances in the database to identify the sample with an exact match. The shape, position, and intensity of the peaks on the right region of the graph are utilized for interpretation. These peaks indicate what functional groups are present in the substance. The functional groups present in the material dictate the pattern of the absorption bands, which indicates the different location and shape of the peaks (Nandiyanto et al 2019). Based on the peaks and the fingerprint region, the suspected microfiber material (Figure 5) was made of chlorinated polyethylene. A similar substance was found by Gui et al (2021), with almost the same peaks, confirming its properties as a microplastic.

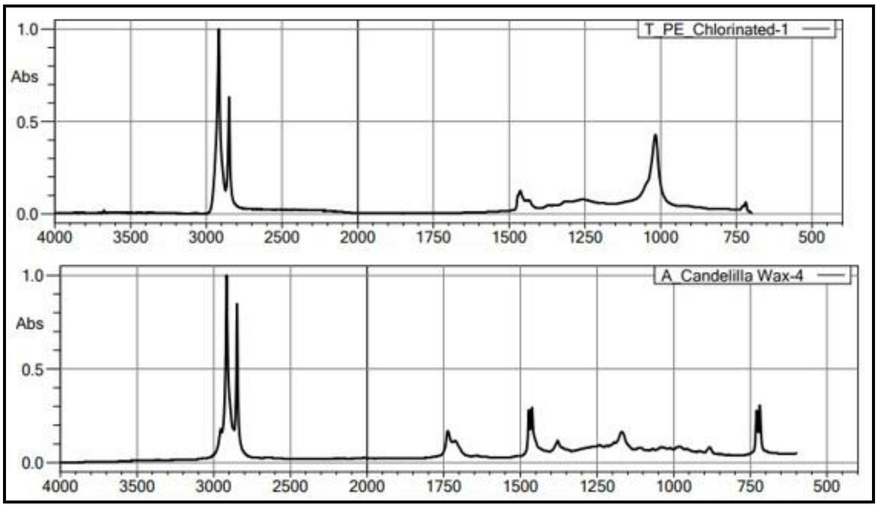

Figure 7. Graphical representation of the results presented as light absorption versus wavenumber.

In order to provide an identification of the suspected microplastic fragment from Figure 6, the raw spectrum data was compared to a list of references in the FT-IR database. Based on the score, the provided spectrum of a polymer of ethylene best matches the material tested. This was followed by an organic microcrystalline wax under the food additives library. The raw spectrum data suggests that the suspected material was made of candelilla wax. In a study characterizing different waxes, similar spectral bands to that described above were reported for candelilla wax (Bucio et al 2021). Thus, the material is not a microplastic. The raw spectrum data was compared to a list of references in the FT-IR database. With a score of 661, the material tested best matches the given spectrum of candelilla wax. This was followed by an organic paraffin wax under the food additives library. The high-density polyethylene (HDPE) only scores 652, ranking fifth among the list in the database. Suaria et al (2018) shows that ingestion of waxes is not rare in marine organisms. Paraffin wax and other petroleum-based products were reported to be present on beaches and causing damages to the European coastal communities. This may pose a threat to marine organisms, though data is still scarce.

Conclusions. Plastic pollution is one of the greatest threats of ocean life. Microplastics are carried along the currents of the ocean as well as other bodies of water and can later accumulate on the shores. From the sampling stations established, it was observed that trash was either dumped or washed up on shore. This may be due to the eddies in the water or simply by anthropogenic activities. Many intertidal gastropods were however observed on the rocky shores of Buru-un. The samples collected were highly abundant on the rocky shores along with other species of snails. The study reveals the bioaccumulation of microplastics in *Echinolittorina*, highlighting the need to utilize the present data as a foundation for implementing comprehensive plastic waste management policies. It is highly recommended to conduct further analyses on additional samples suspected to contain microplastics, with a specific focus on determining abundance. To enhance the accuracy and reliability of the findings, it is advisable to expand the existing sampling stations. Additionally, employing alternative methods for identifying microplastics is suggested due to the limitations of FT-IR spectroscopy in analyzing the data effectively.

Conflict of Interest. The authors declare that there is on conflict of interest.

References

Abreo N. A. S., 2018 Marine plastics in the Philippines: a call for research. Philippine Science Letters 11(1):20-21.

Baker T. J., Tyler C. R., Galloway T. S., 2014 Impacts of metal and metal oxide nanoparticles on marine organisms. Environmental Pollution 186:257-271.

Bucio A., Moreno-Tovar R., Bucio L., Espinosa-Dávila J., Anguebes- Franceschi F., 2021 Characterization of beeswax, candelilla wax and paraffin wax for coating cheeses. Coatings 11(3):261.

Cabansag J. B. P., Olimberio R. B., Villanobos Z. M. T., 2021 Microplastics in some fish species and their environs in Eastern Visayas, Philippines. Marine Pollution Bulletin 167:112312.

D'Ambrières W., 2019 Plastics recycling worldwide: current overview and desirable changes. Field Actions Science Reports, Special Issue 19, pp. 12-21.

De Sá L. C., Oliveira M., Ribeiro F., Rocha T. L., Futter M. N., 2018 Studies of the effects of microplastics on aquatic organisms: What do we know and where should we focus our efforts in the future? Science of The Total Environment 645:1029-1039.

Duncan P. F., 2003 Commercially important molluscs. In: Encyclopedia of food and nutrition. Caballero B., Finglas P. (eds), Elsevier Science and technology Books, pp. 5222-5228.

Dunn J. R., 2001 Nitrile rubbers. In: Encyclopedia of materials: Science and technology. 2^{nd} Edition. Buschow K. H. J., Cahn R. W., Flemings M. C., Ilschner B., Kramer E. J., Mahajan S., Veyssiere P. (eds), Elsevier, pp. 6163.

Fathoniah I., Patria M. P., 2021 Abundance of microplastic in green mussel *Perna viridis*, water, and sediment in Kamal Muara, Jakarta Bay. Journal of Physics: Conference Series 1725:012042.

Gui B., Xu X., Zhang S., Wang Y., Li C., Zhang D., Zhao Y., 2021 Prediction of organic compounds adsorbed by polyethylene and chlorinated polyethylene microplastics in freshwater using QSAR. Environmental Research 197:111001.

Hale R. C., Seeley M. E., La Guardia M. J., Mai L., Zeng E. Y., 2020 A global perspective on microplastics. Journal of Geophysical Research: Oceans. 125:e2018JC014719.

Hasbudin H., Harith M. N., Idrus F. A., 2022 A review on microplastic ingestion in marine invertebrates from Southeast Asia. Songklanakarin Journal of Science & Technology 44(3):609.

Horton A. A., Barnes D. K., 2020 Microplastic pollution in a rapidly changing world: Implications for remote and vulnerable marine ecosystems. Science of The Total Environment 738:140349.

Jambeck J. R., Geyer R., Wilcox C., Siegler T. R., Perryman M., Andrady A., Narayan R., Law K. L., 2015 Plastic waste inputs from land into the ocean. Science 347:768-771

Kaviarasan T., Dhineka K., Sambandam M., Sivadas S. K., Sivyer D., Hoehn D., Murthy M. R., 2022 Impact of multiple beach activities on litter and microplastic composition, distribution, and characterization along the southeast coast of India. Ocean & Coastal Management 223:106177.

Lusher A., Welden N. A., Sobral P., Cole M., 2017 Sampling, isolating and identifying microplastics ingested by fish and invertebrates. Analytical Methods 9(9):1346-1360.

Malaquias M. A. E., Reid D. G., 2008 Systematic revision of the living species of Bullidae (Mollusca: Gastropoda: Cephalaspidea), with a molecular phylogenetic analysis. Zoological Journal of the Linnean Society 153(3):453-543.

Nandiyanto A. B. D., Oktiani R., Ragadhita R., 2019 How to read and interpret FTIR spectroscope of organic material. Indonesian Journal of Science and Technology 4(1):97-118.

Paler M. K. O., Malenab M. C. T., Maralit J. R., Nacorda H. M., 2019 Plastic waste occurrence on a beach off southwestern Luzon, Philippines. Marine Pollution Bulletin 141:416-419.

Poppe G. T., 2008 Philippine marine mollusks. Conch Books, pp. 474-475.
Reid D. G., 1989 The comparative morphology, phylogeny and evolution of the gastropod family littorinidae. Philosophical Transactions of the Royal Society of London B, Biological Sciences 324(1220):1-110.
Reisser J., Slat B., Noble K., du Plessis K., Epp M., Proietti M., de Sonneville J., Becker T., Pattiaratchi C., 2015 The vertical distribution of buoyant plastics at sea: an observational study in the North Atlantic Gyre. Biogeosciences 12(4):1249-1256.
Rochman C. M., Browne M. A., Underwood A. J., van Franeker J. A., Thompson R. C., Amaral-Zettler L. A., 2016 The ecological impacts of marine debris: unraveling the demonstrated evidence from what is perceived. Ecology 97(2):302-312.
Suaria G., Aliani S., Merlino S., Abbate M., 2018 The occurrence of paraffin and other petroleum waxes in the marine environment: a review of the current legislative framework and shipping operational practices. Frontiers in Marine Science 5:94.
Zhang S., Wang J., Liu X., Qu F., Wang X., Wang X., Li Y., Sun Y., 2019 Microplastics in the environment: A review of analytical methods, distribution, and biological effects. TrAC Trends in Analytical Chemistry 111:62-72.
*** Bersales L. G. S., 2015 2015 census of population. Available at: psa.gov.ph
*** http://hyperphysics.phy-astr.gsu.edu/hbase/Organic/alkane.html
*** Infrared Spectroscopy Absorption Table, 2014. Available at: https://chem.libretexts.org/Ancillary_Materials/Reference/Reference_Tables/Spectroscopic_Reference_Tables/Infrared_Spectroscopy_Absorpt ion_Table

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