Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 12 [12] November 2023 : 368-381

Online ISSN 2277-1808
Journal's URL: http://www.bepls.com
CODEN: BEPLAD

REVIEW ARTICLE **OPEN ACCESS**

An Insight into The Toxicological Effects of Microplastics on Earthworms and Their Removal Technologies

Garima Sheoran1, and Vineeta Shukla1*
1 Environment Toxicology and Animal Physiology Laboratory, Department of Zoology, Maharshi Dayanand University, Rohtak- 124001, Haryana, India
***Corresponding author:**
Email- prof.vineetashukla@gmail.com

ABSTRACT

Microplastics (MPs), measuring less than 5 mm, are pervasive environmental pollutants, raising concerns about their toxic effects on terrestrial ecosystems especially earthworms. This review analyzes the sources, distribution, and fate of MPs in soils, emphasizing their ubiquitous presence. It delves into the toxicological impact on earthworms, covering exposure mechanisms and effects on physiology, biochemistry, reproduction, and soil ecosystems. The article also explores various removal technologies, including physical, chemical, phytoremediation, and microbial methods, to combat MPs contamination. Improved risk assessment, long-term studies, and regulatory frameworks are stressed for addressing this pollution. The review underscores the urgency of preserving earthworm populations and soil ecosystems through sustainable practices, urging collaborative efforts in tackling this global environmental challenge.
Keywords: *Microplastic; Earthworm; Polyethylene; Toxicology; Oxidative stress.*

Received 09.09.2023 Revised 11.10.2023 Accepted 23.11.2023

INTRODUCTION

The alarming rise in plastic pollution has become a significant global environmental concern. Among the various forms of plastic debris, microplastics (MPs) have garnered considerable attention due to their widespread presence in ecosystems, including soil [1]. Microplastics are defined as plastic particles with dimensions less than 5 mm, encompassing both primary MPs (manufactured as small particles) and secondary MPs (resulting from the fragmentation of larger plastic items) [2]. Their small size and persistence in the environment have led to their ubiquity in terrestrial systems, raising concerns about their potential toxicological effects on organisms that inhabit these ecosystems [3]. Earthworms, classified as oligochaetes, are an essential component of soil ecosystems, playing a fundamental role in soil health, nutrient cycling, and organic matter decomposition [4]. Their burrowing activities contribute to soil aeration and water infiltration, while their feeding habits facilitate the breakdown and incorporation of organic matter into the soil matrix. Furthermore, earthworms enhance soil fertility through the excretion of nutrient-rich castings, promoting plant growth and ecosystem productivity. Given the crucial ecosystem services provided by earthworms, understanding the impact of MPs on these organisms is of paramount importance [5]. The toxicological effects of MPs on earthworms have emerged as a topic of growing concern in recent years. Several studies have reported adverse effects of MPs exposure on earthworm physiology, behavior, reproduction, and overall fitness [6–8]. The potential pathways through which earthworms interact with MPs include ingestion, absorption through the skin, and physical entanglement. Once internalized, MPs can accumulate within earthworm tissues, potentially causing mechanical damage, hindered digestion, and interference with vital physiological processes [9]. Physical effects of MPs on earthworms include blockage or obstruction of the digestive system, leading to reduced feeding efficiency and nutrient uptake. The accumulation of MPs in earthworm tissues can also impede the movement of coelomic fluid, essential for maintaining physiological homeostasis [10]. Furthermore, the presence of MPs may alter the burrowing behavior of earthworms, influencing soil structure and nutrient cycling dynamics. The potential consequences of MPs exposure extend beyond individual earthworms, potentially affecting population dynamics and overall ecosystem functioning [11]. Apart from the physical impacts, the chemical properties of MPs can also elicit toxicological effects on earthworms. Microplastics have the capacity to adsorb and concentrate various organic pollutants from the environment, such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and heavy metals [12]. When ingested by earthworms, these adsorbed contaminants can be released in the digestive tract, leading to internal exposure and potential toxicity [13]. Additionally, MPs themselves may contain additives and plasticizers that can leach into the surrounding tissues, further exacerbating the toxicological effects on earthworms [14]. The potential implications of microplastic-induced toxicity on earthworms extend to the broader soil ecosystem. Earthworms are vital ecosystem engineers, contributing to soil structure, nutrient cycling, and overall soil health [15]. Their activities promote the development of stable soil aggregates, enhancing water infiltration and reducing erosion. Furthermore, earthworms facilitate the decomposition of organic matter, influencing the availability of nutrients to plants and promoting soil fertility [16]. Any disruption to the earthworm population and their functional roles can have cascading effects on soil health and ecosystem functioning [17]. Given the potential risks associated with MPs on earthworms and soil ecosystems, it is crucial to explore effective strategies for mitigating microplastic pollution. Various removal technologies have been developed to reduce microplastic contamination in soils, aiming to safeguard the health and functionality of earthworms and soil ecosystems. These technologies encompass physical removal methods, chemical remediation, phytoremediation, and microbial remediation [18]. This review provides a thorough analysis of the sources, distribution, and fate of MPs in soils, shedding light on their pervasive presence. The study delves into the toxicological impact of MPs on earthworms, encompassing exposure mechanisms and effects on various aspects of their physiology, biochemistry, reproduction, and soil ecosystems. Furthermore, this article explores and evaluates different removal technologies aimed at combating MPs contamination in soil. These methods include physical, chemical, phytoremediation, and microbial approaches, each with their own merits and challenges. To address the growing issue of MP pollution effectively, the review emphasizes the need for improved risk assessment, long-term studies, and the implementation of robust regulatory frameworks.

SOURCES RESPONSIBLE FOR SOIL MICROPLASTICS

There are notable regional and environmental disparities in the distribution of MPs within soils. Generally, the primary types of polymers found in soils include polypropylene, polyethylene, polyester, and polystyrene, with a prevalence of small-sized MPs [19]. Microplastics exhibit various morphologies, such as fragments, films, fibers, and pellets. However, the specific morphology and polymer composition of MPs tend to differ from one region to another, primarily due to the multiple sources of MPs that enter the soil environment [20]. The lack of comprehensive information regarding soil degradation processes and the wide array of plastic types and sizes make it challenging to establish a realistic timeframe for MP decomposition and the subsequent release of their components. Numerous soil types utilized for various purposes, including agricultural, pasture, forest, industrial, and remote floodplain soils, commonly experience contamination from MPs **(Figure 1)**. In locations heavily affected by pollution, concentrations of MPs can reach levels as high as 6.7% of the soil weight. Primary microplastics refer to plastics manufactured at the microscopic level for both industrial and domestic applications [21]. Examples include plastic pellets, fibers, films, seeds, and powders found in cosmetics like sunscreen, personal care products such as facial scrubs and cleansers, and items designed for children. Additionally, primary MPs may arise from materials used in air-blasting technology or from products manufactured through the ship-breaking process. Over time, larger plastic products, including primary MPs, undergo physical, chemical, and biological breakdown, giving rise to secondary MPs [22,23]. Microplastics can also accumulate due to improper disposal of agricultural plastic films, and film-like MPs are considered a common source in agricultural settings [24]. The application of soil amendments like compost and sludge to agricultural fields can transport and disperse MPs from urban waste drains. Additionally, accidental plastic waste can become a significant contributor. Surprisingly, even washing machines have the potential to generate additional MPs fibers, which may find their way to farms through water treatment facilities [25]. The possibility of tumble dryers being a source of MPs is also intriguing to consider. When minute particles or fibers become airborne, originating from sources like landfills or surface dumps, they possess the potential to disperse over extended distances. Subsequently, these airborne MPs can be transported to terrestrial systems and the soil through atmospheric deposition [26]. Geophagous soil animals, particularly earthworms, may play a role in the formation of secondary MPs. Within their gizzards, earthworms can fragment fragile plastic particles, which are then digested and transformed into MPs. Anecic earthworms, which create vertical burrows but primarily feed near the soil surface, may contribute to the incorporation of surface-deposited plastic fragments into the soil [27]. Moreover, a substantial proportion of plastic mulching residues may persist in the soil due to inadequate management practices, leading to the accumulation of MPs in the soil as a result of soil degradation caused by UV radiation and physical erosion processes [28]. In natural conditions, residual plastic waste undergoes gradual degradation, leading to the formation of small fragments known as MPs. Microplastics can be degraded through various processes such as exposure to UV rays, thermal oxidation, physical abrasion, and biodegradation [29]. During these degradation methods, MPs undergo changes in their polymer's chemical structure, including chain cleaving, disproportionate, and an increase in oxygen-containing functional groups [30]. Soil-bound MPs exhibit mobility, and as a result of agricultural practices and sediment deposition, they can migrate over short distances, for instance during ploughing. Bioturbation, a process involving the movement of soil particles by soil-dwelling organisms, has been found to influence the movement of MPs in the soil [31]. Specific earthworm and collembolan species have been discovered to transport MPs from the topsoil to deeper soil layers. Furthermore, there is evidence suggesting that MPs can be transported over long distances through soil erosion and runoff processes. This can lead to the infiltration of MPs into water bodies, ultimately reaching even the ocean [32]. Microplastics have been detected not only in topsoils but also in deep subsoils. The topsoil, due to its specific characteristics such as direct exposure to UV radiation, relatively higher temperatures, and increased oxygen availability, provides a potential environment for the breakdown of MPs [33]. However, the degradation rate of MPs in soil is generally slow and can be influenced by various factors, including soil-dwelling animals, microbial activity, agricultural practices, and other processes [34]. The physical properties of MPs also play a crucial role in determining their fate and interactions with the environment, including their mobility and pathways. Atmospheric and hydrodynamic factors typically influence the behavior of MPs [35]. Additionally, the sizes, densities, and morphologies of MP particles influence their dispersion, resuspension, and sinking rates. These combined factors contribute to the overall distribution and movement of MPs within the soil and beyond [36].

TOXICOLOGICAL EFFECTS OF MICROPLASTICS

Research on the toxicological impacts of MPs on earthworms is relatively scarce when compared to studies focused on marine organisms [37]. Nevertheless, emerging evidence indicates that MPs can exert detrimental effects on earthworms, which play a vital role as soil-dwelling organisms essential for maintaining soil health and facilitating nutrient cycling [38].

Metabolic and trancriptomic changes

Ingestion and accumulation: Earthworms have the ability to consume MPs found in the soil, either directly or indirectly by consuming organic matter that is contaminated [39]. Once ingested, MPs can gather and accumulate within the digestive tract of earthworms, potentially resulting in physical obstructions and hindering the absorption of nutrients **(Table 1)**. Multiple studies have verified that earthworms can ingest and accumulate MPs [40,41], leading to a reduction in their growth, reproduction rate, and lifespan [42]. The presence of MPs in the diet of earthworms can result in gastrointestinal tissue damage and the dilution of food resources **(Figure 2)**. In a study conducted by Tourinho et al. (2021), it was observed that earthworms exposed to MPs fibers exhibited an increase in lipid, protein, and carbohydrate content. The researchers specifically investigated the effects of silver exposure and attributed the rise in protein content to the binding of metal-associated proteins [43]. This overall increase in metabolite concentration could potentially be attributed to immune-protection or a stress response [44]. Chen and colleagues (2022) conducted a study to examine the effects of polyethylene and propylene MPs on the metabolism and transcriptomics of earthworms [45]. Their findings revealed an elevation in the metabolisms of arachidonic acid and glycerolipids, indicating a disruption in lipid metabolism. In a recent study conducted by Tang and colleagues (2023), the effects of polystyrene nanoplastics on earthworms were examined using advanced multi-omics tools. Their findings revealed an upregulation in the expression of digestive genes, as evidenced by transcriptomics analysis. Additionally, the researchers observed an increase in aldosterone-regulated sodium reabsorption at the transcriptome level and alterations in inositol phosphate metabolism at the proteomic level. Moreover, through transcriptional-metabolic analysis, disruptions in carbohydrate and arachidonic acid metabolisms were identified as responses to exposure to polystyrene nanoplastics. These results highlight the potential adverse effects of nanoplastic pollution on earthworm physiology and metabolism [46]. In a separate investigation, Li et al. (2021) documented a substantial upregulation in the expression of 34,937 genes in response to exposure to high-density polyethylene MPs. Similarly, during exposure to polypropylene MPs, an increase in the expression of 28,494 genes was observed by the researchers [47]. In addition to the metabolic consequences observed in earthworms, MPs also have an impact on their reproductive health. Multiple studies have provided evidence of the detrimental effects of MPs on the population size of subsequent generations of earthworms.

Reproductive health

The toxicological impact of MPs results in growth and reproductive impairments **(Figure 2)**. Research has demonstrated that earthworms exposed to MPs experience detrimental effects on their growth and reproductive performance **(Table 1)**. These effects manifest as reduced body weight, decreased cocoon production, and altered development of offspring. Tourinho et al. (2021) observed that the presence of MPs fibers amplified the toxicity of silver nanoparticles on the reproduction of *Eisenia andrei*. When earthworms were exposed to soil containing MPs fibers and silver nanoparticles, the number of juveniles decreased to 35 individuals [43]. In a separate investigation, Ding et al. (2021) conducted a study to examine the impact of polyethylene and biodegradable MPs on earthworms. Surprisingly, the researchers found that even the biodegradable plastics, such as polylactic acid (PLA) and polypropylene carbonate (PPC), exhibited similar effects as polyethylene on the reproductive health of *Eisenia fetida*. By the 28th day of harvest, a decrease in the number of cocoons was observed with increasing concentrations of MPs. Notably, a significant decline (EC10) in the number of cocoons and juvenile earthworms occurred at concentrations of 53 g kg^{-1} and 97 g kg^{-1}, respectively [48]. Likewise, Sobhani et al. (2021), observed that exposure to polyethylene MPs resulted in a significant decrease of over 70% in earthworm reproduction for both the F0 (parental) and F1 (first filial) generations. Therefore, assessing reproductive health can serve as an initial indicator to detect the toxic effects of MPs on earthworms without the need for conducting extensive physiological studies [49]. In addition to disruptions in reproductive behavior, MPs have been found to induce various other repercussions in organisms. For instance, exposure to MPs can lead to the production of stress-related enzymes, indicating physiological stress responses [50]. Furthermore, organisms may exhibit avoidance responses, attempting to minimize contact with microplastic-contaminated environments. These observations highlight the multifaceted impacts of MPs beyond reproductive effects, emphasizing the need for comprehensive investigations into their ecological consequences.

Other physiological effects

Microplastics have the capacity to alter the behavior of earthworms, leading to modifications in their burrowing activity and movement patterns [51]. Exposure to MPs has been shown to result in reduced burrowing depth, diminished soil mixing, and changes in feeding behavior among earthworms **(Table 1)**. Moreover, MPs exposure can induce significant physiological and biochemical alterations in these organisms [52]. Studies have reported elevated levels of oxidative stress, disruptions in antioxidant defense systems, and modifications in enzyme activities in earthworms subjected to MPs **(Figure 2)**. In a study conducted by Li et al. (2021), the impact of polyethylene MPs on the activity of key antioxidant enzymes, namely superoxide dismutase, catalase, and glutathione peroxidase, was investigated. The researchers examined how exposure to polyethylene MPs influenced the functioning of these enzymes in earthworms [47]. Zhang et al. (2022), studied the combined toxic effect of polyethylene and zinc oxide nanoparticles on earthworms. An increase in the levels of catalase and glutathione synthetase altered antioxidant response in the presence of both polyethylene and zinc oxide nanoparticles. This indicates that the combined exposure to these particles can have a synergistic effect on the oxidative stress response in earthworms [53]. Chen et al. (2020) detected an augmentation in the activity of catalase and acetylcholinesterase in *E. fetida* when exposed to a concentration of 1 g/kg of low-density polyethylene. This observation implies that the presence of low-density polyethylene can induce changes in the enzymatic activities related to antioxidant defense and neurochemical processes in *E. fetida* [54]. Earthworms have a crucial role in preserving soil fertility and structure. However, the presence of MPs contamination in soil can have detrimental effects on earthworm-mediated processes, including nutrient cycling, soil aeration, and decomposition of organic matter [55]. These processes are essential for maintaining a healthy and productive soil ecosystem. Therefore, the impact of MPs on earthworms can have far-reaching consequences for soil quality and ecosystem functioning. As a result, these disruptions can set in motion a series of consequences that have implications for the overall health of soil and the functioning of ecosystems. It is important to highlight that the toxicological effects of MPs on earthworms exhibit variability due to the interplay of multiple factors [56]. These factors include the characteristics of the MPs, such as their type and size, as well as the specific species of earthworms involved. The concentration and duration of exposure to MPs are crucial determinants in shaping the extent of their effects on earthworms and, consequently, the resulting impact on soil health [57]. It should be noted that the presence of other pollutants or environmental stressors can interact with MPs, potentially exacerbating their detrimental effects [43]. These interactions between MPs and other contaminants or stressors can lead to synergistic or additive effects, amplifying the overall impact on organisms and ecosystems. Therefore, considering the potential interactions is essential for a comprehensive understanding of the ecological implications of MPs pollution. Zhang et al. (2022) found that the presence of MPs in soil led to higher bioaccumulation of zinc in *Eisenia fetida* [53]. This observation suggests that MPs can influence the uptake and accumulation of zinc by earthworms, potentially leading to increased levels of this metal within their tissues. In a recent study, Fu et al. (2023) observed that toxicity of polyethylene was enhanced in the presence of imidacloprid. This enhanced toxicity was indicated by alterations in the ferroptosis pathway, a form of programmed cell death, and an increase in the iron content within the tissues of *E. fetida* [58]. Considering the toxic effects of MPs on earthworms, it is crucial to implement measures to minimize their presence in agricultural soil. Therefore, it is imperative to adopt strategies that reduce the introduction and accumulation of MPs in agricultural systems, safeguarding the beneficial role of earthworms and supporting sustainable agricultural practices.

REMOVAL TECHNIQUES OF MICROPLASTICS FROM SOIL

Removing MPs from soil can be a challenging task due to their small size and widespread distribution. However, there are several techniques that can be employed to mitigate and remove MPs from soil [65].

Physical

Physical separation involves the use of sieves or filters to separate MPs from the soil. This method is effective for larger MPs but may not be suitable for smaller particles. Soil washing is a technique where contaminated soil is mixed with water or other solvents to extract MPs [66]. This process relies on the difference in density between the soil particles and MPs. Centrifugation or flotation can be used to separate the MPs from the soil-water mixture. Electrostatic separation utilizes the principle of electrostatic charge to attract and separate MPs from soil. By applying an electric field, the MPs can be charged and subsequently separated from the soil [67].

Physical techniques for mitigating MPs contamination encompass a variety of methods, among which filtration stands out as a representative approach. Filtration includes several distinct methodologies, such as screening, disk filtration, sand filtration, and membrane filtration (comprising microfiltration, ultrafiltration, nanofiltration, dynamic membrane, and reverse osmosis) [68]. Screening finds applications in both conventional wastewater treatment plants (WWTP) and drinking-water treatment plants (DWTP). The screening method efficiently eliminates larger plastic particles through a combination of filtering and sedimentation processes. Extensive studies have confirmed that the screening method can achieve a removal rate of approximately 40% to 80% for MPs [69]. Meanwhile, disk filtration emerges as another widely used technique in WWTP settings. In their study, Simon et al. (2019) demonstrated the high efficiency of the diskfilter in removing MPs smaller than 10 μm, with a remarkable removal rate of up to 89.7%. Additionally, it's worth noting that a sandfilter is a versatile method employed in both conventional WWTP and DWTP for MPs removal [70]. According to the findings of Wolff et al. (2021), rapid sand filtration exhibited impressive MPs removal rates, achieving approximately 99.2% ± 0.29% and 99.4% ± 0.15% [71]. As for membrane filtration, it demonstrated excellent efficiency in removing MPs, especially those larger than 10 μm, with most cases showing removal rates of over 90%. However, it is essential to acknowledge that while membrane filtration effectively removes MPs, it also presents a potential challenge. The deposition of MPs on the membrane surface can accelerate membrane contamination, leading to the contamination of other organic substances present in the membrane [72]. To address this issue and prevent excessive membrane contamination, a pretreatment process becomes necessary when employing a membrane filtration method. This pretreatment process serves to safeguard the membrane from contamination by organic matter and MPs, ensuring its continued effectiveness in removing pollutants [73].

Chemical

Chemical degradation involves the use of chemical agents or enzymes to break down MPs into smaller, less harmful substances. This method can be effective for certain types of MPs but may not be suitable for all polymers [74]. The utilization of chemical methods for MPs removal has been subject to extensive research, with coagulation/precipitation being a prominently employed approach in water treatment. However, the effectiveness of this method can vary significantly depending on several factors, including the specific type and amount of coagulant used, as well as the duration of coagulation retention [75].

Numerous investigations have been conducted to identify the most suitable coagulant type and optimal conditions for efficient MPs removal [76–78]. Despite these efforts, there remains a need for further and more in-depth studies to establish clearer and more comprehensive guidelines for effective MPs removal using coagulation/precipitation methods in the future [79]. Such studies would be valuable for enhancing our understanding of the mechanisms involved and for developing more efficient and targeted approaches for combating MPs contamination in water treatment processes. Lapointe et al. (2020) conducted a comparative analysis of the removal rates of different types of polyester (PE), weathered PE, and pristine PE MPs using a Jar test. They employed aluminum-based coagulants and polyacrylamide (PAA) as part of the treatment process. They found that 2.73 mg of aluminum per liter (Al/L) and 0.3 mg of polyacrylamide per liter (PAM/L) coagulants to water containing 500 MPs per liter (MPs/L), the optimal removal rates were quite similar. The removal efficiency for various microsphere sizes was as follows: 82% for PE microspheres of 140 μm, approximately 80% for PS (polystyrene) microspheres of 140 μm, about 88% for PE microspheres of 15 μm, and an impressive 99% for PEST (polyester) fibers. Moreover, the combination of PAM with aluminum-based and iron-based coagulants could achieve an efficient removal of microplastics, with removal rates reaching up to 99%. The effectiveness of the removal depended on factors such as the size and number of MPs, as well as the specific conditions of the water being treated. Additionally, the study also highlighted the efficacy of electrocoagulation as an effective method for removing microplastics from water [80]. These findings provide valuable insights into the potential application of coagulation and electrocoagulation techniques for efficient microplastics removal, and they underscore the importance of considering different factors when designing effective strategies to combat microplastic pollution in water bodies.

Biological Removal Technology

Bioremediation relies on the activity of microorganisms to degrade or assimilate MPs in soil. Certain bacteria and fungi have the ability to break down or metabolize MPs, leading to their removal from the environment [81]. Phytoremediation involves using plants to extract or degrade contaminants, including MPs, from soil. Some plant species have the ability to take up MPs through their roots and store them in their tissues, thereby reducing the MPs concentration in the soil [82].

Among the techniques employed for the removal of MPs, the biological approaches encompass activated sludge treatment, aerobic and anaerobic digestion, lagoons, and septic tanks have been reported with better removal efficiency [83,84]. In activated sludge systems, bacteria are acknowledged for their ability to capture MPs with a size of less than 0.5 mm. Nevertheless, while the activated sludge system effectively captures MPs from water, its capacity to degrade plastics remains challenging due to the relatively short residence time (7-14 h) in WWTPs [85].

Liu et al. (2019) conducted a study on virgin MPs and revealed that these particles didn't exert a statistically significant influence on the activities of crucial microbial groups such as ammonia oxidizing bacteria, nitrite oxidizing bacteria, and phosphorus accumulating organisms [86]. Conversely, in a separate investigation, Cunha et al. (2020) employed 10 mg/L of fresh *Cyanothece* sp. and observed a noteworthy microplastics removal rate of up to 47% [87]. Canniff and Hoang (2018) examined the growth rate of *Daphnia magna* when exposed to PE beads. They found that the intake rate of PE beads increased with higher particle concentrations and longer exposure times. Additionally, they observed that *Raphidocelis subcapitata* exposed to PE beads exhibited greater growth compared to those without exposure. Despite these findings, the overall efficiency of removing MPs using biological methods was found to be generally low [88]. Moreover, the presence of MPs in sludge or sedimentation can lead to secondary contamination. As a result, it is essential not to overemphasize the impact of MPs on the performance of bioreactor systems. Based on this, it can be concluded that achieving high MPs removal efficiency through biological methods is not very promising [89].

It is crucial to acknowledge that the efficacy of these methodologies can diverge contingent on the nature and dimensions of MPs, alongside the soil's composition. In numerous instances, a synergistic approach encompassing various techniques might be necessary to attain the most favourable outcomes. Furthermore, fostering improved waste management practices and curbing plastic pollution at its source are imperative for establishing enduring solutions.

FUTURE PERSPECTIVES

The extensive presence of MPs in terrestrial ecosystems, including soils, has raised significant concerns about their toxicological effects on earthworms and the overall health of soil ecosystems. This review article provides valuable insights into the impact of MPs on earthworms and highlights the urgent need to address this environmental challenge. It also explores the emerging removal technologies for mitigating MPs pollution in soils, aiming to preserve the health and functionality of earthworms and soil ecosystems. The toxicological effects of MPs on earthworms have been well-documented, encompassing both physical and chemical interactions. Earthworms can ingest MPs, leading to mechanical damage and hindrance of vital physiological processes. The accumulation of MPs in earthworm tissues can disrupt digestion, impair coelomic fluid movement, and affect reproductive and developmental processes. Furthermore, MPs can act as carriers for various organic pollutants, potentially leading to internal exposure and toxicity in earthworms. These toxicological effects have far-reaching implications for the overall health and functioning of soil ecosystems, given the critical roles that earthworms play as ecosystem engineers.

To address the challenge of MPs contamination, several removal technologies have been developed. Physical removal methods, such as filtration, centrifugation, and electrostatic separation, aim to physically separate MPs from soil matrices. Chemical remediation methods, including biodegradation and chemical degradation, utilize biological or chemical agents to break down MPs into less harmful forms. Phytoremediation, involving the use of plants, and microbial remediation, utilizing specific microorganisms, offer promising approaches for removing MPs from soils. Integrated approaches that combine multiple removal technologies are also being explored to enhance the efficiency and effectiveness of MPs remediation. However, it is important to acknowledge that the removal of MPs from soil is a complex and challenging task. The diverse nature of MPs, their varying sizes, shapes, and compositions, as well as their interactions with soil particles, make their complete removal a formidable challenge. Additionally, the long-term effects of these removal technologies on soil ecosystems and the potential unintended consequences need further investigation.

Moving forward, there is a need for improved risk assessment methods to better understand the toxicity of MPs to earthworms and the broader soil ecosystem. Standardized testing protocols should be developed to

ensure consistent and reliable data collection and interpretation. Long-term studies are essential to assess the chronic effects of MPs exposure on earthworm populations, soil health, and ecosystem functioning. Furthermore, regulatory frameworks and policies must be developed to mitigate the release of MPs into the environment and promote the adoption of sustainable practices.
Education and public awareness play a vital role in addressing the issue of MPs pollution. Efforts should be made to raise awareness about the environmental impacts of MPs and the importance of responsible plastic use and disposal. Public engagement and participation are crucial in driving behavioral changes and fostering a collective responsibility towards mitigating MPs pollution.

Table 1. Toxicological effect of microplastics on earthworms.

Sr. No.	Earthworm species and microplastic	Exposure of microplastic	Toxicological effect of microplastic	References
1.	Eisenia fetida; Polystyrene	Earthworms were exposed to 100 µg and 1000 µg of sized 100 nm and 1300 nm polystyrene microplastics per kg of artificial soil for 14 days.	Histopathological study revealed that the intestinal cells were damaged. Higher of MP induced oxidative stress which was confirmed by GSH and SOD levels. The comet assay indicated DNA damage in specimen due to exposure to MP.	[59]
2.	Eisenia andrei; Polyethylene	Earthworms were exposed two size of polyethylene microplastic sphere (180PE and 250PE) at a concentration of 1000 mg/kg dry soil for 21 days.	Exposure to MP caused inhibition of coelomocyte viability. Male reproductive organs were adversely affected. Negligible effects on female reproductive organs were observed.	[42]
3.	Lumbricus terrestris; Polyethylene	Earthworms were exposed polyethylene at a concentration of 0 %, 7 % and 28 % in feeding litter, w/w for 40 days.	Microplastic at 28 % concentration caused 62.5 % mortality and 17.6 % weight loss in earthworms. Reproduction in earthworms was not affected by any treatment.	[60]
4.	Eisenia fetida; Polypropylene carbonate, polylactic acid, and polyethylene	Earthworms were exposed a gradient concentration of microplastics 0, 0.125, 1.25, 12.5, 125, 250 and 500 g/kg of artificial soil for 56 days.	Number of cocoons during reproduction was significantly reduced at 53 g/kg. 15-20 % death rate was observed from various concentration of MP. 27 % in the body weight loss was observed at MP concentration 500 g/kg of artificial soil.	[48]
5.	Metaphire guillelmi; High-density polyethylene and polypropylene	Earthworms were exposed to soil amended with 0.25 % (w/w) high-density polyethylene (25 µm) or polypropylene (13 µm) microplastics for 28 days.	Microplastics exposure did not induce gut microbiota dysbiosis in specimen. Microplastics significantly reduced the bacterial diversity and altered bacterial community.	[61]
6.	Eisenia fetida; Polyethylene and polypropylene	Earthworms were exposed to 0.25 % (w/w) of polyethylene (28–145, 133–415 and 400–1464 µm) and polypropylene (8–125, 71–383 and 761–1660 µm) in an agricultural soil for 28 days.	Microplastic exposure altered SOD, CAT, and GSH activities. Microplastic exposure significantly disturbed several pathways closely related to neurodegeneration, oxidative stress, and inflammatory responses.	[47]
7.	Eisenia fetida; Polystyrene	Earthworms were exposed to a soil amended with polystyrene MPs concentrations 10 mg/kg and 100 mg/kg for 21 days.	Metagenomics sequencing and toxicity tests revealed MP caused toxicity and influenced the abundance of microbial community in specimen.	[62]

			100 mg/kg of 10 µm MP significantly changed the profile of antibiotic resistance genes in earthworms.	
8.	Eisenia fetida; Polystyrene	Earthworms were exposed to pure and commercial polystyrene microplastics (65-125 µm) (0-0.5 % w/w) in artificial soil for 28 days.	No toxicity effect on mortality was observed. Microplastics at 0.5 % concentration reduced 50 % juvenile production. Genotoxicity in terms of DNA damage was observed.	[49]
9.	Eisenia andrei; Polyethylene	Earthworms were exposed to polyethylene microplastic (<100 µm) at a concentration of 100 µg per kg of agricultural soil for 7 and 14 days.	Oxidative stress was observed in earthworms exposed to MP.	[63]
10.	Eisenia andrei; Polystyrene	Earthworms were exposed to microplastic at a concentration of 100 3g per kg of agricultural soil for 28 days.	No mortality could be observed after 28 days. No changes in avoidance behavior were observed. No significant changes of reproduction observed.	[64]

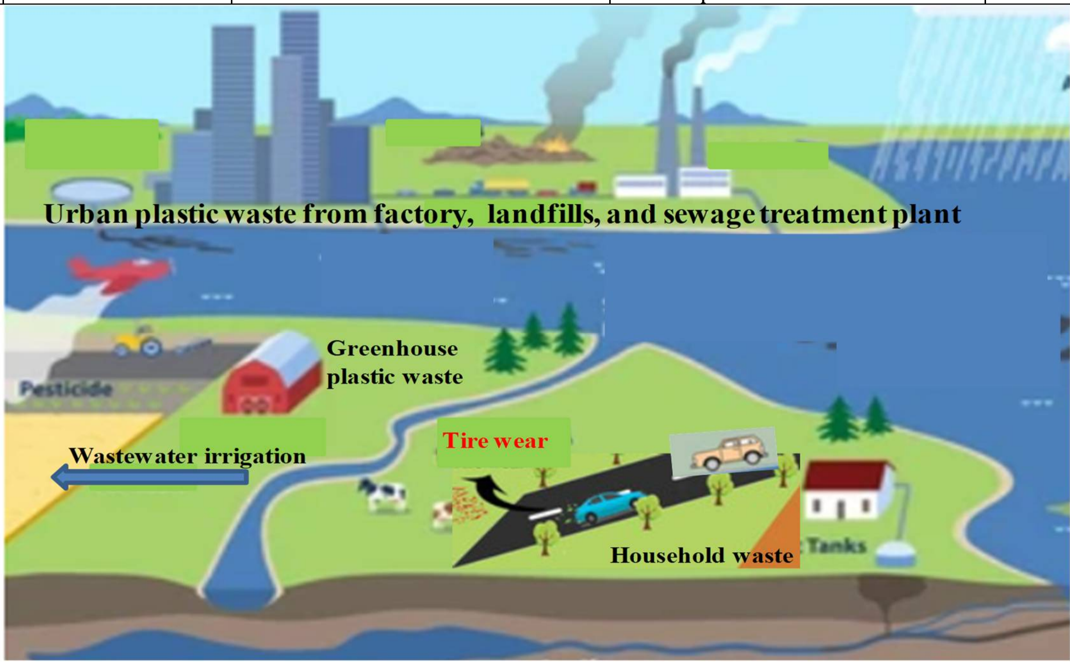

Figure 1. Different sources of microplastics in soil systems and their migration to agricultural soil through irrigation.

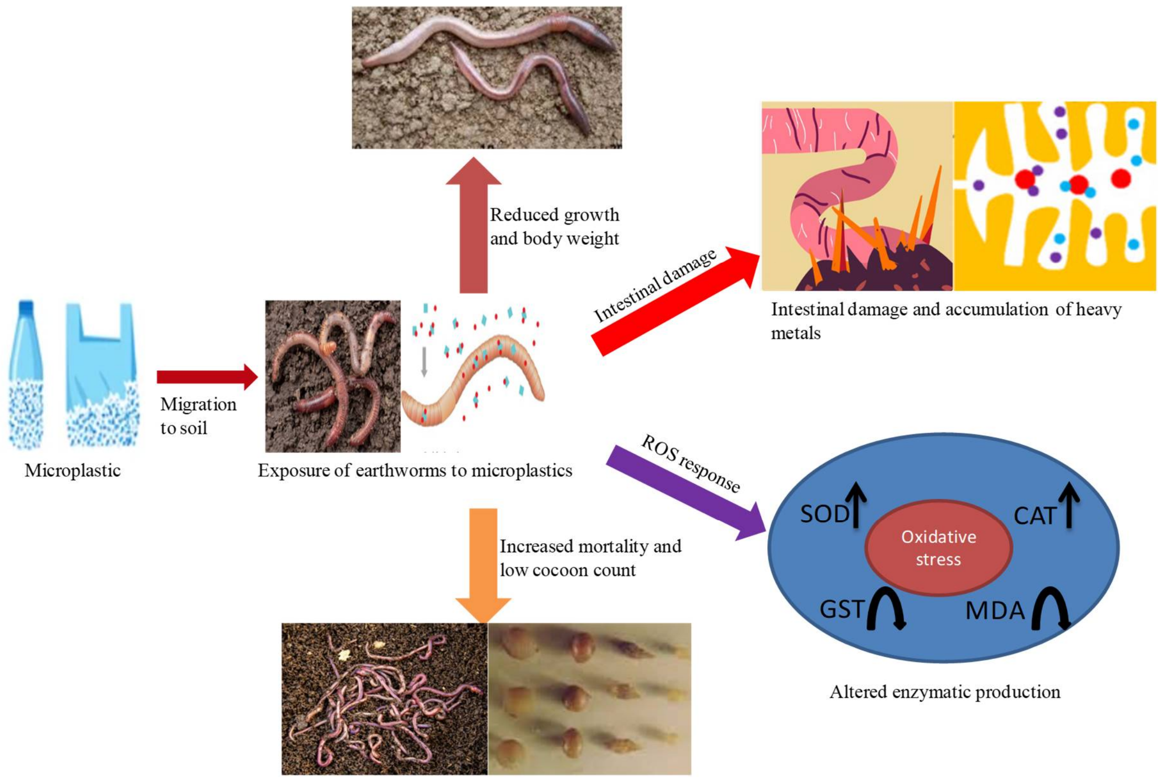

Figure 2. Exposure of earthworm to microplastics and their different harmful effects on growth, metabolism and reproduction.

CONCLUSION

In conclusion, this review article provides valuable insights into the toxicological effects of MPs on earthworms and highlights the urgent need to address this global environmental challenge. The development of effective removal technologies is crucial for mitigating MPs contamination in soils, ultimately preserving the health and functionality of earthworms and soil ecosystems. Future research and collaborative efforts are necessary to further our understanding of MPs toxicity and develop sustainable solutions to combat this pervasive environmental problem. By safeguarding earthworm populations and soil ecosystems, we can ensure the long-term health and sustainability of our planet. Further research is needed to gain a more comprehensive understanding of the mechanisms and long-term consequences of MPs exposure on earthworms and soil ecosystems. Nonetheless, the available evidence suggests that MPs can have detrimental effects on earthworms, underscoring the need to mitigate MPs pollution and protect soil health.

CRediT author contributions: Garima Sheoran: Literature search, Data analysis, Software, Writing - original draft. **Vineeta Shukla:** Conceptualization, Resources, Supervision, Visualization, Writing - review & editing.

Funding: Not applicable

Data availability: All data generated or analyzed during this study are included in this published article

Ethical approval: Not applicable.

Consent to participate: Not applicable

Consent to publication: Not applicable

Conflict of interest: The authors declare that they have no conflict of interest with contents of this article.

Acknowledgement: Authors acknowledge the infrastructural and financial support provided from Department of Zoology, Maharshi Dayanand University, Rohtak-124001, Haryana, India. G.S. is grateful to Council of Scientific and Industrial Research (CSIR) for grant of junior research fellowship (JRF).

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CITATION OF THIS ARTICLE

Garima Sheoran, and Vineeta Shukla. An Insight into The Toxicological Effects of Microplastics on Earthworms And Their Removal Technologies. Bull. Env.Pharmacol. Life Sci., Vol 12 [12] November 2023: 368-381