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Microplastics: Distribution, Isolation, Detection, and Effects on Flora and Fauna – A Mini Review

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ABSTRACT

Plastic use and production have increased significantly since the turn of the 20th century. Because most of the devices and consumables we use are made of plastic, it has become an indispensable component of our daily lives. Plastic waste management practices that result in soil and water pollution have a harmful influence on our planet. Plastics also take longer to decompose in nature and are not biodegradable. A recent issue associated with plastics still being present in the environment is the generation of microplastics. Microplastics are defined as any plastic particles that are less than 5mm in length. These microplastics can easily pollute due to their small size. Such microparticles harm the ecosystem and the life it supports due to their pervasive existence. The main topics of this review are the different sources of microplastics, their classification, the various ways they are dispersed in the environment, their isolation, detection, and characterization from environmental samples, the toxicological effects of microplastics on different life forms, and the control and clean-up of microplastics from the environment.

Keywords: *Microplastics, Eco-toxicology, Clean-up, Human health, Marine pollution*

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INTRODUCTION

Plastics are synthetic organic polymers created when monomers from gas or oil are polymerized. Plastics are favored over other materials because they are corrosion-resistant, enduring, and inert. These characteristics lead to the widespread usage of plastics, which have endless applications (Andrady, 2011). The polymers' chemical inertness boosted their resistance to complete disintegration by the usual degradation process, allowing them to last centuries without completely degrading (Li *et al*., 2018). Millions of metric tonnes of plastic are produced annually for various uses worldwide. The fact that a sizable fraction of these polymers are discarded after usage is concerning (Acquah *et al*., 2021). Zubris and Richards (2005) estimate that only 9% of the plastics produced as waste are recycled. Only 12% of these pollutants are burned, leaving the remaining 79% of these plastics in the environment. The accumulation of plastic garbage occurs directly as a result of being dropped or discarded on land or at sea, as a result of landfills being overfilled, or as a result of accidents and transportation losses. The result is that plastic is finally harming the marine ecosystems around the planet. These plastics are not biodegradable and can persist in the environment for generations (Thompson, 2015; Acquah *et al*., 2021). A variety of plastic types, such as polyethylene, polypropylene, polystyrene, poly (ethylene terephthalate), and polyethylene, are extensively used in industries (vinyl chloride) (Andrady, 2011). Plastic trash has accumulated all over the planet due to the high production volume, high durability of the polymers used, low recycling rates, and improper handling of plastics (Andrady, 2011; Cózar *et al*., 2015; Wang *et al*., 2021). According to recent studies, these plastics may disintegrate under specific conditions despite their strength and inertness. These polymers are broken down into microscopic and nanoscale particles in the environment by atmospheric factors such as UV light, waves, corrosion, and photo-oxidation combined with microorganisms (Braun *et al*., 2021). Due to their small size and low density, these micro-sized plastics can persist in the environment for longer, increasing environmental pollution (Corcoran, 2015). So, microplastics became a problem that affected the entire world, with the main concern being how to get rid of these smaller pieces from the ecosystem (Corradini *et al*., 2019). Microplastics are typically described as a heterogeneous combination of variously shaped materials that range in size from 0.1 to 5,000 µm and are referred to as fragments, fibers, spheroids, granules, pellets, flakes, or beads (Iñiguez *et al*., 2018). Their shape can be flat, oblong, cylindrical, or disk-shaped pellets. Some are spherical to oval with rounded ends (Turner & Holmes, 2011; Coppock *et al*., 2017). Polyethylene terephthalate (PET), nylon, polyvinyl chloride (PVC), and polystyrene are the primary materials found in microplastics (PS) (Issac & Kandasubramanian, 2021).

These microplastics are now easily pushed into the ocean by wind or wave action due to the surface embrittlement and microcracking generated by the prolonged weathering on the beaches (Andrady, 2011). Microplastics discharged into the ocean, or other bodies of water are more likely to inadvertently make their way into the food chain of marine life. As a result, the seafood we consume gets tainted (Iñiguez *et al*., 2018). These microplastics are found in sediments of both marine and lacustrine environments (Martin *et al*., 2022). However, it is unknown whether consuming microplastics has any unfavorable impacts (Rahman *et al*., 2021). It is pretty concerning that microplastics are now causing a more significant threat to the terrestrial ecosystem. These microplastics' interactions with the biota change the geochemical and biophysical environment—the ecology as a whole changes due to this (Windsor *et al*., 2019). Studies have revealed that the continents of Africa, Asia, North America, Europe, and South Africa have been particularly affected by the buildup of microplastics (Li, 2018). Due to their ease of migration from the environment to living things, such as animals, microplastics are at the forefront of environmental contamination (Bessa *et al*., 2019; Braun *et al*., 2021). Identifying and characterizing microplastics in the environment are the most challenging aspects of dealing with microplastics. It is because they can readily be made mysterious using organic or other chemicals with a similar origin. Another challenging task is removing the environmental debris left over from microplastics (Barcelo & Pico, 2019). Additionally, microplastics serve as carriers for germs, harmful compounds, and heavy metals, all of which are risky for human health (Prata *et al*., 2020; Liu *et al*., 2022; Zha *et al*., 2022). The objectives of this review include the classification of microplastics, the variety of sources for microplastics that end up in the environment, the various techniques for isolating microplastics, the impact of microplastics on the environment and the health of living things, and the various controls to stop microplastics from entering the environment.

Classification of microplastics

One of the most pervasive and persistent contaminants is thought to be plastic. The fragmentation and buildup of plastic on the earth's surface have recently raised concerns about its use. Plastics are accumulating because of their poor recycling rate and high durability. Large pieces of collected plastic will gradually break down into microplastics (Yang *et al*., 2021). The size of plastic pollutants was divided into macro-debris, more extensive than 20 mm in diameter; meso-debris, between 5 and 20 mm; and mega-debris, greater than 100 mm (Cózar *et al*., 2015). Most macro-plastics are small, easily observable particles that can be seen with the eye (Cicin-Sain, 1993). Since the turn of the century, microplastics have been classified as pollutants. Microplastic refers to tiny threads, grains, and plastic particles (Andrady, 2011). It is challenging to develop a scientific standard due to study differences, which makes it difficult to describe and classify microplastics.

According to Moore *et al*. (2011), who categorized plastic waste into two classes depending on their sizes, macroplastics are those greater than 5 mm, and microplastics are smaller than 5 mm. Hortan and Barnes (2020) and Betts (2008) described microparticles as having a diameter of less than 5 mm, whereas Thompson *et al.* (2015) defined them as having a diameter of less than 10 mm. Derraik estimated the size to be between 2 and 6 mm, Van Ryan *et al*. (2021) suggested diameters less than 2 mm, and some literary works suggested the size to be less than 1 mm for these particles. According to recent studies, some plastic particles in the environment were assessed to be significantly smaller, or on a nanometer-scale (1 m), and were referred to as "Nano plastics"(Percival *et al*., 2014). Microplastics are divided into three categories based on their source: primary microplastics, secondary microplastics, and nano plastics (Chubarenko *et al*., 2020).

Primary microplastics

Small-sized plastics are referred to as primary microplastics and are displayed as such on purpose. Primary microplastics are made either directly from consumer goods like cosmetics or indirectly from the raw materials used to make virgin resin pellets or nurdles, which are used in the production of plastics (Everaert *et al*., 2018; Li *et al*., 2021; Phuong *et al*., 2021). The washing machine's outflow of wastewater, including acrylic textile fibers from the garments, pre-production pellets, and cosmetic microbeads, were the main sources of primary microplastics (Klages *et al*., 2015; Anderson *et al*., 2016).

The micro debris is made up of two major components, (i) thermoplastic feedstock like resin powders and pellets; and (ii) the broken fragments from larger objects (Koelmans *et al*., 2014). Each country's population and economic state determine how much primary microplastic it releases into the environment. Mainland China was predicted to be the highest microplastic accumulator in a study by Wang *et al*. (2019). Each person in China is estimated to use up to 538 g of microplastics annually. These microplastics are typically by-products of industrial production's discharge of particle pollutants, such as plastic dust from plastic items (Sharma & Chatterjee, 2017). Daily life's most often used items include primary microplastics (Li, 2018; Li *et al*., 2021).

Secondary plastics

Secondary microplastics are produced from more oversized plastic garbage due to chemical, physical, biological, thermal, photic, and chemical effects (Li, 2018; Llorca *et al*., 2020). secondary microplastics are characterized as deteriorating particles of plastic that are worn, embrittled, and irregularly sized (Gregory, 1983). Most fragmented microplastics are angular, rounded, sub-angular, and subrounded in shape. These are also depicted as having pointed, jagged, worn-down, and broken edges (Coppock *et al*., 2017). Secondary microplastics are produced when larger-sized plastics are exposed to high temperatures and UV rays and degrade. It may also be attributed to a confluence of mechanical forces and a photochemical reaction brought on by sunshine (Klages *et al*., 2015). Large-scale plastic material can be broken down into tiny pieces by weathering. Coupled mechanical and chemical weathering processes that result in the embrittlement of primary polymers into secondary microplastics have an even more significant impact on this (Brandon *et al*., 2016; Sharma & Chatterjee, 2017). The disintegration of microplastics increases their surface area, which impacts how biofilms develop on top of them (Oberbeckmann *et al*., 2015). Polymers of the polyethylene type have been discovered to be more vulnerable to mechanical weathering processes than plastics of the polypropylene type (Brandon *et al*., 2016).

Nano plastics

Nano plastics are the smallest polymer with a diameter of less than 100 nm. Nano-plastic debris, originating from manufactured materials, is created as microplastic waste degrades and weathers (Iñiguez *et al*., 2018). Plastic garbage exposed to solar radiation accelerates the photo-oxidation rate, making it more brittle. The polymers are further degraded by abrasion and wave action, which fragments them into micro-sized (0.1 - 1000 m) and nano-sized (0.1 m) particles (Cózar *et al*., 2014). Nanoplastics have been discovered among marine debris, but further research is required to fully understand these particles in this new environment. The fundamental problem with these nano plastics is that they cannot be purified using conventional filtration methods, making it almost impossible to spot them in samples. Because of this, creating standard techniques to identify these nanoparticles in the environment is quite challenging (Gaylarde *et al*., 2021). Another issue is that it is still unclear how these nanoparticles impact human health. A few unanswered questions about these nanoparticles include their impact on food preparation, whether or not they can transform from microplastics to nano plastics after ingestion, and what can happen to them after they enter the body. It is due to the dangers associated with some produced nanoparticles. Additionally, there are no recognized procedures or methods for detecting nano-plastics in food, particularly seafood, at this time (Iñiguez *et al*., 2018).

Distribution of microplastics

The origin of microplastics and their presence in soil are both poorly understood. According to studies, earthworms play a part in producing secondary microplastics. It happens as a result of the worms eating and digesting regular plastics. It causes the plastics to become brittle, which causes smaller fragments to form (Williams *et al*., 2016). Road dust, rubbish, industrial plastics, soil sedimentation, and air deposition were suggested as additional sources of microplastic pollution in agricultural (Chen *et al*., 2020). The main methods that plastic gets into the soil are directly by mulching with plastic or fertilizer applications of compost that contains plastic, as well as indirectly through flooding with river and lake water and untreated sewage and wastewater (Bläsin, & Amelung, 2018). Mulching can result in an enormous accumulation of plastic waste, which eventually settles into the agricultural soil (Huang *et al*., 2020). In terms of content and nature, the various agricultural soil strata contain various microplastics. Highly dense microplastics were deeper in the soil than on its surface (Fu & Wang, 2019). Polyester and polypropylene were the main ingredients in the microplastics that were regularly discovered in the soil (van den Berg *et al*., 2020).

Distribution of microplastic in air

Microplastic contamination in the air poses a new issue. A breathing thermal manikin study found that people would be exposed to many indoor airborne microplastics (Chen *et al*., 2021). The effects of airborne microplastics on people have only been the subject of a few numbers of researches (Jenner *et al*., 2022). A comparison study on the fibers in indoor and outdoor air and interior settled dust was conducted by Dris *et al*. (2017). According to their findings, there were much fewer fiber concentrations in the outdoor air than indoors. According to a study by Trainic *et al*. (2020), microplastics made of polysilicon, polyethylene, polystyrene, and polypropylene were discovered in the isolated maritime environment of the North Atlantic Ocean. Two-thirds of all produced textile fibers are made of synthetic and plastic materials. Fibrous microplastics deteriorate through photo-oxidation. These degraded particles further fragment when wind shear is added. As a result, microscopic particles of microplastics are created and discharged into the atmosphere (Amato-Lourenço *et al*., 2020). There have been reports of microplastic debris in the air over land, beaches, and distant marine ecosystems (Allen *et al*., 2021). Microplastic particles can readily go further than regular dust particles since they are thinner than soil and can be released into the air (Katsnelson, 2015).

Distribution in marine and freshwater ecosystems

Microplastic pollution in the marine environment has become a significant global environmental concern (Jiang *et al*., 2022). The cause of growing concern in the marine environment is the release of plastic garbage. It exists in these ecosystems because of the plastic debris nearby residents and business dumps. Compared to the plastic wastes prevalent on land, plastic breakdown occurs more slowly in the marine ecosystem. More seafood is needed to satisfy the expanding population, accelerating commercial fishing. It infers the increased demand for plastic fishing equipment (Wilber, 1987) obliquely. The coast and ocean ecosystems are negatively impacted by abandoned, misplaced, or discarded fishing gear (Deshpande *et al*., 2020). The three primary materials used in fishing gear construction are nylon, polypropylene, and polyethylene (Mace, 2012). Intense mariculture has been recognized as the primary source of this microplastic contamination, and plastic facilities are the primary microplastics in surface seawater. For instance, it caused Sanggou Bay's microplastic pollution increased by about 62.76% (Jiang *et al*., 2022). According to Yang *et al*. most of the fibers in freshwater sediment samples have a particle size of less than 1 mm. Also, PE or PS is among the most common microplastics. Plastic pollution in the marine ecosystem has recently become a serious global environmental issue affecting all areas of our oceans (Everaert *et al*., 2018). The existence of fiber-type microplastics is widely reported (Rahman *et al*., 2021; Govindaraj *et al.*, 2022).

Engineered microplastics are accidentally spilled after use or are disseminated directly into rivers (Everaert *et al*., 2018). Microplastics have been discovered on the seafloor, sea surface, and shorelines from the coast to the open ocean, including the Arctic Sea and the Atlantic waters to the north of Scotland (Everaert *et al*., 2018; Horton & Barnes, 2020). Globally, surface waterways, shorelines, and seafloor have all been found to contain microplastics (Horton & Barnes, 2020). On the shorelines of South Africa, Chile, the Hawaiian Archipelago, and the Atlantic coast of North America, plastic fishing gears such as buoys, lines, nets, and other fishing gears, as well as plastics such as buckets, bottles, foamed polystyrene, bags/film, and other plastics have been observed (Free *et al*., 2014). According to Rech and colleagues' study, rivers contributed to microplastic pollution in the ocean. According to research by Peng *et al*. (2017), rivers in the southeast Pacific region transported significant volumes of human-made trash from interior sources to the ocean and coastal beaches. Floating spherical microbeads in cosmetic/cleaning goods, wastewater, and wastewater treatment contributed to primary microplastics in freshwater bodies (Van Sebille *et al*., 2015; Meng *et al*., 2020).

Distribution of microplastics in ice

Polar regions, coastal regions, oceans, seas, rivers, and lakes are seriously threatened by terrestrial microparticles that run off into marine environments (Li, 2018). Microplastic contamination in the Arctic Ocean has become a severe issue in recent years due to the discharge of large amounts of microplastic from ice melting (Horton & Barnes, 2020). Compared to surface seawater, Arctic ice has higher quantities of microplastics, which affects the sea ice's characteristics. With an average of 11.71 particles/L, East **Antarctic** fast sea ice is a possible sink for microplastic trash in the Southern Ocean. Polyethylene, polypropylene, and polyamidetype microplastics were commonly identified (Cunningham *et al*., 2020). Geilfus *et al*. (2019) conducted a microcosm experiment to determine how introducing microplastics might change their distribution and affect sea ice conditions. The experiment showed that higher microplastic concentrations affected the depth of light penetration, changing the photochemical and photobiological processes in sea ice, leading to increased ice salinity and changes in sea ice albedo. However, these microplastics did not impact sea ice growth or its total thickness over time. Furthermore, it's thought that microplastics in arctic surface water can easily be incorporated into ice during its initial stages of formation. It remains in the ice until melting takes place.

Distribution in sewage

Washing machine effluents are the principal source of microplastics in surface and municipal water (Barcelo & Pico, 2019). Wastewater effluent usually includes cloth fibers which are synthetic. The sludge and effluent from municipal sewage treatment plants include microplastic fibers due to these sources (Habib *et al*., 1998). Residual effluent usually contains secondary polyester, acrylic, and polyamide-based microplastic fibers at sewage disposal facilities (Meng *et al*., 2020). According to a study, microplastics have been identified in high concentrations in British sewage treatment sludges. According to the forensic analysis, waste effluent from washing machines produced an average of more than 1900 fibers every wash for a single article of clothing (Napper & Thompson, 2016). The purpose of settling tanks is not to collect lighter microparticles like nylon and polyethylene. As a result, at the activated sludge layer, the particles barely settle with flakes.

Additionally, neither the initial settlement process nor adding flocculants or coagulants removes these kinds of particles. The use of microbeads in personal care items and synthetic fibers in the textile industry impacts aquatic ecosystems. Rainwater can wash off roadside plastics, worn tires, sewage, and other microplastics into the sewerage treatment facilities (Hamm *et al*., 2018).

Isolation of microplastics from the sample

Various techniques have been developed to find microplastics in water and other sediments (Klages *et al*., 2015; Morshedizadeh *et al.,* 2022).

Sieving

Microplastics can be distinguished through selective marine environment sampling and direct observation of the sediment surface (Coppock *et al*., 2017). As part of the technique, silt samples from the beach's top layer are sieved using a system of nested sieves. The Tyler sieves were used to separate the smaller plastic particles, and the remaining pieces were then dried in an oven at 65° C. From each filter tray, only the plastic particles with a size between 1 and 15 mm were kept. Plastics can be sorted according to size with preliminary sorting, making it easier to find microplastics. The microplastics are further sorted to create pieces, foams, pellets, lines, and films (Bläsing & Amelung, 2018).

Density separation

Microplastics typically have a density of between 0.8 and 1.4 g/mL. Common microplastics have densities that range from 0.85 to 0.94 grams per milliliter, 0.92 to 0.97 grams per milliliter, and 0.05 to 1.00 grams per milliliter. Still, free-floating plastic films may have a somewhat higher density (Lusher, 2015). The density of the sand and sea sediments is around 2.65 g/mL, which is lower than that of microplastics. These sediment samples can be combined with a saturated solution and shaken for a predetermined period to separate the smaller plastic particles from the denser sediment samples due to their varying densities (Coppock *et al*., 2017). Saline NaCl solution, tap water, sodium polytungstate solution with a density of 1.4 g/mL seawater, and hypersaturated saline with a density of 1.2 g/mL are the solutions utilized for density separations. One of these alternatives is tap water, which can float any plastic items previously floated in the ocean (Lee *et al*., 2017). Microplastics made of polyethylene, polypropylene, and foamed polystyrene float in clean water and saltwater. Nylon, polyethylene terephthalate (PET), and polyvinyl chloride (PVC) are examples of plastics that float in sodium polytungstate solution (Nor & Obbard, 2014; Albrahim *et al.,* 2022). The light-density polyethylene (LDPE) type microplastics were successfully eliminated from soil samples using the flotation method with distilled water. The microplastics were to be removed from the soil using either a three-time flotation process or an ultrasonic technique. Repeated flotation can remove LDPE and polypropylene microplastics from the soil sample (Fu & Wang, 2019). Microplastic separation has been successfully (95.5 1.8%) performed by Munich Plastic Sediment Separator (MPSS) to quantify plastic particles from the sediments (Williams *et al*., 2016; Coppock *et al*., 2017).

Pressurized fluid extraction

Plastic particles of less than 30 microns can be quantitatively extracted using Pressurized Fluid Extraction (PFE) technology. The pressurized fluid extraction is carried out at a pressure of 1500 psi and a cell temperature of 180 oC to detect the presence of microplastics in municipal garbage and soil samples by using dichloromethane and methanol as solvents. The microplastics are distinguished from sample impurities by analyzing the shape change between the pre-heating and post-heating periods. Regarding identifying microplastics, PFE is more reliable than techniques like flotation (Bläsing & Amelung, 2018; Zhang *et al*., 2018). Additionally, the empirical model can be used to evaluate microplastics.

Detection of microplastics

The characteristics of micro- and nano-plastic particles, such as their size, shape, density, kind of polymer, surface characteristics, etc., are incredibly complicated and varied (Liu *et al*., 2020). Micro/macro plastics must be characterized to determine their dispersion and environmental impact. Dense plastic trash in contact with sediment particles has a more significant impact than lighter microplastics. Because microplastics come in a variety of shapes, sizes, sources, and surface features, there are differences between these tiny plastics. Raman, Fourier-transform infrared spectroscopy, microscopy, pyrolysis, thermal desorption by gas chromatography, and other imaging techniques are contemporary instrument approaches for characterizing microplastics (Barcelo & Pico, 2019). Mass-based and particle desorption-gas chromatography/mass spectrometry (TED-GC/MS), pyrolysis gas chromatography/mass spectrometry (Py-GC/MS), matrix-assisted laser desorption/ionization-time of flight-mass spectroscopy (MALDI-ToF-MS), differential scanning calorimetry (DSC), and high-performance liquid chromatography (HPLC) are all used to characterize and identify microplastics. There are several particle-based methods available, such as Attenuated Total Reflection- Fourier Transform Infrared Spectrometry (ATR-FTIR), Micro-Fourier Transform

Infrared Spectrometry (-FTIR), -Raman Spectroscopy, Coherent anti-Stokes Raman Scattering (CARS), Near-Infrared Spectroscopy (NIR), Stimulated Raman Sc (ToF-SIMS) (Liu *et al*., 2020; Li *et al*., 2021). Raman spectroscopy, an FTIR spectrometer, and a laser infrared imaging system are frequently used to investigate microplastics (Chen *et al*., 2021). FTIR spectroscopy makes it possible to distinguish between the many forms of plastic (Gallagher *et al*., 2016). Combining the FTIR approach with a microscope allowed researchers to count the number of microplastics in the sample (Käppler *et al*., 2016). The size and kind of microplastics are determined using the laser direct infrared imaging approach. According to Rummel *et al*. (2016), flow cytometry can identify microplastics in environmental biofilms. Microplastics could be counted using optical microscopy, and the result could be shown in real-time. Following the -FTIR analysis allowed for the accurate counting of microplastics (Jiang *et al*., 2022). Sun *et al*. (2019) employed an AxioCam Hrc linked to a stereomicroscope to look at samples of seawater and zooplankton and image and count the microplastics. Each microplastic's composition, length, and width were manually measured with ImageJ. The FTIR microscope with a detection limit of 10 µm was used to examine the chemical nature of the microplastics. Zhou *et al*. (2020) reported similar reports on utilizing stereomicroscope and -FTIR. Zhang *et al*. method .'s (2018) utilizes a camera attached to a microscope to count and measure the size of microplastic particles.

Microparticles were morphologically identified by Ragusa *et al*. (2021) using a 100x objective (Olympus MPLAN 100x/0.90) lens. Raman spectroscopy was carried out, and the resulting spectra were compared with those from the SLOPP Library of Microplastics and the spectral library KnowItAll software. The Hit Quality Index (HQI) similarity scores of >80% were excellent. Liu *et al*. (2020) confirmed the existence of microplastics by detecting the polymer using micro transformer infrared spectroscopy in the transmittance mode. Using the Hummel Polymer and Additives and Polymer Laminate Films spectrum database, the polymer composition of the microplastics was identified.

Effects of microplastics

Effects on human beings

The ecotoxicological impacts of microplastics include cytotoxicity, neurotoxicity, mortality, reproductive dysfunction, genotoxicity, biotransformation of enzymes, physical and behavioral effects, oxidative stress and damage, and effects on blood and hemolymph (Hamm *et al*., 2018). People ingested, inhaled, and dermally absorbed microplastic particles through drinking water, food, and the air (Barcelo & Pico, 2019; Prata *et al*., 2020; Rahman *et al*., 2021).

The movement of dangerous substances into the organisms is facilitated by the consumption of plastics in the form of microplastics. This type of plastic's increased surface area facilitates the more significant movement of pollutants (Li *et al*., 2018); according to a study, humans may consume between 0.1 and 5 grams of microparticles through various exposure methods (Cortés *et al*., 2020). According to Ragusa *et al*. (2021) these pieces (size 10 m) were found in human placentas. These microplastics were discovered in the membranes of the chorioamnionitis and maternal sides. These microplastics are discolored polypropylene and leftover microplastics from synthetic coatings, paints and finger paints, adhesives and plasters, polymers, cosmetics, and personal care items. Humans have been exposed to a lot of plastic in the environment, as evidenced by the isolation of microplastics from the human placenta (Braun *et al*., 2021).

By evaluating oxidative stress and cell survival, Schirinzi *et al*. (2020) evaluated the cytotoxicity of microplastics. Microplastics of the polyethylene (PE) and polystyrene (PS) types were utilized in the experiment together with human cell lines such as T98G brain cells and HeLa epithelial cells. The information on the impact of microplastics (10 ng/mL to 10 g/mL) on oxidative stress was evaluated on cell lines after exposure for approximately 24 hours. Results that depended on dosage were seen. Reactive oxygen species (ROS) production by polyethylene and polystyrene microplastics greatly impacted T98G brain cells in oxidative stress testing.

Effects on birds

According to research by Zhao *et al*. (2020), 16 out of 17 terrestrial birds had a tiny anthropogenic litter in their gastrointestinal tracts, ranging in size from 0.5 to 8.5 mm. Further investigation revealed that the tiny anthropogenic waste consisted primarily of fragmented plastics (7.7%), natural fibers (37.4%), and plastic fibers (54.9%). Further investigation revealed that the tiny anthropogenic waste consisted primarily of fragmented plastics (7.7%), natural fibers (37.4%), and plastic fibers (54.9%).

The gastrointestinal tracts of red-shouldered hawks had higher concentrations of microplastics. This showed the severe impact of pollution by plastics (Zantis *et al*., 2021).

Effects on the marine ecosystem

The majority of marine plastic trash is made up of microplastics. Over 96.15 kg of microplastics were found in surface waters from Sanggou Bay in China, according to a study on microplastic contamination (Jiang *et al*., 2022). Microplastics are absorbed readily and are pretty small in size.

Collected by aquatic organisms or transferred through trophic interactions, with negative impacts (Chen *et al*., 2020; Jiang *et al*., 2020; Xiang *et al*., 2022). Smaller microplastic particles caused more toxicity than larger ones in the monogonont rotifers, according to Jiang *et al.* (2022) .'s research on the size-dependent toxicity of microplastics. Additionally, aquatic species quickly consumed and collected tiny microplastics, which had negative impacts. More well-known problems with plastic waste include suffocation, entanglement, and ingestion that associated sea creatures, such as zooplankton and cetaceans, marine reptiles, and seabirds, have to deal with (Nizzetto *et al*., 2016).

Effects on agriculture and soil

A global threat is the contamination of agroecosystems with microplastic. Mulched farmlands have been found to have higher concentrations of microplastics than uninhabited areas. Most materials used were microplastics, which come from fibers and films.

CONCLUSION

Microplastics have become one of emerging contaminants in the aquatic environment. The presence has been reported in many places around the world, and has caused great public concerns. However, there is still a lack of sufficient knowledge about microplastics in freshwaters such as their health effects and fast monitoring.

This review article summarized the current status of microplastics contamination in freshwaters, including rivers, lakes, water treatment plants and drinking water.

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REFERENCES

Acquah, J., Liu, H., Hao, S., Ling, Y., & Ji, J. (2021). Microplastics in freshwater environments and implications for aquatic ecosystems: a mini review and future directions in Ghana. *Journal of Geoscience and Environment Protection*, *9*(3), 58-74.

Albrahim, H. A., Alnabulsi, A. K., Assiry, M. M., Aloqbi, M. M., Abdel-Alim, H. M., Al-Sebaei, M. O., & Al-Ghamdi, M. Y. (2022). Confidence of dental post-graduates and general practitioners on performing surgical tooth extraction. *Annals of Dental Specialty, 10*(4), 101-108.

Allen, S., Allen, D., Baladima, F., Phoenix, V. R., Thomas, J. L., Le Roux, G., & Sonke, J. E. (2021). Evidence of free tropospheric and long-range transport of microplastic at Pic du Midi Observatory. *Nature Communications*, *12*(1), 1-10.

Amato-Lourenço, L. F., dos Santos Galvão, L., de Weger, L. A., Hiemstra, P. S., Vijver, M. G., & Mauad, T. (2020). An emerging class of air pollutants: Potential effects of microplastics to respiratory human health? *Science of the Total Environment*, *749*, 141676.

Anderson, A. G., Grose, J., Pahl, S., Thompson, R. C., & Wyles, K. J. (2016). Microplastics in personal care products: Exploring perceptions of environmentalists, beauticians and students. *Marine Pollution Bulletin*, *113*(1-2), 454-460.

Andrady, A. L. (2011). Microplastics in the marine environment. *Marine Pollution Bulletin, 62*(8), 1596-1605.

Barcelo, D., & Pico, Y. (2019). Microplastics in the global aquatic environment: Analysis, effects, remediation and policy solutions. *Journal of Environmental Chemical Engineering*, *7*(5), 103421.

Bessa, F., Ratcliffe, N., Otero, V., Sobral, P., Marques, J. C., Waluda, C. M., Trathan, P. N., & Xavier, J. C. (2019). Microplastics in gentoo penguins from the Antarctic region. *Scientific Reports*, *9*(1), 1-7.

Betts, K. (2008). Why small plastic particles may pose a big problem in the oceans. *Environmental Science & Technology, 42*(24), 8993.

Bläsing, M., & Amelung, W. (2018). Plastics in soil: Analytical methods and possible sources. *Science of the Total Environment*, *612*, 422-435.

Brandon, J., Goldstein, M., & Ohman, M. D. (2016). Long-term aging and degradation of microplastic particles: Comparing in situ oceanic and experimental weathering patterns. *Marine Pollution Bulletin*, *110*(1), 299-308.

Braun, T., Ehrlich, L., Henrich, W., Koeppel, S., Lomako, I., Schwabl, P., & Liebmann, B. (2021). Detection of microplastic in human placenta and meconium in a clinical setting. *Pharmaceutics*, *13*(7), 921.

Chen, X., Chen, X., Liu, Q., Zhao, Q., Xiong, X., & Wu, C. (2021). Used disposable face masks are significant sources of microplastics to environment. *Environmental Pollution*, *285*, 117485.

Chen, Y., Leng, Y., Liu, X., & Wang, J. (2020). Microplastic pollution in vegetable farmlands of suburb Wuhan, central China. *Environmental Pollution*, *257*, 113449.

Chubarenko, I., Efimova, I., Bagaeva, M., Bagaev, A., & Isachenko, I. (2020). On mechanical fragmentation of single-use plastics in the sea swash zone with different types of bottom sediments: Insights from laboratory experiments. *Marine Pollution Bulletin*, *150*, 110726.

Cicin-Sain, B. (1993). Sustainable development and integrated coastal management. *Ocean & Coastal Management*, *21*(1-3), 11-43.

Coppock, R. L., Cole, M., Lindeque, P. K., Queirós, A. M., & Galloway, T. S. (2017). A small-scale, portable method for extracting microplastics from marine sediments. *Environmental Pollution*, *230*, 829-837.

Corcoran, P. L. (2015). Benthic plastic debris in marine and fresh water environments. *Environmental Science: Processes & Impacts*, *17*(8), 1363-1369.

Corradini, F., Meza, P., Eguiluz, R., Casado, F., Huerta-Lwanga, E., & Geissen, V. (2019). Evidence of microplastic accumulation in agricultural soils from sewage sludge disposal. *Science of the Total Environment*, *671*, 411-420.

Cortés, C., Domenech, J., Salazar, M., Pastor, S., Marcos, R., & Hernández, A. (2020). Nanoplastics as a potential environmental health factor: Effects of polystyrene nanoparticles on human intestinal epithelial Caco-2 cells. *Environmental Science: Nano*, *7*(1), 272-285.

Cózar, A., Echevarría, F., González-Gordillo, J. I., Irigoien, X., Úbeda, B., Hernández-León, S., Palma, Á. T., Navarro, S., García-de-Lomas, J., Ruiz, A., et al. (2014). From the cover: Plastic debris in the open ocean. *Proceedings of the National Academy of Sciences of the United States of America*, *111*(28), 10239.

Cózar, A., Sanz-Martín, M., Martí, E., González-Gordillo, J. I., Ubeda, B., Gálvez, J. Á., Irigoien, X., & Duarte, C. M. (2015). Plastic accumulation in the Mediterranean sea. *PloS One*, *10*(4), e0121762.

Cunningham, E. M., Ehlers, S. M., Dick, J. T., Sigwart, J. D., Linse, K., Dick, J. J., & Kiriakoulakis, K. (2020). High abundances of microplastic pollution in deep-sea sediments: Evidence from Antarctica and the Southern Ocean. *Environmental Science & Technology*, *54*(21), 13661-13671.

Deshpande, P. C., Skaar, C., Brattebø, H., & Fet, A. M. (2020). Multi-criteria decision analysis (MCDA) method for assessing the sustainability of end-of-life alternatives for waste plastics: A case study of Norway. *Science of the Total Environment*, *719*, 137353.

Dris, R., Gasperi, J., Mirande, C., Mandin, C., Guerrouache, M., Langlois, V., & Tassin, B. (2017). A first overview of textile fibers, including microplastics, in indoor and outdoor environments. *Environmental Pollution*, *221*, 453-458. doi:10.1016/j.envpol.2016.12.013

Everaert, G., Van Cauwenberghe, L., De Rijcke, M., Koelmans, A. A., Mees, J., Vandegehuchte, M., & Janssen, C. R. (2018). Risk assessment of microplastics in the ocean: Modelling approach and first conclusions. *Environmental Pollution*, *242*, 1930-1938.

Free, C. M., Jensen, O. P., Mason, S. A., Eriksen, M., Williamson, N. J., & Boldgiv, B. (2014). High-levels of microplastic pollution in a large, remote, mountain lake. *Marine Pollution Bulletin*, *85*(1), 156-163.

Fu, Z., & Wang, J. (2019). Current practices and future perspectives of microplastic pollution in freshwater ecosystems in China. *Science of the Total Environment*, *691*, 697-712.

Gallagher, A., Rees, A., Rowe, R., Stevens, J., & Wright, P. (2016). Microplastics in the solent estuarine complex, UK: An initial assessment. *Marine Pollution Bulletin*, *102*(2), 243-249.

Gaylarde, C. C., Neto, J. A. B., & da Fonseca, E. M. (2021). Nanoplastics in aquatic systems-are they more hazardous than microplastics? *Environmental Pollution*, *272*, 115950.

Geilfus, N. X., Munson, K. M., Sousa, J., Germanov, Y., Bhugaloo, S., Babb, D., & Wang, F. (2019). Distribution and impacts of microplastic incorporation within sea ice. *Marine Pollution Bulletin*, *145*, 463-473.

Govindaraj, A., Paulpandian, S. S., & Shanmugam, R. (2022). Comparative evaluation of the effect of rind and pulp extract of *citrullus lanatus* on *streptococcus mutans*. *Annals of Dental Specialty, 10*(4), 34-39.

Gregory, M. R. (1983). Virgin plastic granules on some beaches of eastern Canada and Bermuda. *Marine Environmental Research*, *10*(2), 73-92.

Habib, D., Locke, D. C., & Cannone, L. J. (1998). Synthetic fibers as indicators of municipal sewage sludge, sludge products, and sewage treatment plant effluents. *Water, Air, and Soil Pollution*, *103*(1), 1-8.

Hamm, T., Lorenz, C., & Piehl, S. (2018). Microplastics in aquatic systems–monitoring methods and biological consequences. In *YOUMARES 8–Oceans Across Boundaries: Learning from each other* (pp. 179-195). Springer, Cham.

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

Huang, Y., Liu, Q., Jia, W., Yan, C., & Wang, J. (2020). Agricultural plastic mulching as a source of microplastics in the terrestrial environment. *Environmental Pollution*, *260*, 114096.

Iñiguez, M. E., Conesa, J. A., & Fullana, A. (2018). Author correction: Microplastics in Spanish table salt. *Scientific Reports*, *8*(1), 1-1.

Issac, M. N., & Kandasubramanian, B. (2021). Effect of microplastics in water and aquatic systems. *Environmental Science and Pollution Research*, *28*(16), 19544-19562.

Jenner, L. C., Rotchell, J. M., Bennett, R. T., Cowen, M., Tentzeris, V., & Sadofsky, L. R. (2022). Detection of microplastics in human lung tissue using μFTIR spectroscopy. *Science of The Total Environment*, *831*, 154907.

Jiang, Y., Yang, F., Kazmi, S. S. U. H., Zhao, Y., Chen, M., & Wang, J. (2022). A review of microplastic pollution in seawater, sediments and organisms of the Chinese coastal and marginal seas. *Chemosphere*, *286*, 131677.

Käppler, A., Fischer, D., Oberbeckmann, S., Schernewski, G., Labrenz, M., Eichhorn, K. J., & Voit, B. (2016). Analysis of environmental microplastics by vibrational microspectroscopy: FTIR, Raman or both? *Analytical and Bioanalytical Chemistry*, *408*(29), 8377-8391.

Katsnelson, A. (2015). Microplastics present pollution puzzle. *Proceedings of the National Academy of Sciences*, *112*(18), 5547-5549.

Klages, M., Gutow, L., & Bergmann, M. (2015). *Marine anthropogenic litter* (pp. 57-72). Springer, Cham, Switzerland.

Koelmans, A. A., Gouin, T., Thompson, R., Wallace, N., & Arthur, C. (2014). Plastics in the marine environment. *Environmental Toxicology and Chemistry*, *33*(1), 5-10.

Lee, J., Lee, J., Hong, S., Hong, S. H., Shim, W. J., & Eo, S. (2017). Characteristics of meso-sized plastic marine debris on 20 beaches in Korea. *Marine Pollution Bulletin*, *123*(1-2), 92-96.

Li, J., Zhang, H., Zhang, K., Yang, R., Li, R., & Li, Y. (2018). Characterization, source, and retention of microplastic in sandy beaches and mangrove wetlands of the Qinzhou Bay, China. *Marine Pollution Bulletin*, *136*, 401-406. doi:10.1016/j.marpolbul.2018.09.025

Li, W. C. (2018). The occurrence, fate, and effects of microplastics in the marine environment. In *Microplastic Contamination in Aquatic Environments* (pp. 133-173). Elsevier.

Liu, M., Lu, S., Chen, Y., Cao, C., Bigalke, M., & He, D. (2020). Analytical methods for microplastics in environments: Current advances and challenges. *Microplastics in Terrestrial Environments*, 3-24.

Liu, S., Huang, J., Zhang, W., Shi, L., Yi, K., Yu, H., Zhang, C., Li, S., & Li, J. (2022). Microplastics as a vehicle of heavy metals in aquatic environments: A review of adsorption factors, mechanisms, and biological effects. *Journal of Environmental Management*, *302*, 113995.

Llorca, M., Álvarez-Muñoz, D., Ábalos, M., Rodríguez-Mozaz, S., Santos, L. H., León, V. M., Campillo, J. A., Martínez-Gómez, C., Abad, E., & Farré, M. (2020). Microplastics in Mediterranean coastal area: Toxicity and impact for the environment and human health. *Trends in Environmental Analytical Chemistry*, *27*, e00090.

Lusher, A. (2015). Microplastics in the marine environment: Distribution, interactions and effects. In *Marine anthropogenic litter* (pp. 245-307). Springer, Cham.

Lv, L., Yan, X., Feng, L., Jiang, S., Lu, Z., Xie, H., Sun, S., Chen, J., & Li, C. (2021). Challenge for the detection of microplastics in the environment. *Water Environment Research*, *93*(1), 5-15.

Mace, T. H. (2012). At-sea detection of marine debris: Overview of technologies, processes, issues, and options. *Marine Pollution Bulletin*, *65*(1-3), 23-27.

Martin, J., Lusher, A. L., & Nixon, F. C. (2022). A review of the use of microplastics in reconstructing dated sedimentary archives. *Science of the Total Environment*, *806*, 150818.

Meng, Y., Kelly, F. J., & Wright, S. L. (2020). Advances and challenges of microplastic pollution in freshwater ecosystems: A UK perspective. *Environmental Pollution*, *256*, 113445.

Moore, C. J., Lattin, G. L., & Zellers, A. F. (2011). Quantity and type of plastic debris flowing from two urban rivers to coastal waters and beaches of Southern California. *Revista de Gestão Costeira Integrada-Journal of Integrated Coastal Zone Management*, *11*(1), 65-73.

Morshedizadeh, Z., Ahmadipour, M., & Mahani, S. M. (2022). Investigation of the association between serum HbA1c level and hemodynamic variables in diabetic patients undergoing prostatectomy. *Journal of Advanced Pharmacy Education and Research, 12*(4), 91-96.

Napper, I. E., & Thompson, R. C. (2016). Release of synthetic microplastic plastic fibres from domestic washing machines: Effects of fabric type and washing conditions. *Marine Pollution Bulletin*, *112*(1-2), 39-45.

Nizzetto, L., Futter, M., & Langaas, S. (2016). Are agricultural soils dumps for microplastics of urban origin? *Environmental Science & Technology*, *50*(20), 10777–10779.

Nor, N. H. M., & Obbard, J. P. (2014). Microplastics in Singapore's coastal mangrove ecosystems. *Marine Pollution Bulletin*, *79*(1-2), 278-283.

Oberbeckmann, S., Löder, M. G., & Labrenz, M. (2015). Marine microplastic-associated biofilms–A review. *Environmental Chemistry*, *12*(5), 551-562.

Peng, J., Wang, J., & Cai, L. (2017). Current understanding of microplastics in the environment: Occurrence, fate, risks, and what we should do. *Integrated Environmental Assessment and Management*, *13*(3), 476-482.

Percival, R. V., Lin, J., & Piermattei, W. (Eds.). (2014). *Global environmental law at a crossroads*. Edward Elgar Publishing.

Phuong, N. N., Fauvelle, V., Grenz, C., Ourgaud, M., Schmidt, N., Strady, E., & Sempéré, R. (2021). Highlights from a review of microplastics in marine sediments. *Science of the Total Environment*, *777*, 146225.

Prata, J. C., da Costa, J. P., Lopes, I., Duarte, A. C., & Rocha-Santos, T. (2020). Environmental exposure to microplastics: An overview on possible human health effects. *Science of the Total Environment*, *702*, 134455.

Ragusa, A., Svelato, A., Santacroce, C., Catalano, P., Notarstefano, V., Carnevali, O., Papa, F., Rongioletti, M. C. A., Baiocco, F., Draghi, S., et al. (2021). Plasticenta: First evidence of microplastics in human placenta. *Environment International*, *146*, 106274.

Rahman, A., Sarkar, A., Yadav, O. P., Achari, G., & Slobodnik, J. (2021). Potential human health risks due to environmental exposure to nano-and microplastics and knowledge gaps: A scoping review. *Science of the Total Environment*, *757*, 143872.

Rummel, C. D., Löder, M. G., Fricke, N. F., Lang, T., Griebeler, E. M., Janke, M., & Gerdts, G. (2016). Plastic ingestion by pelagic and demersal fish from the North Sea and Baltic Sea. *Marine Pollution Bulletin*, *102*(1), 134-141.

Schirinzi, G. F. (2020). *Chemical and ecotoxicological assessment of microplastics and emerging risks in the coastal environments*. [PhD Thesis]. University of Barcelona.

Sharma, S., & Chatterjee, S. (2017). Microplastic pollution, a threat to marine ecosystem and human health: A short review. *Environmental Science and Pollution Research*, *24*(27), 21530-21547.

Sun, J., Dai, X., Wang, Q., van Loosdrecht, M. C., & Ni, B. J. (2019). Microplastics in wastewater treatment plants: Detection, occurrence and removal. *Water Research*, *152*, 21-37.

Thompson, R. C. (2015). Microplastics in the marine environment: Sources, consequences and solutions. In *Marine anthropogenic litter* (pp. 185-200). Springer, Cham.

Trainic, M., Flores, J. M., Pinkas, I., Pedrotti, M. L., Lombard, F., Bourdin, G., Gorsky, G., Boss, E., Rudich, Y., Vardi, A., et al. (2020). Airborne microplastic particles detected in the remote marine atmosphere. *Communications Earth & Environment*, *1*(1), 1-9.

Turner, A., & Holmes, L. (2011). Occurrence, distribution and characteristics of beached plastic production pellets on the island of Malta (central Mediterranean). *Marine Pollution Bulletin*, *62*(2), 377-381.

van den Berg, P., Huerta-Lwanga, E., Corradini, F., & Geissen, V. (2020). Sewage sludge application as a vehicle for microplastics in eastern Spanish agricultural soils. *Environmental Pollution*, *261*, 114198.

Van Ryan Kristopher, R. G., Jaraula, C. M. B., & Paler, M. K. O. (2021). The nexus of macroplastic and microplastic research and plastic regulation policies in the Philippines marine coastal environments. *Marine Pollution Bulletin*, *167*, 112343.

Van Sebille, E., Wilcox, C., Lebreton, L., Maximenko, N., Hardesty, B. D., Van Franeker, J. A., Eriksen, M., Siegel, D., Galgani, F., & Law, K. L. (2015). A global inventory of small floating plastic debris. *Environmental Research Letters*, *10*(12), 124006.

Wang, C., Zhao, J., & Xing, B. (2021). Environmental source, fate, and toxicity of microplastics. *Journal of Hazardous Materials*, *407*, 124357.

Wang, Z., Qin, Y., Li, W., Yang, W., Meng, Q., & Yang, J. (2019). Microplastic contamination in freshwater: First observation in lake ulansuhai, yellow river basin, China. *Environmental Chemistry Letters*, *17*(4), 1821-1830.

Wilber, R. J. (1987). Plastic in the North Atlantic. *Oceanus*, *30*(3), 61-68.

Williams, A. T., Randerson, P., Di Giacomo, C., Anfuso, G., Macias, A., & Perales, J. A. (2016). Distribution of beach litter along the coastline of Cádiz, Spain. *Marine Pollution Bulletin*, *107*(1), 77-87.

Windsor, F. M., Durance, I., Horton, A. A., Thompson, R. C., Tyler, C. R., & Ormerod, S. J. (2019). A catchment-scale perspective of plastic pollution. *Global Change Biology*, *25*(4), 1207-1221.

Xiang, Y., Jiang, L., Zhou, Y., Luo, Z., Zhi, D., Yang, J., & Lam, S. S. (2022). Microplastics and environmental pollutants: Key interaction and toxicology in aquatic and soil environments. *Journal of Hazardous Materials*, *422*, 126843.

Yang, L., Zhang, Y., Kang, S., Wang, Z., & Wu, C. (2021). Microplastics in soil: A review on methods, occurrence, sources, and potential risk. *Science of the Total Environment*, *780*, 146546.

Zantis, L. J., Carroll, E. L., Nelms, S. E., & Bosker, T. (2021). Marine mammals and microplastics: A systematic review and call for standardisation. *Environmental Pollution*, *269*, 116142.

Zha, F., Shang, M., Ouyang, Z., & Guo, X. (2022). The aging behaviors and release of microplastics: A review. *Gondwana Research*, *108*, 60-71.

Zhang, S., Yang, X., Gertsen, H., Peters, P., Salánki, T., & Geissen, V. (2018). A simple method for the extraction and identification of light density microplastics from soil. *Science of the Total Environment*, *616*, 1056-1065.

Zhou, Y., Wang, J., Zou, M., Jia, Z., Zhou, S., & Li, Y. (2020). Microplastics in soils: A review of methods, occurrence, fate, transport, ecological and environmental risks. *Science of the Total Environment*, *748*, 141368.

Zubris, K. A. V., & Richards, B. K. (2005). Synthetic fibers as an indicator of land application of sludge. *Environmental Pollution*, *138*(2), 201-211.