Research Article

Microplastic Pollution in Mindanao's Taguibo River Watershed Forest Reserve: Characterization, and Distribution Patterns, and Implications for Freshwater Ecosystem Conservation

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Abstract

Despite increasing awareness of microplastic pollution and the harm it brings to terrestrial ecosystems and human body, few research works have examined how it contributes to freshwater environments, particularly forest reserves. Microplastic presence and characteristics were examined in Mindanao's Taguibo River Watershed Forest Reserve (TRWFR). Three (3) sampling sites along the river stretch were chosen. Analysis of water and sediment samples found microplastic abundances at 477.78 ± 182.83 pa/m^3 and 17.04 ± 14.80 pa/kg, respectively. These microplastics varied in size, shape, and color. A total of 9 and 7 color variations were detected from water and sediment samples, respectively. Brown (43.02%) and black (17.44%), and fibers (39.53%) and films (24.42%) were the most common microplastics from water samples. White (30.43%), and blue and brown (21.74%), and filament (69.57%) were the most common microplastics from sediment samples. FTIR-ATR spectroscopy described the microplastics further. A total of 13 polymer types were identified in water samples, while 8 polymer types were identified in sediment samples. Polymer types such as polyacetylene and regenerated cellulose fibers were mostly found in water samples; and polypropylene and regenerated cellulose fibers from sediment samples. This study shows microplastic pollution in the TRWFR. Microplastic incidence and distribution patterns vary among collection locations, suggesting some areas are more susceptible to pollution. This work improves our understanding of freshwater microplastic contamination and underscores the need to monitor and reduce microplastic pollution to preserve the ecological balance in the Taguibo River and its surroundings.

Keywords: Butuan City, Emerging pollutants, Freshwater ecosystems, Philippines, Watershed forest reserve

1 Introduction

Based on reports, the Philippines is identified as the primary contributor of plastic litter from river sources in the world [1] and ranks 3rd worldwide in terms of plastic litter or macroplastics released into marine environments [2]. Several studies have been done to quantify macroplastics such as the work of [3]–[7]. These studies have shown that most Philippine beaches can be classified as dirty to extremely dirty. Also, studies on the riverine systems showed that macroplastics are transported into the river system [8], [9]. These macroplastics will deteriorate in the environment and can form microplastics.

These minuscule plastic particles, measuring less than 5 mm in size, have been found in various aquatic environments, including oceans, rivers, and lakes. They are the product of the physical degradation of macroplastics. Microplastic pollution has emerged as a pervasive and concerning global environmental issue, with profound implications for ecosystems and human well-being [10]. Microplastics can be categorized as primary or secondary. Primary microplastics are intentionally manufactured to be less than 5 mm in size [11]. Primary microplastics are found in products with “microbeads” such as personal care products, e.g., toothpaste, exfoliating scrubs, and body washes. Secondary microplastics are fragmentation products of larger plastics [12].

Rivers play a critical role in the transport and accumulation of microplastics, serving as major conduits for their entry into marine ecosystems [13]. Despite growing awareness of microplastic pollution, our understanding of its occurrence and impact on freshwater ecosystems, especially within forest reserves, remains limited. Mounting evidence suggests that rivers can act as conduits for the transport and accumulation of microplastics, ultimately leading to their entry into marine ecosystems [14]. Rivers receive inputs of microplastics from various sources, such as urban runoff, industrial discharges, and agricultural activities, which can originate locally or be transported from upstream regions. Once in the river systems, microplastics can be transported downstream, potentially accumulating in sediments, or being discharged into marine environments, where they can persist and impact aquatic organisms [15], [16]. A mean concentration of 300 items/m^3 of microplastics was found in the Cagayan de Oro River, one the largest rivers in Northern Mindanao, Philippines [17]. Additionally, 15 out of 21 sampling sites in Puerto Princesa, Palawan, Philippines, contained microplastics [18]. Microplastics were also found in inland waters, specifically in the largest lake in the Philippines [19]. The recent report on the presence of microplastics in the mangrove sediments [20] of Butuan Bay, Philippines indicates that these microplastics could potentially originate from upstream river sources.

The Taguibo Watershed Forest Reserve, located in Mindanao, Philippines, encompasses a significant freshwater ecosystem that holds immense ecological importance. The intricate network of rivers within this forest reserve serves as vital lifelines, providing water resources, habitat, and food for numerous plant and animal species. Furthermore, the Taguibo Watershed is the primary source of potable water for Butuan City [21], [22], emphasizing the critical need to assess the presence of microplastics in this watershed and its potential implications for human health.

This study documents the first evidence of microplastic occurrence in the water and sediments of the Taguibo River Watershed Forest Reserve (TRWFR), with emphasis on the river systems that not only supply water to Butuan City but also provide habitat for biodiversity which is critical in maintaining ecological balance. Through a multidisciplinary approach combining field sampling, laboratory analysis, and advanced spectroscopic identification techniques, we aimed to quantify the abundance, composition, and spatial distribution of microplastics within this riverine ecosystem. By identifying the presence, sources, and distribution patterns of microplastics, we can develop targeted strategies to mitigate the pollutant's impacts on aquatic organisms, to preserve water resources, and to safeguard the flow of potable water to Butuan City and Butuan Bay. Moreover, the results contribute to a broader understanding of microplastic pollution in freshwater environments, highlighting the urgent need for effective monitoring programs and implementation of sustainable management practices in a river system that is crucial to the life and livelihood of its environs.

2 Materials and Methods

2.1 *Sampling sites and locations*

The Taguibo River traverses from the Mt. Hilong-Hilong Range in Northeastern Mindanao [21]. Recognizing its ecological importance, the Taguibo River was designated as a Watershed Forest Reserve through Proclamation No. 1076 on September 4, 1997, [23]. Spanning an area of 4,367.44 hectares, the Taguibo River Watershed Forest Reserve (TRWFR) encompasses several barangays, including Barangay Anticala, Butuan City; Barangay San Antonio, Remedios Trinidad Romualdez; Barangay Mahaba, Cabadbaran City; and a portion of Sibagat, Agusan del Sur [21], [23]. A barangay is the smallest political unit in the Philippines. This protected area serves as a vital habitat for numerous species and plays a crucial role in maintaining the ecological balance of the surrounding region.

Three sites were strategically chosen to represent the course of the Taguibo river. Three sampling sites chosen along the river stretch were Site 1: Taguibo Bridge/lower stream, Site 2: Pianing Bridge/middle stream, and Site 3: Anticala Bridge/upper stream. Sites 2 and 3 were chosen to represent the tributaries of Taguibo River, while Site 1 is located at the confluence of these tributaries. Sites 2 and 3 are adjacent to a community which is a potential source of pollution. Within each site, three specific sampling points were identified. The chosen sites are situated near bridges, ensuring convenient accessibility for sampling purposes. Sub-points were carefully selected to adequately represent the entirety of the bridge structure in relation to its presence in the river system. Specifically, sub-point 2 was collected beneath the bridge, offering a comprehensive view of its underside and surrounding environment. Additional points were distributed on both sides of the bridge, ensuring comprehensive coverage, and capturing the full spectrum of features and influences associated with the bridge's presence. By selecting these locations, the study aims to obtain a comprehensive understanding of the microplastic presence in the river system while considering practicality and ease of access during the sampling process.

2.2 *Reagents*

A 10% KOH solution was prepared from KOH pellets (JT Baker, Avantor, Sweden) by dissolving the required weight of pellets in an adequate amount of distilled water to achieve the desired concentration. Similarly, a saturated NaCl solution was prepared from NaCl crystals (Duksan Pure Chemicals Co. Ltd., South Korea) by dissolving the required weight of NaCl in a sufficient volume of distilled water. Both prepared solutions were then filtered (Whatman No. 2, 8 µm pore size, Cytiva, China) before use.

2.3 *Sample collection*

Stainless steel buckets were used to collect water samples 0–50 cm from the surface at 60 L per site. Water samples underwent *in situ* filtration using 5 mm sieves, followed by an additional filtration step using 0.25 µm sieves. The residue retained in the 0.25 µm sieves was carefully rinsed with distilled water and collected in a glass jar. The mouth of the jar was covered with aluminum foil prior to sealing it with a lid. Subsequently, the collected samples were securely transported to the laboratory for further analysis.

Stainless Steel Sediment Grab Sampler was used to collect enough sediment. Sediments were collected from three (3) representative points within each site. The collected sediments were carefully placed in glass jars, and like the water samples, the jars were covered with aluminum foil before sealing them with lids. The jars containing the sediment samples were then transported to the laboratory for subsequent analysis.

2.4 *Microplastics extraction*

2.4.1 Water Samples

Microplastics extraction methods were adapted from Inocente *et al.*, [24]. The samples were added with 10% KOH at a 2:1 ratio (sample: KOH). The resulting solutions were digested at 60 °C for 24 h, with periodic stirring every 4–6 h. After the digestion process, the samples underwent flotation. This involved the addition of saturated NaCl solution at a 2:1 ratio (sample: NaCl). Subsequently, the samples were allowed to stand undisturbed for at least 12 h. After flotation, the samples were carefully decanted, and the top solution was filtered using GF/C Filter paper (Whatman GF/C, pore size = 1.2µm). Then, the filter paper was flushed with distilled water and then removed from the filtration apparatus. The filter paper was then dried at 40 °C.

2.4.2 Sediment samples

At the laboratory, the sediment samples were oven-dried. Subsequently, the dried samples were thoroughly homogenized and 150 g of the dried sediments from each point was selected to make up the final sample of 150 g. The final sample of 150 g was soaked with 150 mL of 10% KOH and digested for 24 h at 60 °C. The resulting digestate was then filtered using GF/C filters (Whatman GF/C™, pore size = 1.2 µm). The filter papers were rinsed with distilled water and oven-dried and stored in glass petri dishes.

2.5 *Microplastics characterization*

2.5.1 Visual characterization

The residue obtained from the dried filter paper was subjected to visual characterization under a microscope (iScope Series Euromex, Holland) to identify and separate suspected microplastics. Following the criteria set by Hidalgo-Ruz *et al.*, [25], the color and morphology of the particles were visually assessed. Clean needles and forceps were used to carefully separate and mount the suspected microplastics onto glass slides for further analysis.

2.5.2 Plastic type characterization

Fourier Transform Infrared–Attenuated Total Reflectance (FTIR–ATR) Spectroscopy (Shimadzu IRTracer-100(EN230V), Shimadzu Corporation, Japan) was employed to confirm the identity of the suspected microplastics. The sample's spectra were compared to known polymer types in the library to establish a match. The evaluation of each FTIR spectrum was based on the presence and position of peaks, and if the correlation or similarity index was low (< 20%), the corresponding sample was excluded from the analysis. This correlation score was chosen, considering the size of the sample, emphasizing its associated lower signal-to-noise ratio, and instrumental limitations [26]–[28]. This rigorous approach ensured the reliability and accuracy of the identified microplastic samples through spectral comparison.

2.6 *Data analysis*

The characteristics of the confirmed microplastics were carefully documented and recorded. This included noting the most prevalent color, morphology, and polymer type observed in the samples. Additionally, microplastic concentration was calculated using the following Equations (1) and (2):

$$Microplastic\ Abundance, MP_{(W)} = \frac{number\ of\ microplastic\ particles}{water\ volume\ (m^3)} \quad (1)$$

$$Microplastic\ Abundance, MP_{(S)} = \frac{number\ of\ microplastic\ particles}{sediment\ weight\ (kg)} \quad (2)$$

2.7 *Quality control*

The use of cloth and plastic material was minimized during the whole duration of sample collection and analysis. The glassware used for analysis was washed thoroughly and rinsed properly with distilled water. Prepared solutions were filtered (Whatman No. 2, 8 µm pore size, Cytiva, China) before use. Great care and attention were exercised to prevent any potential airborne contamination, thus maintaining the integrity of the samples, and preserving the accuracy of the analysis.

3 Results and Discussion

3.1 *Confirmation rate and microplastic abundance*

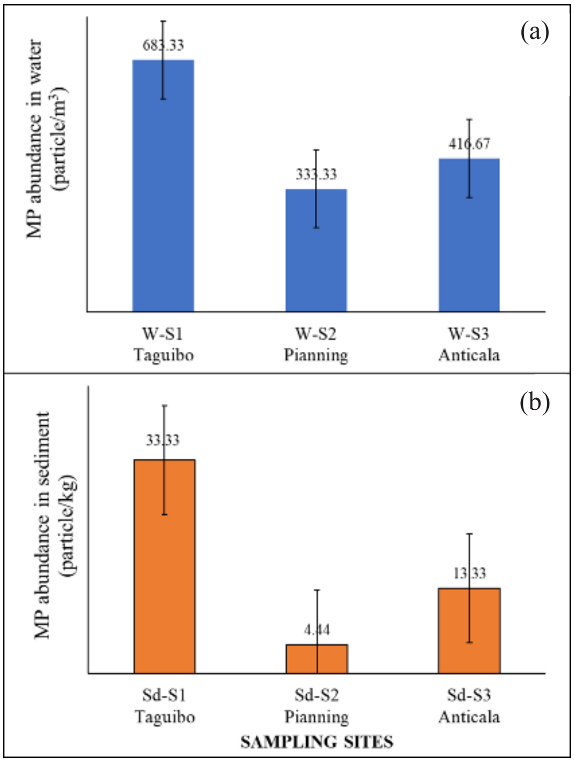

Figure 1: Microplastic abundance per site from water (a) and sediment (b) samples.

The microparticles obtained from the extraction process were subjected to FTIR-ATR analysis to validate their identity as microplastic polymers. These classifications helped track and account for microparticles that either did not meet the criteria of synthetic polymer identification or were unintentionally excluded from the analysis due to transfer-related issues.

A total of 86 confirmed microplastics out of 165 suspected microplastics in surface water were identified in 3 different sites along TRWFR. A total of 41, 20, and 25 were confirmed out of 62, 44, and 59 suspected microplastics from the three sampling sites, respectively. From the sediment samples at Site 1, 15 out of 31 suspected microplastics were confirmed. At Sites 2 and 3, 2 and 6 were confirmed from 15 and 18 suspected microplastics. From sediment samples, Site 1 has the highest abundance at 33.33 particles/kg (Figure 1). Some of the suspected microplastics could not be confirmed for their polymer type by FTIR-ATR with at least a 20% correlation score due to environmental physical degradation, i.e., small size, and loss during transfers. The detection capability of FTIR-ATR may be limited when it comes to extremely small samples [26], [27]. Moreover, microplastics adhering to settling material during extraction, loss during sieving, chemical digestion, and loss during transfers resulted in fewer confirmed microplastics [29].

Microplastics present in the river likely originate from various sources, including rainwater runoff, direct dumping, and sewage disposal into the river. In both water and sediment samples, Site 1 consistently exhibits the highest microplastic abundance among all three sites. This higher abundance may be attributed to the fact that the site is predominantly utilized for residential functions, as evidenced by the existence of dwellings and households near the river.

Most notably, around the sampling site lies a septage area that serves as a discharge point for domestic waste into the river, which also emits an unpleasant odor. The locality is encompassed by arboreal vegetation, undergrowth, and small woody plants.

Another contributing factor could be the comparatively high velocity of the river, especially under high tide conditions, in comparison to other locations. The increased velocity will transport a greater number of particles from upstream. Especially so since Site 1 is located at the confluence of the tributaries from Sites 2 and 3.

In contrast, Site 2 consistently exhibits the lowest levels of microplastic presence in both water and sediment samples. The reduced prevalence could be ascribed to its general well-being. Most residents in this sampling site acquire the required water for their houses either directly or indirectly from the river stream, which is used for numerous activities including bathing and laundry. This indicates that the river at this location is considered by the locals as unpolluted. Furthermore, the area is surrounded by lush flora, including trees, wild plants, and small shrubs.

Site 3 is downstream of a Dam, which is also the main water source of the Butuan City Water District [30]. The locality comprises only of residential dwellings, which are closely situated in proximity to each other [30]. Furthermore, there exists a canal adjacent to the river stream that serves as a repository for domestic waste disposal. The vicinity is characterized by the presence of trees, weeds, and shrubs.

Watkins *et al.*,[31] investigated the difference among microplastic concentrations above, below, and within the reservoirs. Their results show that downstream microplastic concentrations in sediment samples are lower than above and within reservoir sediment samples. In surface water samples, downstream microplastic concentrations are lower than upstream concentrations but higher than reservoir samples [31]. Their study provided evidence that the presence of dams can affect microplastic concentration in surface rivers. The presence of a water reservoir upstream of Site 3 likely influences the microplastic concentration.

In contrast, Site 2 (Pianning Bridge) is located at lower elevations lower than Site 3 but higher than Site 1. These variations in elevation among the sites may contribute to the differences observed in the number of microplastics found in each site. The combination of different sources of microplastics, the presence of a domestic waste discharge point, the presence of a water reservoir, and the geographical characteristics of each site influence the overall distribution and abundance of microplastics in the river.

Generally, microplastics have a lower density than water and are expected to float. However, results show that sediment samples also contained microplastics. Some types of microplastics exhibit a higher density than water, thereby increasing their propensity to sediment and amass within sedimentary environments. The hydrodynamic movement of water within river systems can induce turbulent flow patterns, thereby facilitating the deposition of microplastic particles onto the sediment bed. According to a study conducted by Hurley *et al.*, [32], flooding events can transport and deposit microplastics in sediments. Microplastics also possess the capacity to undergo sorption or adhesion processes with sediment particles. Sediments can serve as a reservoir for microplastics by offering suitable surfaces for adherence and promoting their aggregation. Wang *et al.*, [33] conducted a study that examined the presence of microplastic pollution in river sediments and emphasized the significance of sorption as a mechanism for the accumulation of microplastics in sediments. Moreover, microplastics exhibit variations in size distributions, with smaller particles demonstrating a higher propensity for suspension within the water column, whereas larger particles tend to undergo settling. The presence of sediments within river systems can serve as a mechanism for the entrapment of larger microplastics, thereby resulting in an increased concentration of these particles within the sediment bed. The review by [34] examines the behavior of microplastics in relation to their size and preferential accumulation in sediments. Finally, Biofouling, the process of organisms colonizing and accumulating on surfaces, can contribute to the accumulation of microplastics in sediments. Verster and Bouwman [35] have highlighted that plastic particles in the water column undergo biofouling, leading to the formation of biofilm layers. As these plastic particles accumulate more biotic material, their weight increases, causing them to submerge. This phenomenon significantly affects the displacement and dispersion of plastic particles in freshwater systems, potentially influencing their transportation into marine ecosystems. The length of time required for microplastics to sink, coupled with the dynamic flow of rivers, accounts for the higher count of microplastics observed in water samples compared to sediment samples. Moreover, this phenomenon also explains the greater variation in terms of color, morphology, and

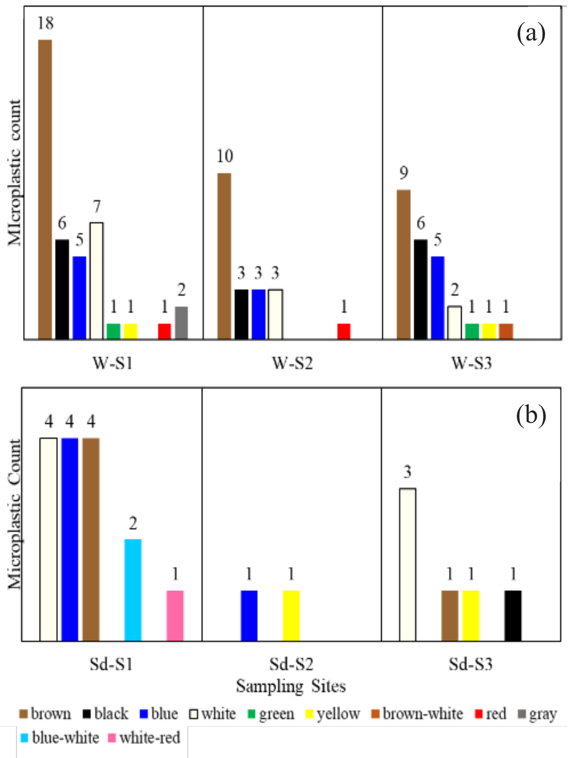

Figure 2: Color variations of microplastics per site from water (a) and sediment (b) samples.

polymer type observed among microplastics derived from water samples, as compared to those found in sediment samples.

3.2 *Color characteristics*

Microplastic particles were observed using a microscope (iScope Series Euromex, Holland). From the confirmed microplastics in water and sediment samples, a total of 9 and 7 color variations were characterized, respectively (Figure 2). Among the observed colors, brown (38.53%), white (17.43%), blue (16.51%), black (14.68%), and yellow (3.67%), were found to be common in both samples. Water samples revealed 4 unique colors: brown-white, gray, green, and red. On the other hand, sediment samples exhibited 2 unique colors: blue-white and white-red. Among the identified colors, brown (42 counts) emerged as the most abundant color in both water and sediment samples, followed by white (19 counts). Brown (43.02%) was consistently prevalent across all sites in the water samples. In sediment samples, white (30.43%) was the most abundant color; however, it was only present in Sites 1 and 3. These findings shed light on the color variations and distributions of microplastics in both water and sediment samples, emphasizing the dominance of certain colors and their associations with specific sampling sites.

Water samples have more unique color variations compared to sediment samples. The diverse color variations observed can be attributed to the fact that water is relatively more mobile than sediments and that microplastics generally float. Notably, Site 1 is situated at the confluence of the tributaries, encompassing both Site 2 and Site 3. This geographical arrangement helps explain the increased abundance of microplastic particles and the corresponding color variations observed in the samples. The convergence of multiple sources in Site 1 likely contributes to the greater diversity and quantity of microplastics, thus influencing the range of colors observed.

For microplastics found from water samples, brown is the most diverse in terms of polymer types (Supplementary Material), with 7 polymer variations. Among microplastics from water samples, Regenerated cellulose fiber (RCF) brand 1 (RCF 1) and RCF brand 2 (RCF 2) are the most diverse in terms of color variations, each displaying 6 different color variations. This variation is expected as RCF 1 and RCF 2 are commonly used in clothing manufacturing. From sediment samples, both brown and white are the most diverse in terms of polymer types with 4 polymer types each. Among microplastics from sediment samples, polypropylene (PP) displays the highest color diversity, with 6 distinct color variations. PP is widely employed in plastic packaging, plastic parts for machinery and equipment, and textiles [36]. These products come in varied shapes and colors, especially plastic packaging. Plastic packaging can be used in food packaging that may accumulate bacteria, undergo biofouling, and eventually lead to the sinking into the sediments of the plastic product [35]. Consequently, plastic products residing in the riverbed sediment gradually degrade and transform into microplastics over time.

White and transparent microplastics seem to be common among the microplastics from water samples from across the Philippines and Asia (Supplementary Material). Different environmental factors, such as temperature and humidity, depositional matrix (e.g., water, soil, sand, terrestrial versus aquatic), and depositional environment, contribute to the fading of colors of macroplastics as they degrade into microplastics [37]. The predominant contributors to the presence of colorless and white particles observed in river samples from the Tamsui River, Dahan River, Keelung River, Xindian River [38] were linked to the following key factors: the gradual fading of color caused by the relentless exposure to UV light and various weathering agents, combined with the initial colorless nature of the plastic upon its production.

White microplastics, which are abundant in sediment samples, are only present in Sites 1 and 3. The research conducted by Yang *et al.*,[39] supports these findings, indicating that microplastics in both aquatic and soil environments exhibit a wide range of color variations. Recent microplastic studies conducted in the Philippines [17]–[20] also exhibit a wide variation of colors of the microplastics. Microplastic pollution is present in different freshwater and marine environments, including lakes (Laguna de Bay) [40], mangrove sediments (Butuan Bay) [20], beach sands (Puerto Princesa, Palawan Island) [18], and rivers (Cagayan de Oro River) [17]. These microplastics are primarily composed of fibers, with blue-colored microplastics being particularly common. Blue, red, white, transparent, brown, and black microplastics were identified from the samples collected from Cagayan de Oro River [17]. It is noteworthy that white microplastics comprise a relatively significant proportion of the samples collected from soil environments. In addition to white microplastics, colored microplastics also make a significant contribution to the composition of sediment. The diversity of colored plastic commodities, designed to enhance market appeal and marketability, contributes to the overall plastic pollution observed in the river system, as highlighted by Thetford *et al.*,[41].

The color characterization of microplastics plays a crucial role in understanding their fate within the food web, particularly concerning unintentional consumption by predatory vertebrates that may mistake synthetic microparticles for prey [42]. In a study conducted by Rios *et al.*, [11], it was observed that fish exhibited a preference for ingesting yellow and blue microplastic fragments while avoiding white fragments. For instance, the fish Decapterus muroadsi from Rapa Nui demonstrated a preference for ingesting

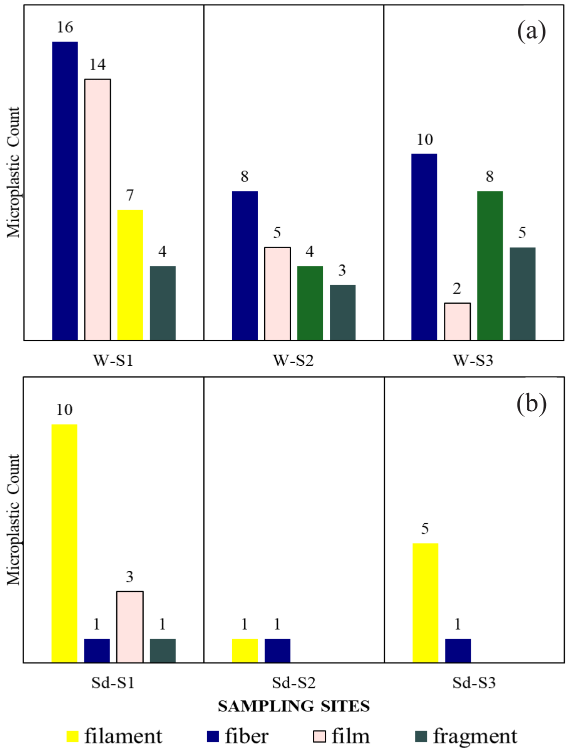

Figure 3: Morphology variations of microplastics from water (a) and sediment (b) samples.

blue polyethylene polymers [43]. Orly *et al.*, further highlighted that fish may mistakenly consume blue microplastics, mistaking them for their copepod prey.

In another study focusing on the microplastic ingestion and egestion by juvenile palm ruff fish (*Seriolella violacea*), which are visually oriented planktivorous species, it was found that fish selectively captured black microplastics that resembled food pellets [44]. Furthermore, [44] revealed that microplastics exhibiting shades of blue, translucent, and yellow were predominantly entangled in the vicinity of food pellets. Conversely, microplastics captured in the absence of food were typically regurgitated, and only ingested when mixed with food particles within the oral cavity of the fish. These findings underscore the importance of considering microplastic colors in understanding their interaction with aquatic organisms and their potential impacts on the food chain, particularly when these colors bear resemblance to natural food sources. Consequently, some fish species present in TRWFR may ingest these microplastics.

3.3 *Morphology*

A variety of morphotypes, including fiber, filament, film, and fragment (Figure 3), are present in both water and sediment samples. Among these morphotypes, fiber (39.53%) is the most abundant in water samples, while filament (69.57%) is the predominant morphotype in sediment samples. In terms of overall abundance, fiber (37 counts) emerges as the most prevalent morphotype, closely followed by the filament morphotype (35 counts). These findings emphasize the presence of different morphotypes in both water and sediment samples, with fiber and filament being particularly prominent in their respective sample types.

Film emerges as the second most abundant morphotype observed in water samples. These particles are likely derived from plastic packaging materials, such as food and grocery bags. It is important to note that such waste items are often disposed of in landfills, presenting a potential source of microplastics in the environment.

Filaments, on the other hand, are thread-like degradation products originating from various sources such as fishing lines, ropes, and hard plastic materials [45]. The presence of fragmented particles in both water and sediment samples may also stem from the degradation of these materials. These results align with the findings of Yang *et al.*, [39], suggesting that microplastics found in soil environments share common sources. This implies that the sources of microplastics, as reflected by their morphotypes, are likely consistent across the studied sites.

Among microplastics identified in water samples, the fiber and filament morphotypes exhibit the highest diversity in terms of polymer variations, with 7 different types identified each. The polymer types found in fibrous microplastics were RCF 1, Cellophane, PET, polyacetylene, polyester, PP, and RCF 2. The polymer types found in filamentous microplastics were RCF 1, CPE, polyacetylene, polyethylene, polyurethane, RCF 2, and VC-AA. Similarly, for microplastics extracted from sediment samples, the filament morphotype demonstrates the greatest diversity with 7 variations in polymer types. These polymer types are RCF 1, CPE, polyethylene, PP, RCF brand 3 (RCF 3), SEBS, and RCF 2.

In terms of morphotype diversity within water samples, RCF 1, RCF 2, and polyacetylene stand out as the most diverse polymers, with all 4 morphotypes present in each. Similarly, for microplastics found in sediment samples, PP displays the highest level of morphotype diversity. These findings highlight the variability in polymer types and morphotypes observed among microplastics in both water and sediment samples, emphasizing the diverse nature of these pollutants in TRWFR.

A comparison of microplastic characteristics from water and sediment samples collected from various rivers and/or watersheds in the Philippines and Asia is provided in Supplementary Material. Among these studies [46]–[58], fiber and fragment morphotypes consistently emerge as the primary type of microplastics found in water samples. Similarly, microplastics extracted from sediment samples are predominantly composed of either fiber [46], [48] or fragments [53], [57], [58]. The sources of fibers in these studies include irrigation water [59]–[65], atmospheric fallout [66], [67], and textiles and clothing [68], [69].

The morphology of microplastics also plays a significant role in determining their fate within the food web. Lehman *et al.*, [70] demonstrated that the shape of microplastic influences soil aggregation and organic matter decomposition. Specifically, regardless of their chemical composition, fibers were found to have a detrimental impact on the formation of aggregates. Additionally, various geometries such as foams and particles, along with the polymer identity, were shown to co-modulate soil responses. A meta-analysis conducted by Salerno *et al.*, [71] examined the impact of microplastics on the functional traits of fish species. The analysis revealed a medium negative effect for microplastic shapes including fibers, fragments, and spheres. Additionally, Li *et al.*, [72] investigated the feeding responses of four fish species to microplastics. The findings indicated that the species did not actively capture microfibers, but instead exhibited a passive tendency to ingest microfibers during respiration. Furthermore, the fish displayed a negative response, voluntarily expelling microfibers that were intermingled with mucus. The researchers concluded that fish unintentionally consume microplastics rather than deliberately seeking them out. These studies collectively highlight the importance of microplastic morphology in influencing ecological responses and interactions within the food web, emphasizing the need for further investigation into the impacts of different microplastic shapes on various organisms.

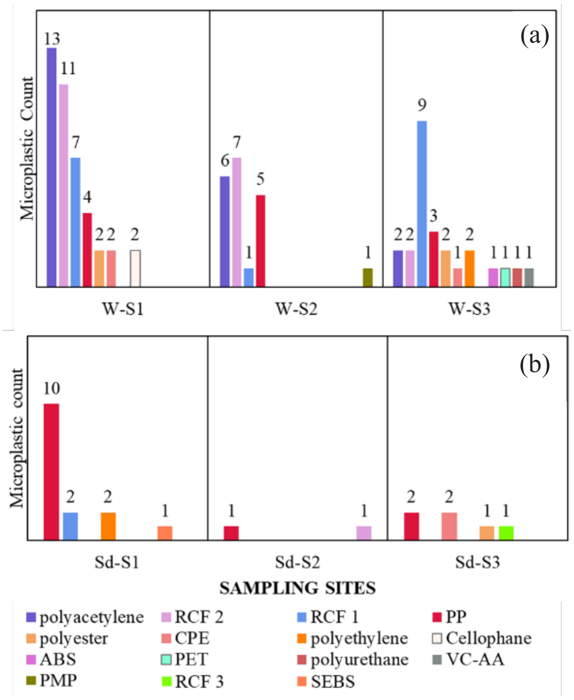

Figure 4: Polymer variations of microplastics that were found in water (a) and sediment (b) samples.

3.4 *Polymer type*

To confirm the polymer identity of particles extracted from water and sediment samples, Attenuated Reflectance Fourier Transform Infrared Spectroscopy (FTIR-ATR) was employed. From the analysis, a total of 13 polymer types were identified in water samples, while 8 polymer types were identified in sediment samples. The polymer types that are common in both sample types include RCF 1, Polyethylene, Chlorinated Polyethylene (CPE), Polyester, Polypropylene (PP), and RCF 2 (Figure 4). Water samples exhibit additional polymer types not found in sediment samples, namely ABS (acrylonitrile-butadiene-styrene), Cellophane, PET (polyethylene terephthalate), PMP (polymethyl pentene), polyacetylene, polyurethane (PU), and VC-AA (vinylidene chloride acrylic acid). On the other hand, sediment samples possess unique polymer types such as RCF 3 and SEBS (styrene-ethylene-butylene). It is important to note that RCF 3 is a plant-based biodegradable fiber [73]. Water polymer type variation indicates that the diversity of polymer types is higher in water samples compared to sediment samples. Among the identified polymer types, Polypropylene (PP) (25 counts) emerges as the most abundant in both water and sediment samples, closely followed by RCF 2 (21 counts). These findings highlight the prevalence of specific polymer types and their abundance in the studied samples, underscoring the importance of PP and RCF 2 as prominent microplastic constituents.

RCF 2 and RCF 1 are examples of relatively new polymer types that fall under naturally sourced regenerated cellulose fibers [74], [75]. During machine wash, these synthetic textiles have the potential to release a significant number of microfibers, with estimates suggesting up to 1,900 microfibers per wash cycle [76]. Urban effluents are recognized as primary contributors to the release of microfibers into waterways [77]. RCF 1, a polymer resembling RCF 2, is likely present in the river due to improper disposal practices or the contribution of the clothing industry to the overall plastic pollution, leading to their entry into waterways and subsequent degradation within the river system.

The primary polymer types of microplastics found in water samples are Polyacetylene, RCF 2, and RCF 1 (Figure 4), at 21, 20, and 7 counts, respectively. RCF 1 and RCF 2 polymer types are commonly used in the textiles and clothing industry (Supplementary Material). However, when comparing the polymer types of microplastics found in water samples from various studies in Asia, no similarities are observed. The identified polymer types in water samples from Asia include PP [46], [48], PVC [51], or PET [49]. Similarly, polyacetylene, polyester, and polyethylene terephthalate were also detected in Cagayan de Oro River [17].

On the other hand, the polymer types of microplastics found in sediments from this study corroborate with those found in other studies in Asia. PP [46], [48] and PE [57] consistently appear as primary morphotypes. Polypropylene microplastics can originate from the disintegration of domestic waste items such as straps, bottle caps, food delivery containers, baby milk bottles, and yogurt plastic bottles [78]. It is noteworthy that domestic runoff contributes to the presence of microplastics in freshwater ecosystems [79].

The chemical identity of microplastics can have a biological effect on organisms and the environment. A study [80] has demonstrated that the presence of microplastics, particularly polyvinyl chloride (PVC) and polyethylene (PE) in the size range of 40–150 µm can induce oxidative stress in leukocytes of Sparus aurata and Dicentrarchus labrax, leading to immunotoxicity. Their empirical evidence [80] suggests that certain types of plastics affect the reproductive capacity of certain types of organisms.

4 Conclusions

This study examined the occurrence and attributes of microplastics within the Taguibo River Watershed Forest Reserve (TRWFR) located in Mindanao, Philippines. A total of three sites located along the river were chosen as representatives, and samples of both water and sediment were gathered for the purpose of analysis.

The findings unveiled the diverse occurrence and spatial dispersion of microplastics in water and sediment specimens across the various sampling locations. Site 1: Taguibo Bridge (lower stretch, confluence) consistently demonstrated the highest rate of occurrence for microplastics, whereas Site 2: Pianning Bridge (middle stretch, tributary) exhibited the lowest rate. Site 3: Anticala Bridge (upper stretch, tributary) exhibited a greater frequency of non-plastic particles, which can probably be attributed to the influence of nearby domestic wastes. Brown (38.53%) was found to be the most prevalent in both sample types, with white (17.43%) being the second most frequently observed color. *Planktivorous* species preferring these colors may mistake microplastics in this color as food. Various morphotypes, such as fiber, filament, film, and fragment, were observed and classified. Fiber (39.53%) and filament (69.57%) were predominantly present in both water and sediment samples. The application of Fourier Transform Infrared Spectroscopy (FTIR-ATR) enabled the identification of distinct polymer types within water and sediment samples, thereby indicating the existence of diverse plastic materials. The identified polymer types included RCF 1 (17.43%), RCF 2 (19.27%), polyacetylene (19.27%), and polypropylene (22.94%). Microplastic morphology and polymer type help to determine the original material and to understand its fate in the environment.

Generally, the study highlights the microplastic's occurrence within the TRWFR and confirms significant observations regarding their properties and spatial dispersion. The findings widen our comprehension of plastic pollution within river ecosystems and underscore the imperatives of efficacious management strategies to alleviate consequential ecological repercussions. The selected sampling sites may be limited in spatial coverage and may not reflect variations in microplastic composition over time and space. Further studies over a longer time duration and more sites along the river and its tributaries are recommended. TRWFR provides water for domestic and industrial purposes, hence, information on its water quality is of utmost importance not only to human health but also to industries.

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Author Contributions

M.H.T.B.: methodology, validation, formal analysis, investigation, data curation, writing-review and editing, visualization, supervision, and project administration; M.C.C.O.: formal analysis, investigation, writing-original draft preparation, and writing-review and editing; S.A.T.I.: conceptualization, methodology, validation, investigation, supervision, and project administration; J.L.M.S.: conceptualization, validation, writing-original draft preparation, and supervision; N.A.B.: writing-original draft preparation; E.V.B.: writing-original draft preparation; A.-N.D.L.: writing-original draft preparation; C.Y.B.: investigation; J.C.C.A.: visualization; H.P.B.: writing-review and editing; A.A.L.: resources; D.M.R.: visualization; R.Y.C.: conceptualization, resources, writing-review and editing, project administration, and funding acquisition

Conflicts of Interest

The authors declare no conflict of interest.

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Supplementary Material

Table S1: Comparison of microplastic characteristics from water samples across different studies conducted in Asia

Location	Sample Type*	Microplastic Abundance	Primary Morphotype	%	Primary Color[T]	%2	Primary Polymer	%3	Source
Taguibo Bridge (Site 1)	water	0.68 items/L	Fiber	39.02	Brown	43.90	Polyacetylene	31.71	This study
Pianning Bridge (Site 2)	Water	0.33 items/L	Fiber	40.00	Brown	50.00	RCF 2	35.00	This study
Anticala Bridge (Site 3)	Water	0.42 items/L	Fiber	40.00	Brown	36.00	RCF 1	36.00	This study
Makati Creeks (Philippines)	Water	833 particles/m^3	N/A	N/A	N/A	N/A	N/A	N/A	[1]
Cañas River (Philippines)	Water	1580 particles/m^3	Fragment	46	White	41.00	Polypropylene (PP)	45.00	[2]
Pasig River (Philippines)	Water	3,405 particles/m^3	Fragment	71	Trans	24	PP	50	[2]
Parañaque River (Philippines)	Water	5,015 particles/m^3	Fragment	55	White	45	PP	49	[2]
Tunasan River (Philippines)	Water	7,547 particles/m^3	N/A	N/A	N/A	N/A	N/A	N/A	[3]
Tullahan River (Philippines)	Water	11,475 particles/m^3	Fragment	54	White	39	PP	36	[2]
Tamsui River (Taiwan)	Water	10.1 ± 5.1 to 70.5 ± 30.6 particles/m^3	Fragment	81.9	N/A		N/A		[4]
Dahan River (Taiwan)	Water	6.7 ± 2.4 to 83.7 ± 70.8 particles per m^3	Fragment	87.2	N/A		N/A		[4]
Keelung River (Taiwan)	Water	2.8 ± 1.2 to 64.4 ± 76.2 Particles per m^3	Fragment	55.2	N/A		N/A		[4]
Xindian River (Taiwan)	Water	2.5 ± 1.8 to 11.7 ± 5.8 particles per m^3	Fragment	48.0	White	25.3	N/A		[4]
Meycauayan River (Philippines)	Water	57,665 particles/m^3	Fragment	61	White	39.00	PP	64.00	[2]
Haihe River (China)	Water	9300 particles/m^3	Fiber	17.4–86.7	white	21.9	Polyethylene	28.2	[5]
Chi River Basin (Thailand)	water	141 items/m^3	Fiber	63	dark blue	28	PP	78.2	[6]

Table S1: (*Continued*) Comparison of microplastic characteristics from water samples across different studies conducted in Asia

Location	Sample Type*	Microplastic Abundance	Primary Morphotype	%	Primary Color[T]	%2	Primary Polymer	%3	Source
Hashilan Wetland National Heritage (Iran)	water	3 particles/L	fiber	91.67	blue/ green	66.67	PP	N/A	[7]
Minjiang River - Minjiang estuary (China)	water	N/A	fiber	59.19	trans	61.84	Polyethylene Terephthalate (PET)	59.8	[8]
Renhuai basin of Chishui River (China)	water	1.77 to 14.33 items/L	fiber	59.4	White/ trans	41.3	N/A	N/A	[9]

Note: [T]trans - transparent

Table S2: Comparison of microplastic characteristics from sediment samples across different studies conducted in Asia

Location	Sample Type*	Microplastic Abundance	Primary Morphotype	%	Primary Color[T]	%2	Primary Polymer	%3	Source
Taguibo Bridge (Site 1)	sed	33.33 items/kg	Filament	66.67	Blue, brown, and white	26.67 each	PP	66.67	This study
Pianning Bridge (Site 2)	sed	4.44 items/kg	Filament and Fiber	50.00 each	Blue and Yellow	50.00 each	PP and RCF 2	50.00 each	This study
Anticala Bridge (Site 3)	Sed	13.33 items/kg	Filament	83.33	White	50.00	PP and CPE	33.33 each	This study
Lawaye River (Philippines)	Sed	75 particle/kg	N/A	N/A	N/A	N/A	N/A	N/A	[10]
Cañas River (Philippines)	Sed	557 particle/kg	Fragment	36	Blue	27	PP	56	[2]
Pasig River (Philippines)	Sed	771 particle/kg	Fragment	65	White	29	PP	50	[2]
Tullahan River (Philippines)	Sed	848 particle/kg	Fragment	48	White	51	PP	36	[2]
Parañaque River (Philippines)	Sed	1,033 particle/kg	Fragment	41	Blue	22	PP	49	[2]
Meycauayan River (Philippines)	Sed	1,052 particle/kg	Fragment	65	White	22	PP	64	[2]
Lihe River Watershed (China)	sed	222.13 ± 54.66 items/kg	Fragment	35.00	white	44.62	PE	37.34	[11]
Sago River (Indonesia)	sed	14,000 particles/kg dry sediment	Fragment	65.42	N/A	N/A	Polystyrene	N/A	[12]
Chi River Basin (Thailand)	sed	9.5 items/kg	Fiber	81.9	dark blue	39.6	PP	73.4	[6]
Hashilan Wetland National Heritage (Iran)	sed	4.44n/kg	Fiber	95.77	black/ gray	47.89	PP	N/A	[7]

Note: *sed – sediment; [T]trans – transparent;
PP – polypropylene; PET – polypropylene terephthalate; PE – polyethylene; CPE – chlorinated polyethylene; RCF 1 – regenerated cellulose fiber brand 1; RCF 2 – regenerated cellulose fiber brand 2

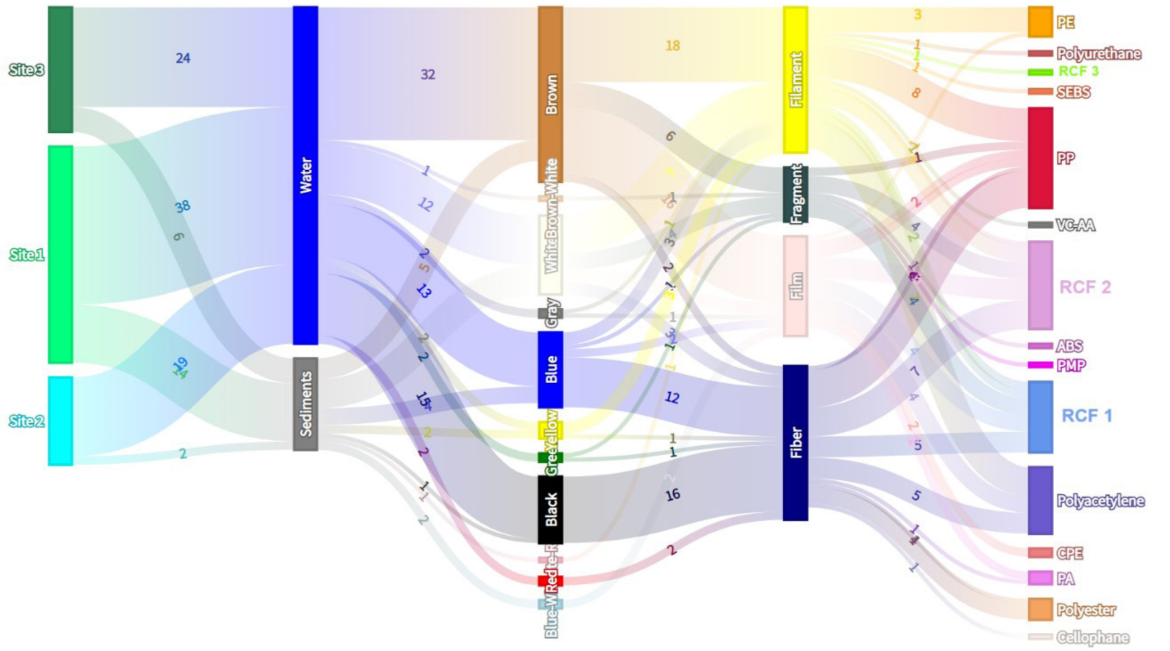

Figure S1: Sankey diagram summary of characteristics of samples from all sites.

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