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Microplastics on Silkworms (Tubifex Spp) in the Brantas River, Indonesia

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ABSTRACT

Microplastics can contaminate water owing to their small size. If aquatic biota consume microplastics, they disrupt their reproductive processes, digestive tracts, and development. This study aimed to identify microplastic waste from silkworms (Tubifex spp.) in the Brantas River. The study was conducted in a descriptive manner by collecting samples of microplastic waste from silkworms and examining the shape, type, amount, and percentage of microplastic abundance in the river. An FTIR test was used to determine the microplastic content. Using a Zeiss Axio Zoom.V16 at 50x magnification, microplastic particles from individual worms and worm samples were visually identified. Then, the 50% hot needle test was used to determine the composition of the plastic. A total of 263 microplastic particles were found in the worm samples. Silkworms (Tubifex spp.) in the Brantas River, Kediri City, were shown to contain four types of microplastics, namely fibers, filaments, fragments, and granules, which were dominated by filament particles with 49% filament content, 45% fiber, 5% fragments, and 1% granules. The microplastic polymers identified via FTIR were polyethylene and ethylene-polypropylene-diene copolymers. These microplastics can originate from plastic bags, used drinking bottles, rope fibers, and pieces of water hose, which are often found around the Brantas River. Silkworms found in the Brantas River contain microplastic waste from various pollution sources.

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INTRODUCTION

Plastics are synthetic materials that are produced and used worldwide. Plastics are synthetic polymers commonly used in everyday life (da Costa et al., 2016)(Laskar & Kumar, 2019)(Rodrigues et al., 2019). The EU Commission statement in 2017 "Microplastics are of particular concern because of their adverse effects on marine and freshwater environments, aquatic life, biodiversity, and possibly human health because of their small size, which facilitates their absorption and bioaccumulation by organisms or the toxic effects of complex chemical mixtures contained in these particles". (Backhaus & Wagner, 2020)

Microplastics are small crumbs, measuring < 5 mm, that originate from certain types of polymers. Microplastics are synthetic solid particles or polymer matrices that are made either primary or secondary and are insoluble in water, either in regular or irregular shapes, with sizes ranging from 1 µm to 5 mm (Frias & Nash, 2019). Primary and secondary microplastics are two forms of microplastics present in the environment. Primary microplastics are the result of plastic dust generated from plastic items and particle emissions released from industrial activities. (Van Cauwenberghe et al., 2015)(Wagner & Lambert, 2018). Large plastic particles are known as secondary microplastics. Large plastic materials disintegrate into smaller pieces because

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of weathering (Arthur et al. 2009). Microplastics can be classified based on their morphology, such as size, shape, and color. Owing to the various effects of exposure to organisms, size is a very important component, and organisms have the ability to release chemicals quickly owing to the high surface-area-to-volume ratio of microscopic particles(de Sá et al., 2018). Based on data from the FAO, microplastics can be classified into five types based on their morphology: fibers, fragments, beads/granules, foam, and pellets (FAO, 2017; Lusher et al., 2017).

A study on polystyrene accumulation in zebrafish (Danio rerio) found microplastics with a diameter of 5 μm in the gills, liver, and intestines, and accumulation of microplastics with a diameter of 20 μm only in the gills and intestines. This causes swelling and fat accumulation in the body of zebrafish. The study also found that exposure to microplastics induced metabolic changes in the liver and disrupted lipid and energy metabolism in fish (Blackburn & Green, 2022). These metabolic changes and disruptions in lipid and energy metabolism can adversely impact the overall health and reproductive success of fish. Additionally, the presence of microplastics in various organs highlights the potential for widespread contamination and its impact on aquatic ecosystems.

Silk worms (Tubifex spp.) are a type of worm that is well known to the public as a fish food. Silk worms (Tubifex spp.) have a reddish body color, slender and smooth body size, and body length of 1-2 cm In aquatic river habitats, and break down living things. Tropical regions are typically home to silkworms (Tubifex spp.) and are also where they are distributed. The silkworms burrow their heads in dirt to search for sustenance. The tail tip was lifted above the mud surface to breathe. The waterways in which these worms lived first appeared as wavy red colonies. They are typically found on the banks of filthy, murky, and shallow rivers where their way of life is focused (Holmquist, 1983).

Silkworms absorb or consume various types of substrates, including inorganic materials such as microplastics, found at the bottom of river waters. Silk worms that are preyed upon by other aquatic biota, such as fish and shrimp, bioaccumulate in the bodies of animals. If consumed by humans, the chemical content of microplastics found in fish and other aquatic biota can cause toxic transfer. Therefore, it is necessary to conduct research to identify microplastic waste in silk worms (Tubifex spp.) in the Brantas River, Indonesia. The novelty of this study is that researchers will examine microplastics in silk worms, where these worms are the main food for fish, which will have a direct impact on humans as consumers of fish.

MATERIAL AND METHODS

Sampling Location

Sampling was conducted along the Brantas River, Indonesia. Sampling was carried out at several points to represent river samples in the research area. Sample 1 was taken at a point near the Insumo Palace Hotel (7°49’55.3”S 112°00’25.6”E), the second point was taken in the area between the Insumo Hotel and Taman Ngronggo Kediri (7°50’11.3”S 112°00’ 27.6”E), the third sample was taken from the Taman Ngronggo Kediri wastewater flow area (7°50’30.4”S 112°00’27.3”E), the fourth sample was taken at the Kediri Islamic University wastewater disposal point (7°50’43.2” S 112°00’15.1”E).

Here is the map link: https://maps.app.goo.gl/nvro5z6RNikN1eWW6 More details can be seen in Figure 1

Study Design

Using a descriptive research methodology, this study aimed to describe the levels of microplastic waste in silk worms (Tubifex spp.) in the Brantas River in Kediri. The obtained data were processed by dividing the number of microplastics by the number of samples. Descriptive analysis was used to obtain data on the shape, type, amount, and percentage of microplastics. The obtained data are presented as tabular and graphical models.

A. The Location of Kediri River (Province of Java, Indonesia)
B. The specific location of sampling
Sampling location 1: 7°49'55.3"S 112°00'25.6"E
Sampling location 2: 7°50'11.3"S 112°00'27.6"E
Sampling location 3: 7°50'30.4"S 112°00'27.3"E
Sampling location 4: 7°50'43.2"S 112°00'15.1"E

Fig. 1. Sampling location (Brantas River, City of Kediri, East Java, Indonesia

Sediment Collection

The UWITEC gravity stripper collected bottom sediment through short cores (<50 cm) from four locations in the Brantas River, Kediri. This allows the sediment sample to remain undisturbed (60 mm internal diameter), which increases the sediment-air interface by compressing the air sample above it. Samples were collected in an upright position and immediately sent to the laboratory for analysis. The sediment sampling method using the UWITEC gravity stripper was very effective in maintaining sample integrity and quality. The collection process was carried out carefully to ensure that the sediment was not disturbed and remained representative of the conditions of the Brantas riverbed in Kediri.

Extraction Of Sediment And Worms

The top layer of the mud was lifted to the surface, and the core was mounted on the UWITEC extrusion equipment. The surface layer of nucleus 5 is often home to the tubifex worms. A small proportion of the worms were juvenile worms that were not positively identified as T. tubifex. However, they were added because they are tubificid and part of the species community. Using stainless-steel tweezers, the worms were carefully removed from the surface sediment and placed in a laboratory dish. Four sites of surface sediment were extruded after the removal of T. tubifex worms. The extrusion process involved carefully pushing the sediment through a screen to separate the remaining worms from the sediment. This ensured that only Tubifex worms were removed and analyzed, allowing for accurate data collection and species identification. In addition, the extruded sediment was carefully examined under a microscope to ensure that no worms were missed before further analysis was performed.

Microplastic extraction

The sediment samples underwent a density-based sequential extraction process after being put in 50-ml polyethylene tubes that had been previously cleaned. Three extracts were used: 1.8 g cm-2 NaI, 1.2 g cm-2 NaCl, and 1.025 g cm-2 104 NaCl. After adding the initial density solution (1.025 g/cm2 NaCl) to the tube, the contents were constantly agitated for three minutes. Subsequently, the sediment was allowed to settle overnight. After settling, the supernatant was poured onto a different petri dish, decanted, and filtered with Whatman GF-C vacuum filter paper. An extract with the same density was used twice to guarantee that all MP particles were extracted. To obtain denser extracts, this process was repeated systematically. The filter paper was then dried in an oven at 40 °C. Microplastics were removed from the worm samples. Whole worms from four different locations were segregated into different containers to investigate the association between worm features and microplastic intake. After thoroughly clearing any outside debris, every worm sample was placed in deionized water. The worms were then allowed to depurate for an entire day. To avoid ingesting the excretions again, water was replaced after 12 h. Whole worms were measured and placed into distinct, previously cleaned 15-ml polyethylene tubes during depuration. These tubes were labeled accordingly to track each worm sample. The worms were then observed and monitored throughout the depuration process to assess any changes in their behavior or appearance. It has been suggested that this process can be used to extract microplastics because it is efficient and does not break down polymers during tissue digestion (Dehaut et al., 2016)(Karami et al., 2017). Full digestion was accomplished. The resultant slurry was vacuum-filtered through Whatman GF-C filter paper for each sample and then dried at 40°C in a Petri dish oven. During the extraction stage, great care was taken to minimize contamination from the laboratory environment. Prior to use, worm samples were examined for external excrement before and after depuration, and KOH solutions were vacuum-filtered (1.2 μm). Each sample was covered at every stage. During filtering, the filter paper was shielded from airborne contaminants using an aluminum foil cover.

Characterization, quantification, and identification of microplastics

Using a Zeiss Axio Zoom.V16 at 50x magnification, microplastic particles from individual worms and worm samples were visually identified. Then, the 50% hot needle test was used to determine the composition of the plastic. Only the particles that responded clearly to the application of the hot needle were collected and tested. Using Zeiss Zen imaging software, plastic particles were measured along their longest axis and classified according to their type (fiber, filament, granule, and fragment). For each extract or worm depuration, the particles were removed from the filter paper into pots that had been previously weighed. Individual microplastic particles that are removed from worms and sediment surfaces are weighed in bulk owing to their extremely light weight. FT-IR spectroscopy was used to determine the polymer composition of the microplastic particles. Fifty% of the microplastics was recovered from the surface sediments, and each worm was examined using FT-IR. FT-IR spectroscopy analysis revealed that the microplastic particles consisted of a variety of polymers, including polyethylene, polypropylene, and polystyrene. This information provides valuable insight into the types of plastics present in marine environments and their potential sources.

RESULTS AND DISCUSSION

The sanitary conditions of the Brantas River in Kediri City at the time of sampling showed that several types of rubbish were stuck in the Gerak Waru Turi Dam on the Brantas River and pillars supporting the bridge. Rubbish consists of pieces of tree branches and inorganic waste, such as plastic bags, leftover bottled drinking water, sacks, leftover pieces of cloth, pieces of tire/sandal rubber, and discarded diapers. Garbage was also found on the banks of the Brantas River. Waste is generated from the daily activities of the residents of the City of Kediri, as well as waste from other locations that are carried away by the currents of the Brantas River. This large amount of waste can cause microplastic pollution in rivers. Microplastic pollution in rivers is a significant concern, as it poses a threat to aquatic life and ecosystems. The accumulation of plastic waste, including plastic bags and discarded diapers, can break down into tiny particles over time, leading to the release of harmful chemicals into water. Efforts should be made to raise awareness about proper waste management and implement effective measures to reduce the amount of waste being dumped into rivers, such as the Brantas River.

The sources of microplastic damage to plastic goods include greater textile friction (Galgani et al., 2021), microbeads used in personal care products such as facial scrubs and body washes, tire particles, road wear (Councell et al., 2004)(Petrucci et al., 2019)(Järlskog et al., 2020), 3d printers (Stabile et al., 2017)(Byrley et al., 2021), and household laundry activities (Yang et al., 2019)(Pirc et al., 2016). Because microplastic particles are thought to be chemically inert, adsorption and surface chemical degradation are of interest. It has been discovered that these particles can adsorb a variety of organic contaminants, including polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs). Additionally, potentially hazardous additives and monomers may be released as a result of surface chemical breakdown of the microplastic particles. These released additives and monomers can pose a risk to the environment and the organisms that come into contact with them (Zhu et al., 2019)(Wang et al., 2015).

Silk worms (Tubifex spp.), which live in riverbeds, survive by decomposing the various types of substrates found in riverbeds, including microplastics. Samples of silkworms from the Brantas River that had been sampled were sent to the Gresik Ecoton Laboratory, East Java, to analyze the amount and type of microplastics. After analysis at the Ecoton Laboratory, silkworms found in the Brantas River were found to contain microplastic waste from various pollution sources. The results showed that the microplastics discovered in the silkworms were from packing materials, fishing gear, and plastic bottles. Concerns concerning the possible effects of microplastics on aquatic life and human health have been raised by their presence in river ecology. These findings highlight the urgent need for stricter regulations on the disposal and management of plastic waste to prevent further ecosystem contamination. Additionally, further research is required to understand the long-term consequences of microplastic ingestion by both aquatic organisms and humans to develop effective mitigation strategies. The microplastic content found in silk worms (Tubifex spp.) in the Brantas River in Kediri City is shown in Table 1.

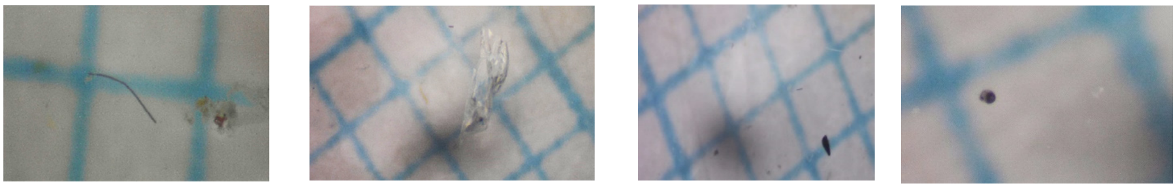
Samples from the Brantas River in Kediri City contained microplastics. The types of microplastics, based on their shapes, are fibers, filaments, fragments, and granules. These four types of microplastics originate from macro-sized plastics, which are broken down into microplastics. Because microplastics that are classified as fibers may come from land, such as residue from washing clothes or fishing gear, they are divided into four categories. Household activities around the Brantas River, such as using plastic bags or containers and disposing of plastic bottles in the river, can produce microplastics in the form of filaments. Fragmented microplastics, such as those found in household appliances made of hard plastic, can also occur because of the use of hard plastic products. Another source of microplastics in the form of fibers is industrial activities such as textile manufacturing and plastic production. These activities can release tiny fibers into the environment, which eventually find their way into water bodies such as the Brantas River. Additionally, microplastics in the form of filaments can also be generated through natural processes, such as weathering and erosion of plastic debris present in riverbanks or nearby coastal areas. More details can be found in Fig. 2.

The difference between filaments and fragments is that filaments appear transparent, whereas fragments do not. Granular microplastics originate from plastic pipes that are dumped into rivers. Gradual deterioration of larger plastic items, such as bottles or bags, can also produce granular microplastics. Although fragments are frequently linked to the mechanical deterioration of plastic objects, filaments can be an indicator of the breakdown of synthetic fabrics in water bodies (A. Lusher et al., 2017). It is important to note that the filaments and fragments have distinct origins and characteristics. Filaments are often linked to the decomposition of synthetic fabrics, whereas fragments are commonly associated with the mechanical breakdown of large plastic objects. Understanding these differences can help researchers identify and address specific sources of microplastic pollution in water bodies.

According to earlier studies from the Baltic Sea, where the average was 0.2 ± 0.2 MP/m3, microplastics appear to have spread around the world (Setälä et al., 2016). The waters of the Gulf of Finland were likewise found to have abundant microplastics (Railo et al., 2018). Microplastics are becoming a worldwide problem rather than being found in some areas. The extensive dispersion of microplastics in many water bodies emphasizes the critical need for additional studies and practical solutions to reduce their negative effects on marine ecosystems. The presence of microplastics in various water bodies has raised concerns regarding their potential impact on marine organisms and ecosystems. Studies have shown that microplastics can be ingested by marine species, leading to adverse health effects and disruption of the food chain. Efforts should be made to develop effective strategies to reduce the release of microplastics into the environment and implement proper waste management practices to

Table 1. Number of Microplastics Identified in Silkworm Samples from the Brantas River, Kediri City

Sample Number	Number and Type of Microplastics			
	Fibre	Filament	Fragment	Granul
1	13	19	3	0
2	42	13	0	0
3	5	7	3	0
4	16	21	1	1
5	9	24	3	1
6	33	45	3	1
Total	118	129	13	3
Percentage	49%	45%	5%	1%



Fibre Filament Fragment Granul

Fig. 2. Forms of microplastics identified from silk worm samples (*Tubifex Spp*)

mitigate their widespread distribution.

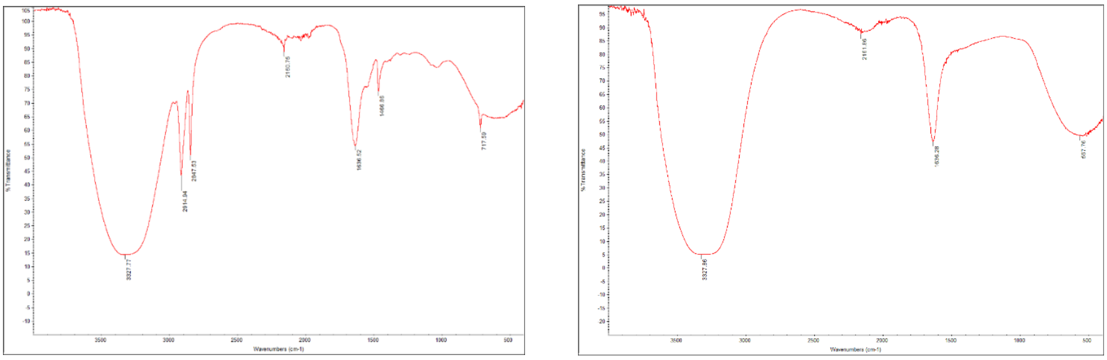
Microplastic concentrations in small, densely populated lakes are higher than those in lakes in less populated areas. Relatively high concentrations were also found in the surface waters of natural Hungary and excavated lakes (3.52–32.05 MPs/m3, particulate matter 100 µm–2 mm) (Bordós et al., 2019). The presence of human activities and population density may contribute to the higher MP concentrations in lakes. Additionally, this study highlights the need for further investigation into the sources and impacts of MPs in these areas to develop effective mitigation strategies. Understanding the sources of microplastics (MPs) in lakes with high human activity and population density is crucial for developing effective mitigation strategies. Further investigations should be conducted to identify specific sources and assess the potential impact of MPs in these areas. This will enable the development of targeted measures to reduce MP concentrations and to protect the health of these ecosystems.

The results of FTIR (Fourier Transfer Infrared) will be used for further identification to ensure that the particles found are microplastics (Particle Suspected as Microplastics, or PSM). FTIR analysis is a widely used technique for determining the chemical composition of materials. By comparing the infrared spectra of the particles with the known reference spectra of microplastics, we can confidently confirm their identity as microplastics. This additional step is crucial to accurately characterize and quantify the presence of microplastics in our environment. The results of FTIR testing of microplastics in samples of silk worms (Tubifex spp.) from the Brantas River are shown in Figure 3.

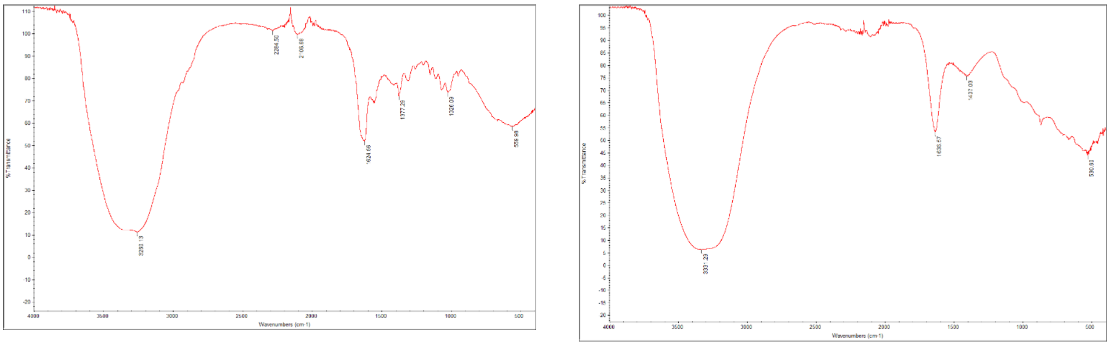
After FTIR analysis was performed on samples of silk worms, the type of microplastic found was polyethylene plastic. Polyethylene is a low-density polyethylene and medium-density polyethylene. Similar results were obtained in a study conducted by Nor and Obbard (2014) in the mangrove forest area of Singapore, namely, the discovery of polyethylene and polypropylene, which are thought to have originated from hard plastic materials (Mohamed Nor & Obbard, 2014). Plastic waste is widespread in various ecosystems, including mangroves. The identification of specific types of plastics such as polyethylene and polypropylene provides valuable insights into the sources and potential impacts of plastic pollution in these environments.

Owing to their large surface-to-volume ratio and chemical composition, microplastics can accumulate waterborne contaminants, including metals and persistent, bioaccumulative, and toxic (PBT) compounds. The interactions between microplastics and chemicals have been studied using adsorption-desorption experiments. Although these interactions are complex, microplastics can act as vectors that transfer environmental contaminants from water to biota. (Wagner et al., 2014). This transfer of contaminants can have detrimental effects on the health and well-being of organisms that ingest microplastics. Additionally, the accumulation of these contaminants in biota can also have cascading effects on entire ecosystems, impacting both aquatic and terrestrial organisms

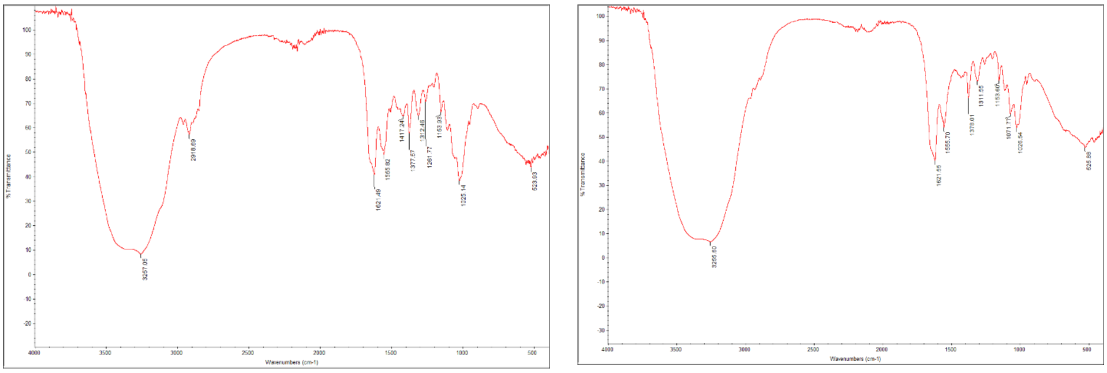
Microplastics are toxic and very dangerous to the environment and humans because they travel in the food chain. Similarly, zooplankton eats microplastics, small fish eats zooplankton,



Sample 1 Sample 2



Sample 3 Sample 4



Sample 5 Sample 6

Fig. 3. FT-IR test results on samples of silk worms (*Tubifex spp*)

oysters, crabs, and predatory fish, all of which end up in human food and enter the digestive system. Microplastics such as PVC can cause the remobilization of small vessels in animals after accidentally eating them. In mammals, small microplastics can move through the digestive tract to the lymphatic and circulatory systems, where they are absorbed into the lungs when inhaled. Microplastics can also affect unborn fetuses because they can travel through the placenta and affect the immune system (Sharma & Kaushik, 2021). Microplastic consumption has been shown to negatively impact the health of several species, including the reduction in immune system function (Segovia-Mendoza et al., 2020)(Liu et al., 2019)(Bhuyan, 2022).

Understanding the extent of adsorption and surface chemical degradation of microplastic particles is crucial for assessing their potential impacts on ecosystems and human health. Adsorption refers to the process by which microplastic particles bind to various substances present in their surroundings such as organic matter and pollutants. This interaction can influence the behavior and fate of microplastics in the environment, affecting their distribution and potential for bioaccumulation. Additionally, surface chemical degradation of microplastics can occur due to exposure to sunlight, water, and other environmental factors, leading to the release of harmful chemicals and further complicating their impact on ecosystems and human health

Nanoparticles in water (waste) treatment process via adsorption. Adsorption is a useful tool to improve the environment. Researchers and businesses are interested in this process. According to Alalwan et al. (2022), several nanomaterials have been investigated for their potential to adsorb organic and inorganic contaminants. Certain nanomaterials have the potential to replace conventional remediation procedures owing to their promising efficacy in eliminating pollutants. However, a few issues prevent these materials from being widely marketed. Process cost-effectiveness, environmental problems, and technological difficulties, such as scaling up to industrial levels and system settings, are some of these shortcomings. In addition, there are several other difficulties. A major issue is the separation of the nanoadsorbent from the aqueous solution, which is related to the size of the material. Furthermore, a major obstacle to the commercial use of nanoadsorbents for water treatment is their low cost and large-scale availability. Furthermore, because nanomaterials accumulate over time, stopping their discharge into the environment is a significant task. Notwithstanding these drawbacks, nanoadsorbents are promising for the treatment of water (waste) and for environmental restoration in the near future. Nanoadsorbents have shown remarkable efficiency in removing various contaminants from aqueous solutions owing to their high surface areas and unique properties. Additionally, ongoing research and development efforts have focused on addressing the challenges of cost-effective production and sustainable disposal of nanomaterials, which will further enhance their potential for widespread use in water treatment and environmental remediation (Alalwan et al. 2022).

Other solutions that can be done In (Ali et al., 2022)s research, remarkable efficacy in eliminating pollutants was noted at a high surface area of the nanoparticle adsorbent. Additionally, the pore volume of the produced nanoparticles reduced the diffusion resistance, which increased the adsorption efficiency. Additionally, Ali's research offers valuable insights into the impact of multiple parameters on the adsorption efficiency. The removal efficiency of the pollutant materials decreased when the pH value was increased to pHpzc Fe3O4/SiO2. However, the removal efficiency and removal percentage of pollutant materials increased significantly when the pH value was increased to six. Ali et al. (2022) suggested that pH plays a crucial role in determining the effectiveness of adsorption using nanoparticles. Furthermore, this research highlights the importance of optimizing the pH conditions to maximize the pollutant removal efficiency in practical applications.

There is insufficient data to make firm judgments about the toxicity of plastic particles in general, and nanoparticles in particular. Microplastics larger than 150 µm are likely to be eliminated by stool according to absorption studies. Although very small microplastic particles, including nanoplastics, may be more widely absorbed and distributed, smaller particles are anticipated to be absorbed to a limited extent. Studies on the toxicology of rats and mice have documented a number of consequences, including liver inflammation. In addition, studies byWthe HO (2019) have shown that plastic particles, especially nanoparticles, can accumulate in various organs, such as the lungs and kidneys. This accumulation can lead to long-term health effects and the disruption of organ function. Furthermore, WHO ((WHO, 2019) suggested that the toxicity of plastic particles may vary depending on their composition and surface properties, highlighting the need for further investigation into their potential risks to human health (WHO, 2019).

CONCLUSIONS

These results demonstrate that microplastic debris from many pollution sources is present in the silkworms discovered in the Brantas River. Based on their form, microplastics can be classified as fibers, filaments, pieces, or granules. These four types of microplastics were produced by the breakdown of macrosized plastics. One type of microplastic is present in polyethylene plastic products. Microplastics are frequently found in packing materials and are easily released into waterways when garbage is improperly disposed. Silkworms contain microplastics, which draws attention to the level of plastic pollution in the Brantas River ecosystem and raises questions about the possible effects on aquatic life and human health. Microplastics are also known to accumulate in the digestive systems of marine organisms, posing a potential threat to their health and survival. Furthermore, the presence of microplastics in the Brantas River ecosystem highlights the urgent need for effective waste management strategies to prevent further contamination of water sources.

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This study did not receive financial support.

CONFLICT OF INTEREST

The authors declare no conflicts of interest regarding the publication of this manuscript. In addition, ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy, were completely observed by the authors.

LIFE SCIENCE REPORTING

No life-threatening threats were encountered during this study.

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