Influence Of Domestic Wastewater Discharges On Microplastic Contamination In Surface Waters And Freshwater Fish At Huai Kho Reservoir, Na Chueak District, Maha Sarakham Province.

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Abstract
The escalation of microplastic pollution in global aquatic environments has become a significant point of concern in recent years. This study delves into the influence of domestic wastewater discharges on microplastic contamination in both surface water and freshwater fish within Huai Kho Reservoir, situated in the Na Chueak District of Maha Sarakham Province. Sampling was conducted meticulously once, specifically during the rainy season, from September to November in the year 2022. The study's findings unveiled a significant revelation. At Station 1 (ST1), located in Khlong Nong Lao, the waterway exhibited the highest influx of microplastic contamination into Huai Kho Reservoir, registering at 65 items per liter. Surface water, comprising 420 pieces, demonstrated a predominant size range of 0.11-1.00 millimeters, constituting 66% of the total. Furthermore, 47% of these microplastics exhibited a blue coloration, with 70% of them being fiber-shaped. In the case of freshwater fish, a total of 999 pieces were examined, with *Henicorhynchus siamensis* (Hesi) displaying the highest average number at 12.25±3.78 pieces per individual. Similarly, the prevalent size range observed in these fish was 0.11-1.00 millimeters, accounting for 58% of the total count. Notably, 70% of these microplastics exhibited a blue color, and an overwhelming 92% were fiber-shaped. The results of this study indicate that microplastic contamination has occurred in domestic wastewater discharges and suggests a relationship between the inhabitants, surface water, and freshwater fish. The analysis revealed that the most prevalent polymer type identified was Polypropylene (PP), found in 50% of the samples, possibly originating from activities such as population density, washing clothes, textiles, agriculture, and fishing.

Keywords: Domestic Wastewater, Microplastic, Surface Waters, Freshwater Fish, Huai Kao Reservoir

Introduction

In recent years, Thailand has experienced a continuous increase in the quantity of waste generated annually. In the year 2022, the country produced 25.70 million tons of municipal solid waste, equivalent to 70,411 tons per day. This waste is distributed across various regions, with a per capita waste generation rate of 1.07 kilograms per person per day, based on the 2022 population data from the Department of Provincial Administration. Upon analyzing the data concerning municipal solid waste generation, it is evident that there has been a notable increase compared to the previous year, which recorded 24.98 million tons. This represents a 3% rise in waste generation (Pollution Control Department, Thailand, 2023).

Microplastics are microscopic plastic grains that are said to be common in discarded plastic fragments (Thompson et al., 2004) goods Microplastics are defined as small particles of plastic less than 5 mm in length, and further categorized according to their origin as primary and secondary. Primary sources include synthetic plastics produced in small sizes for a variety of applications, such as polyethylene microbeads (scrubbers) in household cleaners, cosmetic products, industrial abrasives for sandblasting, and manufactured feedstock pellets. Secondary sources of Microplastics include small fibers or fragments originating from the natural biochemical degradation of large plastic products such as plastic bottles, plastic bags, fishing nets, microwave containers, and plastic household items in the environment (Napper et al., 2015; Horton et al., 2017; Xu et al., 2020)

The direct effects of microplastics on aquatic animals are both physical and nutritional. Plastic ingredients and additives are indirectly aquatic, transferred through aquatic food webs and impact food safety and human health. so microplastics on human health and the toxic effects that may vary depending on the type, size, shape, chemical composition, surface charge, and hydrophobicity of microplastic particles (Lee et al., 2023). When microplastics enter the human body, they can have diverse impacts on several physiological systems, leading to disturbances in the digestive, respiratory, endocrine, reproductive, and immune systems. Studies have shown that exposure to microplastics results in oxidative stress, cytotoxicity, and neurotoxicity. In addition, research indicates the transfer of microplastics to different tissues within the body (Bhuyan, 2022; Lee et al., 2023; Yadav and Agwuocha, 2023).

When fish and aquatic animals ingest microplastics, these particles can obstruct or clog their gills, alimentary canal, and other internal organs, leading to reduced appetite and decreased food intake. This phenomenon, where microplastics infiltrate various tissues such as the skin, gills, muscles, and alimentary canal of aquatic organisms, results in a diminished desire to consume food (Mazurais et al., 2015; Yadav and Agwuocha, 2023). Moreover, exposure to insufficient or nutritionally inadequate food due to these effects has significant repercussions on the population dynamics and overall numbers of aquatic species. MPs (Microplastics) induce an array of detrimental effects, including tissue damage, vascular injuries, inflammation, oxidative stress, alterations in immune-related gene expression, and changes in antioxidant status within fish. Consequently, fish exposed to microplastics exhibit neurotoxicity, growth retardation, and behavioral abnormalities, further underscoring the multifaceted impact of microplastic pollution on aquatic ecosystems (Bhuyan, 2022).

In 2015, the United Nations and its associated members univocally recognized the different actions needed to achieve Sustainable Development Goals (SDGs). Among these, one goal is to assess emerging environmental pollutants such as plastic pollution, their environmental impacts, and different management options both in terms of adaptation and mitigation. Addressing plastic pollution will help to expedite our efforts to achieve various SDG goals, namely Goal 2 (End hunger, achieve food security and improved nutrition, and promote agriculture) Goal 3 (Ensure healthy lives, and promote well-being for all at all ages) because MPs are accumulated in the freshwater fish SDG 12 (Responsible Consumption and Production), SDG 14 (Life Below Water), SDG 15 (Life On Land) (Kumar et al., 2022; Tongnunui et al., 2022)

Huai Kho Reservoir, located in Tambon Khwai Rai, Amphoe Na Chueak, Maha Sarakham province, Thailand, stands as the largest water storage facility in the region. Positioned at coordinates 15°49'35.3"N latitude and 103°02'14.3"E longitude (Figure 1), this medium-sized water storage pond is situated at an elevation of 150 meters above sea level. Covering an expansive area of approximately 2,113 square kilometers or 6,340 acres, Huai Kho Reservoir plays a crucial role in water resource management. With a significant capacity of 31.338 million cubic meters, this reservoir has the ability to store 208.00 square kilometers of rainwater. Huai Kho Reservoir, constructed in 1968, serves multiple purposes including supply water production, flood control, irrigation for agriculture, aquaculture, fisheries, and tourism, making it a versatile and vital infrastructure. The reservoir is surrounded by a residential population and serves as a receptor for untreated community wastewater discharge (CMARE, 2020). Currently, studies have revealed research on microplastic contamination of freshwater fish in stagnant water sources such as reservoirs (Rahmayanti et al., 2022) and wetlands (Neatsingsang, 2020). There are still relatively few studies and research efforts in comparison to those conducted in lakes (Faure et al., 2015; Jabeen et al., 2017; Xiong et al., 2018; Yuan et al., 2019; Adeogun et al., 2020; Xu et al., 2021; Yin et al., 2021; Atici et al., 2021; Mercy et al., 2023; Miranda-Pena et al., 2023) and dams (Turhan, 2021; Kasamesir et al., 2021; Boyukalan and Yerli, 2023).

Freshwater fish serve as a significant protein source for humans residing near Huai Kho Reservoir. The prevalence and ecotoxicological effects of microplastics in fish could potentially impact aquatic food security in the area. Fish caught in Huai Kho Reservoir are not only sold fresh but are also processed into products such as orange roughy and pickled fish. This study investigated the impact of domestic wastewater discharges from activities around Huai Kho Reservoir on the abundance and morphological characteristics of microplastics. The research focused on examining the size, color, shape, and polymer type of microplastics, as well as comparing their abundance in surface water and freshwater fish. The study quantified and assessed the influence of domestic wastewater discharges from Huai Kho Reservoir on microplastic pollution. The results of this study will be instrumental for monitoring microplastics in reservoirs, understanding waste management and water quality concerning microplastics, optimizing the utilization of the reservoir's surrounding areas, and establishing a robust database to bolster fisheries and enhance food security.

2. Materials and Methods

2.1 Ethical statement: This research project has been approved by Ethical Principles and Guidelines for the Use of Animals No. 36/2023 of Mahasarakham University, Thailand. The project leader holds a license number for Ethical Principles and Guidelines for the Use of Animals No. U1-08403-2562 from the Institute of Animals for Scientific Purposes Development (IAD), National Research Council of Thailand (NRCT), Thailand.

2.2 Determination of Microplastics Sampling Points: the selection of microplastics sampling points is a crucial aspect of this study. In defining these points within the reservoir of Huai Kho, the process involves dividing the area into two distinct sections, as follows:

2.2.1 Surface Water Sample Collection: the collection of surface water samples is conducted based on careful consideration of sources of pollutant discharge into the reservoir system of Huai Kho. This selection considers the density of the population in the area and the patterns of water usage surrounding the reservoir of Huai Kho. The delineation of sampling points for surface water collection around the reservoir is categorized into two distinct sections.

Part 1: Surface Water Samples from Canal Areas: this section comprises surface water samples taken directly from the canal areas originating from the activities of the surrounding communities. These communities reside in proximity to the reservoir system of Huai Kho and are directly connected to the inflow channels feeding into the reservoir (ST1-ST5).

Part 2: Determination of Surface Water Sampling Points within the Huai Kho Reservoir (ST6-ST8): For the designation of sampling points within the reservoir system of Huai Kho (ST6-ST8), water samples are collected utilizing a specific method. Samples are extracted using a submersible pump at a depth of 50 centimeters and transferred into 20-liter stainless steel containers. Subsequently, the water is filtered through a mesh with a pore size of 20 micrometers. The filtered water is then transferred into glass bottles. Upon completion, these glass bottles containing the samples are preserved at a temperature of 4 degrees Celsius. This preservation method ensures the integrity of the samples for subsequent laboratory analysis, following the protocol modified from the approach established by Xu et al., 2018.

2.2.2 Fish Sample Collection: During the period between September and November 2022, which coincided with the rainy season, fish specimens were meticulously collected on a monthly basis. These collections took place at three specific sampling stations, namely ST6-ST8, within the Huai Kao reservoir, as illustrated in **Figure 1.** The collection methodology involved the strategic use of gill nets with depths ranging from 1.0 to 1.5 meters. These nets were designed with varying mesh sizes, specifically 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, and 6.0 cm. To enhance efficiency, three sets of gill nets were interconnected in a linear configuration to optimize the sampling process. Six replicates were randomly conducted at each survey point over a duration of 12 hours, commencing at 4:00 p.m. and concluding at 4:00 a.m. Following collection, each fish specimen was subjected to taxonomic identification at the species level, utilizing the methods outlined by Rainboth (1996) and Vidthayanon (2008). Upon completion of the identification process, the individual fish samples were preserved at a sub-zero temperature of -20 degrees Celsius. This stringent preservation method was employed to maintain the integrity of the samples, ensuring their suitability for subsequent detailed analysis within the laboratory setting.

2.3 Microplastic Analysis

2.3.1 Method for Microplastic Analysis in Water Samples: A 1-liter water sample was transferred into a 2000-milliliter beaker. Then, 100 milliliters of 30% hydrogen peroxide solution (H_2O_2) were added to the sample. The sample was left undisturbed for 4 hours. Next,165 grams of salt were added (at a ratio of 150 grams of salt per 1 liter of water).The sample was left undisturbed overnight. The treated water sample was filtered through a glass fiber filter paper with a pore size of 20 micrometers (Whatman GF 4/C 20 µm pore size).The filtered microplastic particles were placed in a glass plate and oven-dried at 55 degrees Celsius for 4 hours. This methodology, adapted and modified from Xu et al. (2018); Barcelo (2020)

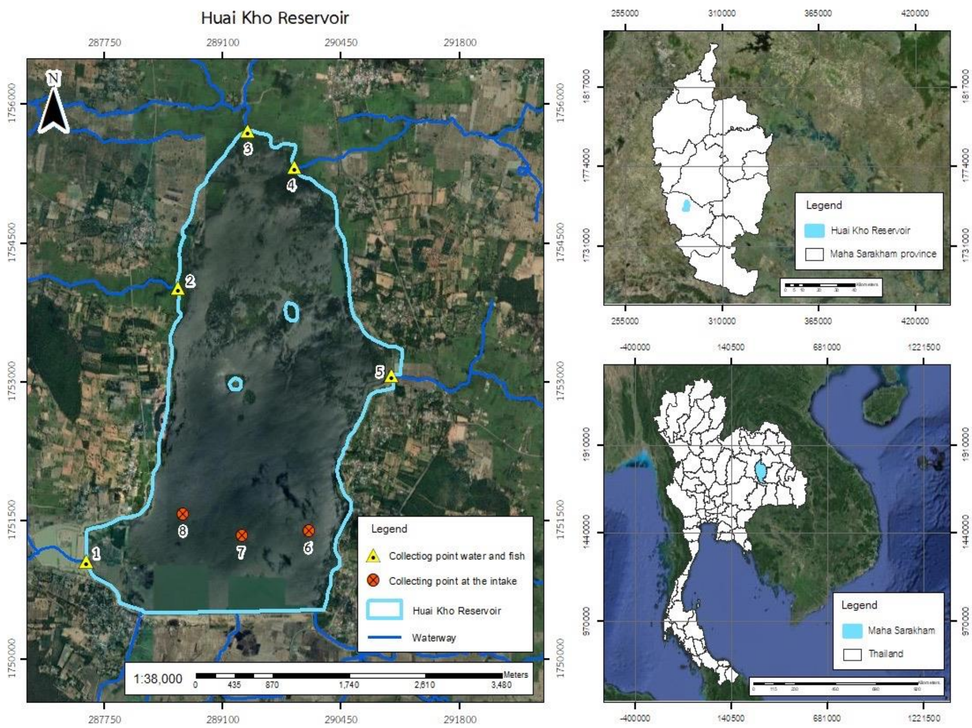


Figure 1. Map and location of the 8 sampling stations (ST) in the Huai Kao reservoir, Na Chueak of District, Maha Sarakham Province, Thailand

Table 1. Sampling Stations (ST) in the Huai Kao reservoir, Na Chueak of District, Maha Sarakham Province, Thailand.

Sampling stations	Site Code	Latitude	Longitude
Khlong Nong Lao	ST1	15°82'9173"N	103°01'59.28"E
Khlong Chum Saeng	ST2	15°85'62.70"N	103°02'69.03"E
Khlong Kud Laeng	ST3	15°87'09.12"N	103°03'30.92"E

Khlong Pha Thung Mah	ST4	15°86'72.96"N	103°03'82.16"E
Khlong Don Loi	ST5	15°84'61.78"N	103°04'62.66"E
Pattaya Na Chauk 1	ST6	15°83'25.43"N	103°03'99.59"E
Huai Kho reservoir	ST7	15°83'26.98"N	103°03'26.62"E
Ban Kao Kwang	ST8	15°83'59.38"N	103°02'47.81"E

2.3.2 Microplastic Analysis in Fish Samples: Individual fish specimens from Section 2.2.2 underwent dissection, and their gastrointestinal tracts were isolated and weighed. Subsequently, the gastrointestinal tracts were finely minced and subjected to an oven-drying process at 70 degrees Celsius for 24 hours. The dried samples were then treated with a digestion solution, comprising 5 times the weight of the sample in 30% hydrogen peroxide (H_2O_2) solution, and stirred for 1 hour. Subsequently, the finely homogenized samples were augmented with 3 grams of salt per 20 milliliters of sample solution. Following this, the samples were filtered using glass fiber filter paper with a pore size of 20 micrometers. After filtration, the residues were placed on a glass plate and then oven-dried at a temperature of 55 degrees Celsius for a duration of 4 hours. This method was adapted from the procedure described by Kasamesiri and Thaimuangphol (2020).

2.3.3 Microplastic Analysis: Upon completion of the filtration process, the dried filter papers were examined under a Stereo microscope. Detailed records were made, noting the quantity, size, shape, and color of all microplastics found in both water and fish samples. Moreover, a random selection of microplastics was subjected to further analysis using Fourier Transform Infrared Spectroscopy (FT-IR) technique. This analysis was performed using the PerkinElmer Spectrum Two FT-IR Spectrometer.

2.4 Statistical Data Analysis: Statistical analysis was performed using percentage values to analyze the percentage distribution of microplastic types based on quantity, size, color, shape, and polymer composition. Microsoft Excel was employed for this analysis.

3. Results

3.1 Microplastic contamination in surface water

3.1.1 Abundance and distribution of microplastic contamination in surface water. The highest average number of microplastics was recorded at station 6, accounting for 34%. It was followed by station 1 at 15%, station 2 at 11%, station 4 at 10%, station 8 at 9%, station 3 at 8%, station 7 at 6%, and station 5 at 6%, respectively.

3.1.2 Size of microplastic contamination in surface water. The most prevalent size range observed was 0.11-1.00 millimeters, constituting the highest proportion at 66%. This was followed by microplastics smaller than 0.10 millimeters, accounting for 14%, sizes between 1.01-2.00 millimeters and 2.01-3.00 millimeters, each at 14%. Microplastics larger than 4.01 millimeters represented 1% of the samples, while sizes between 3.01-4.00 millimeters also constituted 1% (See Figure 2A).

3.1.3 Colors of microplastic contamination in surface water. In terms of color distribution, blue microplastics dominated, comprising 47% of the total observed. Transparent-colored microplastics followed at 9%, while black and sky-colored microplastics each constituted 8% and 7% respectively. Red and orange-colored microplastics were each recorded at 6%, and white-colored microplastics at 5%. Green and pink-colored microplastics each made up 4% of the sample, whereas yellow-colored microplastics accounted for 3%, and brown-colored microplastics represented 1% (See Figure 2B).

3.1.4 Shape of microplastic contamination in surface water: Regarding the shapes of microplastics, fibers constituted the majority, representing 70% of the observed samples. Fragments-shaped followed at 22%, while pellets-shaped accounted for 4%. Rod-shaped microplastics were observed in 3% of the samples, and both foam and film-shaped microplastics each represented 1% of the total (See Figure 2C).

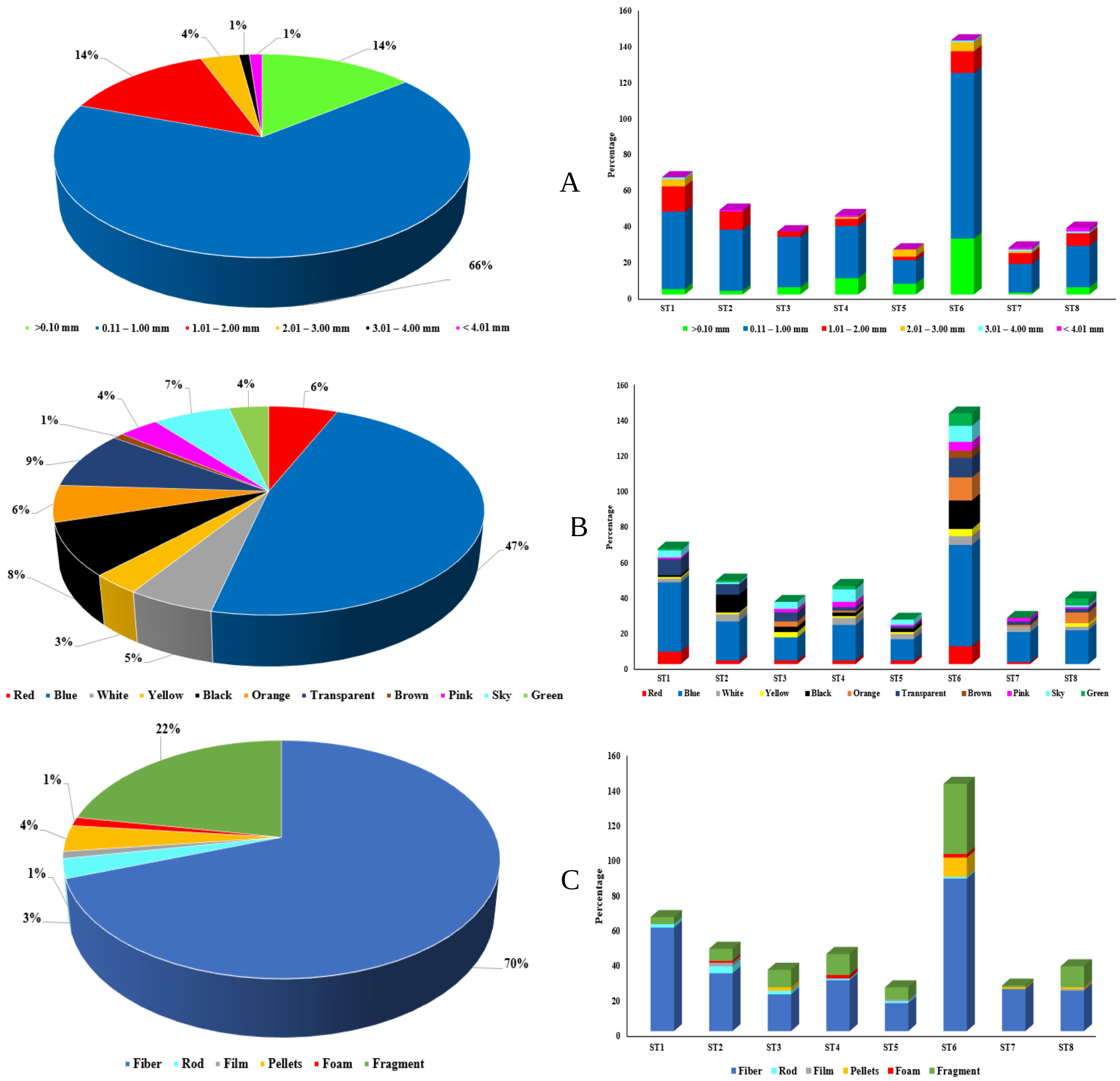


Figure 2. The percentage of the total number of microplastics contamination by surface water of size (A), color (B), shape (C)

3.2 Microplastic contamination in freshwater fish

3.2.1 Freshwater fish species. A comprehensive survey revealed a total of 14 fish species, totaling 184 individuals. The most abundant species observed were *Hampala dispar* (Hadi) with 73 individuals, followed by *Puntius brevis* (Pubr) with 26 individuals, *Henicorhynchus caudimaculatus* (Heca) with 20 individuals, and *Notopterus notopterus* (Nono) with 13 individuals. *Channa striata* (Chst) comprised 10 individuals, while both *Oreochromis niloticus* (Orni) and *Oxyeleotris marmorata* (Oxma) were found in 9 individuals each. *Cyclocheilichthys armatus* (Cyar) and *Anabas testudineus* (Ante) were observed in 5 individuals each, whereas *Henicorhynchus siamensis* (Hesi) and *Clarias batrachus* (Clba) were identified in 4 individuals each. *Monotrete fangi* (Mofa) was found in 3 individuals, *Labiobarbus lineatus* (Lali) in 2 individuals, and *Parambassis apogonoides* (Paap) in 1 individual, as seen in Table 2.

3.2.2 Occurrence of microplastic contamination in freshwater fish. Among the 14 identified fish species, a total of 184 individuals were examined for microplastic contamination. In this regard, 171 individuals (92.93%) were found to have microplastic contamination, while only 13 individuals (8.07%) were devoid of any such contamination. The prevalence of microplastic contamination was highest in species like *Cyclocheilichthys armatus* (Cyar), *Parambassis apogonoides* (Paa), *Henicorhynchus siamensis* (Hesi), *Oxyeleotris marmorata* (Oxma), *Anabas testudineus* (Ante), *Clarias batrachus* (Clba), *Channa striata* (Chst), and *Monotrete fangi* (Mofa), each registering a 100% contamination rate. *Hampala dispar* (Hadi) exhibited a 97.26% contamination rate, *Puntius brevis* (Pubr) showed 96.15%, *Henicorhynchus caudimaculatus* (Heca) exhibited 90.00% contamination, while *Notopterus notopterus* (Nono) showed 92.30% contamination. *Oreochromis niloticus* (Orni) displayed a contamination rate of 42.85%, and *Labiobarbus lineatus* (Lali) exhibited no microplastic contamination (See Table 2).

3.2.3 Abundance of microplastics contamination in freshwater fish. A total of 999 microplastic pieces were discovered, with an average contamination of 5.78±3.26 pieces per individual. Among the species, *Henicorhynchus siamensis* (Hesi) exhibited the highest contamination, averaging 12.25±3.78 pieces per individual. Following closely were *Parambassis apogonoides* (Paap) with an average of 9.00±0.00 pieces per individual, *Anabas testudineus* (Ante) with 8.80±1.96 pieces per individual, *Monotrete fangi* (Mofa) with 8.33±3.12 pieces per individual, *Cyclocheilichthys armatus* (Cyar) with 7.20±2.58 pieces per individual, *Channa striata* (Chst) with 6.70±4.81 pieces per individual, *Hampala dispar* (Hadi) with 5.82±4.37 pieces per individual, *Notopterus notopterus* (Nono) with 5.54±3.74 pieces per individual, *Puntius brevis* (Pubr) with 5.35±3.13 pieces per individual, *Oxyeleotris marmorata* (Oxma) with 4.56±1.33 pieces per individual, *Henicorhynchus caudimaculatus* (Heca) with 3.30±2.17 pieces per individual, *Oreochromis niloticus* (Orni) with 2.00±2.57 pieces per individual, *Clarias batrachus* (Clba) with 2.00±1.15 pieces per individual, and *Labiobarbus lineatus* (Lali) showed no presence of microplastics (See Table 2).

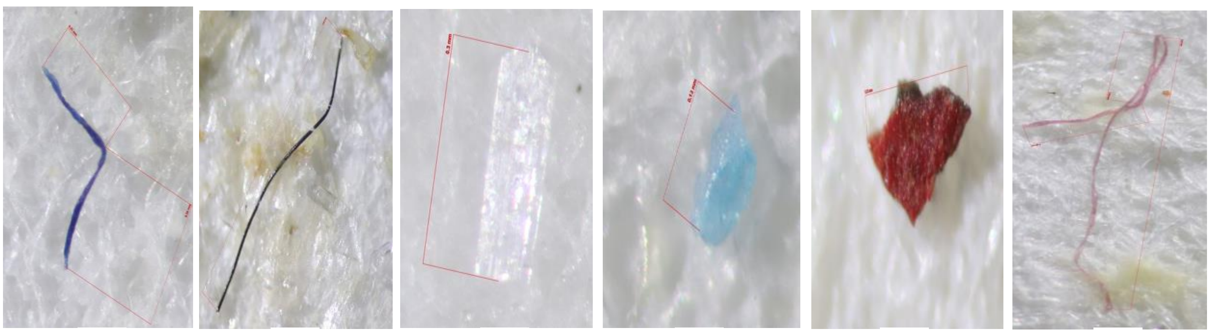
3.2.4 Size of microplastic contamination in freshwater fish. The predominant size range observed was between 0.11 and 1.00 millimeters, accounting for 58% of the total, followed by the range of 1.01-2.00 millimeters at 26%. Sizes ranging from 2.01-3.00 millimeters constituted 8%, while those between 3.01-4.00 millimeters and exceeding 4.01 millimeters each represented 3% of the total. Microplastics smaller than 0.10 millimeters were found in 2% of the samples (See Figure 5A).

3.2.5 Colors of microplastic contamination in freshwater fish. The predominant color among the microplastics analyzed was blue, constituting 70% of the total. Black-colored microplastics accounted for 7%, while Sky-colored microplastics were present at 6%. transparent-colored microplastics were found at 4%., and both red and pink-colored microplastics were found at 3%. Green-colored microplastics and white-colored ones each represented 2% of the samples. Orange and yellow-colored microplastics made up 1% each, while brow-colored microplastics were not detected in the samples. (See Figure 5B and Figure 3).

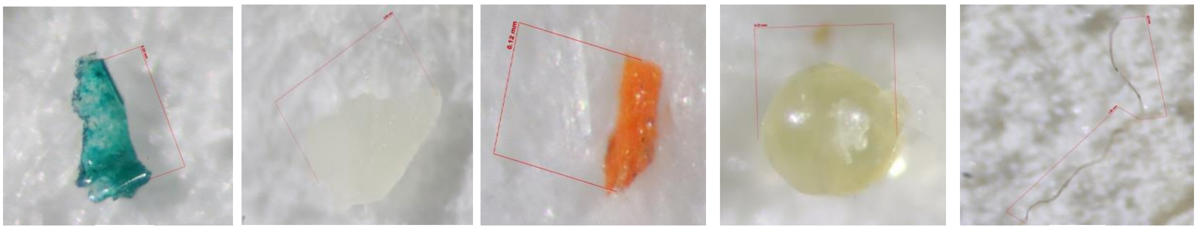
3.2.6 Shape of microplastic contamination in freshwater fish. The predominant shape identified among the microplastics analyzed was fiber, accounting for 92% of the samples. Fragments-shaped were observed in 6% of the samples, while pellets and rods-shaped each represented 1%. Films and foam-shaped were not detected in the samples (See Figure 5C and Figure 4).

Table 2 Fish species, feeding habits, and abundance collected from 3 stations (ST)around the Huai Kao reservoir.

Abbreviation (Ind.)	Feeding habit	Total MPs (pieces)	Total weight (g)	Total length (cm)	Average (pieces /Ind.)	Occurrence of MPs
Nono (13)	Carnivorous	72	42.62±19.49	16.92±3.98	5.54±3.74	92.30
Cyar (5)	Carnivorous	36	20.33±4.97	11.53±0.99	7.20±2.58	100
Hadi (73)	Carnivorous	425	40.26±20.14	14.43±2.28	5.82±4.37	97.26
Paap (1)	Carnivorous	9	6.00±0.00	6.50±0.00	9.00±0.00	100
Orni(9)	Herbivore	18	59.83±23.91	14.33±3.42	2.00±2.57	42.85
Pubr (26)	Omnivorous	139	71.19±27.25	15.91±1.98	5.35±3.13	96.15
Hesi (4)	Omnivorous	49	22.00±0.07	10.63±0.46	12.25±3.78	100
Oxma (9)	Carnivorous	41	51.44±16.34	16.00±1.66	4.56±1.33	100
Ante (5)	Omnivorous	44	40.20±8.91	11.88±1.57	8.80±1.96	100
Heca (20)	plantktivores	66	15.20±3.17	10.34±0.89	3.30±2.17	90.00
Clba (4)	Omnivorous	8	73.50±14.57	20.53±1.76	2.00±1.15	100
Chst (10)	Carnivorous	67	65.90±39.49	6.50±0.00	6.70±4.81	100
Mofa (3)	Carnivorous	25	15.33±1.15	8.10±0.46	8.33±3.12	100
Lali (2)	Herbiivorus	0	23.50±2.12	12.60±0.14	0.00±0.00	-

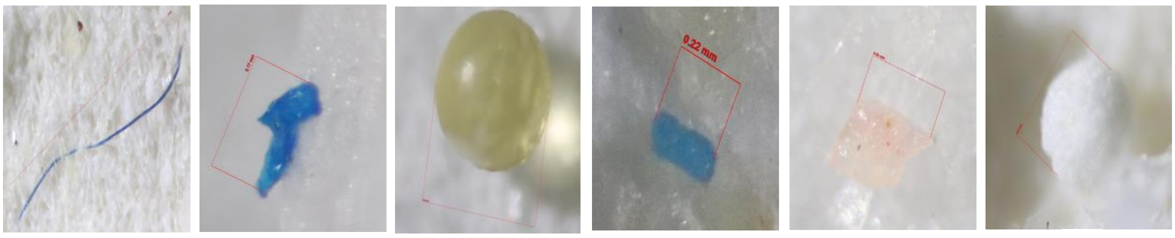


A **B** **C** **D** **E** **F**



G H I J K

Figure 3. Color of microplastics contamination found in gastrointestinal tract of freshwater fish: Blue (A), Black (B), Transparent (C), Sky (D), Red (E), Pink (F), Green (G), White (H), Orange (I), Yellow (J), Brown (K)



A B C D E F

Figure 4. Shape of microplastics contamination found in gastrointestinal tract of freshwater fish: fiber (A), fragment(B), rod (C), pellet (D) film (E) and foam(F)

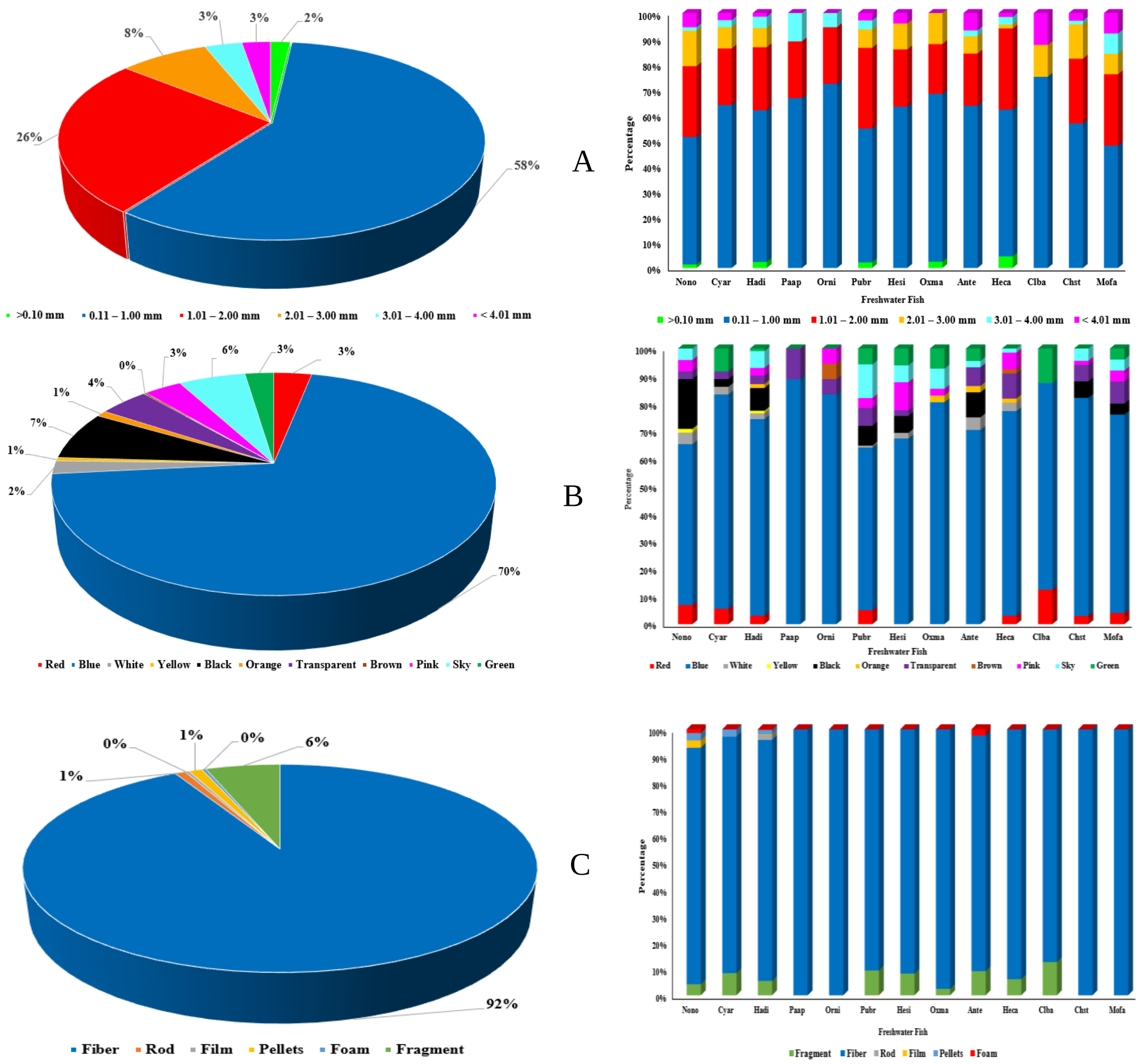


Figure 5. The percentage of the total number of microplastics contamination by freshwater fish of size (A), color (B), shape (C)

3.3 Identification of microplastic. A total of 60 microplastic samples were collected and analyzed using Fourier Transform Infrared Spectroscopy (FT-IR). Among the analyzed polymers, Polypropylene (PP) constituted the majority, accounting for 50% of the samples. Polyethylene Terephthalate (PET) was the second most prevalent polymer, comprising 30% of the samples, followed by Polyethylene (PE) at 15% and Polyamide (PA) at 5% (See Figure 6)

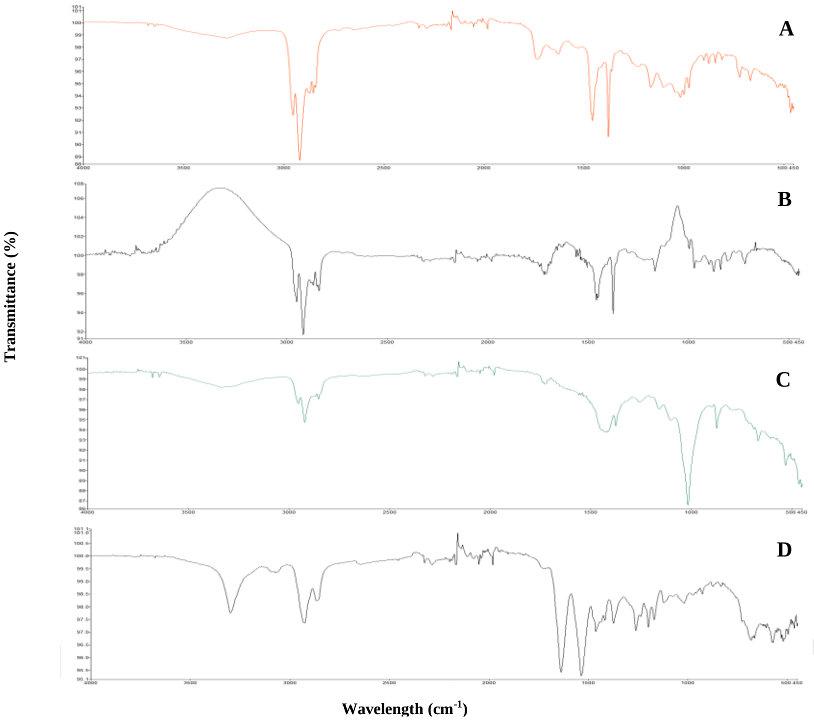


Figure 6. Microplastic fragment polymer types analyzed using FTIR spectra; (A) polypropylene (PP), (B) Polyethylene Terephthalate (PET), (C) Polyethylene (PE) and (D) Polyamide (PA)

4. Discussion

4.1 The abundance of microplastic contamination in freshwater fish

In the investigation of microplastic contamination in freshwater fish, a total of 14 fish species comprising 184 individuals were studied. Out of these, 171 individuals (92.93%) were identified with microplastic contamination, while 13 individuals (8.07%) were free from such contamination. *Henicorhynchus siamensis* (Hesi), classified as an omnivorous species, exhibited the highest average microplastic contamination with 12.25±3.78 pieces per individual. In contrast, *Labiobarbus lineatus* (Lali), identified as herbivores, showed no presence of microplastics (Table 2). This finding is in line with prior research by Mizraji et al. (2017), indicating that omnivorous fish displayed microplastic concentrations significantly higher by an order of magnitude compared to herbivores and carnivores. Consistent with Garcia et al. (2020), fish displaying omnivorous feeding behavior, involving both plant and animal consumption, exhibited a positive correlation with microplastic ingestion. Omnivorous species tend to actively ingest microplastics, possibly due to their diverse foraging habits, spanning various resources throughout the water column (Romeo et al., 2015; Mizraji et al., 2017). This phenomenon could be attributed to the overlapping feeding modes among fish species, including herbivores, planktivores, and detrivores, occupying the same trophic level as primary consumers. These ecological factors, encompassing habitat, feeding behavior, and trophic level, play pivotal roles in determining the frequency and abundance of microplastic ingestion in fish, as highlighted by Adeogun et al. (2020). In this study, samples were collected from five tributaries flowing into the reservoir (ST1-T5) and from the frontal area of the reservoir, specifically near the Huay Kho reservoir (ST6-ST8). These tributaries represented a total of five water channels. The samples were taken in the area characterized by its maximum depth and significant accumulation of debris. The accumulation of debris resulted from the convergence of various pollutants carried by the currents, leading to a high concentration of microplastic contamination in the freshwater fish within this region. The temporal variation in microplastic levels indicates contamination associated with the runoff from tributary streams into the lake during the rainy season (Baldwin et al., 2016; Rojas-Luna et al., 2023), consistent with the findings of Mercy et al. (2023), who identified microplastic pollution in surface water primarily originating from direct domestic wastewater discharges, urban runoff, geographic location and the dispersion of microplastics carried by wind-driven currents (Su et al., 2016; Wibuloutai et al., 2023).

Upon comparison with the reference studies on microplastic contamination in freshwater fish, it was observed that the microplastic ingestion in the gastrointestinal tracts of fish in the Huai Kho reservoir Wildlife Sanctuary's reservoir was higher than other studies. In the present study, microplastic contamination in the gastrointestinal tracts of freshwater fish in the reservoir was found to be 37.96% in 108 fish, with an average of 2.27 pieces per individual. Among the studied species, *Channa striata* exhibited the highest frequency of occurrence, averaging 4.33±4.80 pieces per individual in Bueng Boraphet Wetland, Thailand (Neatsingsang, 2020). Similarly, in Tocagua Lake, Colombia, microplastic contamination was detected in 83.7% of 228 fish, averaging 2.55 pieces per individual, with *Andinoacara latifrons* being the most commonly affected species, having 3.3 pieces per individual (Miranda-Pena et al., 2023). In Surgu Dam, Turkey, 28.6% of 107 fish were found to have microplastics, averaging 1.37 pieces per individual, with *Cyprinus carpi* being the most commonly affected species, exhibiting 1.6 pieces per individual (Turhan, 2021). However, in other studies, microplastic contamination in the gastrointestinal tracts of fish was comparatively lower. For instance, in Gehu Lake, China, only 10.6% of 30 fish had microplastics, averaging 21 pieces per individual, with *Culter dabryi* being the most affected species (Xu et al., 2021). Similarly, in Ubolratana Reservoir, Thailand, 96.4% of 167 fish had microplastics, averaging 2.92±1.30 pieces per individual, with *Parambassis siamensis* exhibiting (4.11±1.08 pieces per fish (Kasamesiri et al., 2021). In Dhanmondi Lake, Bangladesh, 93.3% of 90 fish were found to have microplastics, averaging 8.2 pieces per individual, and *Oreochromis mossambicus* was the most commonly affected species (Mercy et al., 2023) (Table 3). This indicates that fish may ingest microplastics both passively (e.g., through gills) and actively (mistaking them for prey) from the water stream. Consequently, this ingestion leads to the contamination of microplastics in fish organs (Lusher et al., 2013; Barboza et al., 2020). The concentration of microplastics is influenced by various factors, including fish species (Sarijan et al., 2019; Khan and Setu, 2022), fish size (Lestari et al., 2023; Mercy et al., 2023), trophic level (Parker et al., 2021), feeding habits such as herbivory, omnivory, piscivory, and invertivory (Garcia et al., 2020; Kasamesiri and Thaimuangphol, 2020), ecological characteristics (duration of exposure to microplastics-contaminated areas), and physiological differences (e.g., water filtration rate and elimination processes) in fish organisms with active/passive uptake routes (Baalkhuyur et al., 2018; Barboza et al., 2020; Hurt et al., 2020).

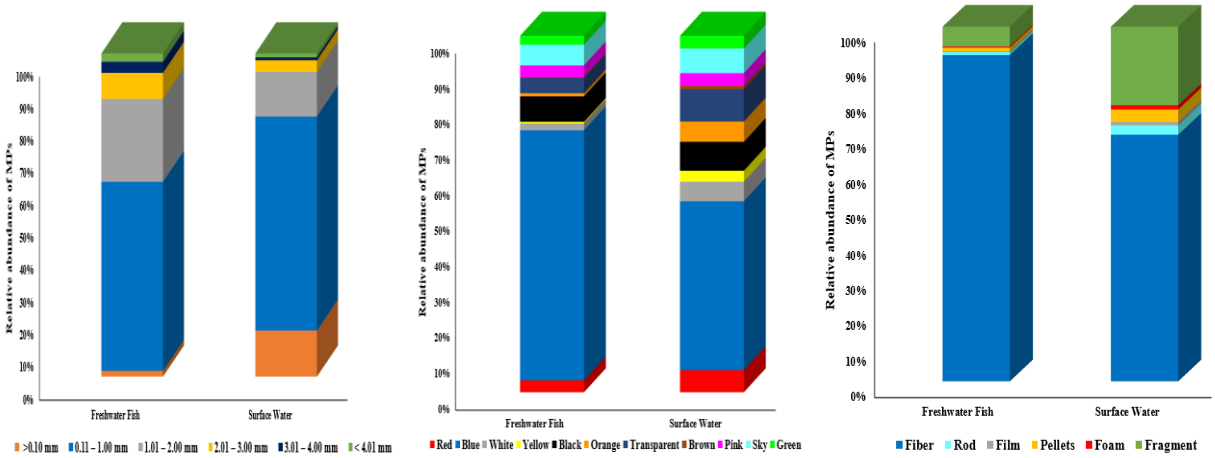
4.2 The Size, Colors, and shapes of microplastic contamination in surface water and freshwater fish

In Huai Kho Reservoir, microplastics smaller than 0.5 millimeters accounted for 99% of the total microplastics. When compared to reference areas, there was no significant difference, indicating that the dominance of small-sized plastics, particularly those less than 0.5 millimeters, is a consistent finding in numerous freshwater studies worldwide. Similar observations have been made in various freshwater bodies, including the Laurentian Great Lakes, USA (Driedger et al., 2015), Lake Hovsgol, Mongolia (Free et al., 2014), Taihu Lake, China (Su et al., 2016), Poyang Lake, China (Yuan et al., 2019), Eleyele Lake, Nigeria (Adeogun et al., 2020), Red Hills Lake, India (Gopinath et al., 2020), Rawel Lake, Pakistan (Irfan et al., 2020) Veeranam lake, India (Bharath et al., 2021), Siberia Lakes, Russia (Malygina et al., 2022), and Phewa Lake, Nepal (Malla Pradhan et al., 2022) The small size of microplastics is a result of the fragmentation or shedding of synthetic fibers from textile products, leading to the formation of microplastics. Consequently, these small-sized microplastics have a higher likelihood of being present in significant quantities. This characteristic allows them to float and persist in the aquatic environment. In addition, the high percentage of small-sized microplastics in the present study suggests a higher probability of contamination due to local activities and land use around the reservoir (Talvitie et al., 2017). These activities might include the use of the surrounding area, leading to the consistent presence of small-sized microplastics in the water body. This finding underscores the need for comprehensive strategies to mitigate the impact of small-sized microplastics in freshwater ecosystems. (Malla Pradhan et al., 2022).

In the conducted study, it was observed that microplastics in freshwater fish predominantly fell within the size range of 0.11 to 1.00 millimeters, constituting 58% of the total microplastics found. This size range was also prevalent in the tributaries (ST1-T5), where microplastics ranging from 0.11 to 1.00 millimeters accounted for 66% of the samples, as shown in Figure 7A. This phenomenon can be attributed to the buoyancy and adhesive properties of small-sized microplastics, allowing them to float effectively on the water surface and adhere to fish food or even to the fish themselves as solid particles suspended in the water. Consequently, this characteristic leads to a higher accumulation of small-sized microplastics in the gastrointestinal tract of fish (Thompson et al., 2004). In the context of human consumption, microplastics smaller than 150 µm, constituting 2% of the microplastics, pose a risk as they can translocate across the human gastrointestinal tract and enter the lymphatic system (Revel et al., 2018). When comparing the microplastic sizes in freshwater fish with the reference areas, consistent patterns were observed. In Ubolratana Reservoir, Thailand, the highest percentage of microplastics in freshwater fish and surface water was found in the size range of 1000-5000 micrometers, constituting 65% of the total (Kasamesir, et al., 2021; 2023). Similarly, in Dhanmondi Lake, Bangladesh, microplastic contamination in both freshwater fish and surface water was most prevalent in the size range of less than 100 micrometers, with 29.53% and 28.88%, respectively (Mercy, et al., 2023). Bueng Boraphet Wetland, Thailand, exhibited the highest microplastic contamination in freshwater fish and surface water in the size range of 355-999 micrometers, constituting 65.9% and 87.5%, respectively (Neatsingsang, 2020). In Gehu Lake, China, microplastic contamination in both freshwater fish and surface water was highest in the size range of 0.1-0.5 millimeters, constituting 66.67% and 51.93%, respectively (Xu et al., 2021). In addition, in Tocagua Lake, Colombia, microplastic contamination was predominantly found in freshwater fish and surface water in the size range of 0.1-0.5 millimeters, accounting for 66.67% and 51.93%, respectively (Miranda-Pena et al., 2023). These findings indicate a consistent correlation between microplastic sizes in freshwater fish and the corresponding surface water in these diverse geographical locations. When comparing microplastic sizes in freshwater fish with the reference areas, no significant correlation was found between the microplastic sizes observed in fish and surface water in Gehu Lake, China. Specifically, the highest microplastic size in freshwater fish was within the range of 0.2-1 millimeters, constituting

45.5% of the total. In contrast, surface water exhibited the highest microplastic size in the range of 1-2 millimeters, accounting for 38% of the total (Xu et al., 2021, Table 3).

Various microplastic colors were identified in the study, with a total of 12 colors, including fundamental colors such as white, black, blue, green, and red, as well as other shades like gray (Yin et al., 2021). In the case of Huai Kho Reservoir, eleven different colors of microplastics were found in both freshwater fish and surface water, including blue, red, black, white, yellow, orange, pink, sky, green, transparent, and brown. Among these colors, blue was the most prevalent, constituting 70% of the total. Specifically, freshwater fish of blue color showed a significant association with the tributary water flowing into the reservoir (ST1-T5) and the water within the reservoir (ST6-T8). Moreover, surface water exhibited a predominantly blue color, accounting for 47% of the total, as reported in Figure 7B. Guo et al. (2021) stated that the composition of microplastic colors is influenced by factors such as concentration, location, and water sampling depth. In the Huai Kho Reservoir area, which is surrounded by households and communities, activities like laundry or textile processing result in water flowing from these activities into the reservoir, causing the water to appear predominantly blue (Li et al., 2018; Yuan et al., 2019). When compared to the reference areas, the relationship between the microplastic colors of freshwater fish was significant. In Dhanmondi Lake, Bangladesh, the highest microplastic contamination of freshwater fish was in transparent color (32.39%), and surface water had a contamination rate of 50% in the same color (Mercy, et al., 2023). Bueng Boraphet Wetland, Thailand, showed the highest contamination of freshwater fish in black color (82.9%), and surface water had a contamination rate of 37.5% in red and black colors (Neatsingsang, 2020). Tocagua Lake, Colombia, had the highest microplastic contamination of freshwater fish in blue color (48.45%), and surface water showed a contamination rate of 43.3% in the same color (Miranda-Pena et al., 2023). Similarly, in Ubolratana Reservoir, Thailand, freshwater fish had the highest contamination rate in blue color (51%), and surface water exhibited the same contamination rate in blue color (51%) (Kasamesir, et al., 2021; 2023). In Gehu Lake, China, both freshwater fish and surface water had the highest contamination rate in transparent color (51%), with surface water showing a contamination rate of 28.91% in the same color (Xu et al., 2021). Being compared to the reference areas, microplastic colors of freshwater fish did not show a significant difference. In Surgu Dam, Turkey, the highest microplastic contamination of freshwater fish was in transparent color (25%), and surface water had a contamination rate of 23.75% in black color (Turhan, 2021) (Table 3).



A B C

Figure 7. Comparison of microplastics by the categories of freshwater fish to surface water of size (A), color (B), shape (C)

The characteristics of microplastics in Huai Kho Reservoir were diverse, including five different shapes: fiber, fragment, rod, film, and foam (Panno et al., 2011). These shapes were also found in both freshwater fish and surface water at the reservoir, with fiber being the most common shape, constituting 92% of the observed microplastics. Freshwater fish exhibited a higher proportion of fiber-shaped microplastics in tributaries (ST1-T5) and the reservoir (ST6-T8), mirroring the prevalence of fiber-shaped microplastics in the surface water, which accounted for 70% (Figure 7C). This high prevalence of fiber-shaped microplastics is indicative of domestic wastewater being directly discharged into the lake, likely originating from human activities in the vicinity of the reservoir (Browne et al., 2011). The presence of fibers in the water, primarily originating from domestic sources such as household wastewater and effluents from community activities, signifies a significant impact on the Huai Kao reservoir. These fibers are associated with various human-related activities, including washing clothes, textiles, and fishing (Meng et al., 2020; Talbot and Chang, 2022). Zhao et al., 2023). The high concentration of fibers highlights the influence of factors like seasonal variations, population density, and fishing activities on microplastic pollution in freshwater ecosystems (Yuan et al., 2019; Hu et al., 2020; Tang et al., 2020; Guo et al., 2021). When comparing Huai Kho Reservoir with reference areas, the analysis revealed that the microplastic shapes in freshwater fish were significantly related to and not significantly different from the microplastic shapes found in surface water. In Bueng Boraphet Wetland, Thailand, the highest microplastic shape observed in freshwater fish was fiber shape, accounting for 93.20%, while surface water exhibited a similar trend with 89.5% of fibers (Neatsingsang, 2020). Tocagua Lake, Colombia, displayed a similar pattern with the highest proportion of fiber-shaped microplastics in both freshwater fish (64%) and surface water (57.24%) (Miranda-Pena et al., 2023). Surgu Dam in Turkey demonstrated a strong correlation between fiber-shaped microplastics in freshwater fish (69.59%) and surface water (69.59%) (Turhan, 2021). In Gehu Lake, China, the highest microplastic shape identified in both freshwater fish (100%) and surface water (93.8%) was fiber shape (Xu et al., 2021). However, when comparing Huai Kho Reservoir with other reference areas, the relationship between the microplastic shapes in freshwater fish and surface water was not significant. For instance, in Dhanmondi Lake, Bangladesh, pellets shape constituted the highest microplastic shape in freshwater fish at 29%, whereas surface water was predominantly film-shaped at 28.0% (Mercy, et al., 2023). Similarly, in Ubolratana Reservoir, Thailand, freshwater fish were primarily composed of fiber-shaped microplastics (98.2%), while surface water was predominantly fragment-shaped at 46% (Kasamesir, et al., 2021; 2023) (See Table 3).

Table 3. Comparison of microplastics concentration in surface water and freshwater fish of reservoir studies around the world

Type of water location	Dominate size	Color	Shape	Height freshwater fish of MPs	Dominate size	Color	Shape	Polymer Type of MPs	Study Areas
Wetland water	355 - 999 µm	red and black	Fiber	*Channa striata*	355 – 999 µm	black	Fiber	Polyester (PES)	Bueng Boraphet Wetland, Thailand (Neatsingsang, 2020)
Lake water	0.1 -0.5 mm	transparent	fiber	*Culter dabryi*	0.1 -0.5 mm	transparent	Fiber	Polyester (PES)	Gehu Lake, China (Xu et al., 2021)
Dam water	1-2 mm	black	fiber	*Cyprinus carpi*	0.2-1 mm	transparent	Fiber	Polyethylene terephthalate (PET)	Surgu Dam, Turkey (Turhan, 2021)
Dam water	1000-5000 µm	blue	fragment	*Parambassis siamensis*	1000-5000 µm	blue	Fiber	Polypropylene (PP)	Ubolratana Reservoir, Thailand (Kasamesir, et al., 2021;2023)
Lake water	<100 µm	transparent	film	*Oreochromis mossambicus*	<100 µm	transparent	Pellets	High Density Polyethylene (HDPE)	Dhanmondi Lake, Bangladesh (Mercy, et al., 2023)
Lake water	<2 mm	blue	fiber	*Andinoacara latifrons*	<2 mm	blue	Fiber	Polyamide (PA)	Tocagua Lake (Miranda-Pena et al., 2023)
Reservoir water	0.11-1.00 mm.	blue	fiber	*Henicorhynchus siamensis*	0.11-1.00 mm.	blue	Fiber	Polypropylene (PP)	This study

4.3 Identification of microplastic

Microplastic polymer types were determined using Fourier Transform Infrared Spectroscopy (FTIR) and categorized into various types, including Expanded Polystyrene (EPS), Polypropylene (PP), Low-density Polyethylene (LDPE), High-density Polyethylene (HDPE), Acrylonitrile-butadiene-styrene (ABS), Polystyrene (PS), Polyamide (nylon) (PA), Polymethyl methacrylate (acrylic) (PMMA), Polycarbonate (PC), Polyethylene terephthalate (polyester) (PET), Cellulose acetate (CA), Polyvinyl chloride (PVC), Polytetrafluoroethylene (PTFE) (Teegarden, 2004). In this study, five types of microplastics were identified in both freshwater fish and surface water: Polypropylene (PP), Polyethylene Terephthalate (PET), Polyethylene (PE), and Polyamide (PA) (Figure 6). Among these types, Polypropylene (PP) was the most prevalent, constituting 50% of the identified polymers. The type and quantity of polymers found most abundantly are correlated with the tributaries that flow into Huai Kho Reservoir (ST1-S5). Specifically, Station 1 (ST1) at Khlong Nong Lao, characterized by densely populated residential communities, exhibited the highest concentration of microplastic contamination, reaching 65 items/liter. This contrasted with other tributaries where population density was lower, as illustrated in Figure 7. Consistently with studies on land use, it was observed that areas densely populated with residential communities had the highest abundance of microplastic contamination. Tibbetts et al. (2018) and Chen et al. (2022) demonstrated that plastic pollution in the Huai Kao Reservoir is a result of tributaries and human activities in the vicinity. The prominent presence of Polypropylene (PP) microplastics, a nonbiodegradable plastic, suggests that disposable single-use plastics from various sources such as tourism, food containers, dishware, ropes, bottle caps, straws, fishing gear, textiles, and face masks for COVID-19 prevention could be the primary contributors to microplastics in the reservoir (Lusher et al., 2017; Singh et al., 2020; Fadare and Okoffo, 2020). This significant presence of Polypropylene (PP) further emphasizes its prevalence in the reservoir.

When compared to the reference areas, our findings align with the previous study on Polypropylene (PP) polymer types. Similar to the study conducted at Ubolratana Reservoir, Thailand (Kasamesir, et al., 2023), Polypropylene (PP) was the most prevalent polymer type, constituting 66% of the microplastics found in our research. However, there are distinctions in our results when compared to other studies. For instance, Polyester (PES) was the dominant polymer type in Bueng Boraphet Wetland, Thailand (Neatsingsang, 2020), accounting for 48.7%. In Gehu Lake, China (Xu et al., 2021), polyester (PES) was the most commonly found polymer. High-Density Polyethylene (HDPE) was prevalent, making up 40.00% of microplastics in Dhanmondi Lake, Bangladesh (Mercy, et al., 2023). Polyethylene terephthalate (PET) was the most common polymer type, comprising 29.1% in Surgu Dam, Turkey (Turhan, 2021), while polyamide dominated with 53.8% in Tocagua Lake, Colombia (Miranda-Pena et al., 2023) (see Table 3). When compared to the reference areas, our findings align with the previous study on Polypropylene (PP) polymer types. Similar to the study conducted at Ubolratana Reservoir, Thailand (Kasamesir, et al., 2023), Polypropylene (PP) was the most prevalent polymer type, constituting 66% of the microplastics found in our research. However, there are distinctions in our results when compared to other studies. For instance, Polyester (PES) was the dominant polymer type in Bueng Boraphet Wetland, Thailand (Neatsingsang, 2020), accounting for 48.7%. In Gehu Lake, China (Xu et al., 2021), polyester (PES) was the most commonly found polymer. High-Density Polyethylene (HDPE) was prevalent, making up 40.00% of microplastics in Dhanmondi Lake, Bangladesh (Mercy, et al., 2023). Polyethylene terephthalate (PET) was the most common polymer type, comprising 29.1% in Surgu Dam, Turkey (Turhan, 2021), while polyamide dominated with 53.8% in Tocagua Lake, Colombia (Miranda-Pena et al., 2023) (see Table 3).

5. Conclusion

This study marks the pioneering investigation into microplastic concentrations within both freshwater fish and surface water at Huai Kho Reservoir, situated in the Na Chueak District of Maha Sarakham Province. The research encompassed an analysis of 14 distinct fish species, totaling 184 individual specimens. Remarkably, out of these, 171 individuals, constituting a significant 92.93%, were found to be contaminated with microplastics. The microplastic concentration in both freshwater fish and surface water exhibited a correlation, particularly in terms of size, color, and shape. The prevalent microplastics found were in the size range of 0.11-1.00 millimeters and exhibited a blue coloration, primarily in a fiber shape, with Polypropylene (PP) composition. In the vicinity of the reservoir, where residential communities directly discharge untreated wastewater into Huai Kao Reservoir, Station 1 (ST1) in Khlong Nong Lao exhibited the highest concentration of microplastics, reaching 65 items per liter. This finding underscores that the influx of untreated water directly influences the microplastics load within the reservoir. The study revealed that the concentration of microplastics in both freshwater fish and surface water is not influenced by factors such as the presence of tributaries, sewage from domestic activities like washing clothes and textiles, population density, or fishing activities. Consequently, our research highlights the significant impact of human domestic sewage on the characteristics of microplastic pollution in Huai Kao Reservoir. This pollution poses potential ecological risks and adverse effects on both the aquatic ecosystem and human health. Given the reservoir's crucial role as a water supply source, as well as its importance for fishing activities and aquaculture, it is imperative to conduct further in-depth and systematic research. Long-term studies are necessary to closely monitor the sources and transport pathways (ST1-ST5) of microplastics. Most important of all, it is crucial to examine the ecological effects of microplastics and assess their potential impact on human health resulting from the consumption of fishery products sourced from Huai Kao Reservoir.

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