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MBA EM GESTÃO DE ÁREAS CONTAMINADAS, DESENVOLVIMENTO URBANO SUSTENTÁVEL E REVITALIZAÇÃO DE BROWNFIELDS

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Bioindicators of the Impacts by Microplastics in Soil: A Systematic Review

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Bioindicators of the Impacts by Microplastics in Soil: A Systematic Review

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RESUMO

Cetrangolo Chirmici, Alyne. Bioindicadores dos Impactos por Microplásticos em Solo: Uma Revisão Sistemática da Literatura. 2024. 40 f. Monografia (MBA em Gestão de Áreas Contaminadas, Desenvolvimento Urbano Sustentável e Revitalização de Brownfields) – Escola Politécnica, Universidade de São Paulo, São Paulo, 2024.

Este estudo apresenta a revisão sistemática da literatura realizada para identificar os principais organismos que podem ser utilizados como bioindicadores dos impactos causados por microplásticos no solo, de forma a subsidiar estratégias de gerenciamento ambiental mais eficazes. A partir de um levantamento inicial nas bases de dados *Scopus, ScienceDirect, ResearchGate* e *Springer*, foram identificados inicialmente 738 artigos, dos quais 29 foram selecionados para análise estatística. Os artigos foram classificados quanto à(s) espécie(s) avaliadas, os efeitos observados decorrentes da exposição a microplásticos, além da distribuição espacial e temporal das informações levantadas. Verificou-se que várias espécies podem atuar como importantes bioindicadores da presença e dos impactos dos microplásticos no ambiente terrestre. Entre essas espécies estão os invertebrados *Eisenia fetida* (minhoca vermelha), *Achatina fulica* (caramujo-africano), *Lumbricus terrestris L.* (minhoca), colêmbolos do solo (*Folsomia candida*), abelhas (*Apis mellifera* and *Apis cerana)*, insetos (*Bombyx mori*), *Enchytraeus crypticus* e o pássaro (*Corvus splendens*); além de impactos na interação da mosca-dos-fungos (*Bradysia difformis*) com plantas da espécie leguminosa lentilha (*Lens culinaris*). Espécies de flora terrestre como *Lepidium sativum* (agrião), *Lolium perene* (azevém), *Triticum aestivum L.* (trigo) e *Vicia faba* (fava) também foram avaliadas nos estudos revisados. Cabe salientar que, embora este seja um tema que vem avançando rapidamente nos últimos oito anos, mais pesquisas científicas são necessárias para que novas espécies terrestres sejam avaliadas e alcancem a análise em larga escala, uma vez que a grande maioria dos estudos ainda descrevem ensaios em escala de bancada.

Palavras-chave: Contaminantes; Solo; Ambiente Terrestre; Bioindicador; Toxicidade.

ABSTRACT

Cetrangolo Chirmici, Alyne. Bioindicators of the Impacts by Microplastics in Soil: A Systematic Review. 2024. 40 p. Monograph (MBA in Contaminated Area Management, Sustainable Urban Development and Brownfields Revitalization) - Polytechnic School, University of São Paulo, São Paulo, 2024.

This study presents the systematic literature review carried out to identify the main organisms that can be used as bioindicators of impacts by microplastics in soil, in order to subsidize more effective environmental management strategies. From an initial survey in the Scopus, ScienceDirect, ResearchGate, and Springer databases, 738 articles were initially identified, 29 of which were selected for statistical analysis. The articles were classified in terms of the species evaluated, the effects observed as a result of exposure to microplastics, as well as the spatial and temporal distribution of the information collected. It was found that several species can act as important bioindicators of the presence and impacts of microplastics in the terrestrial environment. Among these species are the invertebrates *Eisenia fetida* (red earthworm), *Achatina fulica* (African snail), *Lumbricus terrestris L*. (earthworm), soil springtails (*Folsomia candida*), bees (*Apis mellifera* and *Apis cerana)*, insects (*Bombyx mori*), *Enchytraeus crypticus*, and bird (*Corvus splendens*); and the interaction of the fungus fly (*Bradysia difformis*) with plants of the leguminous species lentil (*Lens culinaris*). Terrestrial flora species such as *Lepidium sativum* (garden cress), *Lolium perene* (ryegrass), *Triticum aestivum L*. (wheat), and *Vicia faba* (broad bean) were also evaluated in the studies reviewed. Although this is a topic that has been advancing rapidly over the last eight years, more scientific research is needed for new terrestrial species to be evaluated and achieve large-scale analysis, since the vast majority of studies still describe bench-scale tests.

Keywords: Contaminants; Soil; Terrestrial Environment; Bioindicator; Toxicity.

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SUMMARY

1. INTRODUCTION

Plastic production has shown a substantial increase in recent years, driven primarily by its versatile applications in various products and industries. However, this growth in plastic usage has raised a critical environmental concern: currently, about 40% of all plastic production is destined for packaging, resulting in the ubiquitous presence of plastic contamination worldwide (AL MALKI et al., 2021).

Plastics are produced from fossil fuels and organic materials, with the majority currently synthesized from petroleum (ITRC, 2023). Table 1 summarizes the main polymer types of plastics and their abbreviations.

Table 1 - Main plastic polymers, abbreviations and product examples.

Resin Code	Plastic Type Abbreviation	Plastic Type Name	Product Examples
1	PET	Polyethylene terephthalate	Water and soft drink bottles, salad dressing/peanut butter containers, rope, carpet, polyester fibers
2	HDPE	High-density polyethylene	Milk jugs, juice bottles, freezer bags, trash bags, shampoo/detergent bottles
3	PVC	Polyvinyl chloride	Plumbing and construction materials, pipes, liners, cosmetic containers, commercial cling wrap, siding
4	LDPE	Low-density polyethylene	Squeeze bottles, regular cling wrap, trash bags, shopping bags, furniture
5	PP	Polypropylene	Microwave dishes, medicine bottles, straws, ice cream tubs, yogurt containers, detergent bottle caps
6	PS/EPS	Polystyrene/Expanded polystyrene	PS - CD cases, disposable cups, egg cartons, cutlery, video cases

			EPS - Foam polystyrene, hot drink cups, food takeaway trays, protective packaging pellets
Other	POM	Acetal (polyoxymethylene)	Fan wheels, gears, screws
	PMMA	Acrylic (polymethyl methacrylate)	Aquariums, fiber optics, paint
	ABS	Acrylonitrile butadiene styrene	Car parts, Lego, wheel covers
	PA	Nylon (polyamide)	Air bags, clothing, thread
	P	Polyester	Fibers, rope
	PBT	Polybutylene terephthalate	Keyboards, relays, switches
	PC	Polycarbonate	Eyewear, safety helmets
	PEEK	Polyetheretherketone	Bearings, pump, pistons
	PE	Polyethylene	Mulch, housewares, toys
	PLA	Polylactic acid (bioplastic)	Packaging, syringes, textiles
	PSU	Polysulfone	Appliance parts, filters
	PTFE	Polytetrafluoroethylene	Teflon
	PUR, PU	Polyurethane	Adhesives, coatings, foams
	SAN	Styrene acrylonitrile	Brushes, hangers, printers

Source: ITRC, 2023.

Associated with the emerging issue of plastic usage impacts are microplastics, which consist of particles measuring just a few micrometers, typically less than 5 mm, posing a distinct challenge compared to larger plastic debris. The small size of microplastics facilitates their ingestion by small organisms, allowing for accumulation throughout the food chain. Microplastics primarily originate from paints, personal care products, and synthetic clothing, also being observed in secondary sources from the breakdown of larger plastics, reaching terrestrial environments, particularly soil.

Despite the growing evidence of microplastics presence in soil, research on the contamination of these particles in terrestrial environments is still in its infancy compared to marine ecosystems. According to estimates presented by AL MALKI et al., (2021), approximately 700,000 annual tons of microplastics infiltrate agricultural lands through manure application in Europe and North America, surpassing marine surface waters.

Bioindicators consist of individuals, populations, or even communities of organisms that, through their response to dynamics and changes in the ecosystem they inhabit and/or interact with, act as an important tool for assessing impacts in these environments. These responses include changes in distribution, lifestyle, behavior, physiological changes, among others (PARMAR; RAWTANI; AGRAWAL, 2016).

Considering the importance of terrestrial biota in maintaining ecosystem balance, this study aims to identify and critically evaluate bioindicator organisms sensitive to microplastic impacts in soil, which can be an important complementary tool to traditional chemical and physical analyses, considering the presence of these compounds in the terrestrial environment.

Among these organisms are earthworms, isopods, gastropods, and arthropods, which play vital roles in soil structure and functioning, litter decomposition, and nutrient cycling, and may exhibit adverse effects due to microplastic contamination.

Microplastics can selectively affect microbial communities, both in vital functions such as organic matter decomposition and nutrient cycling, as well as in soil invertebrates and terrestrial flora specimens (RADFORD, 2023). Furthermore, the adsorption of potentially toxic elements to microplastic surfaces and their subsequent bioavailability to animals raise serious questions about pollutant transfer through food chains and their bioaccumulation, including in humans.

Despite this correlation, most studies over the past decade have focused on assessing the presence of microplastics in estuarine and aquatic environments. However, some initial studies already demonstrate the impacts of microplastics on the functioning and dynamics of terrestrial communities, such as microarthropods, earthworms, bees, birds, terrestrial flora, and the interaction between plants and these organisms.

2. OBJECTIVES

This study aims to identify potential bioindicators related to the impacts caused by the presence of microplastics in the soil, as described in scientific literature.

2.1 Specific Objectives

- Identify commonly reported bioindicators for soil impacted by microplastics;
- Evaluate the effectiveness and feasibility of identified bioindicators in reflecting the presence and impacts of microplastics in terrestrial environments through field and/or laboratory experiments;
- Critically evaluate the identified bioindicators according to their application in real-scale studies and projects.

3. BACKGROUND

The impacts of microplastics, including soil contamination, are an emerging environmental issue that has received increasing attention in recent years within the scientific community. The rapid expansion of microplastics production and usage has resulted in widespread dissemination of these particles, impacting terrestrial ecosystems in a manner not yet fully understood.

The presence of microplastics in soil directly affects biodiversity and ecosystem balance, but also poses potential risks to human health. As a result, numerous research projects are currently being conducted worldwide to identify organisms that can serve as bioindicators of soil microplastic contamination.

However, a preliminary literature search did not identify a systematic review and critical evaluation of organisms that can be used as bioindicators of microplastic impacts in soil, in order to support more effective environmental management strategies. Therefore, conducting this work is justified to address this important gap.

4. LITERATURE REVIEW

Microplastics consist of plastic particles smaller than 5mm. Among the main polymers present in microplastics, polyethylene (PE), polypropylene (PP), polystyrene (PS), polyethylene terephthalate (PET), polyvinyl chloride (PVC), and nylon (PA) stand out (ZHU et al., 2019).

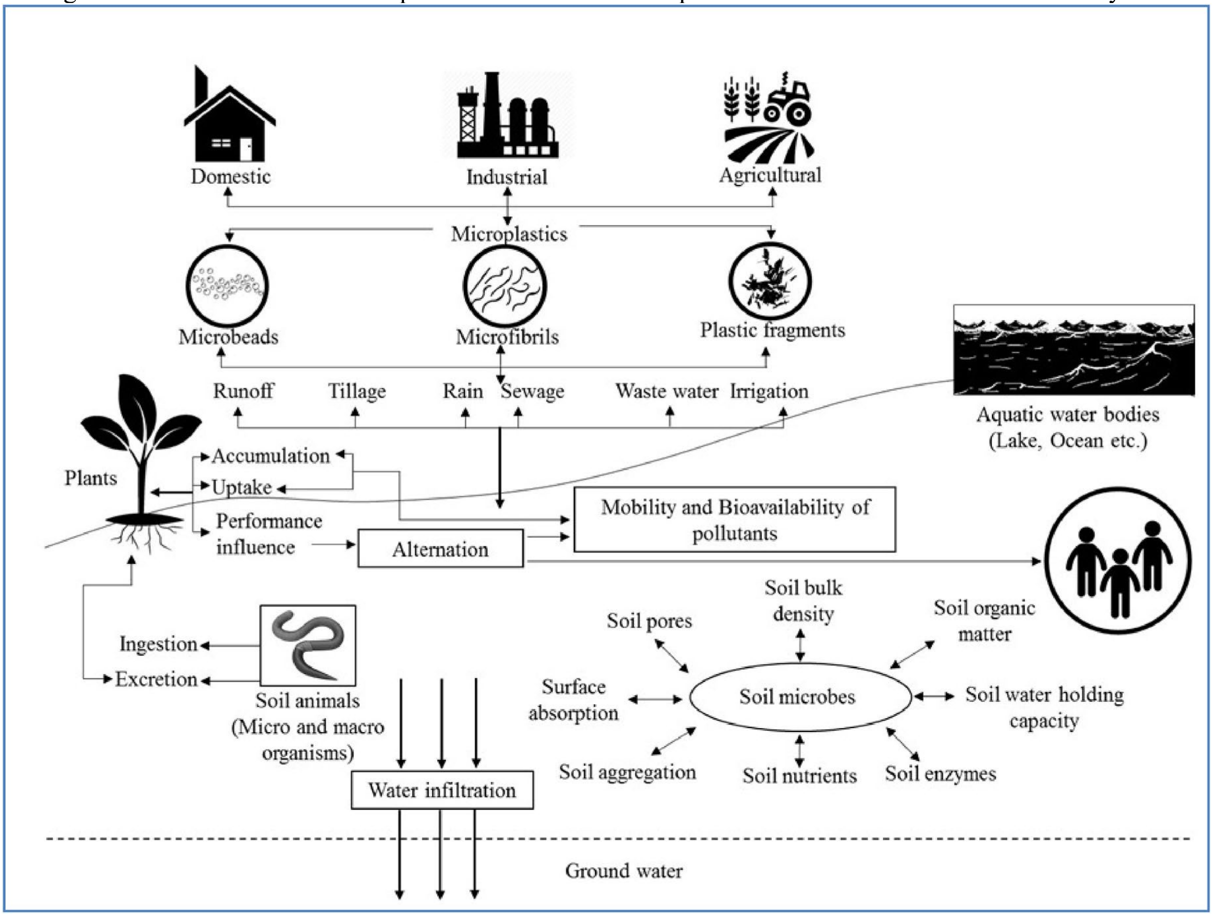
Microplastics are categorized into two main types based on their origin: primary microplastic and secondary microplastic. Primary microplastic refers to those produced in microscopic size, known in the industry as pellets, used in the formulation of various products. The presence of these microplastics in the environment mainly results from improper disposal during industrial processes and accidental losses during transportation, commonly found in areas near ports, industrial complexes, and the open sea. On the other hand, secondary microplastic is generated by the fragmentation of larger plastics discarded in the environment, occurring in both aquatic and terrestrial environments (LASKAR; KUMAR, 2019).

Bioindicators encompass biological processes, species, or communities that act as essential tools for assessing environmental quality and monitoring temporal changes in ecosystems, usually associated with anthropogenic impacts (HOLT; MILLER, 2010).

Bioindicator species are selected based on their tolerance to environmental variability, effectively reflecting the environmental condition. In contrast, less tolerant species may be either very sensitive or rarely found to be used as indicators. Furthermore, the use of environmental bioindicators must follow specific criteria, including a wide geographic distribution, well-known biological characteristics, a fundamental role in the ecosystem, homogeneous response to impacts, and the existence of identifiable toxic effects associated with the degree of impact (LI et al., 2019).

Among the potential sources of microplastics in terrestrial environments is the increasing use of sewage sludge in fertilizers. In addition to containing large amounts of phosphorus and nitrogen, this sludge also contains microplastic particles, promoting their transport to the soil and contamination of planting areas (HORTON et al., 2017). Other potential sources of microplastics in terrestrial environments include plastic covers with the increasing use of polyethylene in agricultural materials, atmospheric deposition, and landfills (DE SILVA et al., 2022). These sources and the main mechanisms of microplastic transport between different environmental compartments are summarized in Figure 1 (SURENDRAN et al., 2023).

Figure 1 - Main sources and transport mechanisms of microplastics contamination in terrestrial ecosystem



Source: SURENDRAN et al., 2023.

5. MATERIALS AND METHODS

The study was conducted using a systematic literature review (SLR) approach with the assistance of the StArt (State of the Art through Systematic Review) software - version 3.0.3, developed by the Software Engineering Research Laboratory (LaPES) at the Federal University of São Carlos (UFSCar). The bibliographic surveys conducted in the Scopus, ScienceDirect, ResearchGate, and Springer databases were used as the basis.

The information used in this study was collected in a three-stage process. The first stage involved retrieving scientific material on the properties of microplastics, their environmental fate and transport, as well as their impacts on the environment and human health from online documents published on the aforementioned platforms. The second stage involved the retrieval and critical evaluation of published studies, based on queries in the databases mentioned above, considering the following strings:

- “Microplastics" OR “Microplásticos”; AND
- “Soil” OR “Solo”; AND
- “Terrestrial” OR “Terrestre”; AND

- “Bioindicator” OR “Bioindicador”.

As a premise of the literature review, only studies published between 2013 and 2024 (the last 11 years) were considered in the analysis. It must be pointed out that this initial survey was updated monthly until February 2024.

From the searches in the 4 cited databases, initially 738 articles were identified. Of these, 21 were duplicate articles.

The remaining 717 articles were selected and categorized in the third analysis stage using the StArt software (version 3.0.3) and considering the following criteria:

- Inclusion: the document provides information on the use of organisms for assessing the presence and impacts of microplastics in soils for terrestrial environments; the article describes a case study, either on a bench scale or a real case, on the application of bioindicators of microplastic contamination in soil;
- Exclusion: the article does not provide information on the use of organisms for assessing the presence and impacts of microplastics in soils for terrestrial environments; other exclusion criteria was if the document was not available in the Integrated Libraries System - University of São Paulo (SIBi USP) and could not be accessed by the author. In addition, review articles were excluded from the analysis.

Based on the aforementioned criteria, 29 articles were included in the SLR, as shown in Table 2 below.

Table 2 - Articles selected from the systematic literature review

Author(s)	Study Title	Publication Year
DE SILVA, Y. S. K. et al.	Effects of microplastics on lentil (*Lens culinaris*) seed germination and seedling growth	2022
AL MALKI, J. S. et al.	Terrestrial biota as bioindicators for microplastics and potentially toxic elements	2021
BOUGHATTAS, I. et al.	Assessing the presence of microplastic particles in Tunisian agriculture soils and their potential toxicity effects using *Eisenia andrei* as bioindicator	2021

Table 2 - Articles selected from the systematic literature review (continuation)

Author(s)	Study Title	Publication Year
COLPAERT, R. et al.	Polyethylene microplastic toxicity to the terrestrial snail *Cantareus aspersus*: size matters	2022
BAEZA, C. et al.	Experimental exposure of *Lumbricus terrestris* to microplastics	2020
DIKE, S.; APTE, S.	Impact of microplastic pollution in terrestrial ecosystem on index and engineering properties of sandy soil: An experimental investigation	2023
FENG, T. et al.	Effect of microplastics on soil greenhouse gas emissions in agroecosystems: Does it depend upon microplastic shape and soil type?	2023
MUHAMMAD, A. et al..	Toxic effects of acute exposure to polystyrene microplastics and nanoplastics on the model insect, silkworm *Bombyx mori*	2021
CHARLES, P. E. et al.	First report on occurrence and characterization of microplastics in feces of *Corvus splendens* (Vieillot, 1817)	2024
DENG, Y. et al.	Microplastic Polystyrene Ingestion Promotes the Susceptibility of Honeybee to Viral Infection	2021
DING, W. et al.	Effect thresholds for the earthworm *Eisenia fetida*: Toxicity comparison between conventional and biodegradable microplastics	2021
LAHIVE, E. et al.	Microplastic particles reduce reproduction in the terrestrial worm *Enchytraeus crypticus* in a soil exposure	2019
ZHU, D. et al.	Exposure of soil collembolans to microplastics perturbs their gut microbiota and alters their isotopic composition	2018
HORTON, A. A. et al.	Accumulation of polybrominated diphenyl ethers and microbiome response in the great pond snail *Lymnaea stagnalis* with exposure to nylon (polyamide) microplastics	2020
BOSKER, T. et al.	Microplastics accumulate on pores in seed capsule and delay germination and root growth of the terrestrial vascular plant *Lepidium sativum*	2019

Source: Prepared by the author.

Table 2 - Articles selected from the systematic literature review (continuation)

Author(s)	Study Title	Publication Year
BOOTS, B.; RUSSELL, C.W.; GREEN, D.S.	Effects of microplastics in soil ecosystems: above and below ground	2019
QI, Y. et al.	Macro- and micro- plastics in soil-plant system: effects of plastic mulch film residues on wheat (*Triticum aestivum*) growth	2018
JIANG, X. et al.	Ecotoxicity and genotoxicity of polystyrene microplastics on higher plant *Vicia faba*	2019
SONG, Y. et al.	Uptake and adverse effects of polyethylene terephthalate microplastics fibers on terrestrial snails (*Achatina fulica*) after soil exposure.	2019
RILLIG, M. C., ZIERSCH, L., & HEMPEL, S.	Microplastic transport in soil by earthworms	2017
PIGNATTELLI, S.; BROCCOLI, A.; RENZI, M.	Physiological responses of garden cress (*L. sativum*) to different types of microplastics	2020
LWANGA, E. H. et al.	Incorporation of microplastics from litter into burrows of *Lumbricus terrestris*	2017
LWANGA, E. H. et al.	Microplastics in the Terrestrial Ecosystem: Implications for *Lumbricus terrestris* (Oligochaeta, Lumbricidae)	2016
MAAß, S. et al.	Transport of microplastics by two collembolan species	2017
YANG, X. et al.	Biogenic transport of glyphosate in the presence of LDPE microplastics: A mesocosm experiment	2019
WANG, H. et al.	Exposure to microplastics lowers arsenic accumulation and alters gut bacterial communities of earthworm *Metaphire californica*	2019
JU, H., ZHU, D., QIAO, M.	Effects of polyethylene microplastics on the gut microbial community, reproduction and avoidance behaviors of the soil springtail, *Folsomia candida*	2019
MACHADO, A. A. S. et al.	Microplastics Can Change Soil Properties and Affect Plant Performance	2019
LIAN, J. et al.	Impact of polystyrene nanoplastics (PSNPs) on seed germination and seedling growth of wheat (*Triticum aestivum* L.).	2020

Source: Prepared by the author.

From this selection, the 29 articles were categorized according to the criteria presented in Table 3 below.

Table 3 - Categories and subcategories used in Systematic Literature Review

Category	Sub-Category
Study Scale	Laboratory Pilot Large Scale
Organism Type	Fauna Flora Plant-Fauna Interaction
Publication Year	-
Microplastic Type	Polyethylene; High-density polyethylene; Polyvinyl chloride; Low-density polyethylene; Polypropylene; Polystyrene/Expanded polystyrene; Acetal (polyoxymethylene); Acrylic (polymethyl methacrylate); Acrylonitrile butadiene styrene; Nylon (polyamide); Polyester; Polybutylene terephthalate; Polycarbonate; Polyetheretherketone; Polyethylene; Polylactic acid (bioplastic); Polysulfone; Polytetrafluoroethylene; Polyurethane; Styrene acrylonitrile
Observed Effects	Biochemical Tissue damage and other pathological alterations Development Reproduction Mutagenicity Toxicological

Source: Prepared by the author.

Regarding the category 'Study Scale', the following premises were adopted for classifying the studies evaluated:

- Laboratory-scale: small-scale study, using small sample size and conducted in controlled environments, where it is possible to establish and have a greater control over the conditions of the process and analysis. Furthermore, this type of study allows to test a wide range of parameters and conditions;

- Pilot-scale: generally uses larger quantities of materials than a laboratory-scale study and is performed before a full-scale. This scale includes greenhouse pot studies;
- Full-scale: large-scale investigation, involving large amounts of data colletion and resources/materials. Offers a comprehensive view of processes in conditions close to reality, evaluating performance in conditions similar to the natural environment.

Based on the systematic literature review, a scientific article was developed and submitted in March 2024 to the journal Science of the Total Environment.

6. RESULTS AND DISCUSSION

The full content of the article developed on the basis of the systematic literature review is presented below.

INTRODUCTION

Plastic production has shown a substantial increase in recent years, mainly driven by its versatile applications in various products and segments. However, this growth in plastic usage has rised a critical environmental concern: currently, about 40% of all plastic production is destined for packaging, resulting in the omnipresence of plastic contamination worldwide (AL MALKI et al., 2021).

Plastics are produced from fossil fuels and organic materials, with the majority currently synthesized from petroleum (ITRC, 2023). Among the main polymers found in microplastics are polyethylene (PE), polypropylene (PP), polystyrene (PS), polyethylene terephthalate (PET), polyvinyl chloride (PVC), and nylon (PA) (ZHU et al., 2019).

Associated with the emerging issue of plastic usage impacts are microplastics, which consist of particles measuring only a few micrometers, generally less than 5 mm, posing a distinct challenge compared to larger plastic debris. The small size of microplastics facilitates their ingestion by small organisms, allowing for accumulation along the food chain. Microplastics have their primary sources in paints, personal care products, and synthetic clothing, also being observed in secondary sources from the degradation of larger plastics, reaching terrestrial environments, mainly the soil (ZIANI et al., 2023).

Microplastics are categorized into two main types based on their origin: primary and secondary. Primary microplastics refer to those produced in microscopic size, known in the industry as "pellets", used in the formulation of various products, such as glitter used in clothing and cosmetics. The presence of these microplastics in the environment mainly results from improper disposal during industrial processes and accidental losses during transportation, commonly found in areas near ports, industrial complexes, and the open sea. On the other hand, secondary microplastics are generated by the fragmentation of larger plastics discarded in the environment, occurring in both aquatic and terrestrial environments (LASKAR; KUMAR, 2019).

Despite the growing evidence of microplastic presence in soil, research on the contamination of these particles in terrestrial environments is incipient compared to marine ecosystems. According to estimates presented by AL MALKI et al., (2021), approximately 700,000 tons of microplastics infiltrate agricultural lands annually through manure application in Europe and North America, surpassing surface marine waters.

Among the potential sources of microplastics in terrestrial environments is the growing use of sewage sludge in fertilizers. Besides containing large amounts of phosphorus and nitrogen, this sludge also harbors microplastic particles, promoting their transport into the soil and contamination of planting areas (HORTON et al., 2017). Other potential sources of microplastics in terrestrial environments include plastic coverings with the growing use of polyethylene in agricultural materials, atmospheric deposition, and landfills (DE SILVA et al., 2022).

Another source of microplastics is the washing water used to clean industrial equipment and facilities, as well as the effluents resulting from production processes, from manufacturing industries that use plastics as raw materials or during the production process. The washing of synthetic textiles also contributes to the release of microplastics into the environment, indicating that the effluent from this type of activity is also a significant source of microplastics to the environment (DE FALCO et al., 2019;HAQUE et al., 2022).

Bioindicators consist of individuals, species, populations, or even communities of organisms that, through their response to ecosystem dynamics and changes, act as an important tool for assessing environmental quality and monitoring impacts in these environments (HOLT; MILLER, 2010). These responses include changes in distribution, lifestyle, behavior, physiological changes, among others (PARMAR; RAWTANI; AGRAWAL, 2016).

Bioindicator species are selected based on their tolerance to environmental variability, effectively reflecting environmental condition. In contrast, less tolerant species may be either highly sensitive or rarely found to be used as indicators. Furthermore, the use of environmental bioindicators should follow some specific criteria, including: broad geographical distribution, well-known biological characteristics, a fundamental role in the ecosystem, homogeneous response to impacts, and the existence of identifiable toxic effects associated with the degree of impact (LI et al., 2019).

In view of the importance of terrestrial biota in maintaining ecosystem balance (DISSANAYAKE et al., 2022), this study aims to identify and critically evaluate bioindicator organisms sensitive to the impacts of microplastics in soil, which can be an important complementary tool to traditional chemical and physical analyses, considering the presence of these compounds in the terrestrial environment.

Among these organisms are earthworms, isopods, gastropods, and arthropods, which play vital roles in soil structure and function, litter decomposition, and nutrient cycling, and may exhibit adverse effects due to microplastic contamination (AL MALKI et al., 2021).

Microplastics can selectively impact microbial communities, both in vital functions such as organic matter decomposition and nutrient cycling, as well as in soil invertebrates and terrestrial flora (RADFORD, 2023). Besides directly affecting biodiversity and ecosystem balance, the adsorption of potentially toxic elements to microplastic surfaces and their subsequent bioavailability to animals raise serious questions about pollutant transfer through food chains and their bioaccumulation, including in humans.

Despite this correlation, most studies over the last decade have focused on assessing the presence of microplastics in estuarine and aquatic environments. However, some early studies already demonstrate the impacts of microplastics on the functioning and dynamics of terrestrial communities, such as microarthropods, earthworms, bees, birds, terrestrial flora, and the interaction between plants and these organisms.

The impacts of microplastics, including soil contamination, are an emerging environmental issue that has received increasing attention in recent years among the scientific community. The rapid expansion of microplastic production and use has resulted in a widespread dissemination of these particles, impacting terrestrial ecosystems in ways not yet fully understood. As a result, many studies are currently being conducted worldwide to identify organisms that can be used as bioindicators of microplastic contamination in soil.

Despite the importance of using organisms as bioindicators of microplastic pollution, studies on the subject have seen a significant increase only in the last decade. This study aims to aggregate and analyze information on the potential use of terrestrial species as bioindicators of the presence and impacts of microplastics. This review should assist in biomonitoring the presence and impacts of microplastics in terrestrial environments, as well as in the development of new research and protocols based on identified gaps.

MATERIALS AND METHODS

The study was developed using a systematic literature review (SLR) approach with the assistance of the StArt (State of the Art through Systematic Review) sof–ware - version 3.0.3, created by the Software Engineering Research Laboratory (LaPES) at the Federal University of

São Carlos (UFSCar). Bibliographic surveys conducted in the Scopus, ScienceDirect, ResearchGate, and Springer databases served as the basis for this study.

The information used in this study was collected in a two-stage process. The first stage involved retrieving scientific material on the properties of microplastics, their environmental fate and transport, as well as their impacts on the environment and human health from online documents published on the aforementioned platforms. The second stage involved the retrieval and critical evaluation of published studies, based on searches in the databases mentioned earlier, considering the following strings:

- Microplastics;
- Soil;
- Terrestrial;
- Bioindicator.

As a premise of the bibliographic survey, only studies published between 2013 and 2024 (the last 11 years) were considered in the analysis.

From searches in the 4 cited databases, initially 738 articles were identified. Of these, 21 were duplicate articles.

The Figure 2 below presents a word cloud generated from the titles of the articles identified in the survey. The size of the letters is proportional to the frequency of occurrence.

Figure 2 – Word cloud of the articles identified in this study. Size of the word is directly proportional to the number of occurrences in the reviewed dataset (n = 717)

Source: Prepared by the author.

The remaining articles were selected and categorized in the next stage of analysis according to the following criteria:

- Inclusion: the document provides information on the use of organisms to assess the presence and impacts of microplastics in soils for terrestrial environments; the article describes a case study, either at the bench scale or a real-world case on the application of bioindicators of microplastic contamination in soil.
- Exclusion: the article does not provide information on the use of organisms to assess the presence and impacts of microplastics in soils for terrestrial environments; the document is not available in the Integrated Libraries S–stem - University of São Paulo (SIBi USP) and could not be accessed by the author.

Based on the aforementioned criteria, 29 articles were included in the SLR, categorized according to the criteria presented in Table 4 below.

Table **Error! Unknown switch argument.** - Categories and subcategories used in Systematic Literature Review

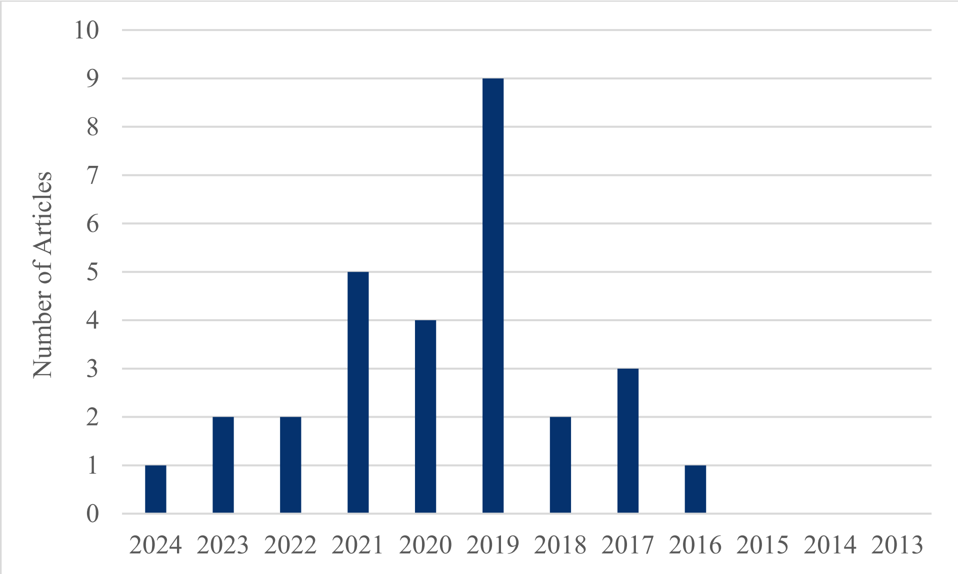
Category	**Sub-Category**
Study Scale	Laboratory Pilot Large Scale
Organism Type	Fauna Flora Plant-Fauna Interaction
Publication Year	-
Microplastic Type	Polyethylene; High-density polyethylene; Polyvinyl chloride; Low-density polyethylene; Polypropylene; Polystyrene/Expanded polystyrene; Acetal (polyoxymethylene); Acrylic (polymethyl methacrylate); Acrylonitrile butadiene styrene; Nylon (polyamide); Polyester; Polybutylene terephthalate; Polycarbonate; Polyetheretherketone; Polyethylene; Polylactic acid (bioplastic); Polysulfone; Polytetrafluoroethylene; Polyurethane; Styrene acrylonitrile
Observed Effects	Biochemical Tissue damage and other pathological alterations Development Reproduction Mutagenicity Toxicological

Source: Prepared by the author.

Temporal trends

Although articles published between 2013 and 2024 (the last 11 years) were used as inclusion criteria in the literature review, the evaluation of the identified studies shows that the majority of articles focus on the last 8 years, with no studies identified prior to 2016 (Figure 3). This demonstrates that the impacts on terrestrial environments resulting from the presence of microplastics have been a growing research topic over the last decade, supported by the expansion of tests and trials with terrestrial organisms.

Figure **Error! Unknown switch argument.** - Distribution by year of the articles evaluated in the Systematic Literature Review



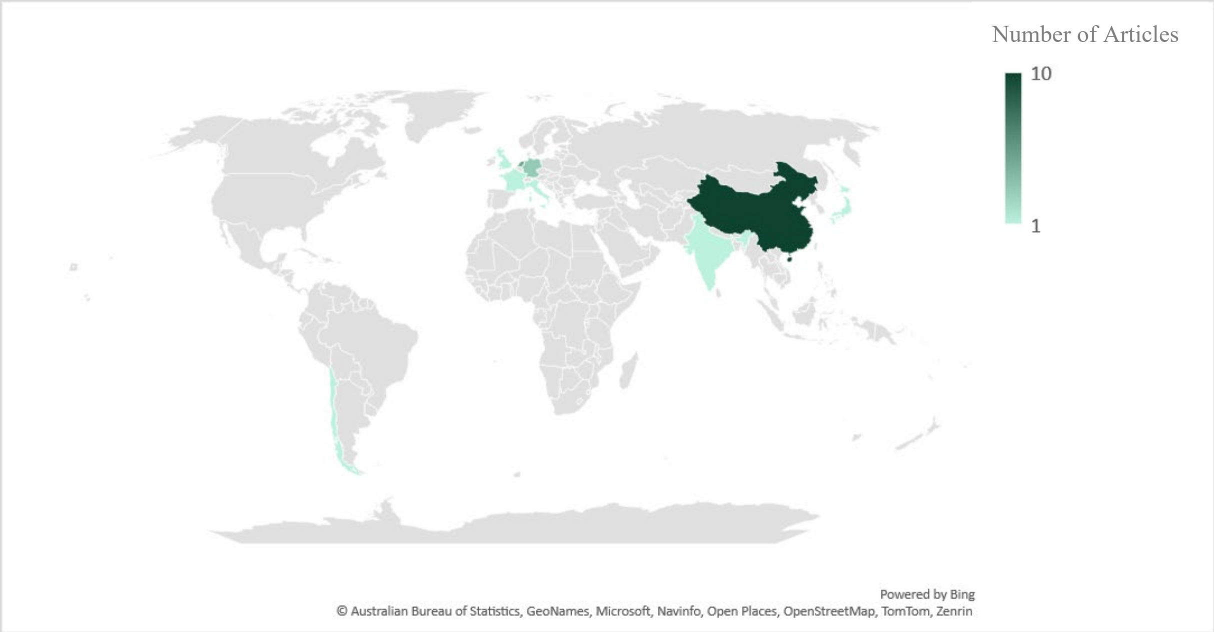
Source: Prepared by the author.

Spatial distribution of results

It was observed that the studies identified in the SLR were conducted on a bench scale, under controlled laboratory conditions, with no studies conducted on a pilot and/or large scale. An exception is the study by DENG et al., (2021) where bee organisms collected in the field were used.

Overall, the evaluated studies are distributed among the countries presented in Figure 4, with the majority of articles coming from China, Netherlands and Germany.

Figure **Error! Unknown switch argument.** - Worldwide distribution of articles evaluated in Systematic Literature Review



Source: Prepared by the author.

RESULTS AND DISCUSSION

What are the impacts of microplastics on terrestrial fauna?

The study by (ZHU et al., 2018) investigated the effects of microplastics on the intestinal microbiota of soil springtails (*Folsomia candida*) exposed over a period of 56 days, suggesting that changes in this microbial community can serve as a sensitive indicator of microplastic contamination. Additionally, exposure to microplastics also significantly inhibited the growth and reproduction of these small arthropods.

Another species of invertebrate evaluated in terms of impacts resulting from exposure to microplastics was the terrestrial snail *Achatina fulica*, also known as the African giant snail. A study conducted by SONG et al., (2019) identified, after exposure of the species to microplastic polyester terephthalate fibers for a period of 28 days, that the contaminant caused a reduction in food intake, with significant damage to the villi in the gastrointestinal walls, and in the excretion of the evaluated organisms.

Several studies also assessed the behavior and dynamics of the earthworm species *Lumbricus terrestris L.* upon exposure to microplastics (RILLIG; ZIERSCH; HEMPEL, 2017;BAEZA et al., 2020). The study conducted by RILLIG; ZIERSCH; HEMPEL, (2017) specifically evaluated the capacity of these organisms to transport microplastic particles from the surface to the soil profile, demonstrating that the species is an important mechanism for the transport and incorporation of microplastics into the soil, consequently increasing the exposure of other organisms of terrestrial biota to these contaminants.

A study conducted by LAHIVE et al., (2019) also indicated direct impacts on the annelid worm *Enchytraeus crypticus*, associated with reduced reproduction, in the presence of nylon microplastics, especially in the smaller size range.

According to DENG et al., (2021), ingestion of polystyrene microplastics by bees increased the susceptibility of these organisms to viral infections and a higher likelihood of mortality after virus infection, compared to bees not exposed to the contaminant. Additionally, microplastics significantly interfered with the immune system of bees, compromising their ability to effectively combat viral infections. These findings highlight the potential negative impacts of microplastics on the health and dynamics of bee populations.

A summary of the main bioindicators in terrestrial fauna related to exposure to microplastics is shown in Table 5.

Table **Error! Unknown switch argument.** - Summary of bioindicators of microplastic impacts on terrestrial fauna

Type of Microplastic	Size	Country	Species	Classification	Observed Effects	Publication Year	Reference
Polyethylene	<50 to 100 µm, >100 µm	Netherlands	*Lumbricus terrestris*	Annelid	Higher concentrations may reduce the rate of growth	2016	HUERTA LWANGA et al., 2016
Low-density polyethylene	<150 µm, <50 µm	Netherlands	*Lumbricus terrestris*	Annelid	Biogenic incorporation of microplastics from the soil surface into burrows walls was observed	2017	HUERTA LWANGA et al., 2017
Urea-formaldehyde	200 to 400 µm	Berlin	*Folsomia candida, Proisotoma minuta*	Microarthropods	Microplastic particles can be moved and distributed by soil microarthropods; *F. candida* transported larger particles and faster than *P. minuta*	2017	Maaß et al., 2017
Polyethylene	2800 to 710 µm	Berlin	*Lumbricus terrestris*	Annelid	The presence of earthworms greatly maximizes the detection of microplastic particles at depth in the soil, acting as transport agents	2017	RILLIG; ZIERSCH; HEMPEL, 2017
Polyvinylchloride (PVC)	250 to 80 µm	China	*Hypoaspis aculeifer, Folsomia candida*	Microarthropods	Significant reduction of weight; significant shift in the microbiome related to nitrogen cycling and organic matter decomposition	2018	ZHU et al., 2018
Polystyrene	0.1 to 0.05 µm	China	*Enchytraeus crypticus*	Enquitreid	Reduction of weight and an increase the breeding	2018	ZHU et al., 2018
Polyvinylchloride (PVC)	80 to 250 µm	China	*Folsomia candida*	Microarthropods	Alteration and inhibition of the microbiota in the gut	2018	ZHU et al., 2018
Low-density polyethylene	<150 µm	China	*Lumbricus terrestris*	Annelid	Gallery weight was adversely affected by the combination of glyphosate and microplastics	2018	YANG et al., 2019
Polyethylene, Polystyrene	<250 µm to <300 µm,	China	*Eisenia fetida*	Oligoqueta	Conc. of HOC (hydrophobic organic compound) in the specie was minimized in the presence of MPs by above 1%	2019	WANG et al., 2019

Source: Prepared by the author.

Table 5 - Summary of bioindicators of microplastic impacts on terrestrial fauna (continuation)

Type of Microplastic	Size	Country	Species	Classification	Observed Effects	Publication Year	Reference
Nylon 6 (polyamide) Polyvinylchloride (PVC)	63 to 90 mm and 90 to 150 mm (nylon 6) 106-150 (PVC)	Netherlands	*Enchytraeus crypticus*	Enquitreid	Reduced reproduction, with smaller size ranges showing a greater effect compared to larger size ranges	2019	LAHIVE et al., 2019
Polyethylene terephthalate	Not specified	China	Terrestrial snails (*Achatina fulica*)	Terrestrial mollusc	Prolonged Exposure to MP can cause reduction of food intake and excretion; villi damage in the gastrointestinal walls was also observed	2019	SONG et al., 2019
Polyethylene	<500 µm	China	*Folsomia candida*	Microarthropods	Inhibited breeding, altered microbial community and decrease of bacterial diversity; reproduction was also inhibited	2019	JU; ZHU; QIAO, 2019
Polystyrene, Propylene, Polyethylene terephthalate, Low-density polyethylene	250 µm	Chile	*Lumbricus terrestris*	Annelid	Physical lesions on the mucus membranes of earthworms; microplastic was observed in all the earthworm segments, with a higher number of particles in the hindgut	2020	BAEZA et al., 2020
Nylon 6	< 50 µm, with a mean size of 13 to 19 µm	United Kingdom	Great pond snail (*Lymnaea stagnalis*)	Terrestrial mollusc	Only subtle effects on weight loss and slight microbiome alterations were observed, showing that the specie is resilient to acute exposures to microplastics	2020	HORTON et al., 2020
Polystyrene	5 to 5.9 µm	China	Silkworm *Bombyx mori*	Insect	Significant changes in the expression of immunity-related genes (*Cecropin A, Lysozyme, SOD, and GST*) and antioxidant-mediated protective response (SOD, GST, and CAT enzymes)	2021	MUHAMMAD et al., 2021
Low-density polyethylene	< 1mm	France	Terrestrial snail (*Cantareus aspersus*)	Terrestrial mollusc	Big sized particles improved growth; small sized particles can trigger oxidative stress but without causing quantifiable cyto- or genotoxic effects	2022	COLPAERT et al., 2022
Polystyrene	0.5, 5 and 50µm	China	Bee *(Apis mellifera* and *Apis cerana)*	Insect	PS affected the *Israeli acute* paralysis virus proliferation, a small RNA virus associated with bee colony decline, enhancing the susceptibility of bees to viralinfection	2021	DENG et al., 2021
Polyester Polypropylene Low-density polyethylene High-density polyethylene Cellulose acetate Nylon	Not specified	India	*Corvus splendens*	Bird	First reported evidence of microplastics in the feces of Indian house crows	2023	CHARLES et al., 2024CHARLES et al., 2024

Source: Prepared by the author.

Plant-fauna interaction

When it comes to the relationship between terrestrial fauna organisms and plant species, high concentrations of high-density polyethylene (HDPE) in the soil (5%) showed a significant impact on plant-herbivore interactions by reducing the attraction of the fungus gnat (*Bradysia difformis*) to lentil (*Lens culinaris*) legume plants (DE SILVA et al., 2022).

The evaluation of plant-fauna interaction, particularly concerning the impact microplastics concentrations on terrestrial fauna attraction to plants, remains a relatively unexplored area in current literature. The study by DE SILVA et al., (2022) stands out as the sole article found to assess this specific interaction, corroborating the scarcity of research addressing this facet of plant-fauna dynamics and highlights the need for further investigations to comprehensively understand the complexities of such interactions.

What are the impacts of microplastics on terrestrial flora?

In a study conducted in 2022 by DE SILVA et al., the impact of microplastic polyethylene particles (PEMP of 740-4990 nm) on lentil seed (*Lens culinaris*) germination and seedling growth was evaluated. During the study, lentil seeds were exposed to different concentrations of microplastics under controlled laboratory conditions for 7 days. The results revealed that the presence of microplastics negatively affected seed germination, reducing its germination rate and even delaying the germination process. Additionally, seedlings exposed to high concentrations of microplastics exhibited impaired growth, evidenced by decreased root and stem length, as well as reduced seedling biomass.

Similarly, in a laboratory study with seedlings of the terrestrial vascular plant *Lepidium sativum*, commonly known as garden cress, the accumulation of microplastics in the pores of seed capsules was observed, negatively affecting seed germination and root growth. Specifically, the presence of microplastics acted physically, slowing down the germination process and decreasing the root growth of *L. sativum* seedlings by inhibiting water and nutrient absorption (BOSKER et al., 2019). These results corroborate the potential harmful impacts of microplastics on the initial development of these plant species - also limiting growth in later stages - and emphasize the need for further research on the effects of microplastics on terrestrial flora species.

Furthermore, the exposure of the species to different types of microplastics (PP, PE, PVC, and a commercial mixture of PE and PVC) in a study conducted by PIGNATTELLI; BROCCOLI; RENZI, (2020), also identified the potential for acute and chronic toxicity produced by these microplastics and the impact on oxidative stress resulting from this exposure, confirming the hypothesis that *Lepidium sativum* species do not have the ability to neutralize the toxicity of microplastics composed of PVC and PE.

Another species that had its development impacted by exposure to microplastics was the perennial ryegrass *Lolium perene*. In a study conducted by BOOTS; RUSSELL; GREEN, (2019), exposure to synthetic fibers (acrylic and nylon mixture) and microplastics containing high-density polyethylene (HDPE) or biodegradable polylactic acid (PLA) resulted in reductions in seed germination and shoot height, as well as alterations in root biomass and shoot/root ratio.

Wheat (*Triticum aestivum L.*) is also an important bioindicator species of microplastic impacts on terrestrial flora. In the study conducted byQI et al., (2018), exposure of wheat to sandy soil with the presence of low-density polyethylene and a type of starch-based biodegradable plastic film resulted in reduced seed germination, root growth, and plant height. Additionally, microplastics were detected in the aerial parts of the plant, indicating their absorption in this species.

Still concerning terrestrial flora, the broad bean (*Vicia faba*) was another species evaluated in terms of impacts resulting from exposure to microplastics. In the specific study conducted by JIANG et al., (2019), the root of this species was exposed to different concentrations of fluorescent polystyrene microplastics (PS-MP) for a period of 48 hours using emulsion solutions containing polystyrene microspheres (5 mm and 100 nm). The emulsion solutions were composed of 100 mg PS-MPs fluorescent microsphere dry powder and 0.05% sodium azide dispersed in 10 mL deionized water with PS-MPs concentration of 100 mg/10 mL, which were diluted to final emulsion concentrations of 10, 50, and 100 mg/L, respectively. The results, even with the short exposure period, demonstrated decreased catalase enzyme activity and increased superoxide dismutase (SOD) and peroxidase (POD) enzyme activity. Additionally, at a concentration of 100 mg/L, impacts on growth were observed, resulting in a significant reduction in seedling development, as well as genotoxic and oxidative damage. Another important aspect observed from the exposure of this species to polystyrene microplastics was the accumulation of this contaminant in the roots, acting to block cellular connections and pores in the cell wall, impacting nutrient transport.

A summary of the main bioindicators in terrestrial flora related to exposure to microplastics is shown in Table 6.

Table **Error! Unknown switch argument.** - Summary of bioindicators of microplastic impacts on terrestrial flora

Type of Microplastic	Size	Country	Species	Classification	Observed Effects	Publication Year	Reference
Low-density polyethylene	1 mm to 50 µm	Netherlands	*Wheat (Triticum aestivum)*	Grass	Both above-ground and below-ground parts of the wheat plant were affected during vegetative and reproductive growth	2018	QI et al., 2018
Nylon (polyamide), Polyethylene terephthalate, Polyethylene high-density PES, Polystyrene, Propylene	20 to 15 µm	Berlin	Spring onions (*Allium fistulosum*)	Herbaceous	Changes were detected in plant biomass, leaf attributes, root traits, and soil microbial activities	2019	DE SOUZA MACHADO et al., 2019
Fluoro-Max Green Fluorescent Polymer Microspheres	50, 500, and 4800 nm	Netherlands	*Lepidium sativum*	Herbaceous	Reduction in germination rate due to physical blockage of the pores in the seed capsule by microplastics, with greater adverse effects as the size of plastics increases; differences in root growth	2019	BOSKER et al., 2019
Polystyrene	100 nm	China	*Wheat (Triticum aestivum L.)*	Grass	Increased root elongation; increases in carbon, nitrogen contents, and plant biomass; reduction in the proportion of shoot to root biomass ratio (S:R ratio) of seedlings	2019	LIAN et al., 2020
Polystyrene	5 µm and 100 nm	China	*Vicia faba*	Leguminosae	Reduction in the activity of biomass and catalase (CAT) enzymes and increase in superoxide dismutase (SOD) and peroxidase (POD) enzymes activity	2019	JIANG et al., 2019
Polyethylene Polyvinylchloride (PVC) Propylene (PP) Mixture of Polyethylene + Polyvinylchloride (PVC)	Não informado	Italy	Garden cress (*L. sativum*)	Herbaceous	Oxidative burst; this study evaluated both acute and chronic exposure and identified that concentration of hydrogen peroxide is always higher in acute than chronic exposure, with the exception of plants treated with PVC	2020	PIGNATTELLI; BROCCOLI; RENZI, 2020
Polyethylene MP	740–4990 nm	Japan	Lentil (*Lens culinaris*)	Leguminosae	PE MP induced reduction in seed germination and was associated with the reduction in the internal biological activity	2022	DE SILVA et al., 2022

Source: Prepared by the author.

Important Considerations and Gaps Identified in the Studies Evaluated

The studies evaluated in this systematic literature review demonstrate that the majority of studies conducted so far on the impacts of microplastics on terrestrial organisms are concentrated in countries such as China, Netherlands and Germany, with studies in tropical countries, particularly in South America, still being scarce. As these regions harbor the greatest diversity of animal and plant species in terrestrial environments on the planet, studies on the impacts of microplastics on terrestrial biota in these regions are an important focus for future environmental research efforts and will aid in identifying other species that may be significant bioindicators of the impact of these contaminants.

Regarding the types of microplastics analyzed, the majority of the studies reviewed focused on assessing the impacts of exposure to polyethylene and polystyrene, which are precisely the most common and widely used types of plastics today. Among plastics in category 7, such as acetal, acrylic, nylon, polyester, polycarbonate, among others, the evaluated studies only covered the impacts of the presence of polyester and nylon, with no studies identified in the survey conducted for the other types of plastics in this category.

Another important aspect that still needs to be better evaluated in terms of identifying indicator organisms of microplastic contamination in terrestrial environments is the joint assessment of the presence of these elements with metallic substances, as it is known that microplastics can act as vectors of organic pollutants in the environment (BRADNEY et al., 2019). From this assessment of exposure to various contaminants commonly found in the environment, it will be possible to establish, for example, whether there are additive or deleterious effects for the exposed biota.

A significant gap in the literature is still the lack of standardization in the selection of bioindicator microorganisms according to the type of microplastic present in the soil. Although there is recognition of the importance of soil microorganisms in responding to microplastics, the variety of microplastic types and their complex interactions with soil organisms require a more specific and targeted approach. The lack of standardized protocols for assessing the effects of microplastics on soil microorganisms also hinders comparison between studies and the identification of consistent patterns. Therefore, advancing in this field requires the establishment of clear guidelines for the selection of bioindicators and testing methods in order to fill this knowledge gap and improve our ability to monitor and mitigate the impacts of microplastics on terrestrial ecosystems.

7. CONCLUSIONS

This study aimed to identify potential organisms of terrestrial biota that can act as bioindicators related to the impacts caused by the presence of microplastics in the soil.

Through searches in the scientific databases Scopus, ScienceDirect, ResearchGate, and Springer, initially 717 articles on this topic were identified. Among these, 29 articles were selected.

The descriptive statistical evaluation of the 29 articles indicated that studies on the topic are recent, with them being concentrated over the past 8 years, with no studies identified before 2016. This fact corroborates that the identification and evaluation of species of terrestrial biota as bioindicators of microplastic impacts is still a growing subject of significant interest for future research.

Considering the set of articles evaluated in this SLR, several species were identified that can act as important bioindicators of the presence and impacts of microplastics in the terrestrial environment, either indirectly through their abundance and lifestyles, or directly through the measurement of contaminants in these organisms. Among these species are the invertebrates *Eisenia fetida* (red worm), *Achatina fulica* (African snail), *Lumbricus terrestris L.* (earthworm), soil springtails (*Folsomia candida*), bees (*Apis mellifera* and *Apis cerana)*, insect (*Bombyx mori*), *Enchytraeus crypticus*, and bird (*Corvus splendens*); and in the interaction of the fungus gnat (*Bradysia difformis*) with plants of the lentil species (*Lens culinaris*). Species of terrestrial flora, some of them used in agriculture and present in Brazil, are also important bioindicators of impacts resulting from exposure to microplastics, including *Lepidium sativum* (garden cress), *Lolium perene* (perennial ryegrass), *Triticum aestivum L.* (wheat), and *Vicia faba* (broad bean). Despite the significant number of studies and the prominent results of bioindicators for monitoring and mitigating the presence of microplastics in terrestrial environments, it is observed that many of these studies were conducted in the laboratory, requiring a better evaluation of promising results and thus paving the way for many subsequent studies to assess these impacts on a pilot and/or large scale, as well as the analysis of the transfer of these microplastics in the food chain.

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