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Recent research advances on heavy metals, microplastics, persistent organic pollutants, and solid waste in aquatic and terrestrial ecosystems

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Published in
Frontiers in Environmental Science

ISSN 1664-8714
ISBN 978-2-8325-4666-6
DOI 10.3389/978-2-8325-4666-6

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Recent research advances on heavy metals, microplastics, persistent organic pollutants, and solid waste in aquatic and terrestrial ecosystems

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Citation
Zhang, Z., Xu, B., Guo, X., Guo, Y., eds. (2024). *Recent research advances on heavy metals, microplastics, persistent organic pollutants, and solid waste in aquatic and terrestrial ecosystems*. Lausanne: Frontiers Media SA.
doi: 10.3389/978-2-8325-4666-6

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frontiers | Frontiers in Environmental Science

TYPE Editorial
PUBLISHED 13 March 2024
DOI 10.3389/fenvs.2024.1391614

Check for updates

OPEN ACCESS

EDITED AND REVIEWED BY
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RECEIVED 26 February 2024
ACCEPTED 04 March 2024
PUBLISHED 13 March 2024

CITATION
Guo Y, Zhang Z and Guo X (2024), Editorial: Recent research advances on heavy metals, microplastics, persistent organic pollutants, and solid waste in aquatic and terrestrial ecosystems.
Front. Environ. Sci. 12:1391614.
doi: 10.3389/fenvs.2024.1391614

Editorial: Recent research advances on heavy metals, microplastics, persistent organic pollutants, and solid waste in aquatic and terrestrial ecosystems

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KEYWORDS

heavy metals, microplastics, persistent organic pollutants, solid waste, aquatic and terrestrial ecosystems

Editorial on the Research Topic
Recent research advances on heavy metals, microplastics, persistent organic pollutants, and solid waste in aquatic and terrestrial ecosystems

Introduction

The global surge in industrialization and urbanization has resulted in the unregulated release of anthropogenic pollutants into both aquatic and terrestrial ecosystems. This aforementioned trend has given rise to a plethora of environmental challenges, including contamination by heavy metal (HMs), the pervasive pollution caused by microplastics, the intricate management required for persistent organic pollutants (POPs), and the urgent concern surrounding solid waste disposal (Quina et al., 2008; Nadal et al., 2015; Zhao et al., 2015; Rillig et al., 2021; Senathirajah et al., 2021; Xue and Liu, 2021; Zhang et al., 2022; Zhang and Zhang, 2022; Zhao and You, 2022). To address these intricate challenges, *Frontiers in Environmental Science* presents a research theme, "*Recent Research Advances in Heavy Metals, Microplastics, Persistent Organic Pollutants, and Solid Waste in Aquatic and Terrestrial Ecosystems*". Scholars from prestigious affiliations contribute their expertise and advancements in this field. This research theme has yielded ten publications, comprising seven research articles and three reviews, each offering unique perspectives on contemporary environmental challenges related to HMs, microplastics, POPs, and solid waste.

Key elements in this Research Topic

The research findings have unveiled the adverse impacts of heavy metals such as arsenic, lead, and bismuth on both plant and animal life, emphasizing the pressing need to mitigate their presence within ecosystems. Sun et al. conducted a study on the spatial distribution and associated health risks of HMs in the soils of the Yangtze River Basin, revealing significant variability in metal concentrations and their corresponding health hazards, particularly in regions with advanced economic development. Pietrini et al. investigated bismuth's effects on garden cress, revealing its detrimental impact on growth, chlorophyll, carotenoid levels, as well as photosynthesis. The findings suggest plants may activate a defense mechanism in response to mitigate the damage caused to photosynthesis, which is indicated by an increase in anthocyanin and flavonol levels. Kadirvel et al. explored the toxic effects of arsenic, lead, and fluoride on boar spermatozoa, uncovering reductions in sperm motility, viability, and mitochondrial membrane potential (MMP) in a dose- and time-dependent manner, both individually and in combination. This study highlights the significant impact of HMs on reproductive health.

Three studies addressed the prevalence of microplastic pollution in marine and terrestrial ecosystems and explored various aspects of this challenge, including management strategies, impacts, and potential solutions. Lou et al. conducted a bibliometric study on global research pertaining to controlling marine microplastic pollution from 2013 to 2022. This study presented a theoretical framework for managing marine microplastic pollution, with a focus on quantification, traceability, and Research Topic. Additionally, it emphasized the crucial need for greater attention to policy implications and technological advancements within the field of marine microplastic pollution control. Ng et al. examined plastic waste in Southeast Asia, highlighting its detrimental effects on marine ecosystems resulting from inadequate waste management practices. This study evaluated the roles played by governments, businesses, and communities in addressing plastic pollution while analyzing the impact of global plastic trade specifically regarding waste transfer from developed to developing countries. It proposed solutions such as bio-based plastics, waste-to-wealth programs, and a circular plastics economy to tackle these challenges effectively. Of these, the production and application of bio-based degradable plastics may play an especially critical role in resolving the global plastic pollution problem at hand. Barbir et al., through their assessment of ecotoxicity associated with polylactic acid-based mulch film usage, found low plant toxicity but observed some adverse effects on earthworm reproduction as well as varying impacts on aquatic life; thus emphasizing that bio-based plastics have complex environmental consequences.

POPs are recognized for their persistence and long-range transmission, exacerbating ecological imbalances as they enter the food chain. Debela et al. conducted an analysis of POPs in Ethiopia, addressing their management, regulations, and environmental impacts. The study underscored the prevalence of POPs, identified regulatory gaps, and emphasized the necessity for enhanced oversight and legislation to mitigate their effects. Saibu et al. investigated the geochemistry and metagenomics of bacteria in Lagos' dumpsites to examine their response to pollution from POPs and HMs. The research revealed a diverse range of bacterial communities that correlated with pollution levels, while also identifying key bacteria involved in pollutant breakdown—suggesting potential bioremediation approaches.

Direct disposal of solid waste in landfills or incinerators not only results in resource wastage but also leads to the generation of secondary pollutants that are detrimental to ecosystem health. The formulation of specific management strategies and the adoption of advanced safe waste treatment technologies can significantly mitigate health risks, while also aiming for economic utilization of waste as a higher objective. Haque et al. investigated the complexities associated with managing medical waste in Rohingya refugee camps, highlighting the absence of tailored guidelines and the prevalent hazardous disposal methods. This study emphasized the need for detailed management strategies to ensure secure waste processing and reduce health hazards. Fianko et al. explored creating phosphorus-rich biochar-compost from maize stover and groundnut husk in Ghana's Guinea Savanna to boost soil fertility. The study assessed biochar's impact on compost quality, especially phosphorus content, highlighting its agricultural and environmental benefits through waste recycling.

Conclusion

This research theme encompasses a compilation of original studies that investigate the impacts of HMs, microplastics, POPs, and solid waste on both aquatic and terrestrial ecosystems. It offers valuable insights into the environmental dynamics of these pollutants and their implications for ecosystems and human health. Encompassing fundamental as well as applied research, these articles enhance our comprehension of such environmental Research Topic while advocating for remedial strategies. The body of work sheds light on the distribution and toxicity of these pollutants, suggesting future research directions such as establishing exposure thresholds, evaluating bioaccumulation processes, and devising mitigation tactics to counteract their adverse effects. These findings are crucial in formulating effective environmental protection policies and contribute significantly to the advancement of environmental science.

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Funding

The author(s) declare that financial support was received for the research, authorship, and/or publication of this article. This work was supported by Natural Science Foundation of China (52270129), Oriental Talent Youth Program, Shanghai Shuguang Program, the Guizhou Provincial Program on Commercialization of Scientific Guizhou Provincial Key Technology R&D Program [QKHZC (2022)-214; QKHZC (2023)-138], and Guizhou Provincial Program on Commercialization of Scientific and Technological Achievements [QKHCG (2023)-027].

Acknowledgments

We thank all the authors, and reviewers who collaborated with this Research Topic.

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TYPE Review
PUBLISHED 07 April 2023
DOI 10.3389/fenvs.2023.1142071

Check for updates

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Toxicology, Pollution and the Environment, a section of the journal Frontiers in Environmental Science

RECEIVED 11 January 2023
ACCEPTED 20 March 2023
PUBLISHED 07 April 2023

CITATION
Ng CH, Mistoh MA, Teo SH, Galassi A, Ibrahim A, Sipaut CS, Foo J, Seay J, Taufiq-Yap YH and Janaun J (2023), Plastic waste and microplastic issues in Southeast Asia.
Front. Environ. Sci. 11:1142071.
doi: 10.3389/fenvs.2023.1142071

Plastic waste and microplastic issues in Southeast Asia

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Plastic pollution on land and in oceans is currently a pressing environmental issue. The accumulation of waste has caused severe, irreversible impacts and consequences on marine life, ecosystems, and the environment due to the lack of good waste collection, treatment, and management systems. Limited resources and infrastructure constantly challenge waste management in Southeast Asia. Therefore, we will examine the current plastic situation and issues in Southeast Asia and gain an understanding of the issues of the existing waste management systems in those countries. Then, we will examine the current practices applied in tackling plastic pollution and review the collective commitment and actions of governments, private sectors, social organizations, stakeholders, and consumers, as the key players in ending plastic pollution.

KEYWORDS

plastic waste, microplastics, Southeast Asia, waste management, global trade

1 Introduction

Plastic material is a convenient and versatile commodity used on a global scale with diverse applications such as in electronics, healthcare, agriculture, transportation, construction, and most significantly, packaging (Huo et al., 2020; Kunwar et al., 2016). With the massive growing population and rapid urbanization, global plastic production reached a cumulative total of 360 million tons in 2018, demonstrating substantial leaps of 1.2-fold from 299 million tons in the short time frame of 5 years (Anuar Sharuddin et al., 2016; Yao et al., 2021). While the use of plastic is swiftly expanding, the accumulation of municipal plastic waste entering the solid waste stream is a major cause of severe environmental issues. The issue is exacerbated as these plastic materials are highly durable due to their unique molecular structures composed of hydrogen, carbon, and other elements that take years to decompose fully. Thus, effective plastic waste management and treatment approaches are urgently needed to solve the environmental problem. Incineration and landfilling are the two most common ways of dealing with plastics disposal, and only 9% of plastic waste is being recycled globally, resulting in an estimated 4–12 million metric tons of plastic waste pilling up in the ocean annually (Geyer et al., 2017a; Jambeck et al., 2015; Wen et al., 2021). The mismanaged plastic waste that enters the ocean forms microplastics, which are tiny plastic particles that originate

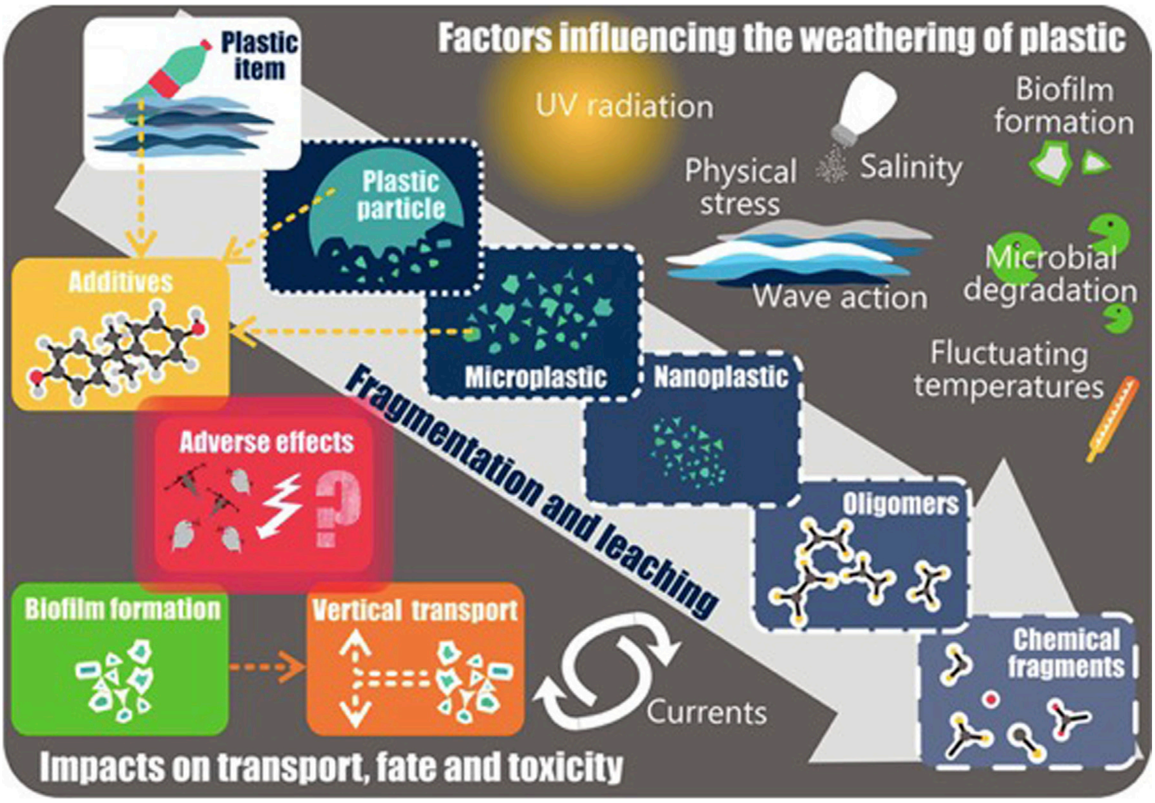

FIGURE 1
Overview of factors influencing the weathering of plastics (Jahnke et al., 2017).

from primary and secondary sources with a size of <5 mm in an infinite shape (pellets, fibers, *etc.*) (Akdogan and Guven, 2019; Horton et al., 2017). When the plastic materials are exposed to ultraviolet radiation, the plastics become brittle and subsequently fragment into microplastics due to the photo-oxidation process (Figure 1). Under the influence of heat, sunlight, and well-aerated conditions, plastic waste undergoes iterative fragmentation processes, and the anoxic conditions of aquatic environments result in the slow degradation of plastics (Zhang, 2017). The primary microplastics originate from synthetic fibers and textiles derived from the abrasion of clothes, in which 1900 fibers per item are estimated to be leached during washing (Napper and Thompson, 2016). Another source of primary microplastics is sedimented microplastics in cosmetic and medical products. On the other hand, secondary microplastics are derived from the fragmentation/degradation of macroplastics into plastic debris due to physical, chemical, and biological processes (Akdogan and Guven, 2019). Light macro- and microplastics can be transported across the land by wind, and the dense ones will be buried deeper in soil layers. The piling up of microplastics poses an ecotoxicological risk, and this hydrophobic debris in water serves as a good absorb heavy metals that affect the water quality (Avio et al., 2017; Wang et al., 2017). Incineration is a common practice in developed countries to resolve domestic plastic accumulation by burning plastic waste at high temperatures (Gupta et al., 2022). However, incineration involves energy-intensive pre-treatment that engenders severe environmental impact, whereas the presence of additives and blends within the plastic lattice may complicate the recycling process (Ragaert et al., 2017; Cao et al., 2020).

As an alternative, global plastic trade flow was triggered in the late 1990s, whereby plastic waste was transferred from developed to developing countries. China, as the primary importer of plastic waste, found that these materials are profitable for goods production; however, the low quality/grade of the plastic waste (contaminated) is the cause of environmental issues. In 2013, China introduced a temporary plastic waste import restriction, which is also known as the "Green Fence" campaign, to combat poor quality and contaminated plastic waste and to reduce illegal foreign smuggling and trading (Brooks et al., 2018). However, this temporary campaign did not entirely halt the illegal transfer of plastic waste, resulting in an annual plastic waste import of 8.88 million tons in China, which triggered a series of environmental problems (Chen et al., 2019). In 2017, China issued a new import policy banning the import of 24 types of solid waste, including plastic waste, which has globally challenged and disrupted the flow pattern of the global plastic waste trade. Following the China ban, the global plastic waste trade volume clearly decreased, as compared to the trade volume prior to the China ban. At the same time, a surge of plastic waste entered developing countries, especially Southeast Asia, making it a major contributor to plastic pollution. Perhaps, these actions could have helped the developed countries in partly managing

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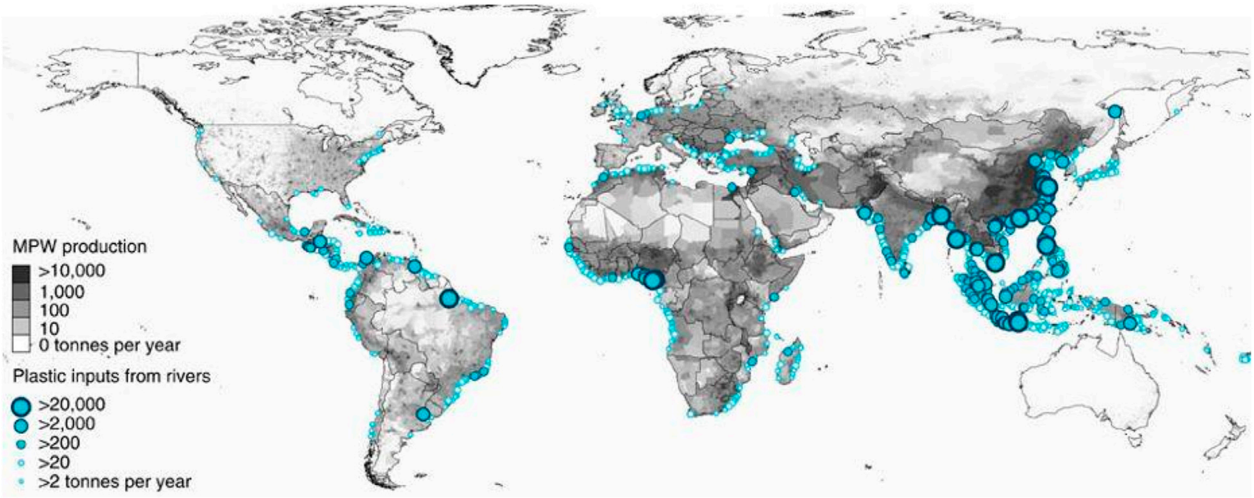

FIGURE 2
Mass of river plastic flowing into oceans in tons per year. River contributions are derived from individual watershed characteristics such as population density (in inhabkm^{-2}), mismanaged plastic waste (MPW) production per country (in kginhab^{-1}d^{-1}), and monthly averaged runoff (in mmd^{-1}). The model is calibrated against river plastic concentration measurements from Europe, Asia, and North and South America (Lebreton et al., 2017).

their domestic waste (waste trading); nevertheless, it poses threats to developing countries. The Southeast Asian countries Indonesia, Thailand, Vietnam, the Philippines, and Malaysia are the top five countries for the production of large municipal solid waste, at 1.14 kg/capita/day worldwide (Arumdani et al., 2021). Mismanaged contaminated and unprocessable waste, poor domestic waste disposal management and facilities and the lack thereof, and insufficient land for proper waste disposal are the main causes of the threat to Southeast Asia's environment (Jain, 2020). As plastic debris is blown by the wind or washed by rain into waterways, plastic materials pile up in the sea, leading to an estimated 14 million tons of waste entering the ocean every year (Bello, 2022). Figure 2 displays the annual flow of river plastic inputs into oceans in tons (Lebreton et al., 2017). This phenomenon is worsening with the illegal waste dumping by developed countries and the smuggling process, which has ensued from the Southeast Asian governments imposing restrictive measures on plastic waste imports. Southeast Asian nations have taken up the challenge in recent years and pledged to curtail the pollution issue. In 2018, Malaysia implemented a roadmap toward zero single-use plastics with the aim of addressing single-use plastic pollution for a cleaner and healthier environment in Malaysia by 2030. In addition, Malaysia has returned 4,120 tons of plastic waste to 13 countries and has officially shut down 200 illegal plastic recycling centers since 2019 (New Strait Times, 2019). Meanwhile, Thailand restricted electronic waste imports and pledged to end plastic waste imports by 2021 (Sasaki, 2021).

Here, we review the issues of plastic waste and microplastics in Southeast Asia by first understanding the current situation surrounding the plastic waste issue in Southeast Asia, followed by identifying the current waste management systems (landfilling, incineration, and recycling) in Southeast Asia. Then, we will identify the potential solutions for tackling the plastic waste crisis in Southeast Asia. In addition, we quantify the cascading impacts of China's import ban and discuss how the ban affects the global trade flow of plastic waste and quantify the magnitude of the environmental impact of trade flow changes and eco-costs of five midpoint indications, namely global warming (GW), fine particulate matter formation (FPMF), freshwater ecotoxicity (FEW), human carcinogenic toxicity (HCT), and water consumption (WC) resulting from the China ban.

2 Plastic waste issues in Southeast Asia

2.1 Current situation around plastic waste in Southeast Asia

Plastic waste is a prevalent issue worldwide. In recent years, due to the COVID-19 pandemic, there has been an alarming increase in the use of single-use plastics throughout Southeast Asia. Due to the lockdown periods, Malaysia, Thailand, and Singapore recorded a spike in plastics such as single-use plastic packaging, bags, and containers (The Japan Times, 2020). Other impacts include the increased plastic medical equipment required due to the pandemic. In Malaysia, there were instances of waste spillage (Yuen et al., 2020) and an increase in household plastic waste (Teoh, 2020). Similar instances were recorded in Singapore, Thailand, Myanmar, the Philippines, and Vietnam (Praveena and Aris, 2021). The total waste generation and management in Southeast Asian regions are tabulated in Table 1.

Southeast Asia is a wealthy and biodiverse region, with almost 150,000 km of coastline and over 25,000 islands including approximately 34% of the world's coral reefs and 25%–33% of the global mangrove forests, which are diverse with tropical marine species (Omeyer et al., 2022). Countries with higher populous density along the coastlines show a higher potential for polluting the ocean with plastics (Ritchie and Roser, 2018). With

10.3389/fenvs.2023.1142071

TABLE 1 **Demographic context, waste generation, and waste management in Southeast Asian countries. Data extracted are based on 2015, unless specified Tun et al. (2020).**

Description		Brunei Darussalam	Cambodia	Indonesia	Laos	Malaysia	Myanmar	The Philippines	Singapore	Thailand	Vietnam
Population		423,188	15,577,899	255,993,674	6,802,023	30,331,007	53,897,154	100,998,376	5,540,000	67,959,259	91,700,000
Per capita GDP (USD)		31,164.6	1162.9	3331.7	2134.7	9955.2	1287.4	3001.0	55,646.6	5840.0	2085.1
Waste generation (tons/year)		210,000	1,089,000 (2014)	22,500,060 (2012)	77,000	10,680,000	1,130,040	14,400,000	7,670,000	26,850,000	12,800,000
Per capita waste generation (kg/capita/day)		0.87	0.6	0.52	0.7	1.52	0.44	0.5	1.49	1.76	1.46
Source segregation	%	<50	<50	<50	<50	<50	50	50–70	<70	<50	<50
Collection rate	%	90	80	56–75	40–70	>70	<50	40–90	>90	>80	80–82
Reused and utilized	%	na	na	7	na	na	na	na	na	17.80	na
Recycling	%	na	20	7	9	5	5	28	47	14	8.20
Compost	%	2	na	-	15	1	na	na	0	10	na
Incineration	%	na	na	na	2	na	1	na	39	5	5.40
	No. of plants	na	na	na	na	4	1	na	4	3	na
Sanitary landfill	%	na	na	na	na	na	na	na	15	na	na
	No. of plants	na	na	10	na	8	na	na	1	91	17
Controlled landfill	%	na	na	na	na	na	na	na	-	na	na
	No. of plants	na	na	70	-	10	-	273	-	20	91
Solid waste disposal	%	70	20	84	61	93	90	65	0	70	na
Others	%	28	60	9	13	6	4	5	8	1	na

Recycling data for Cambodia are based on Phnom Penh only; for Laos PDR, Vientiane; and for Myanmar, Yangon. The disposal method of Vietnam is based on Hanoi. na: not accessible.

multiple countries, including Indonesia, the Philippines, and Vietnam, having large coastal populations, it is highly likely that this would lead to a more significant possibility of polluting the ocean with plastics. These Southeast Asian countries produce over 1.5 million metric tons of mismanaged plastics annually. Malaysia, Thailand, Vietnam, the Philippines, and Indonesia all ranked in the top 10 countries with the highest generation of mismanaged plastics, whereas Indonesia and the Philippines ranked second and third, respectively (Jambeck et al., 2015). These extensive populous coastlines may contribute mainly to the large amount of plastic waste entering marine areas in Southeast Asia. Despite this, many other countries within Asia have a high number of populous coastal regions but do not have a similar issue to the aforementioned countries. These countries have well-established, robust plastic waste management systems despite their proximity to the ocean (Loh, 2020).

It was estimated that 99.5 million metric tons of plastic waste were generated in coastal regions in 2010, and of this amount, around 4.8 to 12.7 million metric tons of plastics ended up in the ocean, which accounts for between 1.7% and 4.6% of the total plastic waste generated by the countries involved (Jambeck et al., 2015). These numbers are already alarming; detrimental effects have already been seen in marine life, in which microplastics have been detected within their bloodstream. Such microplastics have also been detected in humans (Leslie et al., 2022).

Another perspective showed that these large waste-generating countries within Southeast Asia, including Indonesia, the Philippines, and Vietnam, have undergone rapid economic growth over the last 3 decades, which also explains why food waste makes up a significant proportion of waste in Southeast Asia (UN environment, 2017). Considering that ASEAN's urbanization rate is expected to surpass 70% by 2050, waste management issues are likely to worsen in the coming years (Loh, 2020).

2.2 Current plastic waste management system in Southeast Asia

The waste management system preferred in Southeast Asia is open landfill due to the ease of construction and low processing cost. Open landfill, as the name states, is a large land mass area sacrificed to accommodate the large amount of waste produced daily by citizens. Bantar Gebang, Jakarta's largest landfill at around 120 ha, receives nearly 7,000 tons of waste daily. It is estimated to already hold 39 million tons of waste and should reach its capacity of 49 million tons (Raslan, 2019). It has been shown that Southeast Asian countries have produced between 0.21–640 million tons of municipal solid waste, the largest being Indonesia and the smallest being Brunei Darussalam (UN environment, 2017). Reliance on plastics, especially during the pandemic, has increased plastic usage and single-use plastics (Chen et al., 2021).

The prospect of landfills is not sustainable as land mass use would increase daily and would eventually lead to the depletion of usable land mass. Aside from the apparent leachate issues that would be detrimental to the land mass and water sources surrounding the landfill, plastics are a challenging issue since they have a long lifespan, and the issue of microplastics has become more prevalent in recent years. Plastic pollution's impact is visible on land and in the ocean. Landfills are favored, but due to their detrimental effect on the environment, which includes air, water, and land pollution, as well as the change in climate caused by greenhouse gases, they are not sustainable (Arumdani et al., 2021). In Malaysia, approximately 85% of municipal solid waste material goes to landfill sites, and because plastic is not biodegradable, with the current rate at which landfills are being filled, they will soon reach capacity (Chen et al., 2021).

Currently, there is a multitude of ways to manage plastic waste where the Southeast Asian countries use landfills, sanitary landfills, incineration, and composting. Southeast Asian countries use landfills, sanitary landfills, incineration, and composting. Landfills, as mentioned previously are not sustainable in the long run. Only a small percentage of landfills in Southeast Asian countries are sanitary landfills (Arumdani et al., 2021), which provide low-cost waste management compared to other alternatives. Incineration involves higher capital and a management cost of around 80–102 USD per ton, while for sanitary landfills, it is around 10–45 USD per ton (Tun et al., 2020). Despite that, due to the limitations of capacity, this option is not considered sustainable given the amount of waste coming into the site, which is leading to sanitary landfills becoming unsanitary landfills (Loh, 2020).

The current recycling rate for most Southeast Asian countries is below 50% due to the limitations of infrastructure and logistics to provide the necessary operations for it to be profitable (UN environment, 2017). Private companies run most recycling facilities in Southeast Asian countries, and profitability is the main driving factor. In most cases, the waste produced is too dirty to qualify for mechanical recycling (Chen et al., 2021). The current infrastructure is not viable because most recycling practices require sorting and separation processes beforehand. It would be difficult to develop the necessary infrastructure to keep up with the increase in plastic usage, making it a race against time. Most of the recycling infrastructure is located in urban areas; hence, people from outside cities do not have alternatives for recycling their plastics. Regarding plastics, there are limitations to what types of plastics can be recycled. Post-industrial plastics are the easiest to recycle due to their purity. Contrary to that, post-consumer plastics pose many challenges not only due to a lack of waste separation during the initial stages of the waste generation process but also due to the mixture of plastics when producing consumer plastics (Antelava et al., 2019). Due to the ever-increasing load of the recycling process, most plastics that go to recycling plants would be deemed unrecyclable and be directed to landfills (Geyer et al., 2017b).

In addition, the backbone of the recycling process in Southeast Asian countries is often underprivileged citizens. There is no specific unified system to homogenize the retrieval process, which subsequently affects the entire supply chain process of the recycling route. This unreliable route further reduces the profitability of the recycling process, which further decreases the chances of plastics being recycled. In some instances, companies would instead import plastic waste from overseas for recycling purposes (Chen et al., 2021).

Another waste management process in Southeast Asian countries is the waste-to-energy process, or incineration, which focuses on burning waste to create energy. Plastic waste is considered a good source of fuel. A similar problem can be seen

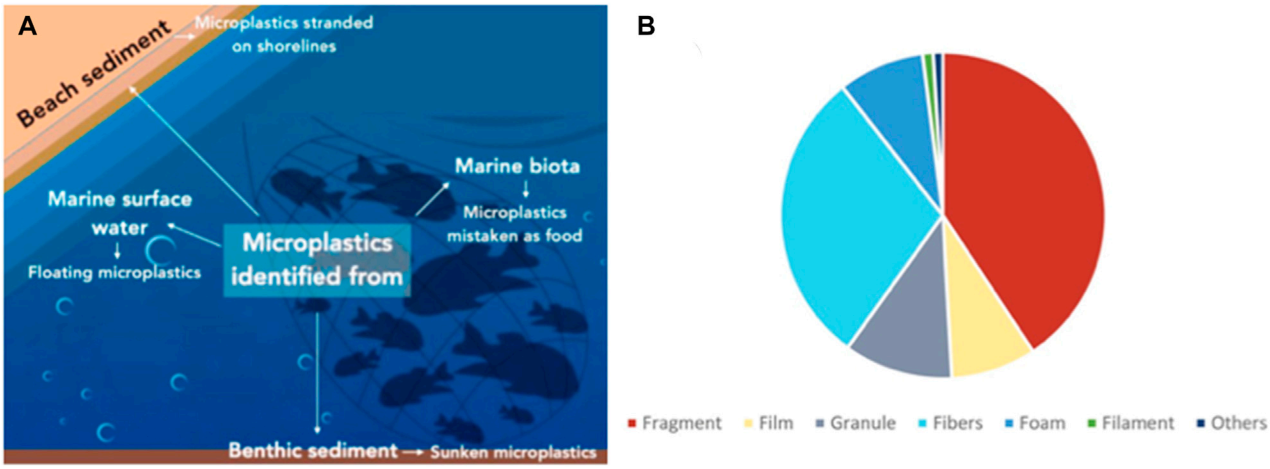

FIGURE 3
(A) Schematic diagram representing the presence of microplastics in the marine environment: in beach sediments, water column, benthic sediments, and marine biota. **(B)** Overall composition of microplastic types found across beach sediments, seawater, benthic sediments, and marine organisms. A total of six main types of microplastic were identified (Curren et al., 2021).

in the implementation of incineration plants in Southeast Asian countries, which is due to the lack of infrastructure and cooperation between governments, municipalities, and private companies regarding the supply of waste. Consistency and quality of waste are crucial for the incineration process to create good quality and reliable energy. Since the majority of waste in Southeast Asian countries is primarily organic waste, this leads to the creation of wet waste, reducing the overall efficiency in producing energy (Tun et al., 2020). The mixture of wastes can produce toxic by-products such as noxious gas emissions and ash by-products that require more advanced after-treatment processes, pushing the cost of incineration plants (Energy, 2020). Hence, similar to the recycling process, sorting is important in order to produce good-quality energy, and hydrocarbon sources such as plastics are more favorable than wet organic waste, which produces lower calorific value energy ranging from 5–11 MJ/kg (Tun et al., 2020).

The problem of plastic waste closely relates to the problem of waste management, as plastic causes further damage due to its long lifespan. Hence, waste management systems are vital to improving the situation around plastic waste destroying land and ocean ecosystems. Major stakeholders, including government bodies, private companies, and international bodies, need to work together internally and externally to create an integrated system to help better manage plastic waste.

2.3 Current microplastic waste and management situation in Southeast Asia

Southeast Asia accounts for a significant proportion of global microplastic pollution, ascribed to the abundance of mangrove, seagrass, and coral habitats in coastal and shallow waters that lead to plastic accumulation by snagging. Microplastics are found in beach sediments, water columns, benthic sediments, and marine biota (Figure 3A) and are accumulated along the high-strand vegetation lines and trapped between plants, according to investigations conducted in Thailand and Singapore (Curren and Leong, 2019).

Microplastics are classified into primary and secondary forms. Primary microplastics are derived from sources such as resin beads, microbeads for facewash or toothpaste, and other products. Secondary microplastics are fragmented macroplastics that originate from coastal and domestic sources and international ocean flows. The fragmentation of macroplastics occurs through environmental weathering, which alters polymer properties due to abiotic factors (light, temperature, air, water, and mechanical forces). Light microplastic debris floats on the water's surface. Over a certain period, the microplastic surfaces can be colonized by microorganisms, which results in denser microplastic particles that eventually sink to form benthic sediment (Riani and Cordova, 2022).

Domestic sources of plastics, such as due to marine litter and fishing activities have caused a substantial environmental impact in the coral reef localities of Darvel Bay, East Sabah Malaysia, where plastic bags (10%), plastic bottles (13%), and fishing nets/lines (21%) have been found in the reef (Figure 4) (Santodomingo et al., 2021). As well as in Malaysia, the presence of microplastics in freshwater ecosystems has also been detected in the river streams of Indonesia, Thailand, and Vietnam. The concentration of microplastics in the seawater in Southeast Asian regions ranges from 0.13–11,100 pieces/L, which is comparable to the figures recorded in the Arctic Ocean and Santa Monica Bay (Curren et al., 2021). Reviewing the case in Thailand, Thailand receives several hundreds of thousands of tons of plastic waste from developed countries every year. At the same time, they have a poor management system, and plastic waste leaks into canals and beaches during heavy flooding. Johansson and Ericsson reported that hard/soft microplastics in the form of foams and beads were found on the water surface of the Chao Phraya River (Ericsson and Johansson, 2018). In

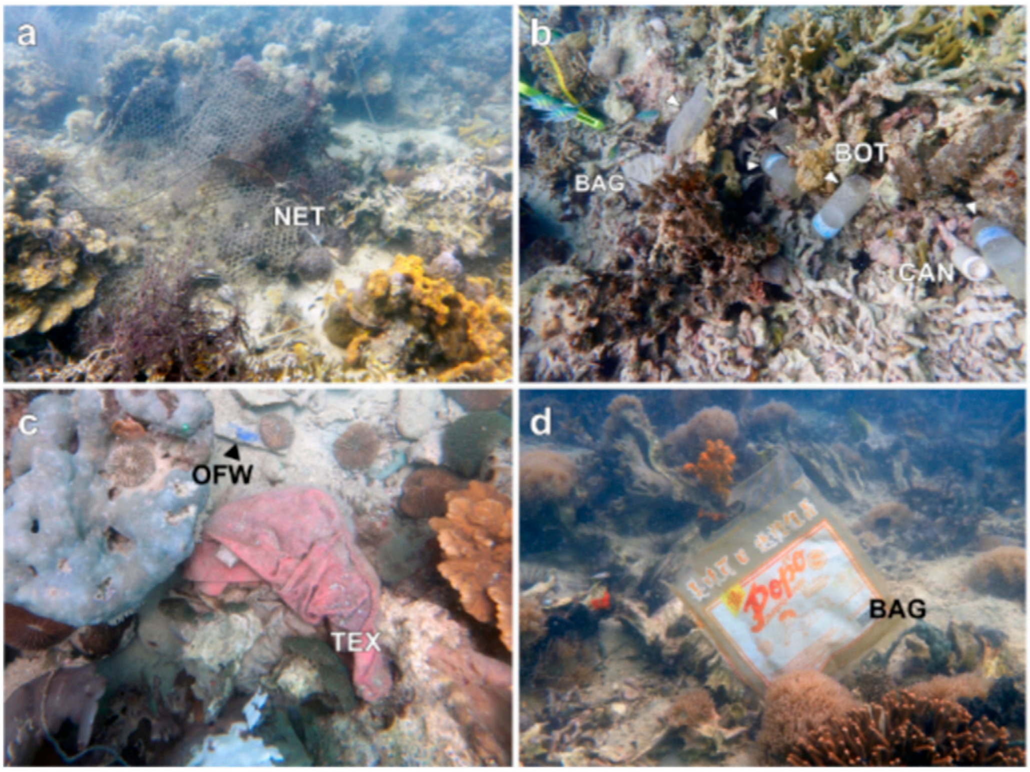

FIGURE 4
Examples of marine litter found in the Darvel Bay reefs: **(A)** Abandoned fishing net in the Triangle Reef at 10 m depth, **(B)** plastic bag (BAG), plastic bottles (BOT), and aluminum can (CAN) in Baik at 5 m depth, **(C)** other food wrap (OFW) and textiles (TEX) in Sakar at 5 m depth, and **(D)** plastic bag (BAG) in Sakar at 10 m depth (Santodomingo et al., 2021).

contrast, 0.04–0.30 particles/L microplastics were discovered on the water surface of the Dungun River in Malaysia (Tee et al., 2020). Microplastics from 50–5000 μm were observed on the water surface and in the sediment of the Citarum River, Ciwalengke River, and Surabaya River in Indonesia. Microplastics can exist in various forms, such as filament, fiber, granule, fragment, film, and foam. Among these forms, Curren and co-workers discovered fragment-type microplastics were dominantly found across beach sediments, seawater, and benthic sediments, whereas the fiber type was discovered in marine organisms (Curren et al., 2021). The overall compositions of microplastic types found across the beach sediments, seawater, benthic sediments, and marine organisms are depicted in Figure 3B.

These microplastics have a negative impact on oceanic carbon cycles, altering the composition of microbial and planktonic communities. In addition, tiny pieces of microplastics can escape from wastewater treatment plants and enter the water stream as domestic effluents (Carr et al., 2016). From there, they can be mistakenly ingested by marine organisms such as sea turtles, whales, and sharks, causing digestive tract injury (Abreo et al., 2019; Coram et al., 2021). The development of coastal and marine pollution, overfishing, aquaculture, *etc.*, endangers 80% of the region's reef species. Hence, it is crucial to identify microplastic pollution hotspots and standardize protocols to better quantify, assess, and monitor microplastic contamination levels.

An integrated waste management system to combat plastic pollution includes efficient collection, processing, and treatment processes. However, these processes still need improvement in most of Southeast Asia. Despite the deployment of 'Interceptors' and 'River Trash Booms' in Indonesia (Jakarta and Bali) and Malaysia (Klang River) to prevent the flow of marine debris into the waterways, they are not a comprehensive solution to marine pollution. In Southeast Asia, the use of microbeads in cosmetic production has been officially banned in Thailand since 2020, as a supportive, collective effort to reduce primary microplastics. We should bear in mind that the fragmentation of macroplastics causes the formation of secondary microplastics; thus, a call to reduce single-use plastics is necessitated in Southeast Asia. Cambodia has banned the import and consumption of single-use plastics. Likewise, Malaysia adopted "The Malaysian Roadmap to Zero Single-Use Plastics" in 2018 and follows the 3R initiative (reduction, reuse, and recycle) (Fauziah et al., 2021) in daily life. Nevertheless, achieving zero single-use plastics is demanding and challenging at this stage because a total ban on plastic bag usage has yet to be implemented throughout Southeast Asia; for example, in Singapore and Malaysia, some supermarkets still provide plastic bags but with certain charges. Hence, educating and changing the public's mindset on plastic use and waste is crucial to realizing a zero single-use plastic nation.

3 Tackling the Southeast Asian plastic waste crisis

3.1 Projection of current trends of plastic waste generation

Following the current trends, the plastics within our oceans are projected to double by 2030 and triple by 2040. Southeast Asian countries are considered significant contributors to the leakage of land-based plastic waste into the seas, with a generation of 31 million tons of plastic waste annually (Julius and Trajano, 2022). It was stipulated that 80% of marine plastic debris originated from the land, which is why it is essential to create or enhance the current waste management system, especially in coastal areas.

The approach used by Southeast Asian countries to tackle waste is regionally blocked and only focuses on specific areas, resulting in significant oversights of an issue affecting the region on a large scale. For example, managing waste through incineration is only available and accessible in some regions, such as Myanmar, Singapore, Thailand, and Vietnam (Table 1). Collaboration between major stakeholders, including government, non-government, and international bodies, is needed in order to tackle this issue. China's ban on plastic imports has resulted in more than double the amount of plastic waste entering Southeast Asia in countries such as the Philippines, Malaysia, and Indonesia (Yoshida, 2022). Countries including Malaysia and the Philippines are returning the plastics to Western countries, while Thailand and Vietnam have restricted the further import of plastic waste. Despite that, Southeast Asian countries are still struggling with the influx of plastic waste generation within their regions. Out of 27.8 million tons of plastic waste generated in Thailand, 27% is improperly disposed of, and similar situations have been seen in bordering countries, including Malaysia (Chen et al., 2021). More than half of Indonesia's landfill is made up of open dumpsites without proper safety measures; these places increase the risk of floods, fires, and refuse avalanches, which have already claimed many lives in places including the Philippines and India (Marks, 2019).

3.2 Enacted solutions to the overall plastic waste issue

The current plastic waste problem is not just a plastic issue, it is a climate problem. The 2021 UNEP report showed that in 2015, the greenhouse emissions from the production, usage, and disposal of fossil fuel-derived plastic emitted approximately 1.7 gigatons of CO_2 equivalent, which will only rise to 6.5 gigatons by 2050, which is approximately 15% of the whole carbon budget (Julius and Trajano, 2022). Some argue that making a large systemic change may not be fast enough in order to deal with this issue; hence, in conjunction with large infrastructure changes, a holistic and community-based approach can be implemented in conjunction with the improvement of waste management infrastructure, especially for places outside of the city center.

In Indonesia, a waste-bank program was introduced in 2012 that encourages households to sort their waste into specific categories, which is then deposited in a central waste bank that provides them with monetary returns (Loh, 2020). There is also the ocean cleanup project under a non-profit organization, which aims to get rid of the plastics in the ocean using innovative solutions such as the interceptor unit utilized along rivers and oceans surrounding Southeast Asian countries (Omeyer et al., 2022). The prospect of utilizing plastic waste in developing communities and converting it into liquid fuel has been explored by Joshi and Seay (2016) and Owusu et al. (2018) in India and Uganda, respectively. Similar environments exist in Southeast Asian regions; therefore, this approach can be helpful in reducing plastic waste, especially in rural areas where waste infrastructure is severely lacking.

Other approaches to reducing plastic waste include banning specific items, deposit return schemes, and biodegradable packaging replacing plastic, which work well in the short term, but long-term systemic changes should be the focus of solutions to plastic waste and waste management in general (Omeyer et al., 2022). Integrated collaboration between the countries and help from international bodies would help implement an integrated waste management system in Southeast Asian countries.

Multiple collaborations and policies have been enacted among Southeast Asian members to solve the issue of plastic waste. This includes the ASEAN Regional Action Plan for Combatting Marine Debris in The ASEAN Member States (2021–2025), which directly addresses the issues of marine plastic waste. The members recognized that there is a lack of capacity with regard to plastic waste management both in the public and private sectors; hence, one of the goals of this process is to help bridge that gap and help improve the overall waste management system (ASEAN Secretariat, 2021).

Additionally, there is the ASEAN-Norway Cooperation project on Local Capacity Building for Reducing Plastic Pollution, which commenced in 2019. This initiative focuses on local municipality-/city-level sustainability and sets of science-based and feasible measures to reduce plastic pollution in crucial sectors. This would help improve the capacity of local actors, including regional governments, non-governmental organizations, and academic institutions. Other initiatives include the ASEAN+3 Marine Plastic Debris Cooperative Active Initiative and the Japan Funded Promotion of action against marine plastic litter in Asia and the Pacific (CounterMEASURE Project), both of which focus on the reduction of marine plastic waste. In addition, there are National recycling associations set up by the companies in Singapore, Malaysia, Vietnam, and the Philippines; although these are exclusively voluntary and don't involve government enactment, so there is a bias (UN environment, 2017).

These initiatives are a step in the right direction, but further development of plastic waste management needs to follow the cradle-to-cradle approach and not just focus on the end-life stage of plastic waste. As opposed to climate change, no global plastic agreement has the power to help push for regional action development to keep up with the increase in waste, and most agreements currently focus on the ocean rather than land-based sources of marine litter (Omeyer et al., 2022). This is an approach that treats the symptoms rather than the source. Moving forward, as mentioned previously, crucial stakeholders, including governmental and non-governmental organizations, need to combine forces and tackle the source of the problem, not just mitigate the after-effects of a larger plastic issue.

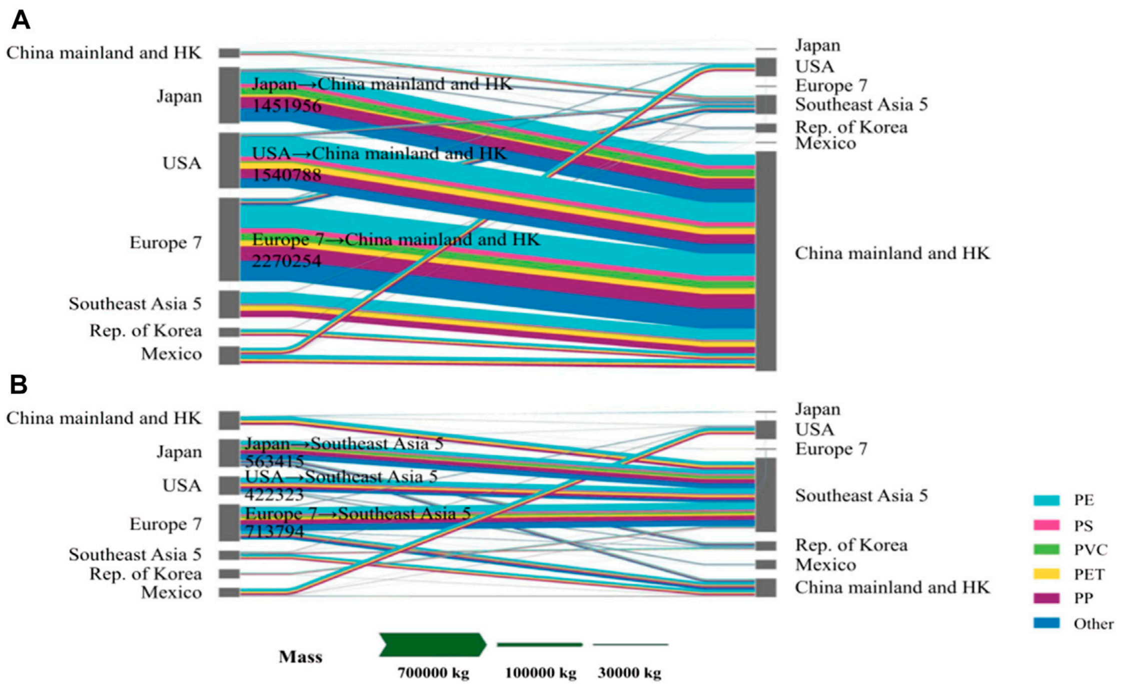

FIGURE 5
Trade flows of six types of plastic waste under two scenarios. **(A)** Global trade flows of six types of plastic waste prior to the ban (Baseline Scenario); **(B)** flows subsequent to the ban (2018 Scenario). The unit of the flows is tons (Wen et al., 2021).

4 Global plastic trade

Plastic waste is a "dirty" material that thrives in the trading of plastic waste worldwide and in the recycling industry. This material is also a significant source of severe environmental issues when it is not adequately treated (Lau et al., 2020). Europe (the EU) is at a crossroads in plastic and plastic waste management and trading plastic waste owing to insufficient recycling capacity. The exportation of plastic waste to Asia has led to substantial growth in international trade from 0.29 million tons (Mt hereafter) in 1988 to 15.99 Mt in 2014. Since 1993, Hong Kong, the USA, Japan, Germany, and the UK have been the largest plastic exporters, and this waste is transported to developing countries for recycling (Brooks et al., 2018; Wen et al., 2021; Tan et al., 2022). China was the world's foremost player in plastic importing, where the Chinese contributed to an annual plastic waste import of up to 8.88 million tons; due to the price of the imported plastics are cheaper for production, rather than using domestic plastic waste (Velis, 2014; Brooks et al., 2018). Among the plastics exporters, Hong Kong exported approximately 3,184,176 tons of plastic waste per annum (approximately 22% of the global trade) to China. Among the types of plastic waste, polyethylene (PE) is top of the list in plastic waste trade flow, which recorded approximately 37% or 11,404,697 tons in total, followed by polypropylene (PP), polyethylene terephthalate (PET), polystyrene (PS), and polyvinyl chloride (PVC) at a lower rate of 23%, 12%, 14%, and <8%, respectively (Figure 5A). The flow of PE from Hong Kong to China is particularly prominent, with a record of 46.2%, while the USA, Japan, and European countries exported 77.9%, 87.6%, and 57.5% of plastic waste to China (Wen et al., 2021). Unexpectedly, China imposed a ban named Prohibition of Foreign Garbage Imports: The Reform Plan on Solid Waste Import Management on 27 July 2017 to ban the import of particular wastes due to the low quality and contamination level of the importer plastics, which triggered severe environmental problems (Qu et al., 2019). China's imports have substantially plummeted by 95.4% (relative to baseline levels) and the world's total plastic waste trade flow declined by 45.5% in 2018 after the ban was imposed, as opposed to the scenario prior to the ban (Baseline Scenario). The China ban has greatly affected all major exporters with total export rates having reduced in Japan, the USA, and Europe by 39.2%, 54.1%, and 29.9%, respectively. This phenomenon has consequently resulted in a surging import of 362% to Southeast Asia, as illustrated in Figure 5B (China Dialogue, 2021).

The proportion of exports from developed countries to Southeast Asia, for instance, Japan, has substantially skyrocketed by approximately 50%, from 4.34% to 55.9%. In contrast, the United States saw an increment of 41.26% (5.24% to 46.5%) in export rates, and Europe saw an increment of 6.1% to 33.0%. At the same time, the import rates of Southeast Asia were increased by 3-fold, approximately 3.62 times higher than the Baseline Scenario, attributed to the contributions from Japan (25.8%), the United States (19.4%), Germany (11.7%), Hong Kong (10.3%), and the UK (9.8%), respectively. The calculated import lg ($TF^{2018}/TF^{baseline}$) of Southeast Asian countries, especially Thailand, the Philippines, and Malaysia, is relatively higher, as illustrated in Figure 6. Upon the Chinese ban, Malaysia grew as the largest plastic waste importer after China by

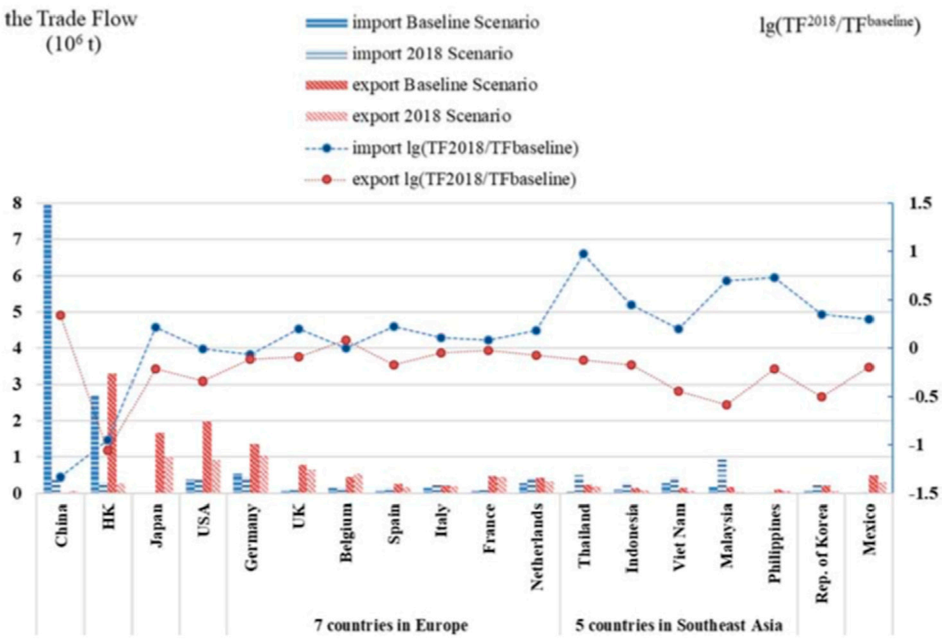

FIGURE 6
Changes in import and export flows after the ban (Wen et al., 2021).

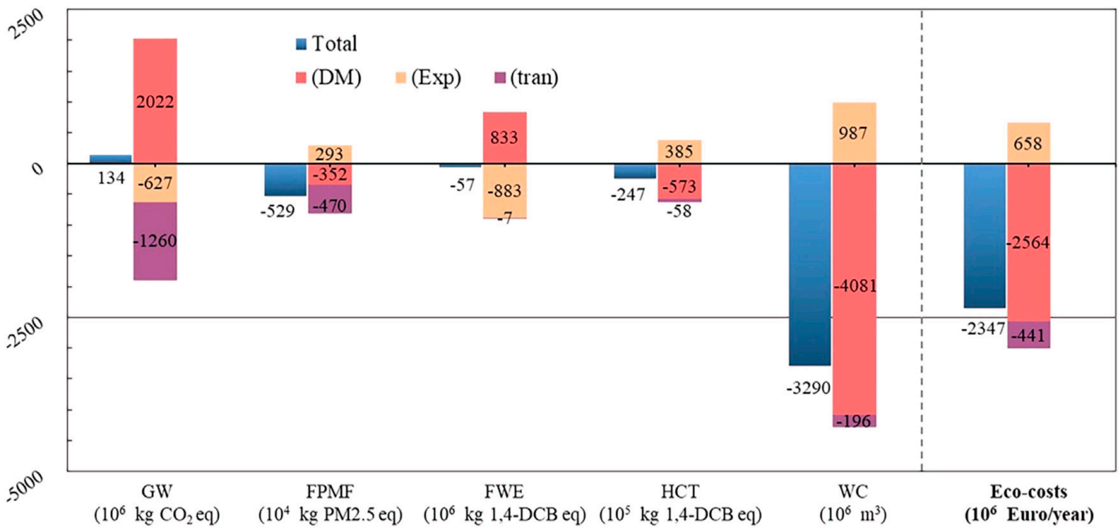

FIGURE 7
EIT and eco-cost of the China ban for the 2018 Scenario. Note that an item has a beneficial environmental impact when its value is negative. To enhance the visibility of midpoint indicator values on the ordinate axis, the unit of each indicator was adjusted as shown in the brackets at the bottom of the figure (Wen et al., 2021).

importing a high volume (105 thousand tons) of plastic waste in 2017, achieving an increasing rate of 68% as of 2016. However, the imported plastic wastes, including illegal imports, are of lower grades (contaminated), resulting in severe environmental issues. As a solution, the Malaysian Government has introduced policies such as issuing plastic waste import permits and close monitoring of permit holders to address the issue mentioned above. Evidently, 62 current permit holders in Malaysia have been monitored closely as of June 2019, and 148 illegal plastic recycling plants were shut down in the same year (Hassan et al., 2000; New Strait Times, 2019; Chen et al., 2021). According to the export data available in Comtrade June 2020, the total exports of plastic waste trade from all countries to Southeast Asia dropped by 32% in 2019, with a total plastic waste trade of 1,331,851 tons, as compared to 1,948,554 tons in 2018. The ban's impact has further intensified the plunging export volumes of the United States, the United Kingdom, and the Republic of Korea to 60%, 37%, and 46% of 2018's volumes, respectively.

In brief, the circumstances of the China ban are expected to result in waste accumulation, or these wastes will be transferred to other low-income countries such as Southeast Asia, consequently leading to undesirable environmental impacts. Figure 7 portrays the environmental impact of trade flow changes (EIT) by considering the environmental indicators, including GW, FPMF, FEW, HCT, and WC, upon the China ban (2018 Scenario). Thanks to the initiative of the China ban, the changes in trade flow have contributed to an improved indicator of FPMF, FEW, HCT, and WC after promoting global environmental sustainability. At the same time, the plummeting export rates resulted in temporal environmental impacts on GW owing to the higher incineration rates of developed countries compared to developing countries because landfilling is the primary waste treatment. In summary, strengthening local management and waste treatment in all countries is essential and is expected to mitigate the environmental issues of the plastic waste trade (Wen et al., 2021).

5 The way forward

5.1 Bio-based and biodegradable plastics as alternative plastics

Conventional plastics derived from crude oil are the major contributor to environmental pollution and global warming, attributed to their non-biodegradable properties where these materials require decades for degradation. The non-biodegradability of plastic refers to the plastic's chemical structure that could not be degraded or broken down by naturally occurring microorganisms, water, carbon dioxide, *etc.* (Babu et al., 2013). In contrast, biodegradable plastics are compostable to form biomass, water, and carbon dioxide or methane *via* microorganisms under specific conditions (Atiwesh et al., 2021).

As per the European Bioplastics Association, bioplastics are composed of materials with partially bio-based renewable raw materials such as biomass and are biodegradable depending on the monomer's characteristics and polymerization processes. Biodegradability implies the conversion of material into natural substances by microorganisms such as bacteria, fungi, and algae. Bioplastics can be bio-based or biodegradable or feature both properties. Bioplastics can be produced using three types of generation feedstock. The first-generation feedstock includes carbohydrate resources based on edible food crops such as sugarcane, potato, and corn, raising concerns over sustainability. In comparison, the second-generation raw materials are derived from lignocellulose-rich feedstock such as wood and non-edible by-products of food crops. Although second-generation raw materials are more eco-friendly than first-generation raw materials, lignocellulose conversion is energy intensive (Singhvi and Gokhale, 2019). Meanwhile, algae and municipal waste are third-generation feedstock for bioplastic production (Singh et al., 2022). Bioplastic is an alternative plastic material derived from all kinds of whole or partial renewable biomass, thus giving rise to bioplastics with different properties (Nandakumar et al., 2021). For instance, PLA, bio-PET, *etc.*, are suitable for packaging, while bio-based succinic acid is used in the automotive and textile industries. There are three ways to prepare bioplastics: (a) thermochemical and catalytic processes, converting biomass feedstock into monomers and then polymerizing them; (b) fermentation processes, fermenting biomass to produce monomers followed by conversion into polymers; and (c) modifying naturally occurring polymers (Singh et al., 2022). In 2019, among the 2.05 million tons of bioplastics produced, merely 54% of them were biodegradable, while 46% were non-biodegradable (IFBB, 2022). The degradability of bioplastics relies on their composition, degree of crystallinity, and environmental factors that result in a degradation time frame that varies from days to years. For example, PLA is the most commercially developed biodegradable plastic, and the biodegradation of PLA bioplastics contributed to a zero net increase in CO_2 and 70% fewer greenhouse gases during the biodegradation in landfills, implying bioplastics are more environmentally friendly than conventional plastics (Elsawy et al., 2017).

Although biodegradable plastics offer significant momentum to end plastic pollution, there are still great uncertainties waiting to be explored, including the complexity of waste management and the presence of contaminants that may trade off the compost quality and the emancipation of toxic chemicals to the environment. Although biodegradable plastics can be degraded under the action of bacteria, fungi, or algae, in some circumstances, degradation can be initiated under the influence of temperature. However, biodegradable bioplastic waste is still processable through mechanical and chemical recycling, thus offering viable waste recovery options that reduce reliance on primary resources, leading to a definite shift of the plastic chain towards sustainability (Fredi and Dorigato, 2021).

5.2 Promoting "waste-to-wealth" initiatives *via* chemical recycling technologies

Waste-to-wealth refers to upcycling and valorizing waste by turning it into valuable/useful products, including refinery feedstock, fuel, and monomers (Jiang et al., 2022). Upcycling plastic waste to make fuel is promising because plastic-derived fuel has a high calorific value comparable to gasoline and diesel in the market. While most countries are still practicing incineration in dealing with plastic wastes to save landfill space, this method merely offers low energy recovery efficiency with the emission of hazardous and greenhouse gases. Another versatile approach worth mentioning is chemical recycling, where plastic waste can undergo gasification and pyrolysis to convert it into valuable products.

Pyrolysis refers to the thermal degrading of complex molecules into smaller molecules at a high temperature (300°C–800°C) in an inert condition, producing liquid oil, char, and gases as value-added products (Fivga and Dimitriou, 2018). It can be performed either with (catalytic) or without the assistance of the catalyst named thermal pyrolysis. The catalysts, such as zeolite and silica-alumina, are often used during the pyrolysis process as the catalyst reduces pyrolysis temperatures, narrows product distribution, and increases product selectivity (Singh et al., 2018; Chen et al., 2021). The actual

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application of the pyrolysis process has been conducted by Muang Sa Ad Co., Ltd., a company that collects, cleans, and converts plastic waste into oil through the pyrolysis process. The pyrolysis oil is refined and used as fuel in refuse trucks in Thailand (MSA, 2018).

Unlike the pyrolysis process, gasification converts the solid fuel to gaseous fuel such as syngas (hydrogen and carbon monoxide) production at high temperatures (usually higher than 800°C) in an oxygen-limited condition. The gasification of plastic waste has caught considerable attention because the produced syngas is an excellent raw feedstock in a fuel cell to generate electricity (Salaudeen et al., 2019; Saebea et al., 2020). On another occasion, researchers from Nanyang Technological University (NTU Singapore) found a way to convert plastic waste to hydrogen based on high-temperature chemical processes. The produced hydrogen, as an alternative clean energy source, helps generate electricity and power fuel cells in electric vehicles (NTU Singapore, 2022).

The establishment of plastic waste upcycling technologies is still in its embryonic stage, but it is an attractive approach to converting municipal plastic into its original monomers, chemicals, and fuel products. In addition, these technologies are promising as a replacement for high-cost plastic waste incineration.

5.3 Establishing a circular economy for plastics

In establishing a circular economy for plastics, it is first essential for society to reconsider plastic as a renewable resource instead of as a waste. Nevertheless, according to the Ellen MacArthur Foundation, merely 14% of plastic packaging is recycled, 40% is left in landfills, 32% is left in ecosystems, and 14% is incinerated for energy recovery. A thriving circular economy would mean the constant flow of plastic around a closed-loop system; looping the used plastic back into the value chain rather than being used once and discarded. This will involve (i) redesigning products for recyclability using new or renewable materials and (ii) closing the loop with chemical recycling. The first case is achievable by substituting fossil-based feedstocks with renewable feedstocks, such as the development of biodegradable plastics (discussed in Section 5.1) that can be degraded in a shorter time frame without contaminating the environment. In addition, the plastic industries should prohibit single-use materials during plastic production and reduce the use of colorants and additives to simplify the recycling process. For instance, Unilever unveiled their new recycling technology, called CreaSolv Process, to recover the plastic from sachets and use it to create new sachets for Unilever products (Unilever, 2017).

Second, chemical recycling (Section 5.2) should be promoted as this process transforms plastic material and additives into their original monomer, which can be the feedstock for a new product. Recently, new and modified pyrolysis pilots have been emerging. For instance, the United Kingdom start-up Recycling Technologies uses a fluidized bed reactor for pyrolysis and found that this reactor could evenly distribute the temperature and modularize, which is more adaptable to a dispersed collection and plastic recycling system (Recycling Technologies, 2018). Moreover, pyrolysis provides high flexibility in terms of feedstock. This thermal process disintegrates polymers, other organic materials, and vulcanized polymers, including automotive rubber tires, which could not be recycled using other methods. In brief, chemical recycling technologies are the alternative methods for processing materials that are hard to be treated with mechanical recycling while producing higher-quality recycled materials (Jahnke et al., 2017). For example, PETRONAS Chemical Group and Plastic Energy Ltd. have successfully developed a new technology to convert non-recyclable and low-quality plastic waste into naphthalene in Malaysia, which can produce virgin-quality polymers (KASA, 2021).

Since 2021, Malaysia adopted the Malaysia Plastics Sustainability Roadmap to set Malaysia on a pathway to plastics sustainability for 2030 and beyond. The first approach to achieving plastic sustainability is improving product design using recycled resin as the raw material to assure environmental friendliness and to be kept in the loop for long without compromising the product's quality and performance. In Malaysia, PP, PET, HDPE, and LDPE are the most common resins used for single-use packaging, which should be phased out and replaced with a new recyclable product with a longer shelf-life and value in the chain. For instance, Thong Guan Industries Berhad, Malaysia's most extensive stretch film manufacturer, has produced nano stretch film to wrap pallets and goods. This new type of film has excellent grip, load stability, and durability that resist wear and puncture compared to the single-use conventional multi-layered stretch film (KASA, 2021). On another occasion, to ensure material circulation, KLEAN Malaysia worked with Shell Malaysia by placing Reverse Vending Machines at certain Shell stations in Kuala Lumpur for the public to deposit/recycle used plastic containers in exchange for a reward (KLEAN, 2021). In Thailand, the Dow Thailand Group and Siam Cement Group collaborated in surfacing roads using recycled plastic waste. They found that asphalt derived from plastic wastes gave rise to enhanced strength and superior erosion resistance of roads (SCG, 2019).

A circular economy is an effective approach to addressing environmental issues such as global greenhouse gas (GHG) emissions and post-consumer waste pollution. Due to the fact that most plastic products (>90%) are produced from virgin petroleum-based feedstock, it is expected that this phenomenon will contribute to 15% GHG emissions by 2050. The Swedish Environmental Protection Agency revealed that recycled plastic saves approximately 1–1.5 kg CO_2/kg resin, and each kg of recycled plastic saves approximately 130,000 kJ of energy (Rahimi and García, 2017). Figure 8A depicts that the total reduction of GHG emissions was achieved by the reuse and recycling of plastic waste between China and trading countries, with a subtle 76-fold increment from 1992 (0.11 million metric tons CO_2e) to 2012 (8.71 million metric tons CO_2e). This phenomenon is credited to the Chinese stakeholders who have imported massive plastic waste from developed countries (EU, US, and Japan) to fulfill the domestic market's needs. The skyrocketing GPWT between China and trading countries through ship transportation from 1992 to 2017 (Figure 8B) led to severe GHG emissions from 0.015 MMT CO_2e to 0.229 MMT CO_2e in 2017, especially during the peak periods between 2009 and 2012. The GHG emissions generated by shipping plunged due to the launch of the Chinese Green Fence campaign by the Chinese government to combat waste smuggling

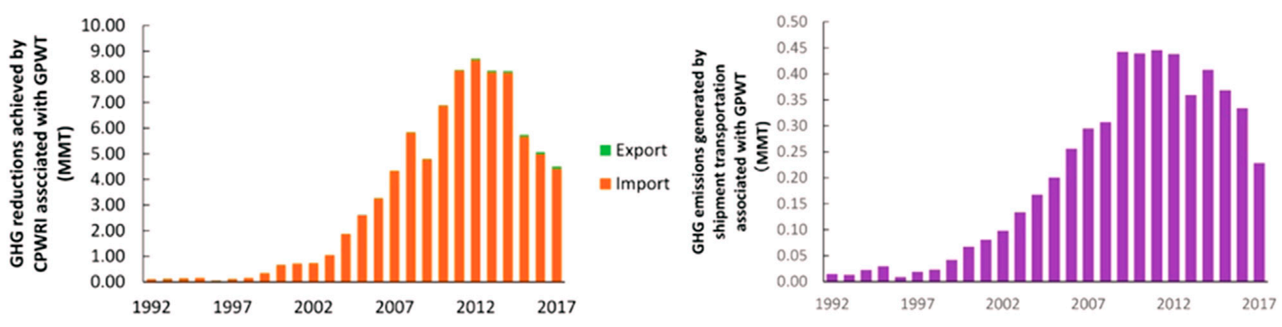

FIGURE 8
(A) GHG reductions achieved by Chinese plastic waste recycling industries (CPWRI) associated with global plastic waste trade (GPWT) between China and trading countries from 1992 to 2017. The import and export of plastic waste are indicated by orange and green bars, respectively. **(B)** GHG emissions generated by shipment transportation associated with GPWT between China and trading countries from 1992 to 2017 (Liu et al., 2021).

activities in China, in addition to the restrictions on allowing low-quality plastic waste to enter China. In 2017, a substantial reduction in GHG emissions was observed due to the ban on 24 categories of recyclables and solid waste by the Chinese government. Multiple efforts to address the issues of GHG emissions and plastic pollution include the execution of Horizon 2020 by the EU, where Canada banned harmful single-use plastic in a bid to reduce ocean waste, and China's ban on non-biodegradable plastic bags that are <25 μm (Walker and Xanthos, 2018; Fraccascia et al., 2019; Liu et al., 2021). Despite CE being proven to be effective in alleviating environmental issues, there are still many obstacles in countries with different standards and operational systems. Thus, the implementation of a robust after-use system for post-consumer plastic materials is vital.

6 Conclusion and future outlooks

The lack of sophisticated plastic waste management systems in Southeast Asia is the prime cause of severe environmental impacts. Southeast Asia is a hotspot for receiving plastic waste from developed countries, yet most of the countries in Southeast Asia lack the infrastructure for sound waste management. Since 2017, Southeast Asian countries such as Thailand, Malaysia, and Vietnam have restricted plastic waste imported from Western countries and imposed various bans to curb the over-usage of single-use plastics and non-biodegradable plastic bags. Moreover, the turning point in winning this battle (ending plastic pollution) is dependent on the individual and collective choices of the people *per se*, as well as the collective efforts and commitments of all interested parties, including the government and NGOs. The Southeast Asian region should raise awareness of the potential environmental risks of waste disposal and, at the same time, formulate related policies to hamper undesirable consequences, which can be done by restricting the production and use of particular plastic products *via* regulations and raising the plastic recycling rate through the construction and improvement of recycling facilities.

In an effort to secure global waste trade, establishing a global extended producer responsibility system is essential to ensure fair and responsible waste trade. This system is aimed toward not only developing nations but also developed countries, who should work hand-in-hand to reshape and rebalance the global CE for plastics to reduce environmental pollution and GHG emissions globally. In addition, it is vital to establish a global standard for the reuse and recycling of plastic waste, such as standardizing the treatment methods and operational systems (mechanical, chemical, and organic recycling) for plastic waste of different kinds to ensure these wastes are properly recycled in other countries. In addition, the transfer of knowledge and technology from developed countries to developing countries helps mitigate potential environmental issues. For example, developed countries could invest in research and development (R&D) and train local employees (employees in the developing countries) in dealing with waste management and recycling technologies.

Author contributions

CN and MM wrote the first draft of the manuscript. CN, AG, AI, and JF contributed to the conceptualization and finding resources for the review paper. CS and JS contributed to visualization. ST and JS contributed to the review and editing of the paper. YT-Y and JJ supervised the progression of the writing and preparation of the manuscript. All authors contributed to the manuscript revision and read and approved the submitted version.

Funding

This work was funded by the Ministry of Higher Education Malaysia Translational Research (TR@M) and Universiti Malaysia Sabah Special Grant Scheme (SDK0321-2021).

Conflict of interest

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frontiers | Frontiers in Environmental Science

TYPE Original Research
PUBLISHED 09 May 2023
DOI 10.3389/fenvs.2023.1197634

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OPEN ACCESS

EDITED BY
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RECEIVED 31 March 2023
ACCEPTED 25 April 2023
PUBLISHED 09 May 2023

CITATION
Sun Y, Liu D, Wu Y, He X, Luo Y, Zhou X, Chen W, Chen W and Li S (2023), Updated spatial distribution and health risk assessment of heavy metals in soils of the Yangtze River Basin, China.
Front. Environ. Sci. 11:1197634.
doi: 10.3389/fenvs.2023.1197634

Updated spatial distribution and health risk assessment of heavy metals in soils of the Yangtze River Basin, China

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Supporting ecological protection and restoration has been at the heart of China's ambitious Yangtze River conservation strategy. Knowledge of the current status of heavy metal distribution is important for planning remediation practices and allocation to waste treatment facilities. Through an extensive and systematic review of literatures, this study depicts the up-to-date spatial distribution and characteristics of typical heavy metals in soils of the Yangtze River Basin, China. A total of 7,694 geo-referenced records of heavy metal in soils of the Yangtze River Basin were compiled from the literatures published between 2000 and 2020. The results show the spatially-heterogeneous concentrations of Zn, Cu, Pb, Cr, Ni, As, Hg and Cd. The degree of heavy metal pollution was relatively higher in the middle reaches, while it was relatively lower in the upstream and downstream. According to the limits set by the state to ensure agricultural production and maintain human health, the average concentration of Cd greatly exceeded its limit. Overall, a certain number of heavily polluted areas were found to occur in regions with frequent human economic activities, posing potential health risks. The carcinogenic and non-carcinogenic risks of children are 1.4 times and 1.6 times higher than those of adults, respectively, and the heavy metal with the highest risk to human health was Cr. This study provides an important basis for the field of soil pollution prevention and control in the Yangtze River Basin. It updates the current understanding of the spatial pattern of major pollutants in a large ecologically protected region in China, which is conducive to the precise prevention and control of public health risks.

KEYWORDS

spatial distribution, heavy metal, health risk, soil, the Yangtze River Basin

1 Introduction

Heavy metals are defined as metals and metalloids with densities greater than 5 g/cm^{-3} (Jarup, 2003; Oves et al., 2012), mainly including zinc (Zn), copper (Cu), lead (Pb), cadmium (Cd), chromium (Cr), nickel (Ni), arsenic (As), mercury (Hg), etc., which are the major pollutants with the characteristics of difficult migration, long residual time, strong concealment and high toxicity (Cai et al., 2012; Yang et al., 2018). Heavy metals come from both natural and anthropogenic sources, of which anthropogenic sources have proven to be the main sources of heavy metals, including mineral resource exploitation, metal processing and smelting, chemical production, factory discharge, and sewage irrigation (Yang et al., 2018). Excessive accumulation of heavy metals in soils can lead to changes in soil composition, structure and function, which can inhibit crop growth and even reduce soil productivity (Cai et al., 2012; Wei et al., 2016). In addition, heavy metal pollution in soils can negatively affect human health, directly or indirectly, through the food chain (Zhang et al., 2012; Cai et al., 2019). For example, minamata disease (Hg pollution) and bone-pain disease (Cd pollution) in Japan were caused by heavy metal pollution (Jarup, 2003).

In the past few years, the central government has conducted several in-depth investigations in regions along the Yangtze River and held symposiums on promoting the development of the Yangtze River Economic Belt, emphasizing that the restoration of the ecological environment of the Yangtze River should be placed in an overwhelming position (Luo et al., 2021; Zhang et al., 2021). The Yangtze River Basin is rich in mineral resources, with mineral species accounting for 80% of the proven mineral species in China. With the rapid development of industry in the Yangtze River Basin, more than 400,000 chemical industries are distributed along the Yangtze River (Ye et al., 2019). Abundant mineral resources would lead to the increase of the background value of heavy metals in soils, and frequent exploitation of mineral resources and industrial activities would lead to the aggravation of heavy metal pollution in nearby soils. Meanwhile, the Yangtze River basin, accounting for 25% of the arable land and 29.1% of the grain output in 2015, is considered a major agricultural production base in China (Xu et al., 2019). Therefore, the spatial distribution, pollution degree and health risk assessment of heavy metals in soils of the Yangtze River Basin are directly related to human health and public wellbeing (Li et al., 2019; Mir et al., 2022), which have received extensive attention from scientific community.

However, at present, most of the studies on heavy metals in soils of the Yangtze River Basin were local and small scale (Yang et al., 2016; Chao et al., 2017; Ni et al., 2018; Yang et al., 2020; Luo et al., 2021; Ai et al., 2022) or a few common heavy metals (Wen et al., 2013; Zhou and Wang, 2019), and there is a lack of comprehensive and systematic studies on soils of the whole Yangtze River Basin. Jiang et al. (2022) studied the spatial distribution of heavy metals in soils and sediments of the Yangtze River Basin, but did not assess the health risk of heavy metals. At present, there are two important scientific issues that require to be resolved: 1) the spatial distribution of heavy metals in soils of the Yangtze River Basin has not been updated; 2) the health risk of heavy metals in soils of the Yangtze River Basin remain unclear.

In this study, we described the spatial distribution and concentration of heavy metals in soils of the Yangtze River Basin by systematically collecting and analysing peer-reviewed literatures from 2000 to 2020, considering the collectability of data, data reliability, research objectives and policy background. We analysed the degree of heavy metal pollution and assessed the health risk of heavy metals in the Yangtze River Basin. This method is highly empirical and practical for studying the spatial distribution of heavy metals in soil, because it makes full use of abundant peer-reviewed literatures in the past 20 years to provide a comprehensive and in-depth data base for our research. Our study fills a research gap in describing the spatial distribution and health risk assessment of major heavy metals in soils of the Yangtze River Basin. Our results could provide fundamental data support for the establishment of well-targeted heavy metal pollution prevention and control strategies, providing new insights into the distribution and potential health risks of soil heavy metals in China's largest river basin.

2 Materials and methods

2.1 Study area

As the largest river in Asia, the Yangtze River is about 6,300 km long, originating in the Tanggula Mountains in Qinghai and entering the East China Sea in Shanghai. The main stream of the Yangtze River flows through 11 provinces (Qinghai, Xizang, Sichuan, Yunan, Chongqing, Hubei, Hunan, Jiangxi, Anhui, Jiangsu and Shanghai), and hundreds of tributaries flow through 8 provinces, including Guizhou, Gansu, Shanxi, Hunan, Guangxi, Guangdong, Zhejiang and Fujian (Figure 1).

2.2 An extensive review and records collection

The schematic overview of the literatures search procedure and results is showed in Figure 2. Since the boundary of the Yangtze River Basin is not clear, it is impossible to limit the scope of publications search through search formulas. Therefore, the spatial scale of data collection was the whole of China, the temporal scale was 2000–2020, the search objects were the information (concentration and location, etc.) of eight heavy metals in soils, including Zn, Cu, Pb, Cr, Cd, Ni, As and Hg, which were listed as priority pollutants by the US Environmental Protection Agency. Considering the wide coverage, strong academic type and high accessibility, the databases of Web of Science (WOS) and China National Knowledge Infrastructure (CNKI) were chosen to search the publications. The keywords used for searching were [(heavy metal OR metal element OR metallic element) AND (concentration OR level OR content OR contamination OR pollution OR spatial distribution) AND soil AND China] with WOS, and [(heavy metal + metal + metal element) * (content + level + concentration + distribution) * soil * China] with CNKI.

Firstly, titles and abstracts of publications were examined and we excluded publications which only described heavy metal resistant plants or heavy metal detection technology not measured heavy metal concentration, or which only focused on human or organism not selected environment matrices, or did not include any target heavy metals, or did not involve any geographic information. Secondly, having intensively read all the full-texts, the publications which failed to report details of occurrence data and geographical information were further excluded, and finally, 1935 publications (736 for WOS and 1,199 for CNKI) were identified to be eligible for extraction.

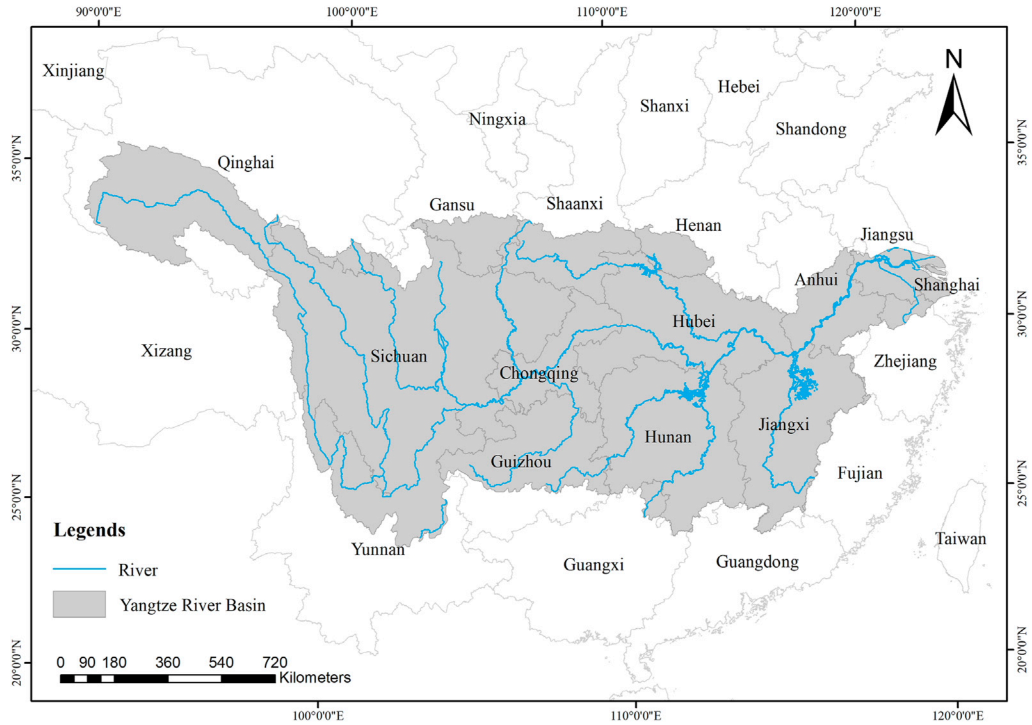

FIGURE 1
Geographic location of the Yangtze River Basin.

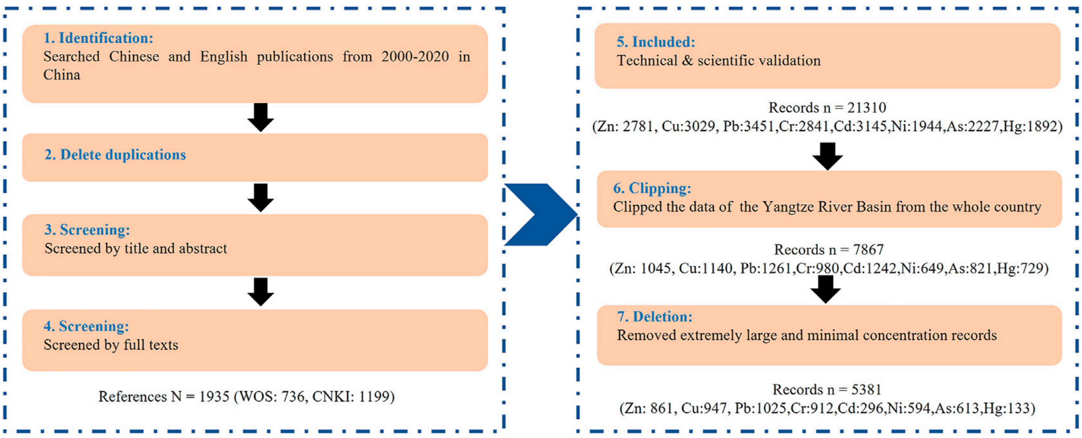

FIGURE 2
The schematic overview of the literatures search procedure and results.

2.3 Georeferencing and data analytical steps

The key information extracted from the publications included sampling location, sampling time, analysis method, concentration of heavy metals. In line with the georeferencing strategy adopted previously (Zhang et al., 2022), the concentrations of heavy metal were processed as follows: 1) the concentration only extracted the total concentration of various heavy metals, ignoring the concentration data that only records one or more forms; 2) the concentration of heavy metals is uniformly converted into standard concentration units (mg/kg). The locations of the sampling points were processed as follows: 1) if the latitude and longitude of the sampling points were recorded in the publication, the latitude and longitude were extracted directly; 2) if the latitude and longitude of the sampling points were not recorded in the publication, we determined the latitudinal and longitudinal using Web APIs (Application Programming Interfaces) to access georeference functions of the most commonly used online location services in China, namely, Baidu Map and Amap. We searched keywords related to the location of each record, for example, the name of specific geographical objects, administrative regions, or water bodies, and recorded the latitude/longitude information. When only maps of the sampling points were provided, we approximated rough coordinates through visual interpretation, mapped these records on Baidu Map or Amap, and then adjusted the coordinates according to the geographical characteristics of the original maps. A total of 21,310 records involving the concentration and location of heavy metals were extracted, one of which contained related information for only one heavy metal, and then we clipped out the data under the Yangtze River Basin. There were 7,867 records of heavy metals in soils of the Yangtze River Basin (Zn: 1,045; Cu: 1,140; Pb: 1,261; Cr: 980; Cd: 1,242; Ni: 649; As: 821; Hg: 729).

We used the data in the X/4~4X (X is the average concentration of each heavy metal) concentration range of each metal in the above records for further pollution degree and health risk accessment to exclude the influence of extremely high and low values of the measured concentrations in the original publication. After a simple processing of the data, a total of 5,381 records (Zn: 861; Cu: 947; Pb: 1,025; Cr: 912; Cd: 296; Ni: 594; As: 613; Hg: 133) were used to assess pollution degree and health risk in the Yangtze River Basin. Descriptive statistics, such as mean, median, minimum, maximum, standard deviation (SD), variation coefficient, kurtosis and skewness, were used to analyze heavy metals. The single factor index and Nemerow's synthetic pollution index were used to estimate the pollution degree for heavy metals. Based on ArcGIS version 10.7, the spatial distribution of heavy metal pollution was visualized by using Inverse Distance Weight (IDW) and Spatial Autocorrelation.

2.4 Pollution index calculation

Single factor index and Nemerow's synthetic pollution index were used to assess the degree of heavy metals pollution in soils (Gong et al., 2008). The calculation (Formula 1ormula –Formula 3) and grading standards (Supplementary Tables S1, S2) of single factor index and Nemerow's synthetic pollution index of heavy metals were as follows (Gong et al., 2008; Chen et al., 2013; Chen et al., 2014; Islam et al., 2023):

(i) Single factor index

$$P_i = \frac{C_i}{S_i} \tag{1}$$

Where P_i is single factor index value of heavy metal i; C_i is the measured concentration of heavy metal i in soils (mg/kg); S_i is the standard of soil environmental quality.

(ii) Nemerow's synthetic pollution index

$$\bar{P} = \frac{\sum W_i P_i}{\sum W_i} \tag{2}$$

$$PI = \sqrt{\frac{\bar{P}^2 + {P_{imax}}^2}{2}} \tag{3}$$

Where PI is Nemerow's synthetic pollution index value of heavy metal; W_i is the weight of heavy metal i; $\bar{P}$ is the weighted average of the single factor index (Cr: 2, Cu: 2, Cd: 3, Pb: 3, Zn: 2, As: 3, Ni: 2, Hg: 3); $P_{i\max}$ is the largest single factor index of heavy metal i.

2.5 Health risk assessment

The carcinogenic risk (CR) and non-carcinogenic risk (NCR) indexes were used to assess the health risk of heavy metals in soils of the Yangtze River Basin to adults and children.

(i) Average daily intake (ADI):

The calculation formulas for the intake of the non-carcinogenic average daily exposure of the three pathways of human exposure to heavy metals were as follows:

$$ADI_{ing} = \frac{C_j \times IR_{ing} \times EF \times ED}{BW \times AT} \tag{4}$$

$$ADI_{der} = \frac{C_j \times SA \times AF \times ABS_j \times EF \times ED}{BW \times AT} \tag{5}$$

$$ADI_{inh} = \frac{C_j \times IR_{inh} \times EF \times ED}{PEF \times BW \times AT} \tag{6}$$

Where ADI_{ing}, ADI_{der}, ADI_{inh} are the average daily intake dose of heavy metal via ingestion, dermal and inhalation absorption (mg/kg/d); C_j is the concentration of heavy metal j (mg/kg); Other parameters are shown in Supplementary Table S3 (UEPA, 2011; USEPA, 2001; HHC, 2004; U. S. DoE, 2011; Varol and Sünbül, 2020; Varol et al., 2020; Jiang et al., 2017).

(ii) The carcinogenic risk (CR) and non-carcinogenic risk (NCR) indexes:

$$HI_j = \sum HQ_i = \sum \frac{ADI_i}{RfD_j} \tag{7}$$

$$NCR = \sum HI_j \tag{8}$$

$$CR = \sum CR_j = \sum \left(LADD_j \times SF_j\right) \tag{9}$$

Where RfD_j is the reference dose of heavy metal j via the ingestion, inhalation and dermal exposure pathways (mg/kg/d); HI_j is the non-carcinogenic risk health risk index of heavy metal j; *THI* is non-carcinogenic health risk index for all exposure pathways and heavy metals; *LADD* is lifetime average daily dose; SF_j is carcinogenic slope factor of heavy metal j; *TCR* is carcinogenic health risk index for all exposure pathways and heavy metals. The RfD_j and SF for different heavy metals and exposure pathways are shown in Supplementary Table S4. The grading standard for carcinogenic health risk recommended by the U.S. Environmental Protection Agency (EPA) is shown in Supplementary Table S5.

3 Results and discussions

3.1 Occurrence and spatial distribution of heavy metals

The spatial distribution of sampling points of heavy metals in soils of the Yangtze River Basin is shown in Figure 3. The boxplots of

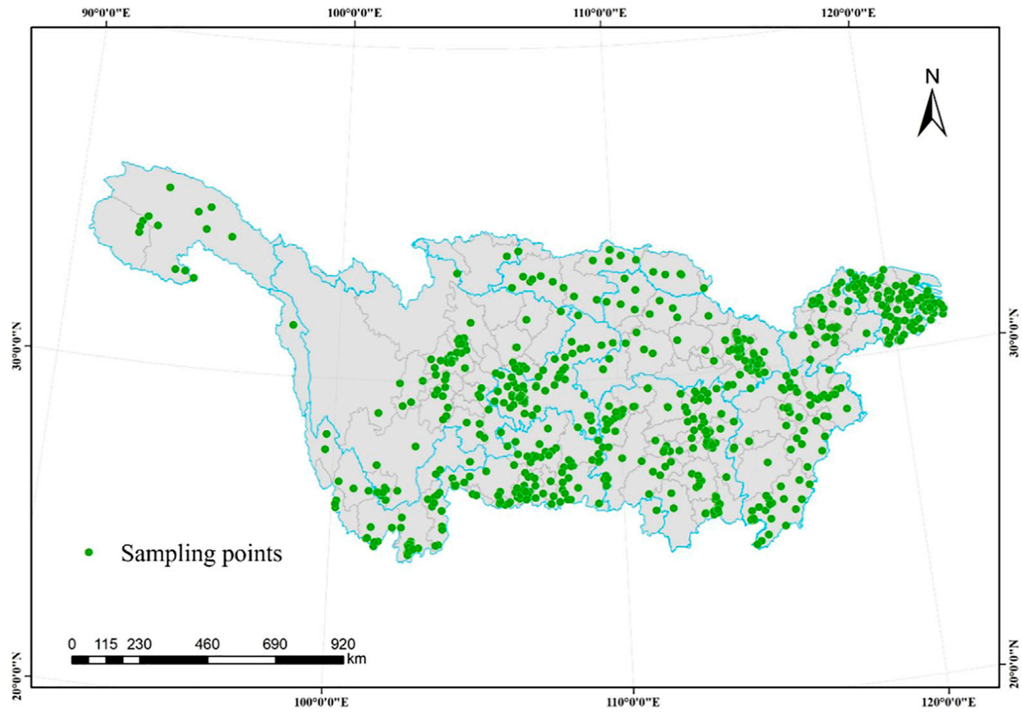

FIGURE 3
Distribution of heavy metal sampling points in soils of the Yangtze River Basin.

heavy metal concentration are shown in Figure 4 and other descriptive statistics are shown Table 1. In these records, the sampling points were mostly located in the surface soil of 0–20 or 0–30 cm, the sampling time involved all months of the year, the treatments of the samples mainly included drying, crushing, digestion and analysis, in which HNO_3–$HClO_4$–HF or HNO_3–$HClO_4$–HCl or HCl–HNO_3–HF–$HClO_4$ were used for the digestion of mixed acids, and the analysis methods were mostly Atomic Absorption Spectroscopy (AAS) or Atomic Fluorescence Spectrometry (AFS) or Auger electron spectroscopy (AES) or Inductively coupled plasma mass spectrometry (ICP-MS).

The distribution of heavy metal sampling points in soils was relatively uniform, and the concentration range of Zn, Cu, Pb, Cr, Cd, Ni, As and Hg was 63.0–893.0 mg/kg, 19.0–291.0 mg/kg, 24.0–331.0 mg/kg, 22.0–305.0 mg/kg, 0.8–12.6 mg/kg, 12.0–191.0 mg/kg, 7.84–116.0 mg/kg, 0.3–4.3 mg/kg, respectively. The average concentrations were 120.6, 41.2, 46.2, 82.0, 2.1, 30.7, 12.8 and 0.5 mg/kg, respectively. With China's Secondary Standard for Soil Environmental Quality as the reference value (GB15618-2018), the average concentration of Zn, Cu, Pb, Cr, Ni, As and Hg were all lower than the reference values, while the average concentration of Cd was 7 times higher than the reference value. In terms of excess rate, all heavy metals had sampling points with excessive concentrations. Most of the sampling points of Cd and half of Cu had excessive concentration. The proportions of sampling points with excessive concentration of Zn, Pb, As, and Hg were less than 20%. The heavy metals with the lowest excess rate were Cr and Ni, which were 7.24% and 7.93%, respectively. The results showed that Cd had a strong tendency to enrich in soils. The variation coefficients of heavy metals were exceeded 50%, which was a strong variation, indicating that the concentrations of heavy metals in soils were not only affected by the local background value, but also by human activities. Kurtosis and skewness were mainly used to measure the steepness and asymmetry of heavy metal concentration distribution. With the exception of Cd, the heavy metals showed a certain degree of steep and positive shift. This may be related to the sampling points range, sampling depth, and the surrounding industrial layout.

Figure 5 shows the spatial distribution of heavy metal pollution in soils of the Yangtze River Basin using Inverse Distance Weight (IDW). It can be seen that the spatial distribution of Cu and Pb was similar to some extent, showing that the concentrations of Cu and Pb were higher in Yunnan, eastern Liangshan Yi Autonomous Prefecture of Sichuan, southern Anhui and southern Hunan, while were lower in Qinghai, Chongqing, central and western Hubei and central and southern Jiangxi. The high value regions of Zn, Cu and Pb were scattered, which may be due to the occurrence of mineral association. The high value regions of Cr, Cd and Ni were dispersed, showing non-point pollution, the pollution of which were mainly from industrial emissions, coal burning and agricultural activities (fertilizers and pesticides). The low value regions of Cd were concentrated in Chongqing and Guizhou, while the low value regions of Ni were distributed in southern Hunan and Jiangxi. The regions with high Hg concentration accounted for the lowest proportion and were distributed in Huaihua, Loudi and Anqing in Hunan, which was because Hg mines in Hunan were mainly distributed in Xiangxi. The spatial distribution of Zn, As and Pb in Hunan were consistent, showing higher in Yongzhou, Hengyang

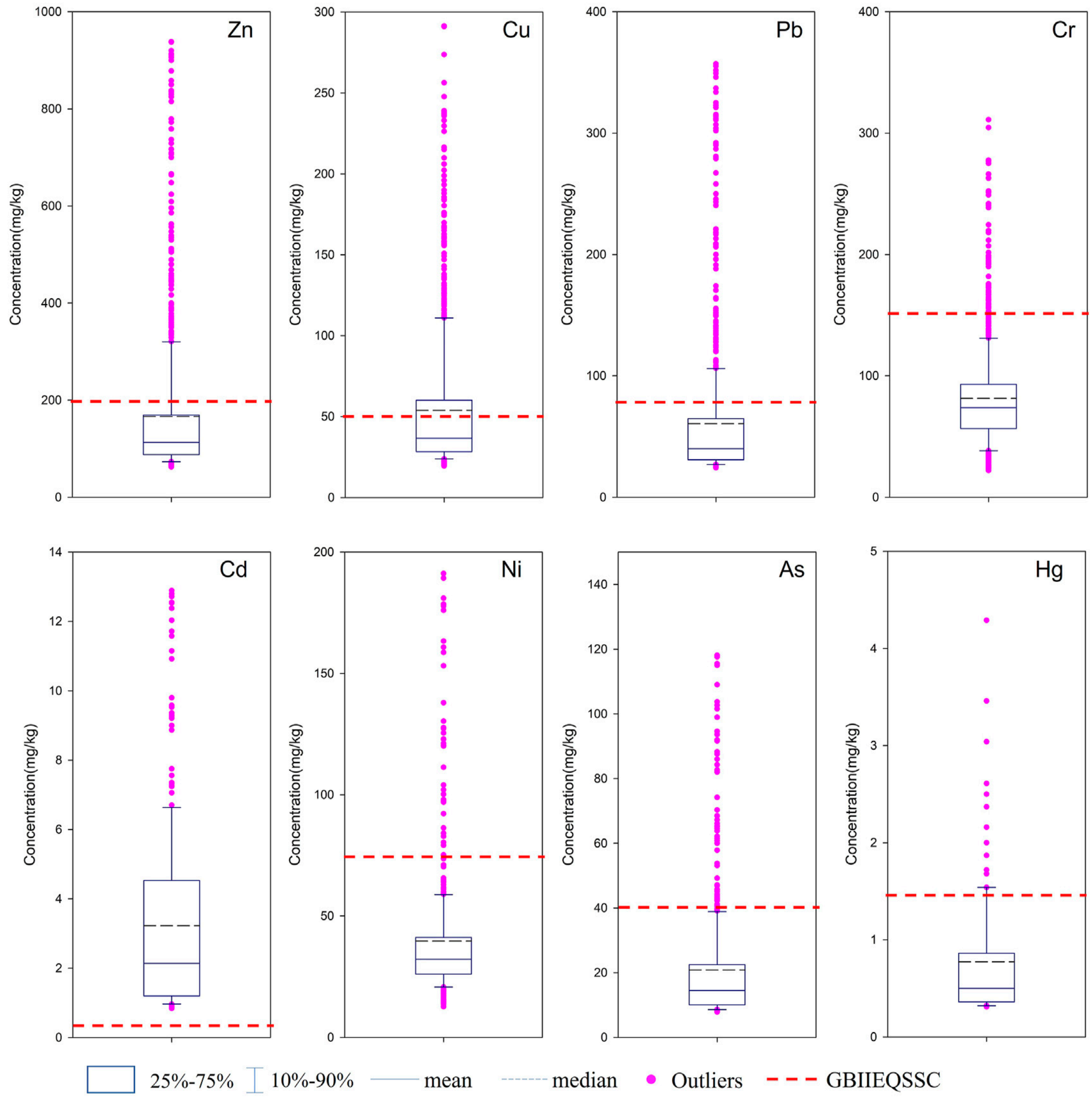

FIGURE 4
Boxplots of heavy metal concentration (mg/kg) in soils of the Yangtze River Basin (GBIIEQSSC: Secondary Standard for Soil Environmental Quality).

TABLE 1 Other descriptive statistics of heavy metal concentration in soils.

Heavy metals	Exceed rate (%)	Standard deviation (mg/kg)	Variation coefficient (%)	Kurtosis	Skewness
Zn	19.74	150.52	90.53	9.68	2.99
Cu	33.26	44.01	81.39	7.13	2.51
Pb	16.68	58.23	95.20	10.71	3.17
Cr	7.24	44.15	53.84	6.40	2.10
Cd	51.58	2.79	85.82	2.59	1.70
Ni	7.93	27.04	68.20	11.83	3.21
As	11.20	18.94	90.97	10.16	3.02
Hg	11.54	0.67	86.41	8.06	2.60

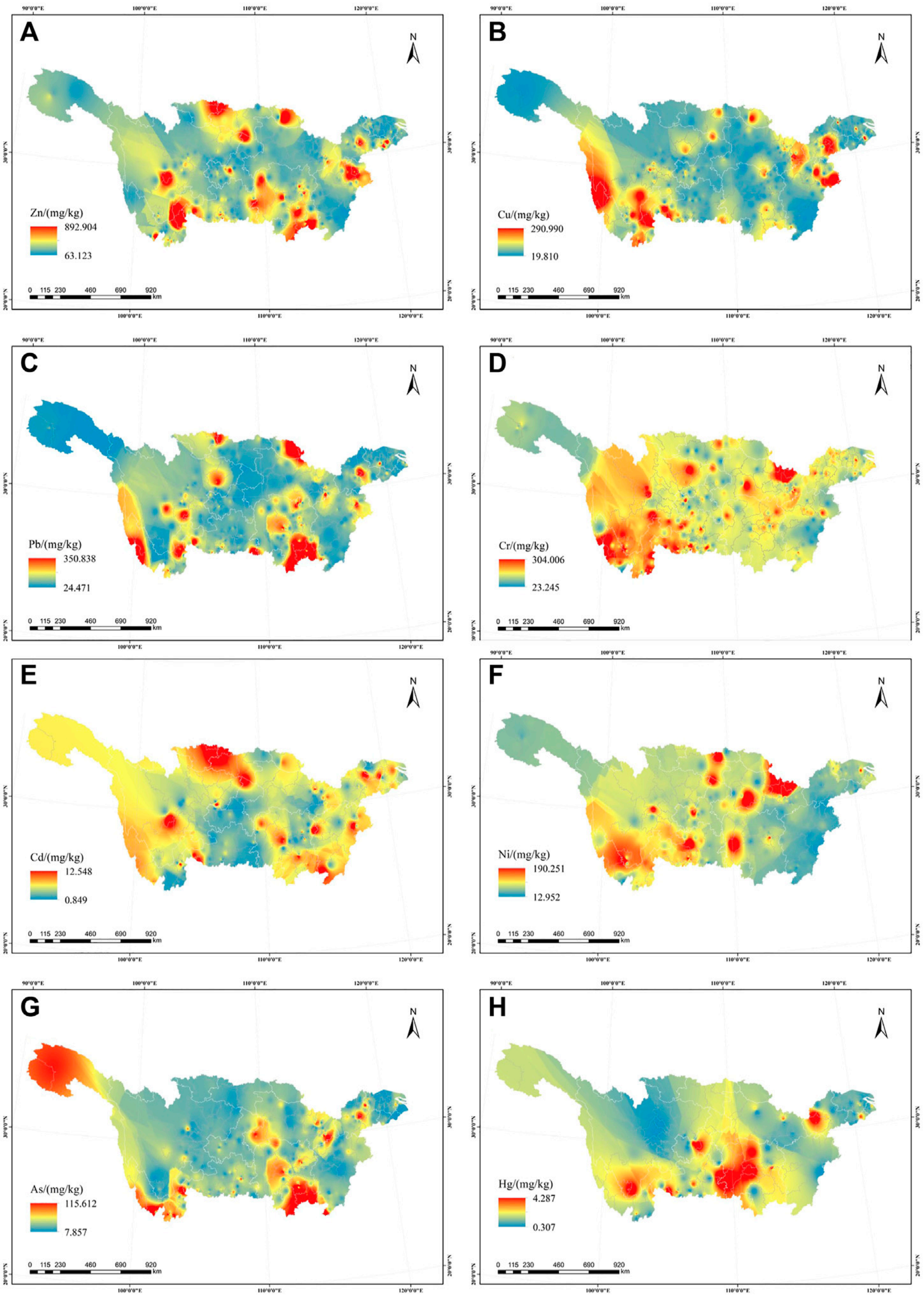

FIGURE 5
Spatial distribution of heavy metal pollution in soils of the Yangtze River Basin [**(A)**: Zn; **(B)** Cu; **(C)** Pb; **(D)** Cr; **(E)** Cd; **(F)** Ni; **(G)** As; **(H)** Hg].

and Binzhou in the south and Loudi and Huaihua in the west. In general, the spatial distribution of heavy metals in soils of the Yangtze River Basin was obviously lumpy, which may be closely related to human activities such as industrial emission and traffic emissions.

3.2 Degree of heavy metal pollution

Figure 6 depicts the spatial distribution of Nemerow's synthetic pollution index of heavy metals in soils of the Yangtze River Basin, and it can be observed that the spatial distribution was uneven, with cities as the dividing point. The soils in the upper reaches of Yushu City and the lower reaches of Nanjing City were mainly clean, and that in the middle were mainly mild or moderate pollution. The serious pollution regions were distributed in plates, accounting for 23.19% of the Yangtze River Basin, mainly distributed in the western Sichuan, the border between Sichuan and Guizhou, the border between Sichuan and Yunnan, eastern Hubei, Hunan, Anhui. Crops and soils in the regions were more seriously polluted, and soil vulnerability was higher. The moderate pollution regions were mostly distributed on the periphery of the serious pollution regions,

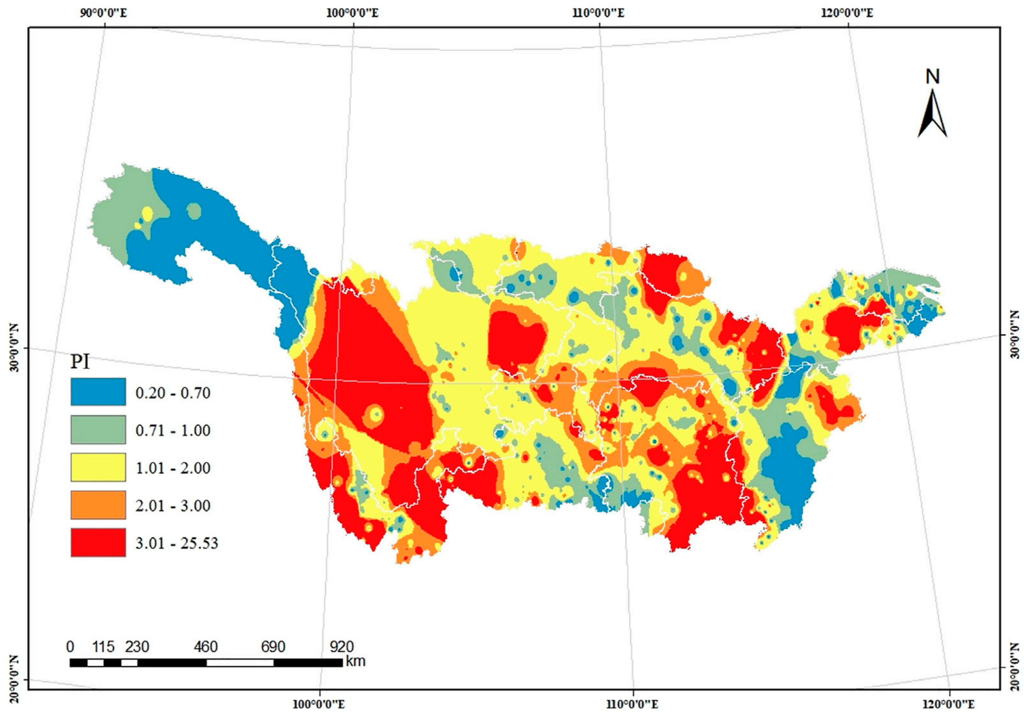

FIGURE 6
Distribution of Nemerow's synthetic pollution index of heavy metals in soil in the Yangtze River Basin.

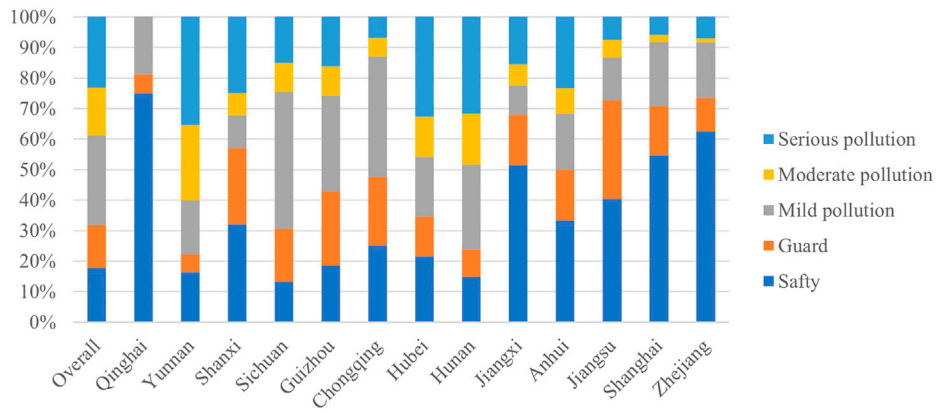

FIGURE 7
Percentage of heavy metal pollution degree for each province in the Yangtze River Basin.

accounting for about 15.58% of the Yangtze River Basin. The mild pollution regions accounted for 29.25%, mainly distributed in the middle reaches of the Yangtze River, including central and eastern Sichuan, Chongqing, northern Hubei, southern Guizhou, southern Gansu and southern Shaanxi. Safety and guard regions were distributed in the upper reaches and Jiangxi, where the soils were in clean state. In short, the proportion of polluted soil in the Yangtze River Basin was 68.01%, while the proportion of clean soil was only about 30%, indicating that the soil in the Yangtze River Basin was seriously polluted by heavy metals.

Figure 7 shows the percentage of heavy metal pollution degree for each province in the Yangtze River Basin. It can be observed that the proportions of serious pollution regions in Yunnan, Hunan and Hubei reached 35.29%, 31.67% and 32.64%, which were higher than the average level of the whole basin, which were related to their rich mineral resources and active human industrial activities. Yunnan is known as the kingdom of non-ferrous metals, with the largest reserves of Zn and Cd and the third largest reserves of Cu and Ni in China. Hunan is known as the hometown of non-ferrous metals, with Pb reserves ranking the third, Zn and Hg reserves ranking the top

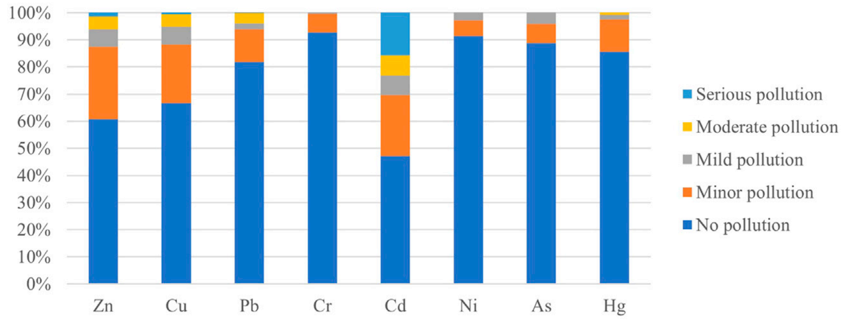

FIGURE 8
Percentage of pollution degree for each heavy metal in the Yangtze River Basin.

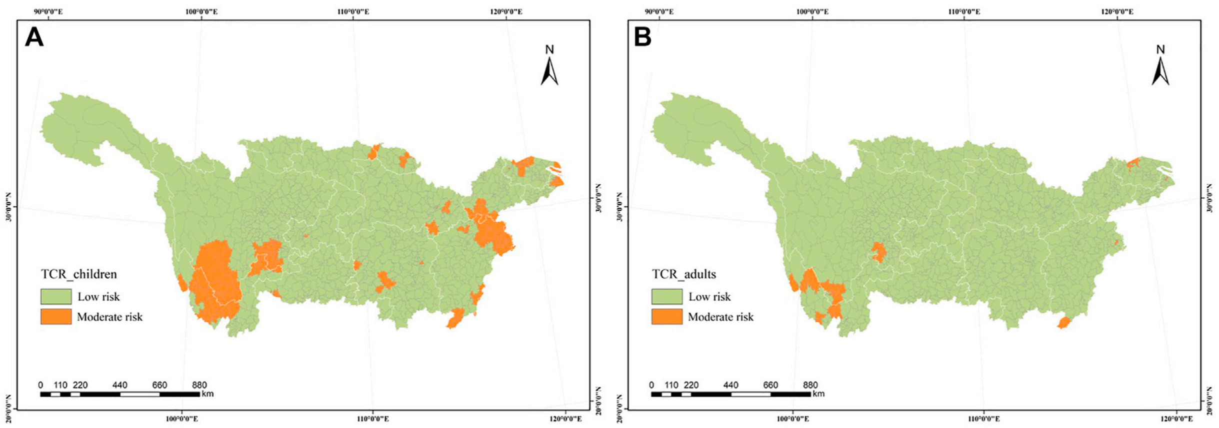

FIGURE 9
Distribution of soil carcinogenic risk of heavy metals at the district or county level in the Yangtze River Basin for **(A)** adults and **(B)** children.

five in China. Huangshi, as a traditional mining city, has the largest mineral resource in Hubei. Human activities such as mineral development, smelting, and waste residue treatment have discharged a large amount of heavy metals. The safety and guard regions in Qinghai, Jiangxi and Shanghai accounted for more than 50%, Jiangsu was 40.16%, Shaanxi and Anhui were 32.14% and 33.33%, and the proportion of clean soil in other provinces was less than 30%. It can be seen that the degree of soil pollution varied greatly in different provinces, and there was a certain relationship between the spatial distribution of polluted region and mineral resources.

Figure 8 shows the percentage of pollution degree for each heavy metal in the Yangtze River Basin. The overall pollution degree of the eight typical heavy metals was Cd > Zn > Cu > Pb > Hg ~ As ~ Ni ~ Cr. The proportion of sampling points with serious pollution for Cd accounted for 15.63% of all sampling points, and Zn, Cu and Pb were 1.40%, 0.53% and 0.10%, respectively. The proportion of sampling points with Cd concentration above the secondary standard value of soil environmental quality accounted for 50%. Therefore, Cd pollution in soils of the Yangtze River Basin was relatively serious.

3.3 Health risk assessment of heavy metal

3.3.1 The assessment of carcinogenic risk (CR)

Supplementary Figure S1 shows the distribution of carcinogenic risk in the Yangtze River Basin using Inverse Distance Weight. Then, the distribution of carcinogenic risk in the Yangtze River Basin at the district or county level using Zonal Statistics was showed in Figure 9. It can be seen that the carcinogenic risks for adults and children were mainly low or medium, of which the low carcinogenic risk for adults was 99.73% and that for children was 97.89%. The regions with moderate carcinogenic risk for adults were mainly distributed in Gucheng District, Ninglang Yi and Yulong Naxi Autonomous County in Lijiang City, Yunnan Province (the carcinogenic risk of Cd was high through dermal absorption), Wuding, Yuanmou and Yao'an County in Chuxiong Yi Autonomous Prefecture (the carcinogenic risk of Cd and Cr was high through dermal absorption), Yanbian and Miyi County in Panzhihua City, Sichuan Province (the carcinogenic risk of Cd was high through dermal absorption), accounting for 0.27% of the total river basin. The moderate risk regions for children included the moderate risk regions for adults and its peripheral regions, accounting for 2.11%

TABLE 2 The carcinogenic risk of heavy metals for adults and children ($\times10^{-6}$).

Object	Type	Min	Max	Medium	Mean	Standard deviation
Adults	Pb	0.026	0.32	0.041	0.054	0.042
	Cr	3.58	49.80	11.50	12.43	6.07
	Cd	0.094	50.10	1.83	4.10	6.48
	As	3.42	44.20	5.94	7.63	5.66
Children	Pb	0.036	0.45	0.057	0.075	0.058
	Cr	4.96	69.00	15.90	17.20	8.40
	Cd	0.13	69.10	2.52	5.65	8.93
	As	4.74	61.20	8.23	12.20	7.83

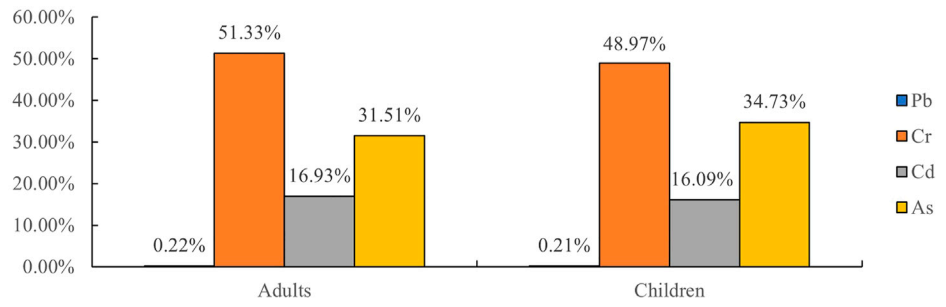

FIGURE 10
The contribution of various heavy metals to carcinogenic risk for adults and children.

of the total basin. The reasons for the larger area of the carcinogenic risk for children are the lower average body weight (BW), lower carcinogenic dose, higher soil ingestion rate (IR_{ing}), higher adherence factor (AF), etc.

The carcinogenic risk of Pb, Cr, Cd and As in soils of the Yangtze River Basin for adults and children are shown in Table 2. Figure 10 shows the contribution of various heavy metals to carcinogenic risk. The total carcinogenic risk for children (3.51×10^{-5}) was higher than that for adults (2.42×10^{-5}), and children were 1.45 times that of adults. However, the carcinogenic risk of soil heavy metals was lower than 10^{-4}, the carcinogenic risk of soil heavy metals to human health was within an acceptable range. The carcinogenic risk of heavy metals for adults and children was Cr > As > Cd > Pb. The contribution rate of Cr for adults and children was the highest, reaching 51.33% and 48.97%, followed by As (31.51% and 34.73%) and Cd (16.93% and 16.09%), while the contribution rate of Pb was less than 0.5%.

3.3.2 The assessment of non-carcinogenic risk (NCR)

Tables 3, 4 shows the non-carcinogenic risk of soil heavy metals in the Yangtze River Basin for adults and children, respectively. It can be found that for adults, the non-carcinogenic risk was manifested as $HQ_{der} > HQ_{ing+inh}$, of which the non-carcinogenic risk through dermal absorption of Pb was 7.98 times that of ingestion and inhalation, the others were more than 10 times, and Cd and Cr reached more than 1,000 times. For children, the non-carcinogenic risk through dermal absorption was also greater. The non-carcinogenic risk through dermal absorption of Pb was 2.26 times that of ingestion and inhalation, followed by As and Zn, and other heavy metals were more than 10 times. The non-carcinogenic risk through ingestion and inhalation were As > Pb > Hg > Ni > Cr > Cu > Cd > Zn. The non-carcinogenic risk through dermal absorption showed the different trend: Cr > Cd > As > Ni > Hg > Pb > Cu > Zn. For adults, the non-carcinogenic risk of Cr was greater than 1, and that of other heavy metals was less than 1, indicating that Cr had obvious non-carcinogenic risk for adults, and Cd and As in some sampling points had significant non-carcinogenic risk. The non-carcinogenic risk for children was generally higher than for adults. The non-carcinogenic risk of Cr and Cd for children was greater than 1, indicating that Cr and Cd had significant non-carcinogenic risk for children and were exposed through dermal absorption. In addition, the maximum value of non-carcinogenic risk of Ni and As was greater than 1, indicating that Ni and As in some sampling points had significant non-carcinogenic risks, and other heavy metals had no significant non-carcinogenic risks.

3.4 Prospect

This study shows the spatial distribution of heavy metals in the soil of the Yangtze River Basin and assesses the health risks.

TABLE 3 The non-carcinogenic risk of soil heavy metals in the Yangtze River Basin for adults.

Risk	Heavy metal	Max	Min	Medium	Mean	Standard deviation	NCR
Zn	$HQ_{ing+inh}$	9.23E-04	6.13E-05	1.11E-04	1.64E-04	1.48E-04	3.43E-03
	HQ_{der}	1.84E-02	1.22E-03	2.22E-03	3.26E-03	2.95E-03	
Cu	$HQ_{ing+inh}$	2.21E-03	3.62E-08	2.52E-04	3.56E-04	3.31E-04	2.69E-02
	HQ_{der}	1.47E-01	9.54E-03	1.80E-02	2.65E-02	2.16E-02	
Pb	$HQ_{ing+inh}$	3.21E-02	2.02E-03	3.37E-03	5.15E-03	4.90E-03	4.62E-02
	HQ_{der}	2.56E-01	1.61E-02	2.69E-02	4.11E-02	3.91E-02	
Cr	$HQ_{ing+inh}$	2.06E-03	1.33E-04	4.61E-04	5.12E-04	2.77E-04	3.20E+00
	HQ_{der}	1.29E+01	8.30E-01	2.89E+00	3.20E+00	1.74E+00	
Cd	$HQ_{ing+inh}$	3.46E-03	3.18E-06	9.39E-05	2.79E-04	4.89E-04	7.80E-01
	HQ_{der}	9.66E+00	8.88E-03	2.62E-01	7.80E-01	1.36E+00	
Ni	$HQ_{ing+inh}$	2.82E-03	1.86E-04	4.75E-04	5.85E-04	3.99E-04	1.52E-01
	HQ_{der}	7.29E-01	4.81E-02	1.23E-01	1.51E-01	1.03E-01	
As	$HQ_{ing+inh}$	1.16E-01	8.17E-03	1.66E-02	2.29E-02	1.95E-02	3.58E-01
	HQ_{der}	1.70E+00	1.19E-01	2.42E-01	3.35E-01	2.85E-01	
Hg	$HQ_{ing+inh}$	4.23E-03	6.68E-05	4.93E-04	7.54E-04	6.60E-04	1.08E-01
	HQ_{der}	6.01E-01	9.51E-03	7.01E-02	1.07E-01	9.38E-02	

TABLE 4 The non-carcinogenic risk of soil heavy metals in the Yangtze River Basin for children.

Risk	Heavy metal	Max	Min	Medium	Mean	Standard deviation	NCR
Zn	$HQ_{ing+inh}$	5.17E-03	3.43E-04	6.23E-04	9.11E-04	8.25E-04	6.04E-03
	HQ_{der}	2.90E-02	1.92E-03	3.49E-03	5.13E-03	4.64E-03	
Cu	$HQ_{ing+inh}$	1.24E-02	3.85E-08	1.41E-03	1.99E-03	1.85E-03	4.37E-02
	HQ_{der}	2.31E-01	1.50E-02	2.83E-02	4.17E-02	3.39E-02	
Pb	$HQ_{ing+inh}$	1.79E-01	3.52E-04	1.89E-02	2.86E-02	2.70E-02	9.32E-02
	HQ_{der}	4.03E-01	2.54E-02	4.22E-02	6.46E-02	6.15E-02	
Cr	$HQ_{ing+inh}$	2.49E-03	1.60E-04	5.56E-04	6.18E-04	3.35E-04	5.04E+00
	HQ_{der}	2.03E+01	1.31E+00	4.54E+00	5.04E+00	2.73E+00	
Cd	$HQ_{ing+inh}$	1.62E-02	1.75E-05	5.13E-04	1.49E-03	2.51E-03	1.22E+00
	HQ_{der}	1.52E+01	1.42E-04	4.09E-01	1.21E+00	2.11E+00	
Ni	$HQ_{ing+inh}$	1.58E-02	1.04E-03	2.66E-03	3.28E-03	2.24E-03	2.41E-01
	HQ_{der}	1.15E+00	7.56E-02	1.93E-01	2.38E-01	1.62E-01	
As	$HQ_{ing+inh}$	6.51E-01	2.88E-05	9.28E-02	1.28E-01	1.09E-01	6.55E-01
	HQ_{der}	2.67E+00	1.88E-01	3.80E-01	5.27E-01	4.48E-01	
Hg	$HQ_{ing+inh}$	2.37E-02	3.74E-04	2.76E-03	4.22E-03	3.69E-03	1.73E-01
	HQ_{der}	9.46E-01	1.50E-02	1.10E-01	1.69E-01	1.48E-01	

However, the collected soil heavy metal data may differ in sampling methods, sample size and analysis methods. The Yangtze River Basin is large and diverse, and the concentration of heavy metals in soil varies significantly depending on soil type, land use, agricultural activities and industrial activities. This study may not capture the full extent of this variability. Therefore, in order to better understand the impact mechanism of heavy metal pollution in the Yangtze River Basin, more research needs to be conducted.

4 Conclusion

In conclusion, this paper depicted the spatial distribution pattern of typical heavy metals in soil of the Yangtze River Basin, evaluated the degree of heavy metal pollution and human health risks, and provided reference for the decision-making of heavy metal pollution control over the region. The results showed that the spatial distribution of heavy metal in soil of the Yangtze River Basin was highly heterogenous. The spatial distribution of Cu and Pb had a certain similarity, the high-value area of Zn was relatively scattered, and the high-value areas of Cr, Cd and Ni were distributed in blocks. The degree of heavy metal pollution in soil of the Yangtze River Basin was low in the east-west and high in the middle. The degree of heavy metal in soil was mainly mild pollution, and the distribution of seriously polluted regions were in blocks, accounting for 23.19% of the total basin. The seriously polluted regions were mainly distributed in the western Sichuan, northern Yunnan, southern Hunan, southern Anhui, which were inseparable from local industrial activities such as mining and smelting, chemical plant production, and human activities such as agricultural production and automobile emissions. The carcinogenic risk of heavy metals was used to measure human health risk, and it was found that the carcinogenic risk for children was about 1.45 times that for adults. The risk areas were mainly distributed in the junction of Yunnan and Sichuan and northern Jiangxi, and the main carcinogenic heavy metal was Cr. From the perspective of the non-carcinogenic risk of heavy metals, dermal absorption was the main exposure pathway, Cr had remarkable non-carcinogenic risk for adults, and Cr and Cd have remarkable non-carcinogenic risks for children. The results have important guiding significance for the control of heavy metal pollution in the Yangtze River Basin.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding authors.

Author contributions

SL, DL, and YS contributed to conception and design of the study. DL organized the database. DL and YS performed the statistical analysis. YS wrote the first draft of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

Funding

This work was supported by the National Key R&D Program of China (2020YFC1908705).

Conflict of interest

Authors YS, XZ, and WnC were employed by Yangtze Ecology and Environment Co., Ltd., Author DL was employed by Huadong Engineering Zhengzhou Corporation Limited Co., Ltd. and Authors YW, XH, YL, and WiC were employed by Yangtze Clean Energy Conservation and Environmental Protection Co., Ltd.

The remaining author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fenvs.2023.1197634/full#supplementary-material

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frontiers | Frontiers in Environmental Science

TYPE Original Research
PUBLISHED 29 June 2023
DOI 10.3389/fenvs.2023.1221573

OPEN ACCESS

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RECEIVED 12 May 2023
ACCEPTED 19 June 2023
PUBLISHED 29 June 2023

CITATION
Pietrini F, Passatore L, Carloni S, Massimi L, Astolfi ML, Giusto C and Zacchini M (2023), Bismuth exposure affects morpho-physiological performances and the ionomic profile in garden cress (*Lepidium sativum* L.) plants. *Front. Environ. Sci.* 11:1221573. doi: 10.3389/fenvs.2023.1221573

Bismuth exposure affects morpho-physiological performances and the ionomic profile in garden cress (*Lepidium sativum* L.) plants

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Environmental pollution caused by heavy metals has long been considered a relevant threat to ecosystem survival and human health. The use of safer substitutes for the most toxic heavy metals in many industrial applications is discussed as a potential way to face this issue. In this regard, Bi has been proposed for replacing Pb in several production processes. However, few literature records reported on the effects of Bi on living organisms, particularly on plants. In this study, garden cress (*Lepidium sativum* L.) plants were exposed to different concentrations of Bi nitrate added to soil in growth chambers for 21 days. Results evidenced the toxic effect of Bi on shoot growth, regardless of the Bi nitrate concentration in the soil, paralleled by a similar reduction in the chlorophyll and carotenoid content, a decrease in the nitrogen balance index values, and an impairment of the photosynthetic machinery evaluated by chlorophyll fluorescence image analysis. The presence of Bi in the soil was shown to affect element accumulation in roots and translocation to shoots, with micronutrient content particularly reduced in the leaves of Bi-treated plants. A dose-dependent plant accumulation of Bi to metal concentration in the soil was observed, even if very low metal bioconcentration ability was highlighted. The reduced Bi translocation from roots to shoots in plants exposed to increasing Bi concentrations in the soil is discussed as a possible defense mechanism likely associated with the observed increase of anthocyan and flavonol contents and the activation of photoprotection mechanisms preventing higher damages to the photosynthetic apparatus.

KEYWORDS

environmental pollution, heavy metals, metal toxicity, photosynthesis, pigments, trace elements

1 Introduction

The increasing occurrence of heavy metals (HMs) in the environmental matrices, resulting from industrial processes and mining activities, and their recognized toxicity on biota are necessitating the adoption of effective solutions for mitigating their impact on the ecosystem and human health. In this context, replacing toxic HMs in industrial processes with safer ones is considered promising. To enhance the environmental sustainability of many industrial applications involving lead (Pb), the utilization of bismuth (Bi) as a Pb substitute has attracted increasing interest (Rohr, 2002; Esquivel-Gaon et al., 2015). In this regard, Wang et al. (2019) reported the use of Bi as a nontoxic replacement for Pb in brass plumbing fixtures, fishing sinkers, free machining steels, and solders, as well as a metallurgical additive in the foundry. Bismuth, a minor metal with a natural abundance 10-fold less than that of antimony (Sb), is considered a “green metal” given its use for treating many health issues since ancient times (Udalova et al., 2008; Wang et al., 2019). The increasing use of Bi among medicinal applications is highly predictable as Bi has been recognized as a theranostic agent (Badrigilan et al., 2020). As the use of Bi-containing products is increasing, a rapid increase in the concentrations of this HM in the environment has to be expected. In this regard, with respect to Bi concentrations in natural soil, ranging from 0.13 to 40 μg g^{-1} D.W. (Das et al., 2006; Fahey et al., 2008), extremely high values were reported by Elekes and Busuioc (2010) for a forest soil close to a highway (Bi concentrations ranged from 930 to 1,891 mg kg^{-1}) and by Wei et al. (2011) near an antimony mine (Bi concentrations up to 1,672 mg kg^{-1}). Soil enrichment in Bi is also predictable as its release in agriculture through phosphate fertilization is suspected. Concerning the aquatic compartment, Bi increase can be the result of the leaching from metal-contaminated soils, as for other metals (Fu et al., 2010), or the release from wastewater (Amneklev et al., 2015; Amneklev et al., 2016). Also, the presence of Bi in the alpine ice was recently reported by Legrand et al. (2023). Finally, Bi can be found in the environment at high relative concentrations due to the use of fireworks (Massimi et al., 2021).

Despite the growing interest in the use of Bi, information on the effects of Bi on biological organisms is not exhaustive. In fact, scarce literature is actually present on the toxicity of Bi on biota, as reported by Badrigilan et al. (2020) in human and animal cells and by Murata et al. (2006) and Omouri et al. (2018) in microbes and earthworms, respectively. Consistently, only few papers recently focused on the effects of Bi on plant species. Seed germination and root elongation were negatively affected by treating perennial ryegrass seeds with Bi nitrate and citrate (Omouri et al., 2019), while the exposure of tomato plants to increasing concentrations of Bi nitrate resulted in enhanced toxicity effects, such as the reduction of shoot and root biomass (Nagata and Kimoto, 2020). Furthermore, the germination and root growth of radish seeds were reduced in soil with 30 and 300 mg kg^{-1} of Bi nitrate (Sudina et al., 2021).

Physiological processes underpinning photosynthetic machinery in plants are very sensitive to metals (Khan et al., 2019). Among them, pigment metabolism and the photochemical efficiency of photosystem II have been long claimed (Aggarwal et al., 2012; Pietrini et al., 2015). In particular, chlorophyll content is recognized as an endpoint to assess ecotoxicity in plants, also in official protocols (ISO/DIS, 20079, 2004; OECD n. 221, 2006a). Carotenoids and anthocyans and flavonols (flavonoids) have also been reported as involved in the plant response to metal stress (Chandra and Kang, 2016; Baskar et al., 2018; Shomali et al., 2022). In this context, it is worth mentioning that pigment content can be effectively evaluated in a non-destructive manner by leaf transmittance and spectral reflectance (Goulas et al., 2004; Croft and Chen, 2017; Pietrini et al., 2023). As a proxy of the photosynthetic efficiency, chlorophyll fluorescence parameters are commonly utilized for detecting toxic effects caused in leaves by stressors (Moustakas et al., 2021), including metals (Pietrini et al., 2015; 2017; 2020). In this regard, the suitability of different chlorophyll fluorescence parameters to highlight the damaging effect of a stressor is debated in the literature. In fact, the photochemical efficiency of photosystem II (F_v/F_m), measured in dark-adapted leaves, is the most used parameter in plant stress studies, but it is often reported as less sensitive and responsive than others, as, e.g., the effective quantum yield of PSII photochemistry (ΦPSII) (Pietrini et al., 2015; Malinská et al., 2020).

Recently, Passatore et al. (2022) observed root growth inhibition, genotoxic effects, and dose-dependent Bi accumulation in *Lepidium sativum* L (garden cress) plantlets after treating the seeds with different Bi nitrate concentrations. In order to get an insight into the toxicity effects and accumulation ability in plants, plants of *Lepidium sativum* were grown in potted soil supplied with three different levels of Bi nitrate, chosen among those previously used (Passatore et al., 2022). *Lepidium sativum* was utilized, as in the previous work, being a model plant for ecotoxicological assay (APAT-RTI CTN_TES 1/2004, 2004; OECD n. 208, 2006b). The effects at biometric and physiological levels, specifically focusing on growth, element uptake and translocation, and photosynthetic performances, were investigated along with the Bi accumulation in roots and shoots. The results were discussed, highlighting the main physiological traits involved both in Bi toxicity in plants and in the defense response likely counteracting the toxic action of the metal.

2 Materials and methods

2.1 Seed germination and plant growth

Garden cress (*L. sativum*) seeds (Ingegnoli seed company, Italy) were sowed in plastic pots (2 L volume, 20 seeds/pot, full seed germination) filled with soil (select black peat substrate, pH 6, EC 0.3 dS/m, apparent density 100 kg/m^3, total porosity 85%, Klasmann-Deilmann, Germany) and placed in a growth chamber (temperature 25°C ± 1°C, 16 L/8 D photoperiod, 70% ± 5% humidity, 350 ± 50 μEm^2s^{-1} PPFD). The soil was added or not with Bi nitrate pentahydrate (Sigma-Aldrich, code 248592, 98% purity) solution to reach four different Bi nitrate concentrations: 0 mg kg^{-1}, control; 30 mg kg^{-1}; 121 mg kg^{-1}; and 485 mg kg^{-1}. Particular care was taken to assure that all the soil volume was homogeneously mixed with the Bi solution. Four pots per thesis were randomly distributed in the growth chamber. Soil humidity was maintained at 70% water holding capacity. At the end of the cultivation period (21 days), after physiological measurements (see Section 2.2), the plants were harvested, gently washed with distilled water, separated into roots

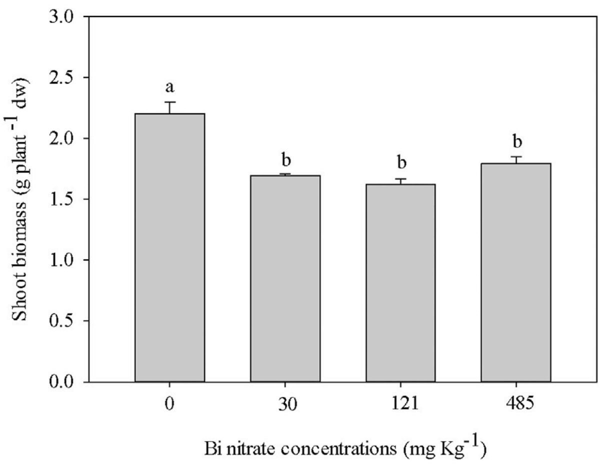

FIGURE 1
Shoot biomass (g plant^{-1} dw) in garden cress (*Lepidium sativum* L.) plants grown in pots filled with soil supplied with bismuth nitrate (0 mg kg^{-1}, control; 30 mg kg^{-1}; 121 mg kg^{-1}; and 485 mg kg^{-1}) for 21 days in controlled conditions in a growth chamber. In each bar, mean data (n = 4, ± S.E.) are shown. Different letters correspond to statistically different values (Tukey's test, $p \leq 0.05$).

and shoots, oven-dried, and weighed for dry weight (DW) evaluation and further processing for elemental analysis (see Section 2.3), as described by Passatore et al. (2022).

2.2 Plant physiological performance analysis

2.2.1 Leaf pigment and nitrogen determination

The estimation of leaf nitrogen and pigment contents was obtained using the leaf-clip portable fluorescence sensor Dualex Scientific+™ (Force-A, France). According to the following equations, it provides four indices: the chlorophyll index (Eq. 1) for leaf chlorophyll concentration in chlorophyll units (Cerovic et al., 2012); the flavonol index (Eq. 2) for epidermal flavonol concentration in absorbance units (Abs. unit); the anthocyanin index (Eq. 3) for epidermal anthocyanin concentration in absorbance units (Abs. unit), and the Nitrogen Balance Index (NBI) index (Eq. 4), as the ratio of chlorophyll to flavonol (Cartelat et al., 2005), which refers to leaf nitrogen content (Cerovic et al., 2012; Cerovic et al., 2015),

$$\text{Chlorophyll index} = (T_{850} - T_{710})/T_{710}, \tag{1}$$

$$\text{Flavonol index} = \log(FRF_R/FRF_{UV}), \tag{2}$$

$$\text{Anthocyanin index} = \log(FRF_R/FRF_G), \tag{3}$$

$$\text{NBI index} = \text{Chlorophyll index}/\text{Flavonol index}, \tag{4}$$

where T_{850} and T_{710} are the leaf transmittance at 850 nm and 710 nm, respectively; FRF is the far-red fluorescence emission (>710 nm) excited by red (R, 650 nm), UV (UV, 375 nm), or green (G, 505 nm) light.

The measurements were taken from at least two representative fully developed leaves per pot. Two Dualex readings were taken from the widest portion of the leaf lamina, avoiding major veins. The two Dualex readings were averaged to represent the leaf value.

2.2.2 Measurements of leaf reflectance and spectral reflectance indices

Leaf reflectance spectra were acquired in the spectral range of 350–1025 nm using an ASD FieldSpec-3 spectroradiometer (Analytical Spectral Devices Inc., Colorado, United States), as reported by Testone et al. (2019), except for respecting a fixed distance (10 cm) to the plant layer to assure a field of view (FOV) of approximately 4 cm (using a 25° bare fiber-optic). Five spectra were then determined to provide a mean spectral value. Three spectral reflectance indices, namely, pigment-specific simple ratio a ($PSSR_a$), pigment-specific simple ratio b ($PSSR_b$), and pigment-specific simple ratio c ($PSSR_c$) (Blackburn et al., 1998), respectively, associated with the chlorophyll *a*, chlorophyll *b*, and carotenoid content, were derived from the collected data and calculated according to the following equations, where R is the reflectance value measured in each band expressed in nm, indicated by the subscript number:

$$PSSR_a = (R_{800})/(R_{680}),$$
$$PSSR_b = (R_{800})/(R_{635}),$$
$$PSSR_c = (R_{800})/(R_{470}).$$

2.2.3 Chlorophyll fluorescence parameters

Chlorophyll fluorescence measurements were performed to assess the efficiency of the plant photosynthetic apparatus. In particular, the maximal quantum yield of PSII photochemistry (F_v/F_m, as the ratio between variable fluorescence and maximal fluorescence), the effective quantum yield of PSII photochemistry (ΦPSII), the quantum yield of regulated non-photochemical energy loss (ΦNPQ), the quantum yield of non-regulated non-

TABLE 1 Chlorophyll index (μg cm^{-2}), anthocyanin index (Abs. unit), flavonol index (Abs. unit), and nitrogen balance index (rel. un.) in leaves of garden cress (*Lepidium sativum* L.) plants grown in pots filled with soil supplied with bismuth nitrate (0 mg kg^{-1}, control; 30 mg kg^{-1}; 121 mg kg^{-1}; and 485 mg kg^{-1}) for 21 days in controlled conditions in a growth chamber (mean data± SE; n = 4). In each column, mean values with different letters are significantly different (Tukey's test, $p \leq 0.05$).

Bi nitrate (mg Kg^{-1})	Chlorophyll index	Anthocyanin index	Flavonol index	NBI
0	19.20 (±0.16) a	0.182 (±0.001) b	0.620 (±0.008) b	31.06 (±0.85) a
30	17.20 (±0.22) b	0.193 (±0.004) ab	0.627 (±0.003) ab	27.51 (±0.80) b
121	16.76 (±0.33) b	0.197 (±0.001) a	0.644 (±0.003) a	26.05 (±0.40) b
485	17.09 (±0.41) b	0.188 (±0.002) ab	0.635 (±0.008) ab	27.08 (±0.82) b

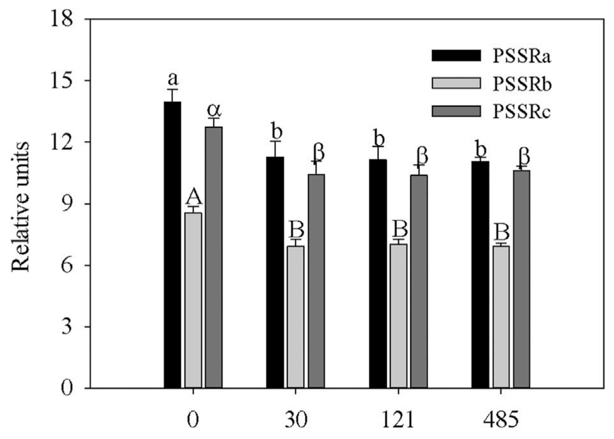

FIGURE 2
Values of spectral indices, $PSSR_a$- pigment-specific simple ratio for Chl_a, $PSSR_b$- pigment-specific simple ratio for Chl_b, and $PSSR_c$- pigment-specific simple ratio for carotenoids, in leaves of garden cress (*Lepidium sativum* L.) plants grown in pots filled with peat substrate supplied with bismuth nitrate (0 mg kg^{-1}, control; 30 mg kg^{-1}; 121 mg kg^{-1}; and 485 mg kg^{-1}) for 21 days in controlled conditions in a growth chamber (mean data± SE; n = 4). Different lower-case letters indicate a significant difference in $PSSR_a$ among different bismuth nitrate concentrations, different capital letters indicate a significant difference in $PSSR_b$ among different bismuth nitrate concentrations, and different Greek letters indicate a significant difference in $PSSR_c$ among different bismuth nitrate concentrations (Tukey's test, $p \leq 0.05$).

photochemical energy loss (ΦNO), the photochemical quenching (qP), the non-photochemical quenching (NPQ), and the electron transport rate (ETR) were measured on fully developed leaves using a MAXI-Imaging-PAM (Walz, Germany). The parameters were measured in 30 min dark-adapted leaves (F_v/F_m) and in leaves adapted to 310 μmol m^{-2} s^{-1} PPFD for at least 5 min to reach a steady-state condition (ΦPSII, ΦNPQ, ΦNO, NPQ, qP, and ETR) and calculated as reported by Di Baccio et al. (2017) and Kramer et al. (2004).

2.3 Ionomic analysis

After collection, shoots and roots were oven-dried at 70°C for 48 h and weighed; 0.02–0.1 g DW of each sample was subjected to microwave-assisted acid digestion for 30 min at 180°C using a HNO_3/H_2O_2 mixture (2:1, v/v). The digested solution was then diluted with deionized water and filtered before elemental analysis.

The concentration of 40 chemical elements, of which only the concentration of 12 macro- and trace elements is reported and discussed, was analyzed in replicate with an inductively coupled plasma mass spectrometer (ICP-MS; model 820-MS; Bruker, Germany). Element concentrations were divided by the DW of each sample to obtain mg kg^{-1} concentrations. Standard deviations of the replicates were all below 10%. Further details on sample preparation, elemental analysis, and instrumental conditions are reported in the work of Passatore et al. (2022) and Astolfi et al. (2020), respectively. As reported by Zacchini et al. (2009), the bioconcentration factor (BCF) was calculated as the ratio between the metal concentration in the plant organ (mg Kg^{-1}) and the metal concentration in the soil (mg Kg^{-1}); the translocation factor (T*f*) was calculated as the ratio between the metal concentration in plant shoots (mg Kg^{-1}) and the metal concentration in plant roots (mg Kg^{-1}).

2.4 Statistics

The trial was arranged following the methodologies of the OECD guidelines for ecotoxicological tests with *L. sativum* (OECD n. 208, 2006b). Data reported in tables and figures refer to at least four replicates for the variant. One-way ANOVA was used to process normally distributed data to evaluate the effects of Bi nitrate concentrations on the different parameters using the SPSS (IL, United States) software tool. The statistical significance of the mean data was assessed by Tukey's test ($p \leq 0.05$), unless otherwise stated.

3 Results and discussion

Shoot biomass produced within 21 days of cultivation in soil supplied with four different Bi nitrate concentrations was evaluated to investigate the effect of Bi on the growth of *L. sativum* plants. The results shown in Figure 1 highlighted that regardless of the Bi concentration, Bi exposure caused a significant growth reduction, ranging approximately from 20% to 26% to control. This finding is in accordance with a previous work on the germination of *L. sativum* seeds, where root growth inhibition was observed (Passatore et al., 2022). To our knowledge, no medium-term experiments with plants grown in Bi-treated soil are present in the literature. In a short trial (7 days) with artificially Bi-spiked soil, Omouri et al. (2019) observed a significant root growth inhibition in ryegrass plants, but did not find any shoot biomass reduction. A toxic effect on shoot growth was instead reported by Nagata and Kimoto (2020) after the treatment of tomato seeds with Bi nitrate in agar. The toxicity of Bi could be related to DNA damage, as recently reported by Passatore et al. (2022) on *L. sativum* seedlings, affecting both gene expression and primary and secondary metabolisms.

To shed light on the effects of Bi at the physiological level, the estimation of both the pigment contents and the nitrogen balance index (NBI, da Silva et al., 2021) was performed using the Dualex sensor (see Section 2.2.1). In Table 1, a reduction in the total chlorophyll content in leaves of plants grown in Bi-added soil is shown. The NBI evidenced a similar trend, being statistically lower in Bi-treated plants compared to control plants, as expected due to the well-studied relationship between chlorophyll and leaf nitrogen content (Sage et al., 1987; Evans, 1989). The reduction of the total chlorophyll content in Bi-exposed plants paralleled the reduction of shoot biomass, indicating this trait as a valuable physiological endpoint for assessing Bi toxicity, as reported for other metals (Pietrini et al., 2015; Chandra and Kang, 2016) and organic pollutants (Kummerová et al., 2006; Pietrini et al., 2022). The suitability of chlorophyll content as a proxy for ecotoxicity assessment in plants has already been proven (ISO/DIS, 20079, 2004; OECD n. 221, 2006a).

As a protective response to the toxic action of Bi, an overall increase of anthocyan and flavonol leaf levels in Bi-treated plants to

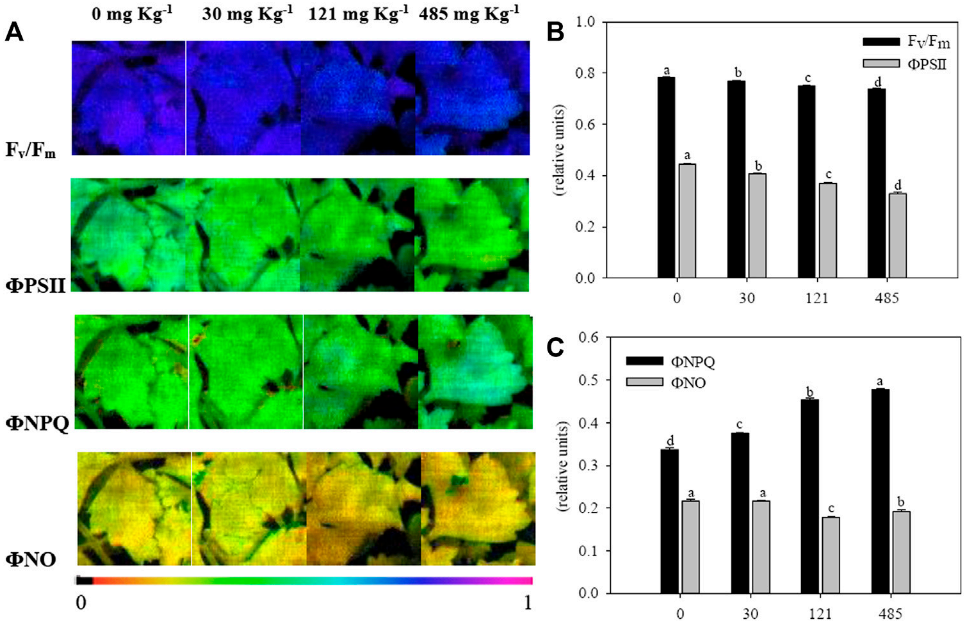

FIGURE 3
Images of chlorophyll fluorescence parameters **(A)** and their associated values **(B, C)** in leaves of garden cress (*Lepidium sativum* L.) plants grown in pots filled with soil supplied with bismuth nitrate (0 mg kg^{-1}, control; 30 mg kg^{-1}; 121 mg kg^{-1}; and 485 mg kg^{-1}) for 21 days in controlled conditions in a growth chamber. Maximum quantum yield of PSII photochemistry (F_v/F_m), the quantum efficiency of PSII photochemistry (ΦPSII), and the quantum yield of regulated (ΦNPQ) and non-regulated (ΦNO) energy dissipation in PSII are measured with an Imaging-PAM M-series system. The false color code depicted at the bottom of each image ranges from 0.000 (black) to 1.000 (pink). Vertical bars represent means (n = 4) ± S.E. One-way ANOVA was applied, and different letters indicate significant differences (Tukey's test, $p \leq 0.05$).

control was observed (Table 1). As reported in the literature, in addition to the long-reported functions as screeners of damaging short-wave solar radiation, flavonoids have been suggested as playing key functions as antioxidants in stressed plants by inhibiting the generation and reducing reactive oxygen species (ROS) once formed (Agati et al., 2012; 2013). In this context, a role for anthocyans (Ai et al., 2018) and flavonoids (Ferdinando et al., 2012) as defense compounds against the oxidative attack caused by HMs has been reported. In this study, the increase in flavonoid content in response to Bi treatment could be due to a stimulation of the ROS production by the metal, as observed by Huang et al. (2022) in the microalga *Chlamydomonas reinhardtii* exposed to nanoscale bismuth oxyiodide (nano-BiOI) at a concentration higher than 25 mg L^{-1}.

Therefore, to further characterize the effects of Bi on pigment content, leaf reflectance spectra of garden cress plants were investigated by analyzing some spectral indices (see Section 2.2.2). As reported by Peñuelas and Filella (1998), leaf surface and internal structure characteristics, in addition to the concentration and distribution of biochemical molecules, are mainly responsible for the leaf reflectance properties. For this reason, leaf reflectance spectra represent a valuable tool to monitor the physiological status of plants under different stress conditions (Croft and Chen, 2017). The values of three spectral reflectance indices ($PSSR_a$, $PSSR_b$, and $PSSR_c$), respectively, associated with chlorophyll *a*, chlorophyll *b*, and carotenoid content, are reported in Figure 2. The reduction in chlorophyll content in Bi-treated plants, previously highlighted in Table 1, was also confirmed by the analysis of $PSSR_a$ and $PSSR_b$ that decreased similarly, regardless of the Bi concentration, with respect to the control. A similar trend was observed for the $PSSR_c$ index (Figure 2). As extensively reported (Sun et al., 2022), carotenoids are not only photosynthetic pigments but also important antioxidants. In Bi-treated plants, the reduction in carotenoid content can be ascribed to a damaging effect exerted by the presence of the metal, likely hampering the plants to eliminate oxygen free radicals in chloroplasts and, thereby, protect chlorophylls from photooxidative damage (Guidi et al., 2017).

To get an insight into the possible mechanisms by which Bi could have affected the photosynthetic machinery in garden cress plants, the analysis of chlorophyll fluorescence parameters and their associated images was utilized. Fluorescence image analysis has been commonly used to evaluate the effects of several HMs on plants, providing information on the photosynthetic apparatus performances (Pietrini et al., 2015; 2017; 2020).

In Figure 3, a representative image of chlorophyll fluorescence parameters (Figure 3A, F_v/F_m, ΦPSII, ΦNPQ, and ΦNO) and chlorophyll fluorescence data (Figures 3B, C) in garden cress leaves are presented. Overall, chlorophyll fluorescence images (Figure 3A) showed that, in dark-adapted leaves, F_v/F_m revealed an almost homogeneous pattern of distribution, both in control and Bi-treated plants. Contrarily, in light-adapted leaves, ΦPSII, ΦNPQ, and ΦNO showed an appreciable heterogeneous pattern of light utilization and photosynthetic activity, especially in plants exposed to 121 and 485 mg kg^{-1} of Bi nitrate. Regarding the photochemical efficiency of photosystem II (PSII), evaluated by F_v/F_m in dark-adapted leaves, results showed a slight decreasing trend as the Bi

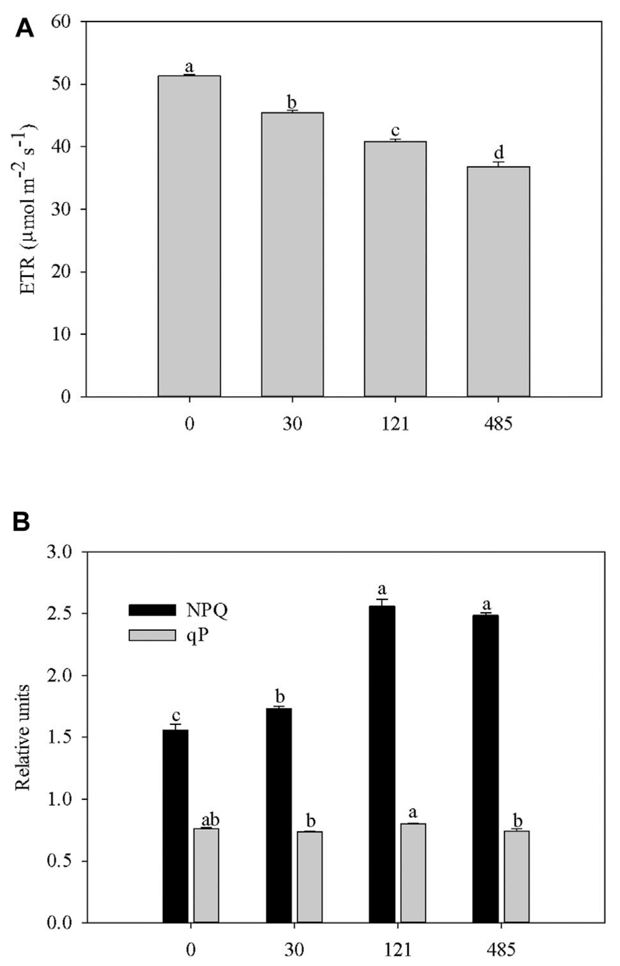

FIGURE 4
Changes in the electron transport rate through PSII (ETR) **(A)** and photochemical (qP) and non-photochemical quenching (NPQ) **(B)** in leaves of garden cress (*Lepidium sativum* L.) plants grown in pots filled with soil supplied with bismuth nitrate (0 mg Kg^{-1}, control; 30 mg Kg^{-1}; 121 mg Kg^{-1}; 485 mg Kg^{-1}) for 21 days in controlled conditions in growth chamber. Vertical bars represent means (n = 4) ±S.E. One-way ANOVA was applied and different letters indicate significant difference (Tukey test, $p \leq 0.05$).

concentration increased (Figure 3B). Compared with control (0), the F_v/F_m ratio at 30, 121, and 485 mg kg^{-1} of Bi nitrate decreased by approximately 1, 4, and 6%, respectively. However, it should be stated that all the F_v/F_m values were in the range of 0.75–0.83, considered optimal values for many plant species (Maxwell and Johnson, 2000). In fact, lower values of this parameter indicate that a proportion of the PSII reaction center is damaged or inactivated, commonly observed under stress (Baker and Rosenqvist, 2004). In this regard, Kearns and Turner (2016) reported that the F_v/F_m ratio was not affected in the macroalgae *U. lactuca* exposed for 48 h to 50 μg L^{-1} of Bi. However, it is worth stating that the F_v/F_m ratio is considered less sensitive and responsive to different stressors in comparison to the effective quantum yield of PSII photochemistry (ΦPSII) (Pietrini et al., 2015; Malinská et al., 2020). Therefore, to deepen the knowledge on the effects of Bi on plant photochemistry, the evaluation of the balance between the light capture and the photochemical energy use in plants was performed by measuring the effective quantum yield of PSII photochemistry (ΦPSII), the quantum yield of regulated non-photochemical energy loss (ΦNPQ), and the quantum yield of non-regulated non-photochemical energy loss (ΦNO) (Figures 3B, C). The analysis of the ΦPSII response, measured in light-adapted leaves, confirmed the higher sensitivity of this parameter with respect to F_v/F_m. In fact, compared with control (0), the ΦPSII values in plants exposed to 30, 121, and 485 mg kg^{-1} of Bi nitrate decreased by approximately 7%, 17%, and 26%, respectively (Figure 3B). Regarding the quantum yield of non-photochemical energy loss, with respect to control, the regulated process (ΦNPQ) increased by 10%, 25%, and 29% in 30, 121, and 485 mg kg^{-1} of Bi-treated plants, respectively, while the non-regulated process (ΦNO) decreased only at the highest Bi nitrate concentrations by 18% and 11%, respectively. Overall, the reported data highlight a quick effective photoprotection mechanism to Bi exposure. In fact, the decrease in the ΦPSII values can result from a photoprotective increase in thermal energy dissipation (ΦNPQ) induced by an excess of absorbed light (Demmig-Adams and Adams, 1992). A non-effective dissipation of excess excitation energy can result in photo-oxidative stress, possibly leading to the production of reactive oxygen species (ROS) (Smirnoff, 1993). Therefore, the increased dissipation by downregulation of the PSII photochemistry, following Bi treatments, seems to overcompensate for the decrease of ΦPSII in such a way that ΦNO decreased. Non-regulated, non-photochemical quenching (ΦNO) consists of chlorophyll fluorescence internal conversions and intersystem crossing, leading to the formation of singlet oxygen ($^1O_2$) (Apel and Hirt, 2004; Klughammer and Schreiber, 2008). The decline or similarity to control of ΦNO values in Bi-treated plants could suggest that ΦNPQ was active in protecting plants from ROS, likely by reducing the $^1O_2$ production, thus limiting the oxidative attack to the photosynthetic apparatus (Moustaka et al., 2018).

The reduction of the electron transport rate (ETR) (Figure 4A), a proxy for photosynthesis, paralleled the response of ΦPSII in Bi-exposed plants, likely being caused by the processes affecting excess light energy dissipation (NPQ) (Kalefetoğlu Macar and Ekmekci, 2009), which, in the form of harmless heat, protect plants from ROS (Hideg et al., 2008; Roach et al., 2020). The overall data assessing the status of the photosynthetic machinery evidenced that the greatest effects in Bi-exposed plants were observed at 121 and 485 mg kg^{-1} of Bi nitrate, concomitantly with the highest decrease of ΦPSII and ETR (Figures 3B, 4A). However, the highest increase in NPQ (Figure 4B) resulted in maintaining a high fraction of open PSII reaction centers (qP), as suggested by the nonstatistical differences between Bi-treated plants and control plants (Figure 4B). According to the literature (Lambrev et al., 2012), this feature is considered a response of maximal photoprotection. Scarce information is reported about the effect of Bi on the plant photosynthetic process. Consistent with the reported results, Prabhavati et al. (2017) highlighted the inhibitory effect on the photosynthesis of *Macrotyloma uniflorum* (Lam.) plants exposed to different Bi concentrations (from 50 to 400 μg g^{-1}).

To evaluate the effects of Bi presence in the soil on the absorption and translocation of nutrients and trace elements, an ionomic analysis was performed by ICP-MS on the roots and shoots of *Lepidium* plants (Figure 5, 6). The study of the ionome has been

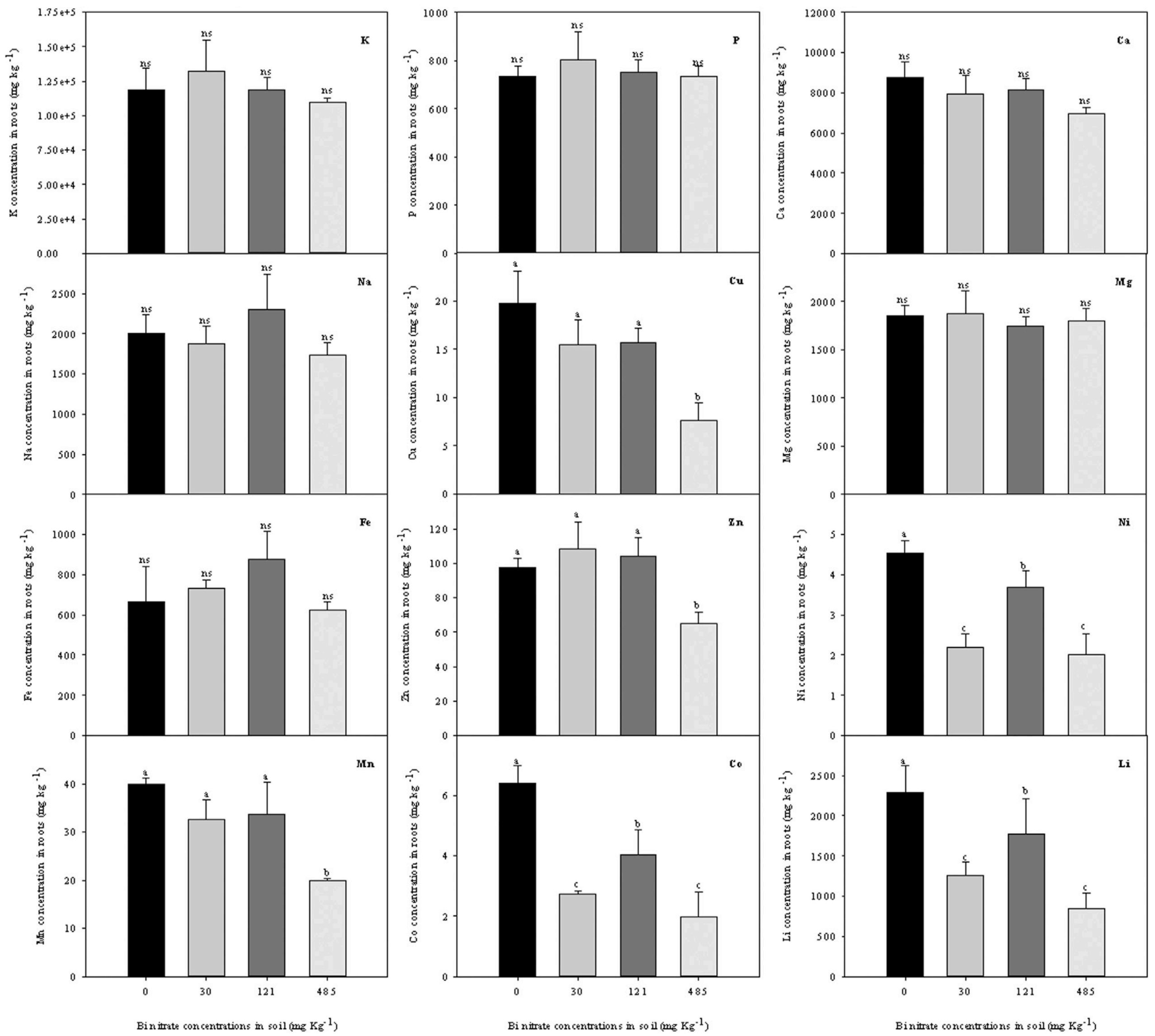

FIGURE 5
Ionomic profile (ion concentration, g kg^{-1} dw) in roots of garden cress (*Lepidium sativum* L.) plants grown in pots filled with soil supplied with bismuth nitrate (0 mg kg^{-1}, control; 30 mg kg^{-1}; 121 mg kg^{-1}; and 485 mg kg^{-1}) for 21 days in controlled conditions in a growth chamber. In each bar, mean data (n = 4, ± S.E.) are shown. Different letters correspond to statistically different values (Tukey's test, $p \leq 0.05$, ns = not significant).

long described as a useful tool to simultaneously assess the elemental composition and its change in response to physiological stimuli in plants (Salt et al., 2008). Ionomic analysis was performed by evaluating the concentration of 40 chemical elements in Bi-treated plants (data not shown). The dataset evaluation allowed highlighting the modification of the concentration of 12 chemical elements (9 macro/micronutrients plus non-essential elements such as Na, Co, and Li) in the roots and shoots of Bi-treated *Lepidium* plants. Regarding roots (Figure 5), results put in evidence how the concentrations of the main macronutrients analyzed (e.g., K, P, Ca, Mg) were not substantially modified after Bi treatment, while the uptake of most micronutrients was negatively affected by all the Bi concentrations tested (e.g., Ni, Cu) or by the highest Bi concentration in the soil only (e.g., Zn, Mn). Contrary to the work of Nishimura and Nagata (2021), Fe concentration in roots was not altered by the presence of Bi in the substrate, likely due to the differences in both the experimental approaches and the plant species used. Concerning not essential elements, while Na concentration was not modified, the uptake of Li and Co was altered in Bi-exposed plants, with a particular reduction at the highest Bi concentration.

A data overview on macro-, micro-, and non-essential nutrients in shoots of Bi-treated plants (Figure 6) highlighted a reduction in the concentrations of all the elements, except for Na. In particular, macronutrients P and Ca revealed a near dose-dependent relationship between content in shoots and Bi level in soil, while K and Mg concentrations were negatively affected only in plants grown in soil with 485 mg kg^{-1} of Bi nitrate. A reduction of the

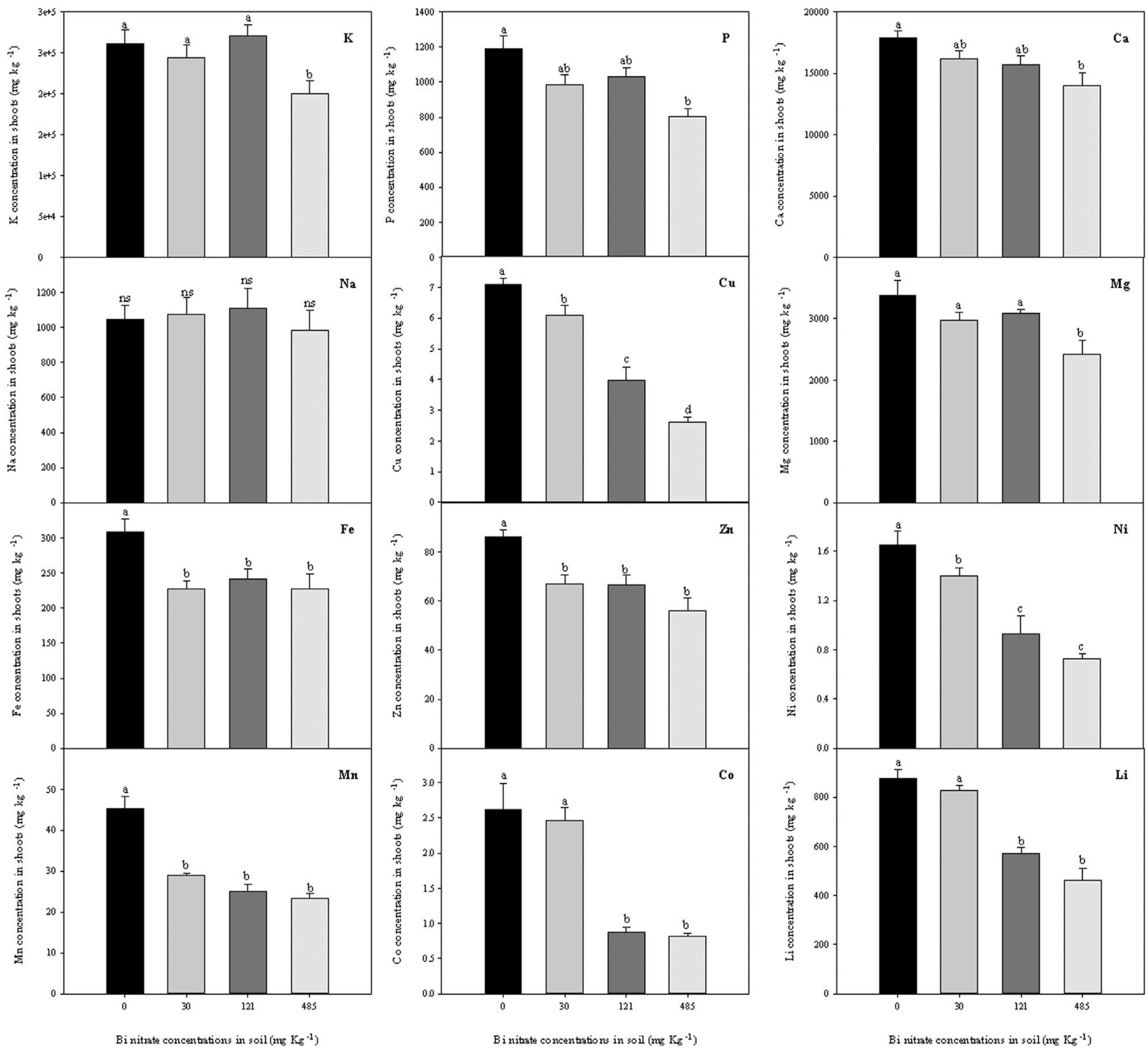

FIGURE 6
Ionomic profile (ion concentration, g kg^{-1} dw) in shoots of garden cress (*Lepidium sativum* L.) plants grown in pots filled with soil supplied with bismuth nitrate (0 mg kg^{-1}, control; 30 mg kg^{-1}; 121 mg kg^{-1}; and 485 mg kg^{-1}) for 21 days in controlled conditions in a growth chamber. In each bar, mean data (n = 4, ± S.E.) are shown. Different letters correspond to statistically different values (Tukey's test, $p \leq 0.05$, ns = not significant).

micronutrient (Cu, Fe, Zn, Ni, and Mn) concentrations in the shoots of Bi-exposed plants was also observed, even with different extents among elements. Finally, non-essential element concentration in shoots was drastically reduced at the highest Bi level in the soil, except for Na, which, as observed in roots, was not affected by Bi treatment.

The analysis of other 28 elements in *Lepidium* plants exposed to different Bi concentrations was also performed, and results for roots (Supplementary Table S1) and shoots (Supplementary Table S2) were reported. Interestingly, a decreasing trend of element concentration in roots as a consequence of Bi treatment was observed for Al, As, and Cd (Supplementary Table S1). At the shoot level (Supplementary Table S2), this trend was evidenced for As and Cd. Notably, Al is the third most abundant element on Earth's crust, while As and Cd are among the most toxic elements occurring in metal-contaminated sites. Therefore, further investigations will be aimed to shed light on the interference of Bi with the accumulation of these elements in plants.

Taken all together, data on ionomic analysis revealed that Bi presence in the soil had a higher impact on the uptake and translocation of micronutrients rather than macronutrients, where a relevant effect could be observed only for the lower translocation of K, P, Ca, and Mg from roots to shoots in plants exposed to the highest Bi concentration. In this regard, it is worth mentioning the work by Nagata (2015), which highlighted how the exposure to Bi of *Arabidopsis thaliana* seedlings notably affected the expression levels of genes involved in the absorption of several ions, including Fe, Cu, and Zn. Interestingly, Nagata also put in evidence

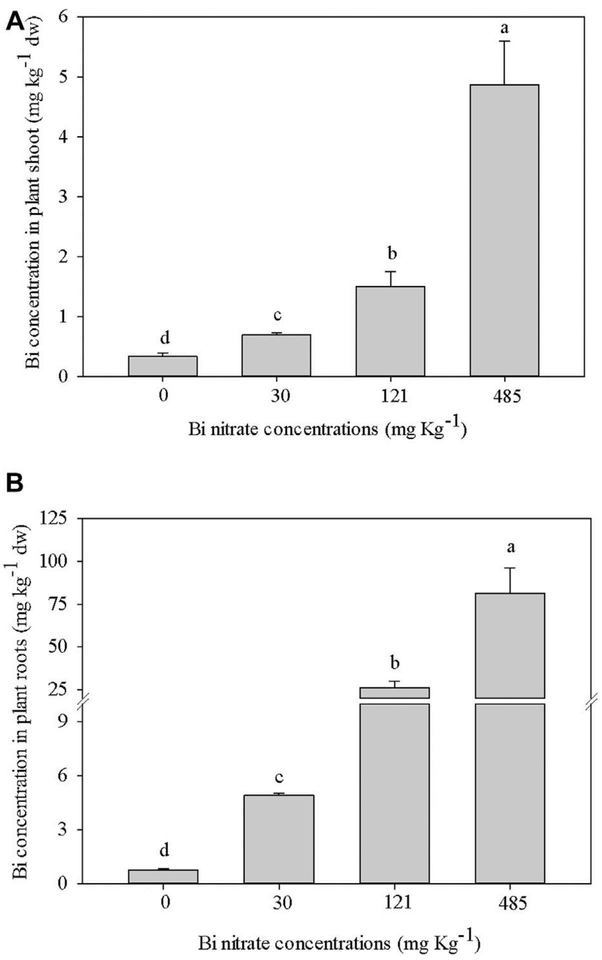

FIGURE 7
Bismuth concentration (g Kg^{-1} dw) in garden cress (*Lepidium sativum* L.) plants (A = Bi concentration in shoot; B = Bi concentration in roots) grown in pots filled with soil supplied with bismuth nitrate (0 mg Kg^{-1}, control; 30 mg Kg^{-1}; 121 mg Kg^{-1}; 485 mg Kg^{-1}) for 21 days in controlled conditions in growth chamber. In each bar, mean data (n = 4, ±S.E.) are shown. Different letters correspond to statistical different values (Tukey test, $p \le 0.05$).

TABLE 2 Bioconcentration factor (shoots and roots) and translocation factor in garden cress (*Lepidium sativum* L.) plants grown in pots filled with soil supplied with bismuth nitrate (0 mg kg^{-1}, control; 30 mg kg^{-1}; 121 mg kg^{-1}; and 485 mg kg^{-1}) for 21 days in controlled conditions in a growth chamber (mean data; n = 4).

Bi nitrate (mg Kg^{-1})	BCF shoots	BCF roots	*Tf*
0	—	—	0.432
30	0.053	0.379	0.141
121	0.028	0.502	0.057
485	0.023	0.388	0.060

that the Bi treatment might disturb iron homeostasis in seedlings, resulting in a significant increase of iron content in plant roots. Therefore, even if the experimental conditions of Nagata's work were quite different from those utilized in the present experimental trial (plant species, substrate, and Bi concentrations), the alteration of the expression profile of genes involved in the element transport caused by Bi exposure could have been involved in the modification of the uptake and translocation of some elements, especially micronutrients, in Bi-treated plants (Figure 5, 6).

The exposure of *Lepidium* plants to Bi could have negatively affected the growth of plants by interfering with different physiological processes. In fact, the reduction of the macronutrient content in shoots could be associated with the biomass decrease in Bi-treated plants, as reported in Figure 1, considering the primary role of macronutrients for plant growth and development (Tripathi et al., 2014). In this context, the Mg content reduction observed in shoots of Bi-treated plants could have affected the functionality of the photosynthetic process by altering the pigment biosynthetic pathway (Table 1). Moreover, the decrease in micronutrient concentration, especially occurring in the shoots of Bi-treated plants, could have affected the physiological status of plants by altering the enzymatic activity, given the role of these elements (*i.e.*, Cu, Fe, Zn, Ni, and Mn) as cofactors in many fundamental enzymatic reactions, especially those linked to the oxidative status of plants (Pandey, 2018). In addition, a role for the micronutrient content reduction in the impairment of the photosynthetic machinery can be suggested, given their participation in many electron chain-related reactions, with a particular role of Mn in the oxygen-evolving complex (Raymond and Blankenship, 2008). In the literature, to our knowledge, a role for Bi in interfering with plant nutrient homeostasis was put in evidence by Nagata (2015) and Nishimura and Nagata (2021) for iron. Therefore, in this work, a first report on the possible effect of Bi in altering the nutrient uptake and translocation in plants was shown. In this context, a similar feature about the shoot micronutrient content decrease after metal exposure was reported by Metanat et al. (2019) in plants treated with Pb, a metal belonging to the same chemical group (post-transition metals) of Bi.

In Figure 7, the results of ICP-MS analyses revealed that in both roots and shoots of Bi-exposed plants, a dose-dependent accumulation of Bi to metal concentration in the soil was observed. Bi concentration in roots of Bi-treated plants (Figure 7A) was more than 10-fold higher than in shoots (Figure 7B). A detectable level of Bi was also observed in control plants, likely due to metal uptake from impurities of the peat substrate (data not shown). The low but significant Bi concentrations found in the shoots (Figure 7B) demonstrated that, at least in part, Bi entered the root cells, reaching the vascular tissues to be transported to the aerial parts, possibly through the transpiration flow. As underlined by Babula et al. (2010), the mechanisms of Bi transport in plants are still unknown, and to date, no studies dealing with it have been reported in the literature. Consistently, few papers discussed the accumulation of Bi in plants. An increasing concentration of Bi in garden cress young seedlings to metal concentration in the solution was shown by Passatore et al. (2022). Similarly, Nagata and Kimoto (2020) showed that Bi accumulation occurred in tomato seedlings, probably disturbing iron homeostasis by altering the primary Fe^{2+} uptake transporter in the roots, as reported for *A. thaliana* (Nishimura and Nagata, 2021). Low Bi detection in plants grown in soil enriched by Bi pellets was instead found by Fahey et al. (2008). Even if the Bi uptake processes in plants are not fully clarified, it is known that a suite of soil characteristics can affect the uptake of Bi by plants, such as pH, organic matter, cation exchange capacity, and soil texture (Fahey et al., 2008).

The bioconcentration factor (BCF) and the translocation factor (T*f*) are commonly used indices to evaluate the ability of plant species to accumulate metals in plants and transfer them in the aerial part (Zacchini et al., 2009). In Table 2, BCF values for both shoots and roots highlighted the low capability of garden cress plants to accumulate Bi from the soil, as evidenced in other plant species (Fahey et al., 2008). As markedly depending on both metal concentration and agronomical properties of soil, BCF comparison among different studies is not easy. Anyway, BCF values in above-ground organs for some non-metal-hyperaccumulating plants grown in metal-enriched soils have been reported to be higher than 1 (Wang et al., 2002), even if decreasing with the increase in the metal concentration in soil, as also observed in our experiment. Also, at the root level, a 2 to 10 fold higher BCF compared to that reported in Table 2 has been highlighted for Cd, Cr, Cu, Ni, Pb, and Zn in barley plants grown in soil amended with sludge containing different HM concentrations (Soriano-Disla et al., 2014). Interestingly, the authors reported the root to shoot metal transfer, fully comparable to the T*f* shown in Table 2, ranging from 0.14 to 0.010 for the different metals. Therefore, such data evidenced that the T*f* of Bi found in our experiments is very similar to that reported for Cr, Ni, and Pb, commonly described as low-mobile elements in plants. Remarkably, in Table 2, T*f* values highlighted that the translocation of Bi from roots to shoots is progressively lower with the increase in Bi accumulation in plants. This mechanism could be ascribed to a protective response possibly limiting the harmful action exerted by Bi on the physiological processes occurring in leaves, as observed by Seregin and Kozhevnikova (2006) for other metals and described as a defense response against metal toxicity.

4 Conclusion

The overall results of this study put in evidence that plants grown in Bi-enriched soil underwent a toxicity status, as revealed by the growth reduction, the lower chlorophyll and carotenoid contents and nitrogen level (NBI), and the decrease in the effective quantum yield of PSII photochemistry (ΦPSII). The reduction in nutrient accumulation in shoots in Bi-treated plants could be associated with these toxic effects, likely affecting the balance within primary metabolism between growth and defense processes. Protective mechanisms were also highlighted, including the overall increase in anthocyan and flavonol levels in leaves, photoprotection mechanisms, and, finally, the restriction of Bi transport from roots to leaves in order to preserve the fundamental physiological processes occurring in leaves. Interestingly, chlorophyll content, NBI, and ΦPSII emerged as valuable proxies of Bi toxicity in plants as their values paralleled the extension of the damaging effects at the growth level. Overall, the results of this study showed that the extent of damage in *L. sativum* is not related to the Bi concentrations both in soil and plant tissues, providing evidence of the lower toxicity of Bi compared to other metals. Finally, even if some information about the adverse effects of Bi in plants is given in this study, the overall mechanism of Bi phytotoxicity is far from being clarified and requires further studies (Pietrini et al., 2009).

Data availability statement

The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation.

Author contributions

FP: conceptualization, methodology, validation, formal analysis, investigation, data curation, and writing—review and editing; LP: investigation, resources, data curation, and visualization; SC: investigation and resources; LM: methodology, validation, formal analysis, and writing—review and editing; MA: methodology, validation, formal analysis, and writing—review and editing; CG: investigation; and MZ: conceptualization, validation, investigation, writing—original draft, writing—review and editing, supervision, and project administration. All authors contributed to the article and approved the submitted version.

Funding

This project was funded under the National Recovery and Resilience Plan (NRRP), Mission 4 Component 2 Investment 1.4—Call for tender No. 3138 of 16 December 2021, rectified by Decree n.3175 of 18 December 2021 of the Italian Ministry of University and Research funded by the European Union—NextGenerationEU; Award Number: Project code CN_00000033, Concession Decree No. 1034 of 17 June 2022 adopted by the Italian Ministry of University and Research, CUP B83C22002930006, Project title "National Biodiversity Future Center—NBFC."

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fenvs.2023.1221573/full#supplementary-material

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TYPE Review
PUBLISHED 29 June 2023
DOI 10.3389/fenvs.2023.1182048

OPEN ACCESS

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RECEIVED 08 March 2023
ACCEPTED 15 June 2023
PUBLISHED 29 June 2023

CITATION
Debela SA, Sheriff I, Daba C, Tefera YM, Bedada D and Gebrehiwot M (2023), Status of persistent organic pollutants in Ethiopia.
Front. Environ. Sci. 11:1182048.
doi: 10.3389/fenvs.2023.1182048

Status of persistent organic pollutants in Ethiopia

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Over the years, the chemical market has shown significant growth, but the hazardous impact of chemical use and disposal on the environment and human health is a growing concern. Persistent organic pollutants (POPs) are among the most dangerous chemicals with widespread effects on the environment and living organisms, including humans. This study aimed to assess the current status of POP management and regulatory infrastructure in Ethiopia by collecting information from stakeholders responsible for recording the import, export, use, management, and regulation of POPs both in government and private sectors. Additionally, a comprehensive literature review was conducted using Boolean operators from international databases and libraries. The results indicated that Ethiopia banned the import of pesticides listed under Annex A (i.e., those to be eliminated), except endosulfan, which was imported at a quantity of 199,767 kg lt^{-1} from 2011 to 2015. There are 2,435 PCB-containing transformers in Ethiopia, of which 2,242 (92%) are currently in use. These transformers and capacitors contain 1,031,661 kg and 1,255 kg of dielectric fluids, respectively. As for Annex B POPs (i.e., those to be restricted), there are between 928,509 kg and 1,383,095 kg of active and obsolete dichlorodiphenyltrichloroethane found in different stores across the country. Ethiopia imported approximately 337,000 kg of products containing perfluorooctane sulfonic acid from 2000 to 2020, with an annual average of 16,850 kg of photographic film, paper, and plates. POPs were also detected in different environmental matrices (soil, waterbodies, sediments, food items, and air) as well as human blood. Despite these findings, regulation and management of POP waste and stockpiles are largely inadequate in Ethiopia. Therefore, it is crucial to improve the monitoring, management, and regulation of POPs in the country. This could be achieved by strengthening the collaboration among different regulatory bodies, harmonizing the fragmented laws on POP management and control, and building institutional capacity.

KEYWORDS
persistent organic pollutant, national implementation plan, Ethiopia, Stockholm Convention, chemicals

1 Introduction

Persistent organic pollutants (POPs) refer to a group of chemicals, including industrial chemicals, pesticides, and unintentional by-products, that share common characteristics, such as environmental persistence (Aljerf and AlMasri, 2018; Sathishkumar et al., 2021), bioaccumulation (Weltmeyer et al., 2021), and long-range transport potential. These chemicals are ubiquitous in the environment (Scheringer, 2009; Hageman et al., 2015; Kallenborn et al., 2015), and exposure to them has been linked to significant changes in the human gut microbiome, resulting in toxicity (Popli et al., 2022) as well as an increased risk of type 2 diabetes and hypertension (Hernández-Mariano et al., 2022). Prenatal exposure to POPs has also been associated with an increased risk of developing gestational diabetes in pregnant women (Zhang et al., 2018) and causing cardiometabolic effects on offspring not only during infancy but also up to adolescence (Güil-Oumrait et al., 2021).

The negotiation process for the Stockholm Convention, the main multilateral environmental agreement regulating POPs, started in the 1990s, and it was eventually adopted in 2001, before being enforced in 2004 (Scheringer, 2009, Secretariat of the Stockholm; Convention, 2019a). The convention initially targeted 12 POPs, known as the 'dirty dozen,' which included polychlorinated biphenyls (PCBs) and some organochlorine pesticides (OCPs) (Secretariat of the Stockholm Convention, 2019b). However, over the years, the list of restricted compounds has grown (Secretariat of the Stockholm Convention, 2022a), with six additional compounds (Dechlorane Plus, methoxychlor, UV-328, chlorpyrifos, chlorinated paraffins, and long-chain perfluorocarboxylic acids, their salts, and related compounds (LC-PFCAs)) currently under review for inclusion to the list (Secretariat of the Stockholm Convention, 2022b). The Stockholm Convention categorizes POPs into different groups, such as Annex A, B, and C, and mandates that parties take measures to eliminate and restrict the production and use of chemicals listed under Annex A and Annex B, respectively. Annex C chemicals are generally considered unintentional by-products.

The Stockholm Convention has 152 signatories and 186 parties, including Ethiopia, which submitted its first National Implementation Plan (NIP) on POPs in 2006 (EMEFCC, 2006). However, the plan has not been updated despite additional POPs being included in the convention, which puts Ethiopia behind other countries in the region (e.g., Kenya, Burundi, Tanzania, and Seychelles) that have updated their NIPs. Several reviews have been conducted on the POP situation in different countries, focusing on research trends and regulation (Sheriff et al., 2021), environmental levels and human exposure (Minh et al., 2008; Mazlan et al., 2017; Bruce-Vanderpuije et al., 2019; Helou et al., 2019; Olisah et al., 2021; Rezania et al., 2022), sources (Olisah et al., 2021), and levels in fish and food (Mazlan et al., 2017; Uzomah et al., 2021). However, recent information on the POP situation in Ethiopia is lacking. This study seeks to address this gap by providing a comprehensive assessment of the status, management, and regulatory infrastructure (legal, institutional, administrative, and technical) of POPs in Ethiopia. The outcome can be used to develop evidence-based policies and strategies to reduce POP exposure and contamination in Ethiopia.

2 Methodology

2.1 Data collection

The study used two methods of data collection. First, a checklist was developed to obtain information on the use, import, export, releases, alternative future use of POPs, institutional collaboration, and management of POP stockpiles. The data were collected from organizations responsible for recording the import, export, use, management, and regulation of POPs both in private and government sectors. Table 1 presents the list of these organizations and the nature of the information collected from each.

Second, a comprehensive literature review on POPs in Ethiopia was conducted. Scopus, Science Direct, Web of Science, Google Scholar, and PubMed were searched using Boolean operators and keywords, such as Stockholm Convention, National Implementation Plan, and Ethiopia, to locate published articles. The search was conducted without any time restrictions to find relevant studies on POPs. The review included studies conducted in Ethiopia that reported on the level of POPs in environmental media, as well as articles published in scientific journals and gray literature. All papers and reports were critically evaluated based on their title, abstract, and full-text, and only full-text articles written in English were considered.

2.2 Data analysis

The data were transferred to and analyzed using STATA software (StataCorp. 2021. Stata Statistical Software: Release 17. College Station, TX: StataCorp LLC). The STATA software was also used to generate maps that illustrate the storage and regional distribution of chemicals.

3 Results and discussion

3.1 Annex A pesticides

Based on an inventory conducted by the Ethiopian Revenue and Customs Authority since 2000, Ethiopia has not yet produced any pesticide listed under Annex A. Moreover, with the exception of endosulfan, the country has not imported any old or new Annex A pesticide. Therefore, this study solely focuses on endosulfan, which is utilized in high quantities for controlling cotton pests in Ethiopia's agricultural sector. Table 2 shows that approximately 200,000 L of endosulfan were imported between 2015 and 2020, although there are no data available for 2019. Farming activities may lead to the direct release of endosulfan to the environment. Apart from endosulfan, the first NIP in 2006 identified four types of obsolete Annex A pesticides: aldrin, dieldrin, heptachlor, and chlordane, with respective quantities of 2,159, 2,822, 7,043, and 2,591 kg Lt^{-1} (EMEFCC, 2006). In the northern region of Ethiopia, stockpiles of aldrin, heptachlor, and dieldrin were 1,398, 1,523, and 621 kg, respectively (Haylamicheal and Dalvie, 2009b; Debela et al., 2020b).

3.2 Annex A PCBs

The data provided by the Ethiopian Electric Utility reveal that there are 2,435 transformers in Ethiopia that contain PCBs, out of which 2,242 are currently in use. In contrast, the 2006 NIP documented 2,505 PCBs containing electric transformers, and nearly 2,415 of them were in use (EMEFCC, 2006). The difference in these two reports (−70 in the first) could be due to

TABLE 1 List of organizations and the nature of the information collected on POPs in Ethiopia.

S. no.	Name of organizations	Information obtained
1	• Environmental Protection Agency (EPA)	Regulation of all POPs, including unintentional by-products
	• Ethiopian Chemical Institute	
2	• Ministry of Agriculture	Supply, distribution, and marketing of agricultural inputs and the proper administration and control of pesticides
3	• Ministry of Health	Usage of some POPs (e.g., DDT) for malaria control and other POPs contained in medical equipment
4	• Ministry of Trade and Regional Integration	Transportation, provision, and control of commercial registration and licensing of POPs
	• Ethiopian Revenue and Customs Authority	
5	• Ministry of Water and Energy	Information on various industrial POPs, such as PCBs, perfluorooctane sulfonic acid (PFOS), polybrominated diphenyl ethers (PBDEs), and perfluorooctanesulfonyl fluoride (PFOSF), which are used in a range of industries, including electric utilities, plastics and rubber products, foams, aviation, photography, sealants and adhesives, metal plating, and leather production
	• Ethiopian Electric Utility	
	• Ministry of Industry	
	• Ethiopian Airlines	
	• Ethiopian Leather Development Institute	

TABLE 2 Imported quantities of endosulfan (trade name—Thionex) from 2015 to 2020 in Ethiopia (source: the Ethiopia Revenues and Customs Authority).

Trade name	Purpose	Quantity (liters)	Year of import
Thionex 35%EC	For farm use	55	2015
Thiodan 25%ULV	Commercial	20,000	2015
Thionex 35%EC	Commercial	9,240	2015
Thionex 35%EC	Commercial	15,200	2016
Thionex 35%EC	For farm use	160	2017
Thionex Technical	For formulation	120,000	2017
Thionex 35%EC	Commercial	8,316	2017
Thionex 35%EC	Commercial	17,556	2018
Thionex 35%EC	Commercial	9,240	2020

the initial inventory's incompleteness in terms of assessing suspected locations. The total quantities of suspected PCB-containing dielectric fluids in these transformers and capacitors are 1,031,661 kg and 1,255 kg, respectively. The 2006 NIP reported that the weight of PCB-containing dielectric fluids was 1,181,667 kg and 1,255 kg for transformers and capacitors, respectively (EMEFCC, 2006). For capacitors, the weight reported in 2006 and in the current assessment remains unchanged. The slight variation in the weight of transformer dielectric fluid implies that the responsible bodies either did not update the data or the fluids were not disposed-off due to limited capacity. It is also essential to note that the time/year when the Ethiopian Electric Utility updated its PCB inventory for this study remains unclear.

Western Ethiopia has the highest number of PCB-containing transformers, with 497, followed by the southern Addis Ababa Region, with 357 (Figure 1 and Supplementary Table S1). This is in contrast to the 2006 NIP report, which reported the highest number of PCB-containing transformers in the Central Region as 674, followed by 309 in Western Ethiopia (EMEFCC, 2006). The Ethiopian Electric Utility reports that some PCB-containing transformers are currently in use and are kept in workshops for maintenance, while others have been discarded. All PCB-containing transformers were manufactured before 1989, and there is a workshop for PCB transformer maintenance in the Central Region.

The Ethiopian EPA has identified three current PCB hotspot areas across the country: Gofa central warehouse, Kotobe auxiliary maintenance workshop, and METEC-EPEI (a newly established transformer refurbishing and manufacturing factory). Gofa central warehouse was the only hotspot identified in the 2006 NIP (EMEFCC, 2006). Discarded transformers, suspected to contain PCB oil, were stored in steel tankers at the Kotobe maintenance workshop (Debela et al., 2020a; Debela et al., 2021). Transformers, capacitors, and PCB-suspected dielectric fluids and oil are located in highly populated areas in the cities. These materials are stored in an unlocked fenced area. According to the information obtained from the Ethiopian Electric Utility, 11,232 L of dielectric fluid and oil were sold to a lime-producing factory in 2016, which used it as an energy source for lime processing.

3.3 Annex B pesticides

DDT was formulated in Ethiopia by the Adami Tulu Pesticide Processing Share Company for the purpose of controlling vector-borne diseases, such as malaria. However, there are no available data on the importation and exportation of DDT. A previous assessment reported 160, 573 Kg Lt^{-1} active and 55, 720 Kg Lt^{-1} obsolete DDT in different storage sites of the country (EMEFCC, 2006) (Figure 2, Figure 3). According to the Ministry of Agriculture, there are a total of 1,383,095 kg of both active and obsolete DDT in Ethiopia, whereas data from the Ethiopian EPA indicate that only 928,509.3 kg are present (Supplementary Table S2). It could be

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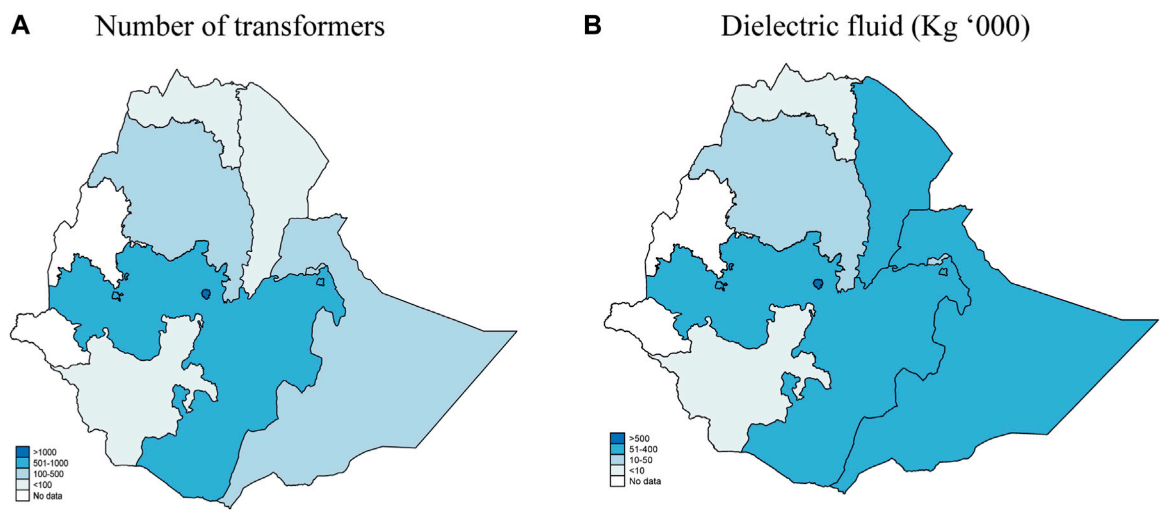

FIGURE 1
(A) Number of transformers and **(B)** dielectric fluid (Kg '000) in different regions of Ethiopia (detailed reports are indicated in Supplementary Table S1; source: the Ethiopian EPA and Ethiopian Electric Utility).

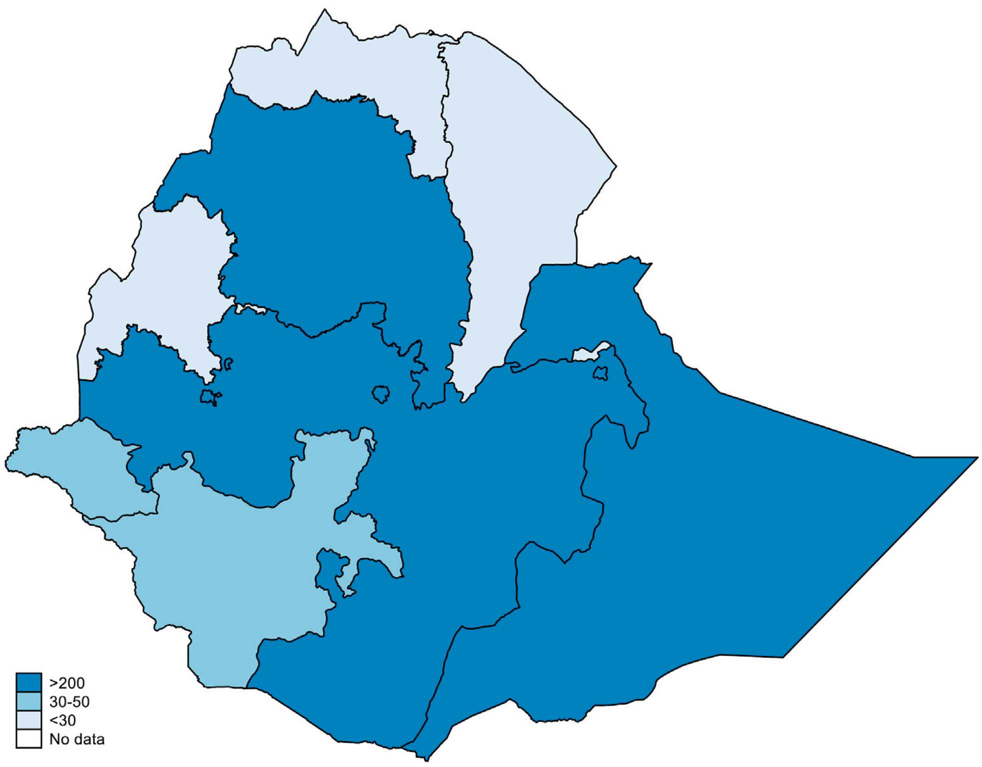

FIGURE 2
DDT amount (Kg '000) in different regions of Ethiopia (detailed reports are indicated in Supplementary Table S2; source: the Ethiopian EPA).

seen that the DDT data provided by these two government institutions that are in charge of pesticides were not the same. This discrepancy highlights the need for greater collaboration among government ministries, departments, and agencies. The Adami Tulu Pesticide Processing Share Company warehouse currently stores 454,586 kg of DDT, and there are approximately

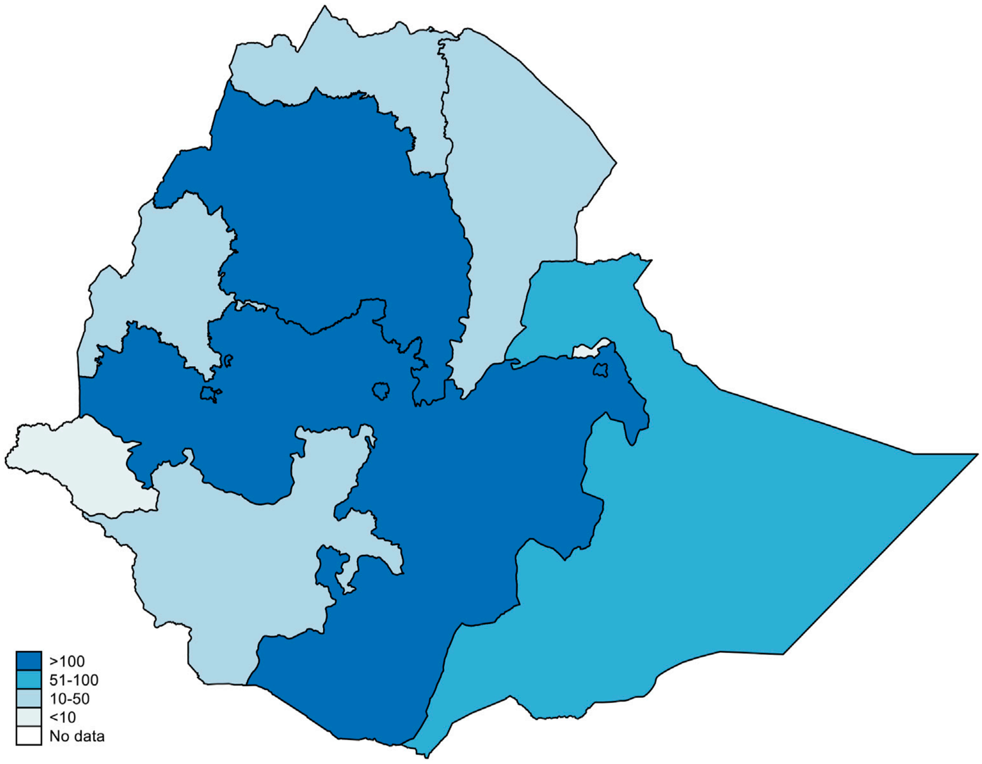

FIGURE 3
Number of DDT stores in different regions of Ethiopia (detailed reports are indicated in Supplementary Table S1; source: the Ethiopian EPA).

460 sites suspected to be contaminated with DDT. Production of DDT for disease vector control was permitted under the Stockholm Convention (UNEP, 1997); however, studies have shown that disease vectors in Ethiopia are developing resistance to DDT (Alemayehu et al., 2017; Demissew et al., 2022). As a result, the Ministry of Health has been using alternative non-POP chemicals, such as piperonyl butoxide, pyrethroid, and carbamates, since 2018 (Gari and Lindtjorn, 2018). Despite being officially discontinued for vector control, there are reports of smallholder farmers purchasing DDT from the illegal market for other purposes. This has resulted in the unintentional release of DDT into the environment due to improper storage and illegal use (Mekonen et al., 2017; Afata et al., 2022; Endalew et al., 2022).

3.4 Annex B perfluorooctane sulfonic acid

The information on the production and use of PFOS and its related substances and articles suspected for PFOS were collected from Ethiopian Airlines, the Ministry of Trade and Regional Integration, the Ministry of Industry, and the Ethiopia Chemical Institute. PFOS is used in different sectors, including photo imaging, metal plating, mining, plastic and rubber products, textile, leather, synthetic carpet, pulp and paper, paints, varnishes, electric and electronic parts, firefighting foam, hydraulic fluids, and others. From 2000 to 2020, Ethiopia imported approximately 337,000 kg of PFOS-containing products, including photographic film, paper, and plates, with an annual average imported quantity of 16,850 kg. According to the Ethiopian EPA, emission of PFOS from these sectors is 2.2 kg per year (Supplementary Table S1 and Supplementary Table S2). METECH Engineering, the Ethiopian Air Force, and Ethiopian Airlines are among the large companies that operate their own maintenance workshops and utilize PFOS for plating purposes. However, the Ministry of Mines, Petroleum, and Natural Gas has reported that there is no mining industry in Ethiopia that uses PFOS. Similarly, the respective industry bodies of plastic and rubber production, textile, and leather manufacturing have reported that they do not use PFOS or PFOS-containing products in their operations.

Other potential sources of PFOS in Ethiopia may include imported carpets and pulp and paper production. To estimate the potential use of PFOS in imported carpets, data on the total amount of imported synthetic carpets were collected. Between 2000 and 2020, Ethiopia imported 105,764 kg of synthetic carpets, with an average annual import quantity of 5,288 kg. This indicates a potential consumption of 3.3–33.6 kg of PFOS per year, assuming that all imported carpets contain PFOS. However, the pulping industry in Ethiopia is still in its infancy and not yet at the stage of using PFOS chemicals. Most of the paper used in Ethiopia is imported, and some paper plants use recycled paper and cartons for pulp production. Packed food materials are also imported, which may contain PFOS. These packing materials are typically disposed-off as garbage in open fields and dump sites, which can lead to the entry of PFOS-containing materials into the environment.

Although Ethiopia's aviation hydraulic fluid and firefighting foam industries are not using PFOS, they may use PFOS derivative chemicals. For example, hydraulic fluid Skydrol LD4 contains a minimal amount of PFOS. However, these industries do not have any stockpile of PFOS-containing waste under their control. As municipal solid waste in Ethiopia is collected and dumped without segregation (Akele and Tarekegn, 2017; Amare et al., 2022; Lewoyehu et al., 2022) and industries discharge their wastewater with little or no treatment to the surrounding river, PFOS-containing waste materials from industries are likely to cause environmental contamination (Ahrens et al., 2016).

3.5 Polybrominated diphenyl ethers

Ethiopia has imported PBDEs through electrical and electronic equipment and in-vehicle products. The country has experienced a rapid transformation in Information and Communication Technology (ICT) in recent years, resulting in the importation of computers, mobile phones, and TV sets from developed countries. This has contributed to the increasing amount of electronic waste. There is only one TV assembly company in Ethiopia, and it uses PBDEs.

3.6 Annex C POPs

There are no reliable data on the status of other POPs in Ethiopia. However, the Ethiopian EPA believes that there is unintentional production of Annex C POP chemicals from different categories of sources, such as uncontrolled combustion process of waste incineration, metal and mineral production, transport, power generation, and household heating with biomass and charcoal. During data collection, we found that the level of awareness concerning unintentional POPs among government officials was very low.

3.7 POP disposal facilities in Ethiopia

In Ethiopia, there is no dedicated chemical disposal facility, potentially leading to the accumulation of chemicals in the county. Except for POP pesticides, there have been no efforts to dispose-off other POP chemicals at the time of this assessment. In 2015, various donors and/or partners disposed-off 1,502, 1,093, and 450 tons of chemicals containing 25%, 10%, and 2% POP pesticides, respectively. Furthermore, the United States Agency for International Development (USAID) recently disposed-off 85 tons of DDT as well as 15 tons of DDT-contaminated packing cartons. The Enkoken company in Finland disposed-off 1,511 tons of obsolete pesticides from 220 stores in collaboration with the Food and Agriculture Organization (FAO) and the Ministry of Agriculture between 2000 and 2003 (Haylamicheal and Dalvie, 2009b; Debela et al., 2020b). In addition, Veolia Environmental Services in England removed 1,000 tons of obsolete pesticides during the second phase from 2006 to 2007 (Haylamicheal and Dalvie, 2009a). In 2015, the African Stockpile Project and USAID also disposed-off 3,050,000 kg of DDT.

3.8 POP-contaminated sites in Ethiopia

In the current study, there are no data and figures on POP-contaminated sites in Ethiopia. In the 2006 NIP, 220 contaminated stores and sites were identified across the country. It consisted of 178 contaminated stores (where 41 stores were contaminated by spilled chemicals from the store), 23 burial sites, and 19 open-container stores (EMEFCC, 2006), with the Oromia region having the highest number (102) of contaminated sites.

3.9 Monitoring of POPs in environmental matrices and human blood

This section presents POP concentrations in different environmental matrices, such as soil (Table 3), waterbodies (Table 4), sediments (Table 5), food items (Table 6), and air (Table 7), as well as human blood (Table 8), from different locations in Ethiopia.

3.9.1 Soil

Soil from agricultural land, urban areas, and industrial areas is identified as a major source of organic pollutants, including pesticides (DDT, aldrin, dieldrin, heptachlor, and others) and PCBs (dioxin and non-dioxin) (Table 3) in Ethiopia. A study performed in a transformer workshop and dumpsite in Addis Ababa industrial soil found that PCB levels in the soil ranged from 1.027 to 4.862 mg kg^{-1} and dioxin-like (DL) Σ12PCBs ranged from 1.6036 to 0.56128 mg kg^{-1}, while non-dioxin-like (NDL) Σ6PCBs ranged from 0.166 to 4.5 mg kg^{-1} (Debela et al., 2020a). In another study, slight PCB levels in the urban soil of Addis Ababa Σ 16PCB ranged from 0.4 to 18.5 μg kg^{-1} (Prasse et al., 2012).

In the upper Awash agricultural soil, different POP pesticides, such as endosulfan derivative, aldrin, dieldrin, DDT derivative, and heptachlor, were also detected (Westbom et al., 2008). The highest levels of endosulfan were found in a concentration range of 4.6–28,900 ng g^{-1}. The concentration of PCBs in an urban area is lower than in an industrial area. For the past 40 years, the industrial area has been used for PCB maintenance and disposal. Furthermore, in Ethiopia, eight regions have been identified as potentially contaminated by PCBs, and in all areas, PCB-containing equipment is kept in open filled storage (EMEFCC, 2006).

3.9.2 Water

Four studies investigating the presence of POPs in lakes and rivers were identified. POPs, such as organochlorine pesticides, PCBs, polyfluoroalkyl substances (PFASs), and PFOS, have been found in the Akaki River, Lake Awassa (Hawassa), Lake Tana, and Lake Ziway in recent studies (Table 4). In the Akaki River, the concentrations of ΣDDTs were in the range of 5.33–30.58 ng L^{-1}, which is higher than other POP chemicals (PCBs, PFASs, and PFOS) detected in the lakes of Tana, Ziway, and Awassa. The Akaki River is a river that drains across the capital city (Addis Ababa) and has the potential to be contaminated by different pollutants (Kassegne et al.,

TABLE 3 Concentration of POP chemicals detected in soil.

Location	Type of POPs	Extraction type	Instrument	Concentration	References
Upper Awash agricultural soil	a-Endosulfan	SPLE	GC-ECD	4.6–28900 ng g^{-1}	Westbom et al. (2008)
	b-Endosulfan			1.8–25900 ng g^{-1}	
	Endosulfan sulfate			1.2–1,140 ng g^{-1}	
	Aldrin			Nd–3.8 ng g^{-1}	
	Diedrin			Nd–2.4 ng g^{-1}	
	Heptachlor			Nd–1.8 ng g^{-1}	
	α-HCH			Nd–5.5 ng g^{-1}	
	β-HCH			Nd–0.9 ng g^{-1}	
	γ-HCH			Nd–3.3 ng g^{-1}	
	p,p'-DDT			0.7–4.9 ng g^{-1}	
	p,p'-DDE			20–225 ng g^{-1}	
Addis Ababa	Σ6PCBs	ASE	GC–MS	0.3–7.5 μg kg^{-1}	Prasse et al. (2012)
	Σ16PCBs			177–1,640 μg kg^{-1}	
Transformer area	Σ6PCBs	PLE	GC–MS	403–4050a μg kg^{-1}	Debela et al. (2020a)
	Σ12PCBs			561–1,186 μg kg^{-1}	
	Σ7PCBs			1,027–486 μg kg^{-1}	

Nd, not detected; SPLE, solid-phase liquid extraction; PLE, pressurized liquid extraction; ASE, accelerated solvent extraction; GC–MS, gas chromatography–mass spectrometry; GC-ECD, gas chromatography electron capture detector.

TABLE 4 Concentration of POP chemicals detected in Ethiopian waterbodies.

Location	Type of POPs	Extraction type	Instrument	Concentration	References
Akaki Catchment River	ΣDDT	LLSE	GC–MS	5.33–30.58 ng L^{-1} (mean 11.39 ng L^{-1}).	Kassegne et al. (2020)
	Lindane			15.4 ± 0.25 ng L^{-1}	
	Dieldrin			4.85 ± 0.007 ng L^{-1}	
Lake Ziway	Endosulfun	EPE	GC-ECD	.095–59 ug L^{-1}	Teklu et al. (2018)
Lake Tana	PFAS	SLE	HPLC	0.073–5.6 ng L^{-1}	Ahrens et al. (2016)
Lake Awassa	PFOA	SPE	UPLC–MS/MS	6.24–10.7 ng L^{-1}	Melake et al. (2022)

LLSE, liquid–liquid and Soxhlet extraction; EPE, eichloromethane and petroleum ether; SLE, solid–liquid extraction; SPE, solid-phase extraction; GC–MS, gas chromatography–mass spectrometry; GC-ECD, gas chromatography electron capture detector; HPLC, high-performance liquid chromatography; UPLC–MS/MS, ultra-high-performance liquid chromatography–tandem mass spectrometry.

2020). The detection of POPs in waterbodies may eventually lead to human exposure through different routes.

3.9.3 Sediment

Water is one of the primary transport channels for substances with hydrophilic functions, such as POPs, whereas sediments typically serve as repositories for these substances because they also have hydrophobic functionalities (Ssebugere et al., 2020). Most POPs have been detected in sediments. According to Kassegne et al. (2020), DDT was the most prevalent pesticide found in all sediment samples collected from the Akaki Catchment River and Aba Samuel (Table 5). DDT concentrations ranged from 1.91 to 1,076.73 g kg^{-1}. In addition, other OCPs were detected in this study area. PCBs have been detected in Addis Ababa urban sediment (Σ16 PCBs ranged from 712 to 3,040 mg kg^{-1}), which is higher than studies conducted by other authors in the sediment of the upper Awash and Lake Tana (Table 4). PCDD and PCDF have also been detected in Lake Tana and the Koka Reservoir. Sediment from Lake Tana has higher concentrations of both PCDF and PCDF than that from Koka Reservoir. PFOA was also detected in Awassa Lake and ranged from 0.1 to 0.6 ng g^{-1} dw (Ahrens et al., 2016).

3.9.4 Air

The literature on the levels of POPs in the air in Ethiopia is very limited. To date, only two studies have investigated the occurrence of organochlorines in the air. However, the available data show that the levels of POP chemicals are low and vary widely among the two identified study locations. In Ziway, three POPs, namely, heptachlor, DDT, and

TABLE 5 Concentration of POP chemicals detected in sediments.

Location	Type of POPs	Extraction type	Instrument	Concentration	References
Akaki Catchment River and Aba Samuel	ΣDDT	LLSE	GC–MS	1.91–1,076.7 μg kg^{-1}	Kassegne et al. (2020)
	Lindane			120.51 μg kg^{-1}	
	Dieldrin			78.56 ± 0.14 μg kg^{-1}	
	Abendosulfan			127.7 ± 0.01 μg kg^{-1}	
	Heptachlor			12.65 ± 0.01 μg kg^{-1}	
	ΣPCBs			Nd–63.76 μg kg^{-1}	
Addis Ababa	Σ6PCBs	ASE	GC–MS	3.4–13.7 mg kg^{-1}	Prasse et al. (2012)
	Σ16PCBs			712–3,040 mg kg^{-1}	
Lake Tana	PFAS	SLE	HPLC	0.22–0.55 ng g^{-1}	Ahrens et al. (2016)
Upper Awash	Σ 7PCB	Soxhlet	GC-ECD/MS	0.85–26.56 ng g^{-1}	Dirbaba et al. (2018)
	Σ 7PBDEs			3.71–18.95 ng g^{-1}	
	Σ 8DDT			1.99–139.68 ng g^{-1}	
Lake Awassa	PFOA	SPE	UPLC–MS/MS	0.1–0.6 ng g^{-1}dw	Melake et al. (2022)
Lake Awassa	PCDD	ASE	HRGC-HRMS	120.8 ± 17.7 pg g^{-1}	Urbaniak and Zalewski (2011)
	PCDF			149.56 ± 9.79 pg g^{-1}	
Koka Reservoir	PCDD			37.29 ± 9.80 pg g^{-1}	
	PCDF			25.88 ± 3.01 pg g^{-1}	
Lake Tana	PCBs	PLE	GC–MS	10.8–14.4 ng g^{-1}	Zebeaman and Schmid (2019)

LLSE, liquid–liquid and Soxhlet extraction; SLE, solid–liquid extraction; SPE, solid-phase extraction; ASE, accelerated solvent extraction; PLE, pressurized liquid extraction; GC–MS, gas chromatography–mass spectrometry; GC-ECD, gas chromatography electron capture detector; HPLC, high-performance liquid chromatography; UPLC–MS/MS, ultra-high-performance liquid chromatography–tandem mass spectrometry; HRGC-HRMS, capillary column high-resolution gas chromatography–high-resolution mass spectrometry.

TABLE 6 Concentration of POP chemical detected in the air of the towns Ziway and Assela.

Place	Type of POPs	Extraction type	Instrument	Concentration	References
Ziway (City)	Heptachlor	SSE	GM-MS	151.1 mg m^{-3}	Mesfin (2019)
	DDT			19.61 mg m^{-3}	
	Benzene hexachloride			4.64 mg m^{-3}	
Asella	PCB	SE	GC–MS	1.2–2.5 ng m^{-3}	UNEP (1997)
	Adrin		GC–APCI–MS	LOQ–1.4 ng m^{-3}	
	Diedrin		GC–APCI–MS	LOQ–1.4 ng m^{-3}	
	DDT		GC–MS	61.5–152.4 ng m^{-3}	
	HCB		GC–MS	2.8–4.1 ng m^{-3}	
	PCDDs		GC-HRMS	80–102.2 ng m^{-3}	
	PCDFs		GC-HRMS	205.1–286.3 ng m^{-3}	

SSE, Soxhlet solvent extraction; SE, Soxhlet extraction; LOQ, limit of quantification; GC–MS, gas chromatography–mass spectrometry; GC–APCI–MS, gas chromatography–atmospheric pressure chemical ionization–mass spectrometry; GC/HRMS, gas chromatography/high-resolution mass spectrometry.

benzene hexachloride, were determined with average concentrations of 151.1, 19.6, and 4.64 mg m^{-3}, respectively (Table 6). The reports are higher than the study findings conducted by UNEP in Asella City, which found that PCDFs are the predominant OC in Assela City and ranged from 205.1 to 286.3 ng m^{-3}. Ziway is a place where a large number of pesticides are manufactured and stored at the country level.

3.9.5 Food items

POP pesticides and PFOS and its derivatives have been detected in different food items, such as in the residue of rice (Mekonen et al., 2017), cattle meat (Letta and Attah, 2013), fish, fish liver and muscles (Sjöholm, 2015; Ahrens et al., 2016; Melake et al., 2022), *khat* (Daba et al., 2011), and cow and goat milk (Deti et al., 2014) (Table 7).

TABLE 7 POP chemicals in different food items in Ethiopia.

Food items	Type of POPs	Extraction type	Instrument	Concentration	References
Residue of rice	Endosulfan sulfate	AEH	GC–MS	0.076 ± 0.025*	Siraj et al. (2021)
	p,p'-DDE			0.035 ± 0.013*	
	p,p'-DDT			0.046 ± 0.020*	
	Aldrin			0.06 ± 0.033*	
Meat	o,p'-DDT	SPE	GC–MS	3.24 ± 0.042*	Letta and Attah (2013)
	p,p'-DDT			4.32 ± 0.003*	
	Endosulfan-I			0.06 ± 0.004*	
	Chlorothanolin			0.004 ± 0.001*	
	Aldrin			0.012 ± 0.002*	
	Dieldrin			0.04 ± 0.003*	
	Lindane (ã-HCH)			0.05 ± 0.001*	
Fish muscle	PFAS	SLE	HPLC	Nd–5.8 ng g^{-1}	Ahrens et al. (2016)
Gelemso *Khat*	p,p'-DDT	SPE	GC–MS	141.2–973 ug Kg^{-1}	Daba et al. (2011)
	o,p'-DDT			139.3–399 ug Kg^{-1}	
Aseno *Khat*	p,p'-DDT	SPE	GC–MS	194.3–999 ug Kg^{-1}	
	o,p'-DDT			62.2–224.8 ug Kg^{-1}	
Cow milk	Aldrin	CRE	GC–MS	Nd–11.6 L ug Kg^{-1}	Deti et al. (2014)
	a-Endosulfan			Nd–47.8 ug Kg^{-1}	
	b-Endosulfan			Nd	
	DDT			259–1,230 ug Kg^{-1}	
Goat milk	Aldrin			Nd	
	a-Endosulfan			ND–142.1 ug Kg^{-1}	
	b-Endosulfan			Nd	
	DDT			82.7–874.4 ug Kg^{-1}	
Fish	PFAS	AUB	LC–MS	Nd–5.78 ng g^{-1}	Sjöholm (2015)
Fish liver	PFOA	SPE	UPLC–MS/MS	0.1–0.6 ng g^{-1}	Melake et al. (2022)
	PFDA			0.5–3.2 ng g^{-1}	
	PFOS			<LOQ–1.2 ng g^{-1}	
	PFUnDA			0.5–3.9 ng g^{-1}	

AEH, acetone/ethylacetate/n-hexane; SPE, solid-phase extraction; SLE, solid–liquid extraction; CRE, centrifuge and rotary evaporator; AUB, acetonitrile and ultrasonic bath; GC–MS, gas chromatography–mass spectrometry; HPLC, high-performance liquid chromatography; LC–MS, liquid chromatography–mass spectrometry; UPLC–MS/MS, ultra-high-performance liquid chromatography–tandem mass spectrometry; LOQ, limit of quantification; * is mg kg^{-1}; Nd, not detected.

3.9.6 Human serum

Samples from blood serum can be used as a marker to monitor human exposure to organic pollutants. In a study conducted in Western Ethiopia, small-scale farmers who were exposed and non-exposed to these pollutants were examined. The blood samples of exposed small-scale farmers showed the highest mean concentrations of p,p'-DDT (0.28 ± 0.4 mg L−1), with high levels of p,p'-DDE, p,p'-DDT, and heptachlor also identified (Afata et al., 2021).

3.10 Weaknesses of regulatory and non-regulatory measures for chemical management in Ethiopia

Except for the proclamation aiming at controlling and regulating pesticides, no legislation specifically addresses other POP chemicals in Ethiopia (Ministry of Agriculture, 2010). As this could be considered a clear gap, it is important to have specific legislation on the management of other POP chemicals. In addition, there is

TABLE 8 POP chemicals detected in human blood.

Location	Type of POPs	Extraction type	Instrument	Concentration	References
Jimma	DTT	EEH	GC-ECD	5.20 ± 4.8[a] μg L^{-1}	Mekonen et al. (2021)
				4.37 ± 2.8[b] μg L^{-1}	
	Heptachlor	EEH	GC-ECD	6.90 ± 4.3 μg L^{-1}	
				9.15 ± 3.8 μg L^{-1}	
	Aldrin	EEH	GC-ECD	1.20 ± 1.1 μg L^{-1}	
				1.45 ± 1.4 μg L^{-1}	
	Endrin	EEH	GC-ECD	3.44 ± 0.9 μg L^{-1}	
				1.75 ± 2.2 μg L^{-1}	
	Endosulfan	EEH	GC-ECD	1.33 ± 0.65 μg L^{-1}	
				3.72 ± 2.04 μg L^{-1}	
Western Ethiopia	p p′-DDT	SEM	GC-ECD	0.28 ± 0.4[c] mg L^{-1}	Afata et al. (2021)
				0.25 ± 0.45[d] mg L^{-1}	

EEH, ethyl ether/hexane; SEM, Soxhlet extraction method; GC-ECD, gas chromatography electron capture detector.
[a]Case.
[b]Control.
[c]Exposed farmer.
[d]Non-exposed farmer.

weak coordination among and within responsible institutions. This is a concern in Ethiopia when it comes to the implementation of regulatory measures in chemical management (Akele and Tarekegn, 2017). For instance, there is no active collaboration among the Ministry of Agriculture, the Customs and Revenue Authority, and the EPA. The existing institutional framework does not have a way for coordination because there is no strong line of communication and information sharing among the responsible regulatory bodies. There is a challenge in handling contraband and adulteration, and the shortage of inspectors and vehicles for monitoring purposes bedevils the adequate control of POP chemicals (Debela et al., 2020b). There is also no structure and practice of information exchange on the movement or management of POP chemicals with the customs authority, different ministers, and industries.

Data on chemicals and their safety are available from a variety of governmental and non-governmental organizations, including the ministries of agriculture, trade and industry, customs, and mines and energy. The available information on chemicals in Ethiopia is not systematically organized and easily accessible despite the fact that some institutions have country-wide data on certain chemicals. There is a lack of information on industries suspected of using POP chemicals, their production levels, and the estimated or modeled amount of such chemicals released into the environment.

The current laws related to the management and control of POPs in Ethiopia are unclear, especially in terms of the roles and responsibilities of the relevant organizations. Various government offices have their own regulations concerning the chemicals originating from their respective sectors. Moreover, there are no laws regulating the manufacturing, importing, handling, transport, storage, or disposal of hazardous chemicals, whether industrial or otherwise. Currently, legal frameworks to address these issues are either under development or pending adoption.

3.11 Gaps in institutional capacity

The overall technical infrastructure concerning chemical management within the country is very weak. Industries dealing with chemicals do not have the required experts for the handling of chemicals, and professionals are not well versed with the knowledge and skills that are required for dealing with chemicals. In addition, chemicals are not properly labeled (Ejigu and Mekonnen, 2005; Mequanint et al., 2019; Debela et al., 2020b).

4 Conclusion

This study provides an overview of the status of POPs in Ethiopia by gathering data from various governmental and private institutions involved in the use or regulation of POPs in the country. The findings indicate a significant accumulation of Annex A, B, and C POPs, such as PCBs from electric transformers and POP pesticides in Ethiopia. Notably, the review of the existing literature demonstrates that various environmental matrices, including soil, waterbodies, sediments, food items, and air, as well as human blood, have substantial levels of different POPs. This study has identified several regulatory and non-regulatory gaps in chemical management in the country. Specifically, there is insufficient information available on various POP pollutants, except for endosulfan, at the national level, and no legislation directly addresses other POP chemicals except the pesticide proclamation. Therefore, it is crucial to have specific legislation targeted toward the management of POP chemicals in Ethiopia, achieved by enhancing collaboration and coordination among different regulatory bodies, harmonizing fragmented laws on POP management and control, and building institutional capacity.

Author contributions

SAD, IS, and MG were involved in conceptualization and data Curation; SAD, IS, CD, YT, DB, and MG wrote the original draft, reviewed and edited the manuscript. All authors contributed to the article and approved the submitted version.

Acknowledgments

The authors acknowledge both government and private organizations for all their genuine support. They are also grateful to the managers of the aforementioned institutions for their cooperation.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fenvs.2023.1182048/full#supplementary-material

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10.3389/fenvs.2023.1182048

Glossary

DDT	Dichlorodiphenyltrichloroethane
EPA	Environmental Protection Agency
EMEFCC	Ethiopian Ministry of Environment, Forest, and Climate Change
FAO	Food and Agriculture Organization
LC-PFCAs	Long-chain perfluorocarboxylic acids, their salts, and related compounds
NIP	National Implementation Plan
HCB	Hexachlorobenzene
HCH	Hexachlorocyclexane
o,p'DDT	1-Chloro-2-[2,2,2-trichloro-1-(4- chlorophenyl) ethyl]-benzene
OCPs	Organochlorine pesticides
p,p –DDE	p,.p -Dichlorodiphenyldichloroethylene
p,p –DDT	p,.p -Dichlorodiphenyltrichloroethane
USAID	United States Agency for International Development
PBDEs	Polybrominated diphenyl ethers
PCBs	Polychlorinated biphenyls
PCDD/Fs	Polychlorinated dibenzo-p-dioxins/dibenzofurans
PFASs	Polyfluoroalkyl substances
PFOA	Perfluorooctanoic acid
PFOS	Perfluorooctane sulfonic acid
PFOSF	Perfluorooctanesulfonyl fluoride
PFUnDA	Perfluoroundecanoic acid
PFDA	Perflurodecanoic acid
POPs	Persistent organic pollutants
α-HCH	alpha-Hexachlorocyclohexane
β-HCH	beta-Hexachlorocyclohexane
γ –HCH	gamma-Hexachlorocyclohexane (lindane)

frontiers | Frontiers in Environmental Science

TYPE Original Research
PUBLISHED 07 July 2023
DOI 10.3389/fenvs.2023.1171261

OPEN ACCESS

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RECEIVED 21 February 2023
ACCEPTED 27 June 2023
PUBLISHED 07 July 2023

CITATION
Barbir J, Arato E, Chen C-Y, Granberg M, Gutow L, Krång A-S, Kröger SD, Leal Filho W, Liwarska-Bizukojc E, Miksch L, Paetz K, Prodana M, Saborowski R, Silva Rojas R and Witt G (2023), Assessing ecotoxicity of an innovative bio-based mulch film: a multi-environmental and multi-bioassay approach.
Front. Environ. Sci. 11:1171261.
doi: 10.3389/fenvs.2023.1171261

Assessing ecotoxicity of an innovative bio-based mulch film: a multi-environmental and multi-bioassay approach

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Among the highly diverse range of biobased polymers, polylactic acid (PLA) received vast attention in recent years due to its versatility for different applications and being the first commercially used polymer produced from renewable sources. Production and application of bio-based, biodegradable plastics will have one of the most crucial roles in tackling worldwide plastic pollution.

Methods: This study is based on integrative ecotoxicological assessment of an innovative PLA-based agricultural mulch film (BPE-AMF-PLA), developed under the H2020 EU project "BIO-PLASTICS EUROPE", towards organisms from different environmental compartments (soil, fresh water and marine) and from different trophic levels. Such comprehensive evaluation has an overarching goal to promote environmentally safe and sustainable use of these PLA-based plastics for agricultural and other potential applications.

Results: Low-to-no phytotoxicity was obtained in both single-species standardized bioassays, and in a multi-species microcosms experiment. Earthworm reproduction was negatively affected at the lowest test concentration of 0.1% w/w of PLA-based plastic particles. For freshwater Daphnia, reproduction was found a sensitive endpoint, upon exposure to the leachates of the PLA-based plastic. However, the reported toxicity seemed to be caused by the presence of 2-methylnaphthalene, which can be avoided in the production process. As for the marine organisms, algae growth was inhibited with a LOEC = 25 g L^{-1}, whereas test with brine shrimp only revealed stimulation of lipase upon digestion of micro-sized PLA-based plastics. Marine lugworm ingested pristine and UV pre-treated micro-sized plastics, yet without impact either on biological activity, or on the health of the test individuals.

Discussion: The approach used in the present work will contribute to product development, environmental safety and sustainable applications of the PLA-based mulch film BPE-AMF-PLA, in the scope of project BIO-PLASTICS EUROPE. Furthermore, the tools and results obtained in this work are a relevant contribution in the framework development for additional support in the certification of the bio-based polymers, being aligned with European zero waste and non-toxicity strategies, certification, and regulations.

KEYWORDS

toxicity, bio-based plastics, mulch films, bioassays, PLA, environmental toxicity

1 Introduction

Bio-based polymers (or bio-based plastics) are one of the most suitable resources to tackle the large environmental challenge produced by plastic pollution (Narancic & O'Connor, 2019). Because of their origin from renewable sources, agricultural byproducts, or microbial sources, bio-based plastics can have the property of renewability, and in some cases biodegradability (Reddy et al., 2013; Madadi et al., 2021), which implies fewer greenhouse gas (GHG) emissions and possible reduced plastic debris generation (European Bioplastics, 2021a). Moreover, biodegradation rate of both fossil-based and bio-based plastics depend on their chemical formation and conditions such as the presence of additives, crystallinity and the presence of proper microorganisms, temperature, moisture, and pH of the environment (Mohee & Unmar, 2007). Currently, bio-based plastics comprise only about 1% of all plastic production, but is expected to grow from 2.2 Mt (million tonnes) in the year 2022 to approximately 6.3 Mt by 2027 (European Bioplastics, 2020). From the variety of biopolymers used currently, polylactic (PLA) is one of the most commercialized in a global context (Rezvani Ghomi et al., 2021).

1.1 Benefits of bio-based plastics

Nevertheless, the largest benefit gained from the use of bio-based plastics is the contention that they provide against climate change (Filiciotto & Rothenberg, 2021). The use of fast-growing microorganisms, such as bacteria or algae may result in a considerable annual reduction in CO_2 emissions (Spierling et al., 2018). Additionally, the implementation of agricultural wastes in the production of bio-based plastics could reduce pressure on food supply and security, since the crops would not be used for that purpose (Koul et al., 2022). Thus, with the use of feedstocks such as lignocellulosic or agro-based, only 0.01% of the agricultural area of a total of 5 billion hectares is occupied (European Commission, 2018). Furthermore, provided that there is proper and efficient reuse and recycling, bio-based plastics can strongly contribute to the process of a circular economy with their use, and as a result, contribute to reduce the amount of plastics debris reaching the oceans (Di Bartolo et al., 2021).

1.2 Benefits and constraints behind biodegradation process of novel bio-based plastics

Since conventional plastics are persistent, they will not biodegrade in nature, but disintegrate into microplastics, therefore the removal of plastics once they have entered the ecosystem is often either prohibitively expensive or impossible (Gall and Thompson, 2015; Bråte et al., 2017). For these reasons, the production and use of biodegradable bio-based plastics is an important component to combat worldwide plastic pollution. Something all biodegradable polymers have in common is that the monomers are connected by enzymatically degradable linkages, which can be hydrolysed by various enzymes (Luyt and Malik, 2019). During biodegradation, the biopolymers disintegrate to smaller fragments until, ideally, the polymer is completely mineralized to CO_2, H_2O and new biomass. Further, during the degradation process, bio-based as well as conventional, fossil-based plastics may leach harmful accompanying and metabolite compounds, like plasticizers and other additives, lubricants, non-intentionally added substances, oligomers and monomers (Asiandu et al., 2021; Zimmermann et al., 2020). Several of these compounds are of high environmental concern because they may exhibit properties like persistence, bioaccumulation, endocrine disruption and toxicity (Stibany et al., 2017). Consequently, during degradation, bio-based and biodegradable plastics may release micro-fragments and harmful compounds into terrestrial and aquatic environments where they can accumulate in organisms via suspended particles, from the consumption of contaminated sediment and foods or directly from the water.

1.3 PLA-based plastics

Among bio-based polymer materials individuated as a potential and suitable replacement for traditional plastics, polylactic acid (PLA) has received much attention in recent years (Castro-Aguirre et al., 2016) due to its versatility for different applications and for being the first commercially used polymer produced from renewable sources (Henton et al., 2005). PLA is an aliphatic polyester that can be obtained from agricultural products and the synthesis takes place through a multistep process starting from the production of lactic acid, followed by the intermediate step of lactide formation, and ending with the polymerization reaction (Hartman, 1998). It shows good processability in standard equipment and a much lower environmental impact in comparison to fossil plastics. However, it has also some disadvantages as far as low toughness, slow degradation rates and hydrophobic characteristics (Farah et al., 2016). The biodegradation rate of PLA (measured as loss of weight at high soil moisture content and air temperature of 40°C) was higher when used as a composite in combination with other biopolymers (i.e., starch), then that of pure PLA (Yu et al., 2020). Improved biodegradability was also observed in combination with chitosan (Vasile et al., 2018). Baltrán-Sanahuja et al. (2021) emphasize that the environmental factors crucial for the process of biodegradation of bio-based plastics (including PLA-based) in soil can have higher variability than those in aquatic, therefore urging for inclusion of reference to the performance under specific

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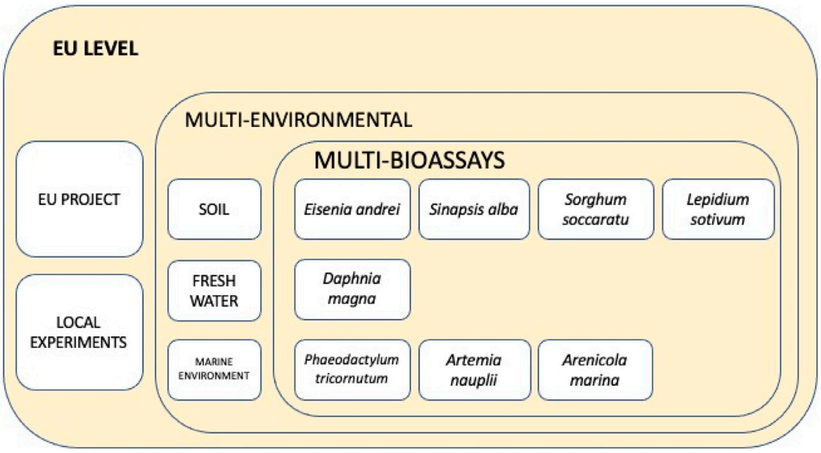

FIGURE 1
Approach used for assessing toxicity of BPE-AMF-PLA compound.

environmental conditions within the biodegradability certification. Virgin PLA usually can be blended with different kinds of fillers (Haave et al., 2019; Moliner et al., 2020) to improve their mechanical properties and expand their range of applications (Bledzki and Jaszkiewicz, 2010). Biodegradability, recyclability, compostability, and possible toxicity of PLA-based compounds have been studied in detail by the partners of the BIO-PLASTICS EUROPE funded project, performing specific experiments within different working packages.

1.4 Application of mulch films

The use of PLA-based materials in agriculture for mulch applications has developed only in recent years (Serrano-Ruiz et al., 2021). Studies on the effects of individual bio-based mulches on the growth and development of plants and on the impact on soil microorganisms still need to be thoroughly investigated in order to produce sustainable mulches and assure environmental safety. This work intends to contribute to the study of PLA based compounds for plasticulture applications, helping to shed light on doubts about their toxicity, in order to replace fossil plastics with environment-friendly biobased compounds.

The aim of the current study is to provide a comprehensive ecotoxicological assessment of an innovative PLA-based agricultural mulch film material, BPE-AMF-PLA, developed under the EU H2020 project "BIO-PLASTICS EUROPE," towards organisms from different environmental compartments such as soil, fresh water and the marine environment, and from different trophic levels, including primary producers, and first and second level consumers (Figure 1). The integrative evaluation of and the discussion on the ecotoxicity of the PLA-based mulch film material will promote its safe and sustainable use as a polymer for agricultural and other potential applications.

Each project partner involved in toxicity testing contributed specific experimental and analytical data, which together form a comprehensive picture on the various aspects of ecotoxicity in a multi-environmental approach. No consistent test was possible due to variety of environments.

2 Materials and methods

2.1 Test material

The material examined in the present work is a PLA-based compound blended with polybutylene adipate terephthalate (PBAT) from a producer NaturePlast SAS (Ifs, France). The ratio of PLA:PBAT is 30:1, as confirmed with nuclear magnetic resonance (NMR) spectroscopy. More detailed characterization regarding ^{1}H- and ^{13}C-NMR spectra can be found in Miksch et al. (2022), the study also carried out in the scope of BIO-PLASTICS EUROPE. This material is intended to be fully degradable *in situ*, and non-toxic in soil, freshwater, and marine environments, yet its biodegradability in nature is not documented. The enzymatic degradability in seawater of the material was very low under environmentally relevant conditions, whereas hydrolysis rate is 30 nmol·min^{-1} when incubated with lipase at 30°C (Miksch et al., 2022).

2.2 Ecotoxicity towards soil organisms

2.2.1 Toxicity towards *Sorghum saccharatum*, *Lepidium sativum* and *Sinapis alba*

Phytotoxicity of the bio-based plastics was evaluated according to ISO Standards 18763 (ISO 18763, 2016) with the commercially available Phytotoxkit Solid Samples (order no. TK 61) provided by Microbiotests Inc. (Gent, Belgium). The test included three species of higher plants: one monocotyledon, *Sorghum saccharatum* (Sorghum, series no. SOS041019), and two dicotyledones, *Lepidium sativum* (Garden Cress, series no. LES260820) and *Sinapis alba* (Mustard, series no. SIA020719).

The tests were run for 72 h according to ISO Standards 1873. For the control tests the reference OECD soil prepared in agreement with OECD method no. 207 (OECD, 1984) was used only, while for the other tests the plastic particles (3 mm × 2.5 mm) were added to the OECD soil. A 105 g of soil was used in each replication. The soil was saturated up to 100% with deionised water. The concentrations of plastics in the soil were 0.02, 0.095, 0.48, 2.38, and 11.9% w/w. The wide range of concentrations of plastic particles in the soil was selected based upon the literature data concerning the quantity of plastics in the terrestrial compartment (Fuller and Gautam, 2016; Piehl et al., 2018; Scheurer & Bigalke, 2018). Each concentration was tested in three replications for each plant species, while the control test was conducted in six replications per each species test. The lengths of roots and stems as well as Germination Index (GI) were determined. The detailed description of the procedure is presented elsewhere (Liwarska-Bizukojc, 2022a). One-way analysis of variance (ANOVA) was applied to determine statistical differences in the lengths of roots and stems, respectively, exposed to the bio-based plastics and the controls without bio-based plastics at a confidence level of 95% ($p = 0.05$).

2.2.2 Toxicity towards earthworms *Eisenia andrei*

The effects of bio-based plastic particles on earthworms were evaluated according to the method OECD 222 (OECD, 2004). In this test, specimens of the earthworm *Eisenia andrei* were used. They originated from the synchronized culture of Institute of Environmental Protection - National Research Institute (Warsaw, Poland). Ten earthworms were put into the container with 600 g of the reference OECD soil (three replicates) with the bio-based plastic particles (3 mm × 2.5 mm), or without (representing control).

The reproductive output of the earthworms exposed to the test material (in this case the bio-based plastic particles BPE-AMF-PLA) was compared with a control of pure soil. The final concentrations of plastics in the dry soil were 0.1, 0.5, 2.5 and 12.5% w/w that corresponded with the literature data (Fuller and Gautam, 2016; Piehl et al., 2018; Scheurer & Bigalke, 2018). The test comprised two stages. In the first stage, the mortality and the body mass of adult earthworms was determined after 28 days. In the second stage, which lasted for another 28 days, the number of cocoons was counted and the effect on the reproductive ability of earthworms was assessed. Relative changes in body mass of earthworms (R_M) exposed to BPE-AMF-PLA were calculated for each concentration tested (Eq. 1).

$$R_M = \left[\left(M_{28} - M_{28,\mathrm{control}} \right) / M_{28,\mathrm{control}} \right] \cdot 100 \qquad (1)$$

where: M_{28} is the mean body mass of the individual earthworm exposed to BPE-AMF-PLA after 28 days of the test and $M_{28,\mathrm{control}}$ is the mean body mass of the individual earthworm not exposed to BPE-AMF-PLA (control run) after 28 days of the test. The positive values of R_M indicate the increase in earthworm body mass, while the negative values show its decrease. One-way ANOVA was used to determine statistical differences between the number of cocoons found in the soil containing BPE-AMF-PLA and the number of cocoons in the control tests at a confidence level of 95% ($p = 0.05$).

2.3 Toxicity towards freshwater organisms

The toxicity of bio-based plastic (BPE-AMF-PLA) towards freshwater invertebrates was investigated with the crustacean *Daphnia magna* applying acute and chronic tests according to the guidelines OECD 202 and OECD 211, respectively. The test medium, ISO water, was prepared according to OECD 202 (2004) and sterilized by autoclaving (Systec, DE-23). Toxicity of mulch film (thickness ≥ 150 μm towards *D. magna* was tested in two different approaches:

1) Contact test: mulch film pieces of 10 mm × 10 mm were introduced directly into the test,
2) Leaching test: mulch film pieces of 10 mm × 10 mm were incubated for 14 days in ISO water on a horizontal shaker at 20°C in the dark with a shaking frequency of 200 rpm. The leachates were decanted before application to avoid plastic pieces in the test, and the organisms were exposed only to the liquid fraction.

The mulch film concentrations in both experimental approaches ranged from 1.5625 to 50 g L^{-1}. The concentration range was chosen in order to determine the dose-response curves under realistic conditions as close to natural conditions as possible. Lithner et al. (2009, 2011) observed toxic effects (immobility) for *D. magna* in 9 of 32 products for conventional plastics, with 24-h and 48-h EC_{50}-values ranging from 5 to 80 g L^{-1}. Incubation (KBF 240, Binder) of the *D. magna* took place under defined conditions at 20 °C ± 1°C and a light-dark rhythm of 8:16 h. The test duration was 48 h for acute and 21 days for chronic test, respectively. During chronic tests daphnids were fed with 16×10^6 cells per day of the algae *Chlorella vulgaris*. No feeding was performed in acute tests. In accordance with OECD 202 and OECD 211, potassium dichromate was used as the positive control. Data were tested for normality (Kolmogorov–Smirnov test with Lilliefors significance correction) by software GraphPad Prism (Version 9.3.1). The results of BPE-AMF-PLA were compared to determine the toxic effects of treatment using one-way analysis of variance (ANOVA) with a significance level of 0.05.

2.3.1 Acute toxicity towards freshwater invertebrate *Daphnia magna*

Acute contact and leaching tests were performed according to OECD 202 (2004) using 6-well flat bottom polystyrene plates (Macro plate PS 6 F with lid, Boettger GmbH, Bodenmais, Germany). For contact testing the same concentrations as for leaching tests were used. Four groups of 5 daphnids and 10 mL medium, each, for the respective mulch film concentration were placed on one well-plates. Mulch film leachates were diluted with ISO water in steps of two until the level 1:32 was reached (test concentrations: 50, 12.5, 6.25, 3.125, and 1.5625 g L^{-1}). To prevent evaporation of the medium during acute testing, the well-plates were covered with a non-sterile polyester film (Adhesive Film for Microplates, VWR) and lids. Immobilization of daphnids was recorded after 24 and 48 h pH and oxygen content were measured at the beginning and end of each test (Al15, AQUALITIC).

2.3.2 Chronic toxicity towards freshwater invertebrates *Daphnia magna*

Chronic tests of mulch film toxicity towards *D. magna* were carried out in 100-mL glass beakers with ten replicates of the respective concentration. Therefore, one daphnid in 50 mL medium was incubated according to OECD, 2012. The concentrations of mulch film contact and leachate tests were 50, 12.5, 6.25, 3.125, and 1.5625 g L^{-1}. Observation of offspring was performed 5 times a week (Monday till Friday). Medium was renewed three times a week and the daphnids were fed with 16 × 10^6 cells per day of the microalgae *Chlorella vulgaris*. pH levels and oxygen content were monitored before and after every medium exchange.

2.4 Toxicity towards marine organisms

2.4.1 Toxicity towards marine microalgae *Phaeodactylum tricornutum*

Leachate of mulch film BPE-AMF-PLA (thickness ≥ 150 μm) was prepared by cutting the film without pre-washing into pieces of 10 mm × 10 mm according to Lithner et al. (2009). Five g of mulch film pieces were placed into a 250-mL glass bottle and 100 mL artificial ISO standard seawater (ASW) were added (DIN, 2015). The bottle was placed on a horizontal shaker (neoLab-Orbital-Shaker, Plattform 409 mm × 297 mm, 10 mm Amplitude) at 200 rpm for 14 days at 20°C ± 1°C in the dark. Afterwards, the leachate was diluted with ASW in a two-step procedure to the lowest concentration of 0.39 g l^{-1} (1:128). Concentration of leachates ranged from 0.39 g l^{-1} to 50 g l^{-1} since Luo et al. (2019) observed growth inhibition of marine algae at 1.6 g microplastic per l. To ensure the observation of effects the concentration range was increased compared to Luo et al. (2019). Algae toxicity tests with the marine algae *Phaeodactylum tricornutum* were performed according to Ratte et al. (2016) in a miniaturized form on 24-well plates with 3,5-Dichlorophenol (3,5-DCP) as positive control and ASW as negative control. During the test period the well-plates were placed on shakers (IKA MTS 2/4) at 120 rpm and incubated at 20°C ± 1°C with a constant light intensity of 80 μmol m^{-2}·s^{-1} (Climate chamber ICH750L, Memmert). Growth inhibition of algae was observed after 24, 48 and 72 h by measuring the fluorescence (Tecan infinite F200Pro, Software i-control 1.8 SP1). Kolmogorov–Smirnov test with Lilliefors significance correction was used to test for normality (GraphPad Prism, Version 9.3.1). For the determination of toxic effects of BPE-AMF-PLA an ANOVA with a significance level of 0.05 was used. Organic contaminants in the test media were analyzed by gas chromatography-mass spectroscopy (GC: 7890A GC system, Agilent Technologies; quadrupole MS: 5975C Inert XL MSD with Triple-Axis Detector) from the leaching media and from the two highest concentrations (50 and 25 g L^{-1}) at the end of each test.

2.4.2 Toxicity towards brine shrimp *Artemia* nauplii

Brine shrimp, *Artemia* spec., are established model organisms in ecophysiological and ecotoxicological research (Nunes et al., 2006). Brine shrimp may either bear live nauplii or produce stress-resistant dormant eggs ("cysts") from which the larvae hatch under favorable conditions. For our experiments, nauplii were raised from cysts of *Artemia persimilis* (Art. no, 10745, REBIE-Zoologischer Versandgroßhandel, Bielefeld, Germany) as per the supplier's instructions. The medium was natural seawater (32 PSU) filtered through 0.45 μm membrane filters, hereafter referred to as filtered seawater (FSW).

Maintenance and exposure experiments were carried out in non-pyrogenic and non-cytotoxic 24-well tissue culture plates (Sarstedt, NC 28658, USA). Before the start and between experiments, the tissue culture plates were stored submersed in FSW for 24 h to leach out any soluble chemicals. The 24-well plates were incubated in a KBS-E400 Incubator (RUMED, Rubarth Apparate GmbH, Germany) at 24°C. A LED panel (Tween Light, 16 W, 30 cm × 30 cm × 5 cm) provided continuous and homogeneous illumination.

2.4.2.1 Preparation of microparticles

BPE-AMF-PLA pellets (5 mm) were ground in liquid nitrogen with a cryogenic mill (6775 Freezer/Mill, SPEX SamplePrep, USA). The protocol involved 15 min of pre-cooling and 4 cycles of milling (2 min each) with 2 min of cooling in between. One gram of the plastic material was processed per run. The ground particles were separated with a stainless-steel sieve to obtain the fraction smaller than 200 μm.

2.4.2.2 Ingestion of microplastics

To test whether *Artemia* nauplii ingest microplastics, specimens were incubated with fluorescent polymer beads (Fluoro-Max™, Fremont, CA 94538 USA, 9.9 μm diameter) and BPE-AMF-PLA microparticles, respectively. Up to ten freshly hatched *Artemia* nauplii each were transferred into the wells of the cell-culture plates containing 3 mL FSW. Five μl of the microplastic suspensions (0.1% w/v) were added to each well and the *Artemia* nauplii were left to feed for 2 h. Thereafter, the nauplii were examined and photographed under a fluorescence microscope (Nikon SMZ 25). The high concentration of particles was chosen to clearly observe and document ingestion or avoidance by the nauplii and to test whether or not these particles may induce biochemical reactions in the digestive tract.

2.4.2.3 Exposure of *Artemia* nauplii

Artemia cysts were incubated in seawater for 24 h at 24°C to hatch. About 300 of the hatched *Artemia* nauplii were transferred to each well of a 24-well plate, which contained 300 μL FSW and 1.5 mL of plastic particle suspension (3 g l^{-1}) per well. The control contained only FSW without micro-particles. The well-plate was incubated for 24 h under permanent illumination. After incubation, 75 randomly taken *Artemia* were transferred from each well to separate 1.5-mL reaction tubes with 300 μL of the incubation fluid. The *Artemia* were homogenised with a micro-pestle and centrifuged for 10 min at 20,000 g and 4°C. The supernatant was pipetted into new 1.5-mL reaction tubes and stored at −80°C until further use.

2.4.2.4 Enzyme assays of *Artemia* nauplii

MUF (4-methyl-umbelliferone) derivatives of butyrate (C4) and oleate (C18) were used as fluorogenic substrates for esterase and lipase enzymes. The substrates were dissolved in dimethyl-sulfoxide (DMSO) and then diluted with 0.1 M Tris/HCl-buffer (pH 7.5). The stock solution contained a substrate concentration of 0.1 mmol L^{-1}

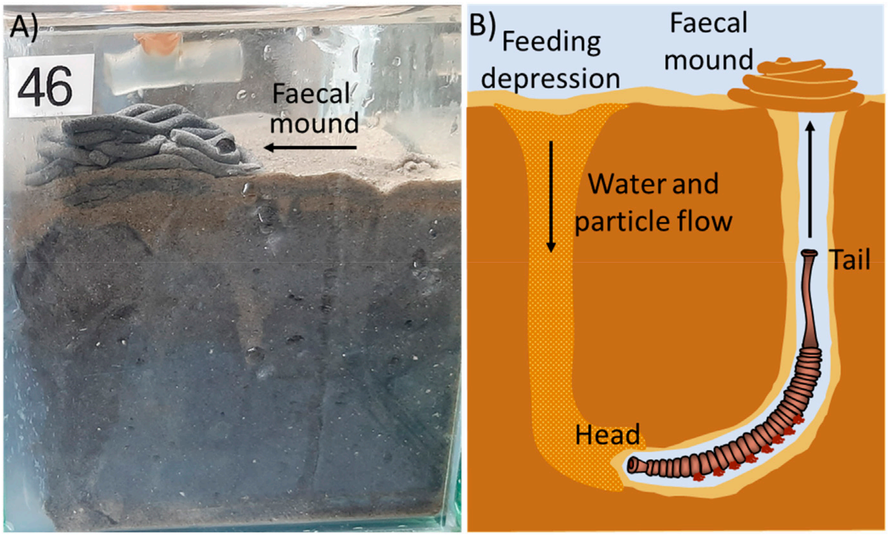

FIGURE 2
Experimental set up with the lugworm *Arenicola marina* in individual aquaria with sediment surface layer spiked with microplastics **(A)**. The lugworm creates a U-shaped burrow in the sediment, feeding and defecating on the sediment surface **(B)**.

and 2% DMSO. The assay was run in triplicate in 96-well plates (3 wells per plate). The *Artemia* extract (20 µL) was given into the wells of the plate and 250 µL of the substrate stock solution subsequently were added. The fluorescence was measured every 30 s for 20 min at 25°C (Fluoroskan Ascent FL, Thermo Fisher Scientific). The MUF standard curve was prepared from 0 to 35 µmol l^{-1} and contained 2% DMSO. Statistical analyses were done with two-tailed t-tests on data sets of three replicates.

2.4.3 Toxicity towards the marine infaunal lugworm *Arenicola marina*

The effects of microparticles from BPE-AMF-PLA and LDPE, a conventional, fossil-based plastic used for agricultural mulch films, was investigated on the marine infaunal polychaetae lugworm *Arenicola marina*. *A. marina* is a non-selective deposit feeder, found in high densities in shallow, sandy to muddy bays around Europe, likely to ingest large amounts of microplastics accumulated in sediments in polluted areas. *A. marina* and sediment were collected from mudflats on the Swedish west coast (mean worm weight: 4.1 ± 1.0 g, $n = 50$; no significant differences between treatments; One-way ANOVA, $F = 0.39$, df = 4, $p = 0.81$). Sediment was dry sieved (2 mm) for removal of macro fauna and worms were acclimatized on sieved sediment before the start of the experiment. Flow-through of surface seawater was used at all times, with experimental conditions resembling the worms' natural environment (temperature 15°C, oxygen level 9.7 ± 0.4 mg L^{-1}, with ambient salinity fluctuation at 26–31 PSU and a 10:14 h light-dark regime). Microplastics prepared with a cryogenic mill as described in 2.4.3.1, were sieved through 100 and 300 µm nylon filters (Bopp Utildi, Sweden) to attain 100–300 µm particles used in these experiments, i.e., within the size range of natural food particles ingested by *A. marina*. Pristine BPE-AMF-PLA and LDPE microplastics were compared with those pre-treated with UV-A (350–400 nm, peak at 370 nm, intensity ca. 12 W/m^2) and UV-B (290–315 nm, peak at 300 nm, intensity ca. 1.8 W/m^2) light for 7 days, to be able to compare whether ecotoxicological effects are affected by this simulated weathering process (by use of Philips fluorescent lamps; Actinic BL TL-K 40W/10-R for UV-A light, and TL 20 W/12 RS SLV/25 with a pre-burnt cellulose acetate UV-C filter (Nordbergs Tekniska AB, Vallentuna, Sweden) for UV-B light, at 10 cm distance). All pristine and UV treated microplastics were stained with Nile Red (Sigma Aldrich) fluorescent dye (dissolved in methanol (Merck, for analysis EMSURE® ACS,ISO,Reag. Ph Eur) at 10 µg per mL^{-1}) for 30 min at 37°C, to enhance subsequent microplastic identification and analysis.

A. marina was exposed to either of five microplastic treatments; i.e., surface sediment spiked with pristine BPE-AMF-PLA and LDPE, or UV-treated BPE-AMF-PLA and LDPE, at a concentration of 0.1% per dry weight sediment, or to sediment without added microplastics for the controls. The experimental set up comprised 10 replicate aquaria per treatment with one worm per aquarium (in total 50 aquaria and worms) (Figure 2). Each aquarium (15 cm × 11 cm × 12 cm) was filled with a 6 cm bottom layer of clean sediment (i.e., no microplastics added) and a 2 cm-layer of surface sediment spiked with microplastics, or clean control sediment. A thin layer of 0.5 cm clean sediment covered the spiked or control surface sediment, to prevent microplastics from floating away, and sediment was let to settle for several hours before introducing a 3.5 cm layer of gentle flow-through of surface seawater. Sediment and microplastics were pre-incubated for 4 days before the introduction of lugworms, to enable formation of natural biofilm. At the start of the experiment, one *A. marina* (pre-purged of gut content for 24 h) was added to each aquarium. Lugworms were exposed to microplastics for 15 days, to assess effects on their health and biological activity.

The effect of microplastics was tested, using the following effect endpoints: a) time to initiate borrowing (the time it took for *A. marina* to start burrowing into the sediment), b) time to complete burrowing (time from start burrowing until completely buried in the sediment), c) overall feeding rate (averaged volume of faecal mound

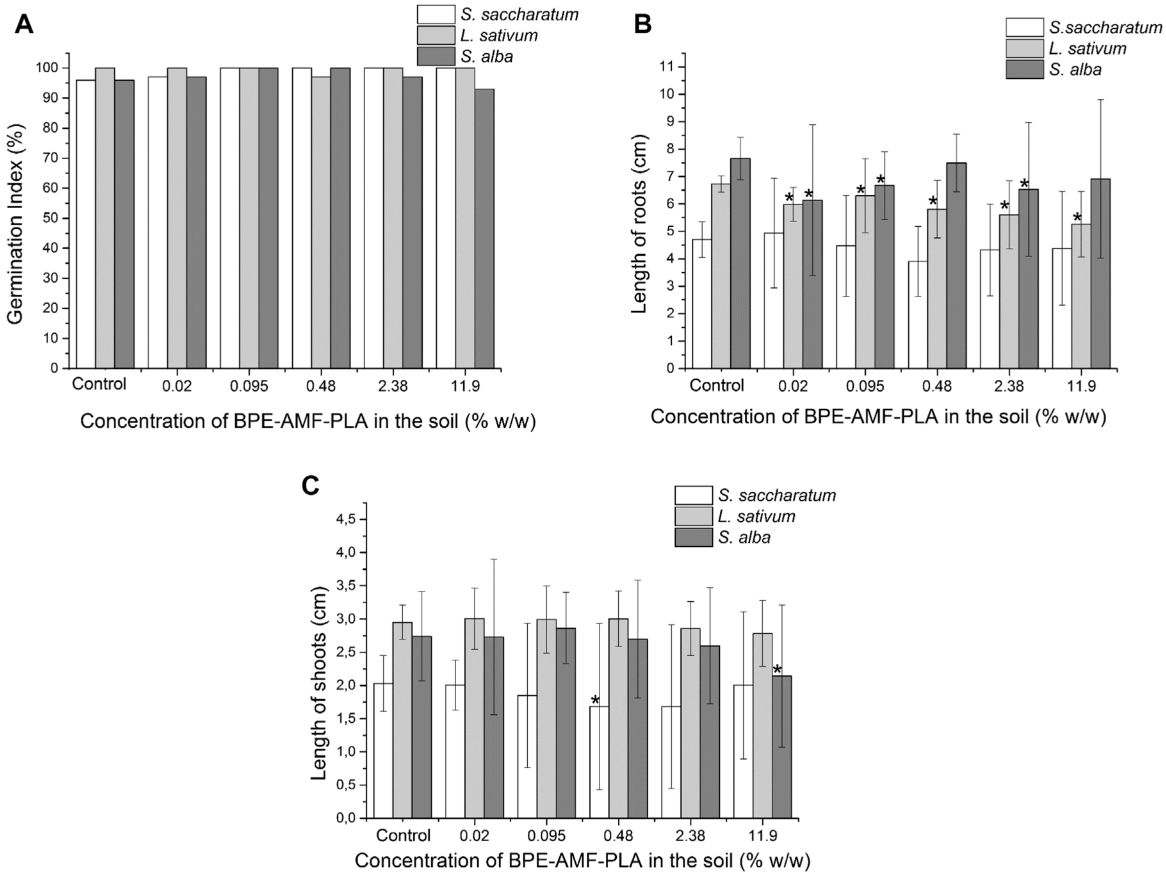

FIGURE 3
(A) Effect of BPE-AMF-PLA on seed germination of three higher plants (*S. saccharatum L. sativum, S. alba*). Standard deviation (SD) was below 2% in each case. **(B)** Effect of BPE-AMF-PLA on root growth of three higher plants (*S. saccharatum, L. sativum, S. alba*). The error bars reflect the values of SD. The asterisks refer to the statistically significant difference compared to control. **(C)** Effect of BPE-AMF-PLA on shoot growth of three higher plants (*S. saccharatum, L. sativum, S. alba*). The error bars reflect the values of SD. The asterisks refer to the statistically significant difference compared to control.

produced per ww lugworm and hour), d) change of weight {[(final ww-initial ww)/initial ww] ×100}, and e) induction of oxidative stress (lipid peroxidation, LPO). Induction of LPO was measured as an increase in malondialdehyde (MDA) and 4-hydroxyalkenals (4-HNE) concentrations (i.e., toxic by-products of LPO) in lugworm soft body tissue homogenate as an indication of oxidative stress (using G-bioscience LPO assay kit, combined with DetectX bicinchoninic acid (BCA) protein assay kit to quantify total protein content of the samples). To test for statistical differences between treatments, one-way analysis of variance (ANOVA) was applied, after log or arcsin transformation of data if required, followed by Tukey HSD *post hoc* test, using SPSS v. 26 (IBM) and statistical significance set to $p < 0.05$. Sediment and faecal mound samples were collected at the end of the 15-day exposure period and stored at 4°C for subsequent microplastic analyses for confirmation of microplastic ingestion. Lugworms were collected, rinsed and gut purged in filtered (0.45 μm) seawater for 24 h at 15°C, weighed and stored at either −80°C for oxidative stress assay, or at −20°C for microplastic analysis. Microplastics were extracted by gentle enzymatic treatment according to the modified method of von Friesen et al. (2016). Briefly, using 1 pancreatic enzyme capsule (Creon® 25000 pankreatin, BGP Products AB, Stockholm) per 15 mL 1 M Tris buffer, pH 8.0 (Biotechnology Grade, VWR Life Science), incubating samples on shaker at 37.5°C for 24 h. Sediment and faecal mound samples from all treatments were density separated by use of saturated sodium iodide (NaI) solution (1.8 kg L^{-1}) in glass funnels, subsequently filtering the supernatant onto 20 μm nylon filters (Bopp Utildi, Sweden), and were analysed for microplastic >20 μm content by use of fluorescence stereo microscope (Leica MZ FLIII).

3 Results

3.1 Toxicity of bio-based agricultural mulch towards soil organisms

3.1.1 Effect of bio-based agricultural mulch on germination and early growth tests with *Sorghum saccharatum, Lepidium sativum* and *Sinapsis alba*

BPE-AMF-PLA did not affect seed germination of any of the three higher plants studied. The germination index (GI) ranged

TABLE 1 **Relative change in earthworm body mass, presented as mean ± standard deviation of the mean.**

Concentration of BPE-AMF-PLA (% w/w)	Relative change in body mass (%)	Standard deviation (%)
0.1	−1.8	0.9
0.5	3.9	1.9
2.5	−3.5	2.1
12.5	2.9	1.7

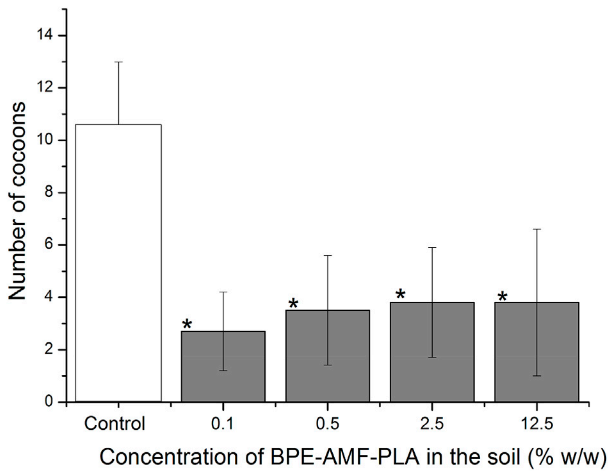

FIGURE 4
Number of cocoons from the earthworm species *E. andrei*exposed and not exposed to BPE-AMF-PLA. Error bars represent the values of SD ($n = 3$). The asterisks refer to the statistically significant difference compared to control.

from 93% to 100% depending on the plant and the concentration of the bio-based plastic particles in the soil (Figure 3A). The values were at the same level as those determined for the control runs.

Neither root growth nor shoot growth of the monocotyledonous plant *S. saccharatum* were affected in the presence of BPE-AMF-PLA in the soil (one-way ANOVA, $p > 0.05$, $n = 3$, Figure 3B). The same was found for the shoot growth of both dicotyledonous plants *L. sativum* and *S. alba* (Figure 3C). However, the root growth of both dicotylodonous species was inhibited (Figure 3B). The length of roots exposed to BPE-AMF-PLA was statistically lower than that of the control without BPE-AMF-PLA material ($p < 0.05$). The reduction in the length of roots varied from 6.4% to 21.8% in the case of cress (*L. sativum*) and from 2.1% to 19.9% in the case of mustard (*S. alba*).

3.1.2 Toxicity towards earthworms *Eisenia andrei*

No mortality of earthworms appeared after 28 days and after 56 days, irrespective of the concentration of BPE-AMF-PLA particles in the soil. BPE-AMF-PLA did not contribute to the decrease of the body mass of earthworms tested (Table 1).

However, presence of BPE-AMF-PLA in the soil significantly affected the reproduction ability of *E. andrei*. Compared to the controls, the number of cocoons decreased by 63.8%–71.4% depending on concentration of the bio-based plastic in the soil (Figure 4). The differences in the number of cocoons between the tests with BPE-AMF-PLA and the control tests were statistically relevant (one-way ANOVA, $p < 0.05$). The 'lowest observed effect concentration' LOEC is equal to 0.1% w/w of BPE-AMF-PLA particles.

3.2 Toxicity of bio-based agricultural mulch towards freshwater invertebrates

3.2.1 Acute *in vitro* toxicity to *Daphnia magna*

Effective concentrations 50 (EC_{50}) values of *D. magna* exposed to potassium dichromate for 48 h (positive control) ranged from 0.8 to 0.9 mg L^{-1}. The pH values were between 7.6 and 8.5. These values are in accordance with OECD 202 (2004), confirming the validity of the assay. The immobilization in standard reference water (negative control) was 0%. The acute contact and leaching tests showed no immobilization of *D. magna* after 24 and 48 h, respectively.

3.2.2 Chronic toxicity to *Daphnia magna*

The mortality of daphnids in the negative control of the first tested BPE-AMF-PLA charge ($n = 10$) of the chronic contact tests was 0% with an offspring of 7.4 ± 0.45 neonates per daphnid during 21 days. The pH ranged between 7.8 and 8.6 during the test period, which is in accordance with validity requirements (OECD, 2012). The chronic contact tests with first charge of the bio-based mulch film (BPE-AMP-PLA) showed no significant deviation from the negative control (Table 2). The mulch film leaching toxicity tests showed a decreasing number of offspring by increasing concentrations of BPE-AMF-PLA and the observed LOEC was 1.5625 g L^{-1}. Therefore, leachates of the first charge of BPE-AMF-PLA provoked adverse effects towards *D. manga* and influence the reproduction of the limnic invertebrate. The second charge of BPE-AMF-PLA leachates did not provoke toxic effects towards *D. magna.*

3.3 Toxicity of bio-based agricultural mulch towards marine algae and invertebrates

3.3.1 Toxicity towards marine algae *Phaeodactylum tricornutum*

The tests with *P. tricornutum* were performed after the results of the first test with *D. magna* were available. For the second batch, the production process was changed, thus avoiding contamination of the material. Only the second batch was used for the tests. The initial pH of the BPE-AMF-PLA leachates was between 7.9 and 8.0 for all

TABLE 2 Offspring and survival of *D. magna* after exposition to AMF-PLA contact (C) and leachates (L) for 21 days.

Concentration AMF-PLA [g·L^{-1}]	Offspring per daphnid C	Dead daphnids C	Offspring per daphnid L first trial	Dead daphnids L first trial	Offspring per daphnid L second trial	Dead daphnids L second trial
0	7.4 ± 4.5	—	7.4 ± 4.5	—	10.8 ± 1.0	1
1.5625	7.2 ± 4.6	—	3.1 ± 2.45*	—	13.5 ± 1.0	1
3.125	7.2 ± 3.5	1	2.4 ± 3.4*	—	17.2 ± 0.8	—
6.25	7.1 ± 5.5	—	1.2 ± 2.7**	1	19.6 ± 1.3	1
12.5	6.9 ± 2.5	—	1.8 ± 2.3**	—	17.4 ± 0.8	1
50	7.0 ± 3.0	1	-***	4	13.5 ± 0.8	1

*$p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

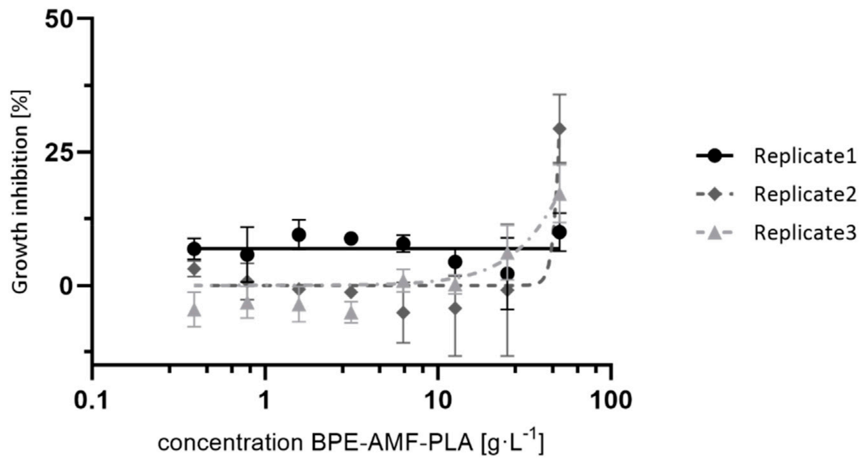

FIGURE 5
Concentration-response curves for bio-based mulch film in chronic marine algae test with *P. tricornutum* after 72 h of exposure. Plotted are the mean growth inhibition and standard deviations of three independent replicates.

dilutions, which is within the optimal pH range according to DIN, 2015 (optimal 8.0 ± 0.2). During the tests, the pH value increased up to 8.4, which also complies with the guideline (the pH value must not have increased by more than 1.0). The cell density in the negative control increased exponentially by a factor of 16 ± 2 (DIN, 2015). EC_{50}-values ranged from 1.27 to 1.33 mg L^{-1} for 3,5-DCP as positive control. Growth inhibition was observed for BPE-AMF-PLA mulch film material (F (7, 64) = 11.65, $p < 0.001$). The daily growth rate of *P. tricornutum* was 0.9–1.0. NOEC was 12.5, and LOEC was 25 g of BPE-AMF-PLA per liter (Figure 5). No harmful substances were detected by GC-MS in the leaching medium.

3.3.2 Effects on digestive enzyme activities of *Artemia persimilis* nauplii

Artemia nauplii ingested both types of micro-particles. Compared to the empty gut (Figure 6A), ingested fluorescent microbeads appeared bright green (Figure 6B) and ingested BPE-AMF-PLA particles appeared densely dark packed in the gut (Figure 6C).

The average esterase activity of the control group (FSW without micro-particles) was 30.3 ± 5.8 mU ind^{-1} (Figure 7A). *Artemia* nauplii exposed to BPE-AMF-PLA showed similar activities of 30.8 ± 1.1 mU ind^{-1} (df = 4, t = 0.164, p = 0.878). Lipase activity increased significantly from 0.48 ± 0.13 mU ind^{-1} in the control group to 0.94 ± 0.18 mU ind^{-1} after ingestion of BPE-AMF-PLA (Figure 7B, df = 4, t = 3.604, p = 0.022).

3.3.3 Effects on biological activity and health of *Arenicola marina*

No adverse effects on *Arenicola marina* biological activity or general health were detected after 15 days exposure to BPE-AMF-PLA microparticles (100–300 µm, at 0.1% per sediment dry mass).

A. marina ingested all types of microplastics, shown by the presence of microplastics in the faeces of lugworms from all experimental treatments but the controls. Lugworms from the different treatments showed no significant difference in the time it took to initiate borrowing (One-way ANOVA, F = 1.88, df = 4, p = 0.13; Figure 8A), however, the time it took to complete burrowing varied significantly between treatments (One-way ANOVA, F = 3.95, df = 4, p = 0.008; IVL Figure 8B).

Lugworms on sediment with pristine LDPE microplastics took significantly longer time to bury, compared to those on sediment

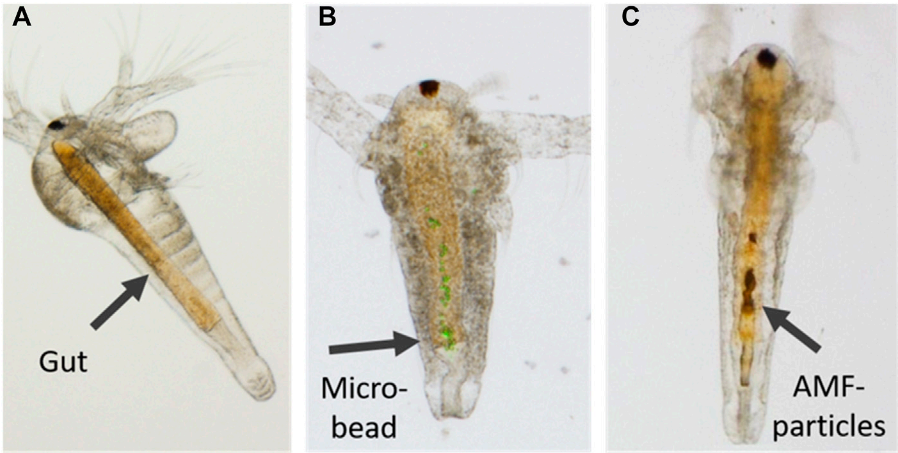

FIGURE 6
Freshly hatched *Artemia persimilis* nauplii with **(A)** empty gut, **(B)** ingested fluorescent microbeads (9.9 μm), and **(C)** ingested BPE-AMF-PLA microplarticles.

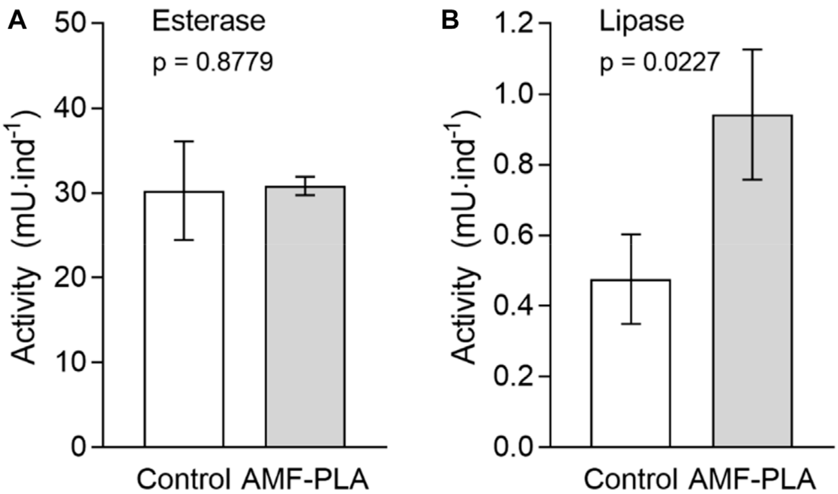

FIGURE 7
Activities of **(A)** esterase (C4) and **(B)** lipase (C18) in *Artemia persimilis* nauplii of the control and nauplii exposed to BPE-AMF-PLA (mean ± SD, n = 3).

with UV-treated microplastics of both kinds (Tukey HSD, $p < 0.05$). There was no significant difference in feeding rate between treatments, investigated by measuring the volume of faecal mounds produced per wet weight lugworm and hour, average over the 15 days of exposure (One-way ANOVA, $F = 0.22$, df = 4, $p = 0.92$; Figure 9).

The mortality was low throughout the experiment (0–1 individuals per treatment) and there were only minor changes in weight of the lugworms after 15 days of microplastic exposure (mean weight change: 0.039 ± 0.32 g, corresponding to a 0.36% weight change), with no statistical differences between treatments (One-way ANOVA, $F = 1.32$, df = 4, $p = 0.28$).

A minor increase of lipid peroxidation (LPO) could be detected in lugworm soft body tissue, but there was no indication of oxidative stress at microplastic exposure, instead MDA and 4-HNE concentrations were similar or even lower compared to the controls (One-way ANOVA, $F = 1.70$, df = 4, $p = 0.21$; Figure 10).

3.3.4 Summary of the results

As a summary of the results of this comprehensive ecotoxicological study, Table 3 provides the list of the experiments carried out in the three environmental compartments (soil, freshwater, marine), stating the organisms and/or test species, names of the tests/bioassays (standardized or well-established), indication if works are previously published or not in the scope of BIO-PLASTICS EUROPE project, test endpoints and concentration ranges.

The table also reports the main (adverse) effects obtained in each bioassay, including the derived ecotoxicity parameters, where applicable (LOECs and NOECs; Table 3). The plant responses in a single-species

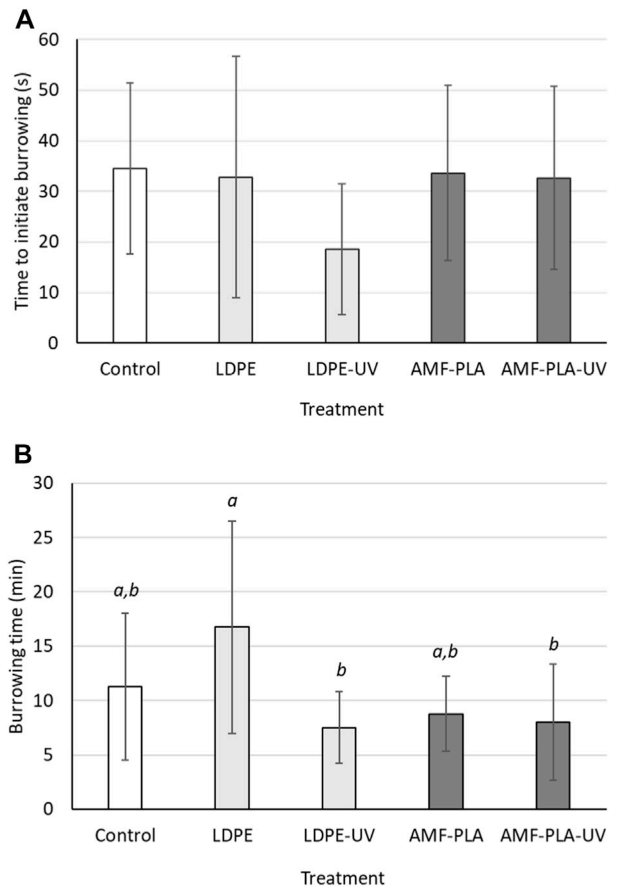

FIGURE 8
Time to initiate burrowing (s) **(A)** and time to complete burrowing (min) **(B)** for lugworms added to sediment spiked with different microplastic treatments, i.e., pristine or UV-treated LDPE and AMF-PLA, or to untreated control sediment. Significantly separated groups ($p < 0.05$) indicated with different letters above bars (One-way ANOVA and HSD Tukey post doc test; bars show means ± SD, $n = 10$, except AMF-PLA-UV in A where $n = 9$).

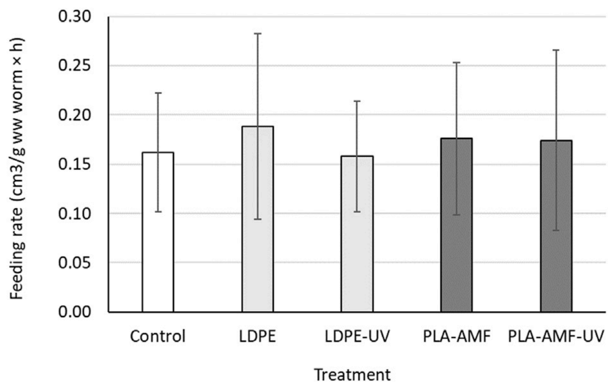

FIGURE 9
Feeding activity of *Arenicola marina* averaged over 15-days of exposure to sediment spiked with LDPE and AMF-PLA microplastics (0.1% based on dw sediment), either pristine or pre-treated with UV-light, or to untreated control sediment. Feeding activity is defined as average volume faeces produced per hour, divided by weight of each lugworm (bars show means ± SD, $n = 9$, except AMF-PLA-UV, which had $n = 10$).

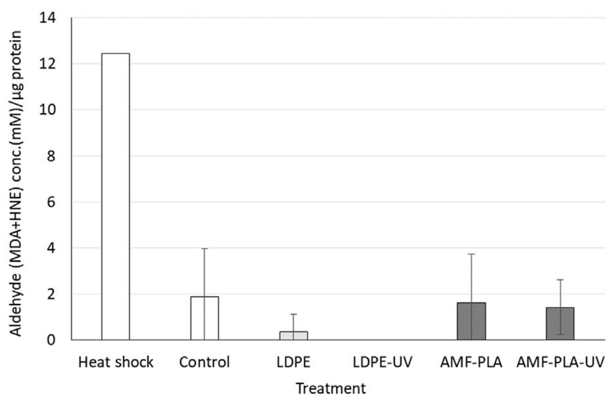

FIGURE 10
Oxidative stress in *Arenicola marina* soft body tissue after exposure to microplastics, measured as concentration (mM) of malondialdehyde (MDA) and 4-hydroxyalkenals (4-HNE) (i.e., toxic by-products of lipid peroxidation) per µg protein. Heat shock represents tissue homogenate from a lugworm not included in the experiment but pre-heated at 50°C for 1.5 h, used as a positive control (bars indicate means ± SD, $n = 4$ for LDPE, LDPE-UV, AMF-PLA-UV, $n = 3$ for Control, AMF-PLA).

bioassays were not validated with the microcosms approach, the latter showing no adverse impact of BPE-AMF-PLA on the tested species. However, the earthworm responses suggest a negative impact on the population level, including the risk to the habitat function of soil in the presence of the tested bio-based plastics. Reduction in daphnid reproduction when subjected to the leachates in comparison to the no-effect when exposed in the contact assay, emphasize the need to further address various environmental scenarios and develop methodologies that could be proxies for weathering or ageing of bio-based plastic films. Adverse effects on algae as primary producers raises concern on the effects on marine ecosystem, as an indirect sink of bio-based plastic and/or bio-microplastics.

4 Discussion

4.1 Toxicity towards soil organisms

The current results are in accordance with the published study from the higher tier approach with the terrestrial microcosms, being the integrative part of this ecotoxicological assessment of BPE-AMF-PLA within BIO-PLASTICS EUROPE. BPE-AMF-PLA did not deteriorate seed germination processes of any of the two plants (sorgho and cress) used in the microcosm tests (Liwarska-Bizukojć, 2022b). Also, the shoot fresh mass and shoot length of these plants were not affected. The differences between the fresh mass or the length of shoots exposed to BPE-AMF-PLA and the fresh mass or the length of shoots not exposed to this material were not statistically significant ($p > 0.05$) (Liwarska-Bizukojć, 2022b). BPE-AMF-PLA did not contribute to the mortality of *E. andrei*, resulting in

TABLE 3 Summary and the main outcomes of the ecotoxicity experiments conducted with BPE-AMF-PLA bio-based plastics in the scope of BIO-PLASTICS EUROPE project. LOEC—lowest observed effect concentration, NOEC—no observed effect concentration.

Environmental compartment	Organism/ species tested	Test name	Result previously published	Endpoints evaluated	Test concentrations/ ranges of BPE-AMF-PLA	Adverse effects observed	Derived ecotoxicity parameters
Soil	Plants *Sorghum saccharatum, Lepidium sativum, Sinapsis alba*	ISO 18763	No	Germination, root and shoot length	0.02, 0.095, 0.48, 2.38, 11.9% w/w	Root reduction in *L. sativum* and *S. alba*	*L. sativum* LOEC = 0.02% w/w of BPE-AMF-PLA particles
	Earthworm *Eisenia andrei*	OECD 222	No	Survival, body mass change, reproduction	0.1, 0.5, 2.5, 12.5% w/w	Reduction in offspring	LOEC = 0.1% w/w of BPE-AMF-PLA particles
	Multi-species *Eisenia andrei, Sorghum saccharatum, Lepidium sativum*	Microcosms	Yes (Liwarska-Bizukojc, 2022b)	Plant biomass and germination, earthworm survival, earthworm avoidance/ preference behaviour	2.5% w/w	Avoidance behaviour of earthworms	
Freshwater	Crustacean	OECD 202	No	Immobilization	1.5625, 3.125, 6.25, 12.5, 50 g L^{-1}	First trial: decreasing number of offspring by increasing concentrations Second trial: None Reduction in offspring in the leachate test	First trial: LOEC = 1.625 g L^{-1}
	Daphnia magna	OECD 211 - Contact test and leachate test approaches		Survival & reproduction			
Marine water	Alga *Phaeodactylum tricornutum*	DIN EN ISO 10253; leachate test	No	Growth	0.39–50 g L^{-1}	Inhibition of growth	NOEC = 12.5, LOEC = 25 g BPE-AMF-PLA per L
	Shrimp *Artemia persimilis*	Effects on digestive enzymes of *Artemia* nauplii	No	Digestive enzymes activity	3 g L^{-1}	Increased activity of lipase	
	Lugworm *Arenicola marina*	Toxicity towards the marine infaunal lugworm *Arenicola marina*	No	Burrowing activity, feeding rate, change of weight, induction of oxidative stress (lipid peroxidation - LPO)	100–300 μm at 0.1% per sediment dry mass	Presence of the microparticles in faeces; No adverse effects	

survival of all test individuals. Also, the body mass of earthworms exposed to BPE-AMF-PLA was not affected. The significant differences in the depth distribution of earthworms between the small-scale terrestrial model eco-systems (STMEs) containing the particles of BPE-AMF-PLA and the control STME were observed. The presence of the bio-based plastics favoured the downward movement of earthworms Liwarska-Bizukojć, 2022b). This is an indication on the need for the integrative approaches that allow for assessment of organisms' interactions under bio-based plastics application, and additional endpoints, including the impact on soil habitat function (i.e., using avoidance behaviour as indicator).

4.2 Toxicity towards freshwater invertebrates

The bio-based mulch film did not provoke acute toxic effects towards *D. magna*. These results are in accordance to Lithner et al. (2009, 2011) as most of the conventional plastics tested in the studies had EC_{50} values higher than 250 g L^{-1}. There was a lack of the adverse effects in the acute test, but the reduction in offspring is aligned with the study of Schrank et al. (2019).

A strong effect was provoked by mulch film leaching tests since the leaching of accompanying and metabolite compounds like plasticizers might be harmful for daphnids. Therefore, the chemical analysis of the leaching medium detected an unusually high concentration of 186 μg 2-methylnaphthalene leaching from 1 g mulch film. Since 2-methylnaphthelene provoked immobilization of *D. magna* in acute tests (Bobra et al., 1983), mortality during chronic leaching tests was probably caused by the contamination of the mulch film with 2-methylnaphthlene. However, 2-methylnaphthalene was not an additive of the mulch film material but could be traced back to a contamination of the material with lubricating oil in the manufacturing process of the film. By repeating the chronic contact and leaching tests of bio-based mulch film with a new charge, no toxic effects were observed and no 2-methylnaphthalene could be detected by GC-MS.

4.3 Toxicity towards marine invertebrates

Artemia nauplii are suspension feeders, which ingest a wide range of digestible and indigestible particles (Bour et al., 2020). Similar to our study, the closely related *Artemia franciscana* ingested particles in the size range of 6.8–27.5 μm (Kokalj et al., 2018). Most of the particles were egested after 24 h. Only a small amount remained in the intestine after 72 h (Eom et al., 2020).

Digestive enzymes play a crucial role in the utilization of food and, thus, energy metabolism. Changes in diet or starvation can affect digestive enzyme activity in various invertebrate species, including crustaceans and molluscs (Jones et al., 1997; Johnston & Freeman, 2005; Kreibich et al., 2008; Koussoroplis et al., 2017, Trestrail et al., 2021). Likewise, ingestion of microplastic particles has been shown to alter digestive activities in, e.g., crustaceans and fish (Gambardella et al., 2017; Romano et al., 2018; Korez et al., 2019; Han et al., 2021).

Esterases are a diverse group of enzymes, capable of hydrolysing ester bonds with wide substrate specificity. Herbivorous, omnivorous, and detritivorous organisms use esterases to degrade tannins and phenolic compounds (Hübner et al., 2015). An increase in esterase activity has been reported in the marine isopod *Idotea emarginata* after ingestion of food enriched with PMMA particles (Korez et al., 2019). Binding sites of esterases are present in the PMMA polymer. However, the biochemical background of the hydrolysis reaction is unknown. Exposure of *Artemia* nauplii to BPE-AMF-PLA caused no change in esterase activity as compared to the control animals although the polymer is linked by ester bonds.

Lipases hydrolyze longer-chained substrates than esterases. They usually split triacylglycerides into glycerol and fatty acids by hydrolyzing the ester bonds (Rivera-Pérez et al., 2011). Lipase activity in *Daphnia magna* increased when food of poor quality was given (Koussoroplis et al., 2017). The authors hypothesized that digestive enzyme secretion might be homeostatically controlled to ensure a sufficient uptake of the most limiting nutrients.

BPE-AMF-PLA is a blend of PLA and polybutylene adipate terephthalate (PBAT). Both PLA and PBAT contain ester groups. However, exposure to pure PLA does not enhance lipase activity in *Artemia* nauplii (data not shown). Therefore, degradation of PBAT within the BPE-AMF-PLA bio-based plastics may be more likely. Even though these results were unexpected, lipases were previously reported to degrade plastics. Several studies showed that some aliphatic polymers are degraded by bacterial lipases (Tan et al., 2021).

The results of the current study complement *in-vitro* observations by Miksch et al. (2022) who found that BPE-AMF-PLA is readily hydrolyzed by isolated lipase but not by esterase from micro-organisms. Apparently, ingestion of BPE-AMF-PLA microparticles by *Artemia* nauplii selectively activates the digestive system. Probably, the liberation of bio-based plastic oligomers stimulates the expression of lipase to enzymatically degrade the biopolymer. Whether the resulting oligo- or monomers can be metabolized as valuable energy source or the elevated enzyme activity is a false and metabolic costly reaction remains to be investigated.

Mulch films accidently or deliberately released to the environment will eventually generate microplastic particles, induced by weathering and fragmentation processes. Microplastics that end up in marine waters will likely sink to the bottom and accumulate in sediments that act as a sink, with possible impacts on benthic fauna. Indeed, high concentrations of conventional microplastics have been measured in sediments along the Spanish coast in areas with intense agricultural industry (Dahl et al., 2021). The marine lugworm *A. marina* is a non-selective deposit feeder, occurring at high densities in shallow, sandy to muddy bays around Europe, likely to ingest large amounts of microplastics in such polluted areas. Lugworms are important bioturbating bioengineers, as well as important food item for fish and seabirds and thus functions as a vector for the transfer of plastics and chemicals from sediments to higher trophic levels (Cadée, 1976). Thus, investigating effects of microplastics of BPE-AMF-PLA and conventional mulch film plastics on *A. marina* is of high ecological relevance. Here, we found no adverse effects on *A. marina* biological activity or general health at exposure to BPE-AMF-PLA or fossil-based LDPE microplastics mixed with surface sediment to 0.1% dw. *A. marina* ingested all microplastics, as shown by their presence in lugworm faeces, but there were no effects on burrowing or feeding behaviour of the pristine microplastics, and none of the microplastics affected the body mass of lugworms or induced oxidative stress or mortality, contradicting any negative impacts related to chemical exposure or dilution of edible organic material of the sediment at this concentration. We used induction of lipid peroxidation (LPO) as a measure of oxidative stress at microplastic exposure. LPO is a well-established example of oxidative damage in cell membranes, lipoproteins and other lipid-containing structures, often used as a biomarker related to pollutants in marine invertebrates (Lesser, 2006; Hannam et al., 2010). The great induction of LPO by heat shock treatment, used here as a positive control, validates the bioassay for this species, but other biomarkers may be more sensitive for stress responses induced by microplastic pollution not detected here.

Furthermore, microplastics that are affected by different abiotic and biotic processes in the environment undergo alterations in their physical and chemical characteristics that might affect their toxicity. Both increases and decreases in toxicity have been observed after UV-irradiation of different microplastics (Bejgarn et al., 2015; Simon et al., 2021). We found no effect of UV-weathering on BPE-AMF-PLA. However, it took significantly longer time for lugworms to bury in sediment mixed with pristine LDPE microplastics, compared to sediment mixed with UV-aged microplastics (Figure 8B). A possible explanation could be that *A. marina* senses and therefore avoids sediment contaminated with LDPE to minimize exposure, but that this effect is rescinded by an increased biofouling of microorganisms on plastic surfaces affected by weathering.

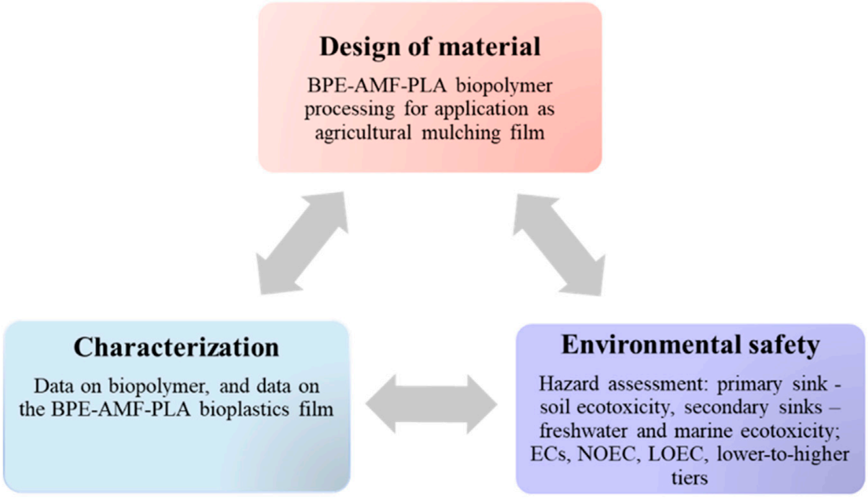

FIGURE 11
The scheme presenting a preliminary framework towards safe-by-design bio-based plastic mulch film development, using the BPE-AMF-PLA case study.

The exposure concentration used for the *A. marina* experiment represents high but still environmentally relevant levels of microplastics in sediments of highly polluted areas (Carson et al., 2011; Haave et al., 2019). Microplastics have previously been shown to affect *A. marina* by decreases in weight (Besseling et al., 2013), depletion of energy reserves (Wright et al., 2013), increased oxygen consumption (Green et al., 2016) and reduced feeding activity (Besseling et al., 2013; Wright et al., 2013; Green et al., 2016), although at higher concentrations (5%–10%) and longer expsoure time (ca. 4 weeks) compared to the current study. Although no adverse effects of BPE-AMF-PLA on *A. marina* were detected here, toxic effects may still exist at higher concentrations or after longer exposure times, but this remains to be investigated.

4.4 Development of the cross section and framework representing necessity of this kind of studies

As BPE-AMF-PLA is not yet commercially available, the ecotoxicity tools used in the current study are directly serving to the safe-by-design product development. The key iterative elements of preliminary framework for such product development are 1) design of bio-based plastic, 2) characterisation of the material (biopolymer itself, and the product—bio-based plastic film) and 3) environmental safety evaluation (Figure 11). Beyond this, the present case study of BPE-AMF-PLA can be used as a testing scheme for hazard assessment, or impact of bio-based plastics' disposal to soil in support of EU regulations and strategies on environmental quality and biodiversity, such as EU Green Deal, EU Action Plan on zero pollution, EU Soil Strategy 2030 (EU Commission, 2019; EU Commission, 2021a; EU Commission, 2021a).

In the current work, the environmental safety aspects of the framework are demonstrated by: 1) conducting integrative ecotoxicity assessment in three environmental compartments while taking into account the leaching potential of bio-based plastics—soil (as primary media, i.e., sink of bio-based plastics when using in agriculture as mulching film), fresh water and marine water (as secondary sinks of bio-based plastics and/or bio-based microplastics); 2) using organisms with different exposure routes to potentially toxic compounds from the bio-based plastics, and/or from different trophic levels within the same compartment; 3) development of different sample preparation procedures and simulation of different exposure scenarios; 4) evaluation of acute and chronic endpoints, and extrapolation of ecotoxicity parameters especially relevant for regulatory risk assessment (NOEC, LOEC); 5) the approach from lower-to higher-tier, where the latter can be applied as an intermediate tool between the laboratory single species standardized bioassays and field studies; 6) evaluation of sublethal endpoints by targeting different levels of biological organisation—from effects on organisms to biochemical-level responses, therefore providing mechanistic understanding and fundamental knowledge on the impact of bio-based plastics/ micro-bio-based plastics on non-target organisms. Finally, the results and recommendations of the current study may provide additional support for the certification of the biopolymers and bio-based plastic materials, thus contributing evidence for their safe and sustainable use in line with European strategies and regulations (EU Commission, 2018; EU Commission Eu, 2021c).

5 Conclusion

Overall, organisms' responses to PLA-based plastics were endpoint- and species-specific. Low-to-no phytotoxicity was observed upon exposure to the soil amended with particles of BPE-AMF-PLA. The exception was the growth reduction effect observed in the roots of dicotyledon plants. This result emphasizes the need to better understand and contextualize the use of bio-based mulching films with different plant/crop species. The earthworms were the most sensitive species tested in the current study, with the reproduction (number of cocoons) as the most sensitive endpoint (LOEC = 0.1% w/w of BPE-AMF-PLA particles), followed by the avoidance of the soil containing BPE-AMF-PLA in the microcosms experiment (2.5% w/w). Toxicity to freshwater crustacean *D. magna* was possibly linked to the presence of 2-methylnaphthalene, which can be avoided in the material production process. The reproduction response was dependent on the sample preparation method, revealing the reduction in offspring when exposed to the leachate of BPE-AMF-PLA. No adverse effects of BPE-AMF-PLA were observed in the contact assay. Growth of the marine algae was significantly inhibited at the concentration of 25 g BPE-AMF-PLA l^{-1}. Lugworm *A. marina* ingested both bio-based and conventional, fossil-based microplastics, as shown by the presence of microplastics in the faeces. Despite no adverse effects on the organisms' biological activity and health were reported in this study, a risk for trophic transfer cannot be excluded. Further contextualization of risks under relevant environmental conditions and range of abiotic and biotic factors remains to be addressed prior to safe use of novel bio-based plastic mulching films. Digestive enzyme activity, namely, lipase, was increased in brine shrimp *Artemia nauplii.* Although benefits or costs of such response need to be elucidated, the reported results indicate potential degradation of biopolymers within the bio-based plastic film by these organisms. This study represents an early ecotoxicological safety evaluation of the PLA-based plastic film BPE-AMF-PLA, destined to prevent development and production of toxic alternatives. Beyond this, the study approach and results provide a solid platform for the framework development for safe use of novel bio-based materials. A comprehensive risk assessment needs to consider the conditions of product use, the potential release of toxic substances, and their environmental accumulation. Consideration of these factors allows for estimating the Predicted Environmental Concentration (PEC), which can then be tested to target organisms. Additionally, biodegradabilty of biopolymers is a critical property that needs to be contextualized alongside the ecotoxicological approaches and environmentally relevant scenarios. These factors should be considered in follow-up studies from the BIO-PLASTICS EUROPE project, which will be based on a broader knowledge regarding the novel compounds.

Data availability statement

The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation.

Author contributions

JB: Conceptualization; Project administration; Resources; Supervision; Roles/Writing—original draft; Writing—review and editing. EA: Supervision, Roles/Writing—original draft; Writing—review and editing. C-YC: Data curation; Investigation; Methodology. MG: Conceptualization, Data curation; Formal analysis; Investigation; Methodology; Supervision; Validation. LG: Conceptualization, Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Resources; Supervision; Validation; Writing—review and editing. A-SK: Conceptualization, Data curation; Formal analysis; Investigation; Methodology; Supervision; Validation; Writing—original draft. SDK: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Supervision; Validation; Writing—review and editing. WLF: Supervision; Validation. EL-B: Data curation; Investigation; Methodology; Roles/Writing—original draft. LM: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Supervision; Validation; Writing—review and editing. KP: Conceptualization; Data curation; Formal analysis; Methodology; Validation; Writing—original draft. MP: Conceptualization; Methodology; Writing—review and editing. RS: Conceptualization; Formal analysis; Funding acquisition; Investigation; Methodology; Supervision; Validation; Visualization; Writing—original draft. RSR: Methodology; Writing—review and editing. GW: Roles/Writing—original draft; Writing—review and editing. All authors contributed to the article and approved the submitted version.

Funding

This study has received funding from the European Union's Horizon 2020—Research and Innovation Framework Programme through the research project BIO-PLASTICS EUROPE, under grant agreement No.860407. Thanks are due to financial support to CESAM by FCT/MCTES (UIDP/50017/2020 + UIDB/50017/2020 + LA/P/0094/2020), through Portuguese national funds.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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TYPE Original Research
PUBLISHED 13 July 2023
DOI 10.3389/fenvs.2023.1149314

OPEN ACCESS

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RECEIVED 21 January 2023
ACCEPTED 16 June 2023
PUBLISHED 13 July 2023

CITATION
Haque MR, Chowdhury FN, Hossain A, Akter R and Rahman MM (2023), An emerging concern of medical waste management in Rohingya refugee camps at Cox's Bazar, Bangladesh: existing practice and alternatives.
Front. Environ. Sci. 11:1149314.
doi: 10.3389/fenvs.2023.1149314

An emerging concern of medical waste management in Rohingya refugee camps at Cox's Bazar, Bangladesh: existing practice and alternatives

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The plight of humanity in refugee camps is an age-old issue, as is the ever-increasing issue of waste management, especially medical waste. Though situations have improved in recent times for refugee camps, the same cannot be concurred about medical waste management, as it increases in amount every day. This is the first study on the refugee camp, which was conducted to assess the status of medical waste management and to quantify and characterize medical wastes disposed of in the Rohingya refugee camp at Cox's Bazar, Bangladesh. A cross-sectional, qualitative, and quantitative study was done. A total of 499 households, 30 solid waste collectors, 30 HCF workers, and 21 solid waste management (SWM) plant workers were interviewed by KoBo Toolbox. Monthly medical waste collection data from February to June 2022 was also collected. Data collection, processing, and statistical analysis were done using Origin Pro and SPSS software. It was found that the camps did not follow any specific guidelines for medical waste management except for a few segregations in healthcare facilities (HCF). Though 88% of the respondents were aware of the hazardous nature of medical waste, most of them disposed of these wastes in open places (49%) and drains (44%), and 70% did not segregate it from regular waste at the household level. Moreover, 73% of solid waste management workers found medical waste daily in communal bins. The HCF did not keep any records on the production of medical waste. Different types of medical waste were found in communal bins; glass bottles containing medicines were common among them. Data from the material recovery facility (MRF) of this camp showed that the highest amount of medical waste found in the communal bins was in April (65 kg) during the seasonal change from spring to summer and the lowest in February (12.7 kg). Moreover, HCF's existing medical waste management practices were analyzed with SWOT and DPSIR framework. Based on all the findings, a comprehensive on-site and off-site management plan for medical waste is also proposed here. That will help the concerned prepare a camp medical waste management guideline.

KEYWORDS

health care facility, medical waste management, SWOT, DPSIR, WHO, health risk

1 Introduction

"Nothing on Earth is more international than disease," said Paul Russel. Health and disease have no political or geographical boundaries (WHO, 1980). Health care facility has been established worldwide to save us from different diseases (Alam, 2019; Barua and Hossain, 2021; Khalid et al., 2021). This facility creates a special kind of waste that is called medical waste (MW). Environmental protection and public health are seriously threatened by medical waste. Due to its rise in volume, medical waste management (MWM) difficulties that exist worldwide have been made worse in the COVID-19 pandemic (Barua and Hossain, 2021; Khalid et al., 2021). The World Health Organization (WHO) defines medical waste as "Waste that is produced in the diagnosis, treatment, or immunization of individuals or animals in research related there, or in the manufacturing or testing of biologicals" (Windfeld and Brooks, 2015). WHO predicts that hazardous compounds that may be infectious, poisonous, or radioactive comprise about 20% of these medical wastes (Birchard, 2002). Medical waste contributes to the second-largest volume of hazardous waste in the nation, according to Asian Development Bank's hazardous waste inventory from 2008 (Hasnat and Sinha, 2010). MWM is one of the many intricate and demanding problems that humankind is currently experiencing as the world's population grows and the need for medical services rises (Windfeld and Brooks, 2015). When medical waste is not managed and is therefore disposed of inappropriately, there is a significant danger of infection or injury for medical staff as well as risk for the general public due to the release of microorganisms from medical institutions and hazardous properties of medical waste into the environment (Mohee, 2005; Chauhan and Singh, 2016; Mitiku et al., 2022; Wassie et al., 2022). So MWM is an important event for all countries. Most industrialized countries have laws governing medical waste, but there is typically minimal guidance on campsite medical waste management (Mbongwe et al., 2008; Prem Ananth et al., 2010). As a result, medical waste in different campsites is not adequately managed, and various pathogens and infectious agents can frequently spread (Kwikiriza et al., 2019).

Bangladesh is a small developing country. The population of Bangladesh is higher than its area. This large number of people in Bangladesh requires many healthcare facilities to get proper health treatment. This establishment also produces a large number of medical waste. In Bangladesh, medical waste generation is estimated to be roughly 0.5 kg/patient/day under typical circumstances (Hassan et al., 2008; Biswas et al., 2011). In opposition to this, during the COVID-19 pandemic, waste production increased to 3.4 kg/patient/day, around 6.8 times more than usual (ADB, 2020). Medical waste management was a predicament in Bangladesh even before the pandemic ensued (Barua and Hossain, 2021). After the pandemic, the condition becomes more worst. In 2004, the first environmental assessment and action plan for the country's sector addressing MWM in the areas of health, nutrition, and population was made public. It was later upgraded in 2011 (Barua and Hossain, 2021). Bangladesh published its first MWM rules in 2008. Later, from 2009 onwards, some Non-Government Organizations (NGOs) came forward for MWM. However, Bangladesh has yet to demonstrate proper implementation of the MWM system's rules. Failure to implement adequate MWM might endanger the country's ecology and biodiversity (Hassan et al., 2008; Barua and Hossain, 2021).

On the 25th of August in 2017, a previously unprecedented surge of Rohingya refugees from Myanmar's Rakhine State began arriving in Cox's Bazar of Bangladesh. They are predominantly Forcibly Displaced Myanmar Nationals (FDMNs) from Myanmar who have fled to Bangladesh. In the Cox's Bazar area, as of October 2019, there were an estimated 911,566 Rohingya refugees, 905,754 of whom were housed in 34 refugee camps (Security and Management, 2019). This created a humanitarian disaster and necessitated considerable cooperation among several stakeholders to face this. (Alam, 2019; Lewis, 2019; MacLean, 2019; Jeffries et al., 2021). This demographic group also has significant and diverse physical and mental health requirements, including issues with sexual and reproductive health, infectious diseases, chronic illnesses, physical impairments, injuries, and emergencies (Kwikiriza et al., 2019; Al-Khatib et al., 2020; Andrew et al., 2021; Jeffries et al., 2021). To give them medical support, non-governmental organizations such as the United Nations High Commission for Refugees (UNHCR), International Organization for Migration (IOM), Bangladesh Rural Advancement Committee (BRAC), Gonoshasthaya Kendra (GK), and United Nations International Children Emergency Fund (UNICEF) establish health camp facilities. These procedures create massive medical waste containing infectious and pathogenic contaminants. Exposure to hazardous medical wastes can cause infections, infertility, genital deformities, hormonally triggered cancers, mutagenicity, dermatitis, asthma, and neurological disorders in human beings (New WHO Handbook on Healthcare Waste Management, 2013). Typhoid, cholera, hepatitis, AIDS, and other viral infections can be transmitted through sharps contaminated with blood (Oli et al., 2016; Kenny and Priyadarshini, 2021). Considering the issue, managing medical waste is necessary for the Rohingya camp (Chauhan and Singh, 2016). However, there is very little specific and detailed literature on medical waste management in humanitarian campsites (De Montclos and Kagwanja, 2000; Oka, 2014; Ekezie et al., 2019; Karsu et al., 2019; Zarei, 2022). As a result, medical waste poses considerable health and environmental risks to camp people and the surrounding locality. This study has been conducted to eradicate this issue and ensure more efficient medical waste management in campsites. It will also critique existing management facilities and show possible mitigation alternatives. That will help the concerned prepare a camp medical waste management guideline and ensure SDG 3.

The specific objective of this study is (1) to characterize medical waste generated with regular solid waste in the context of the refugee camp, (2) to find out the efficiency of existing medical waste management with SWOT analysis and DPSIR framework, (3) to recommend an alternative for more efficient medical waste management in the humanitarian context.

2 Methodology

2.1 Study area

The study area is mainly conducted in the Rohingya camps. The refugee camp is situated in the southeast part of Bangladesh, in Cox's

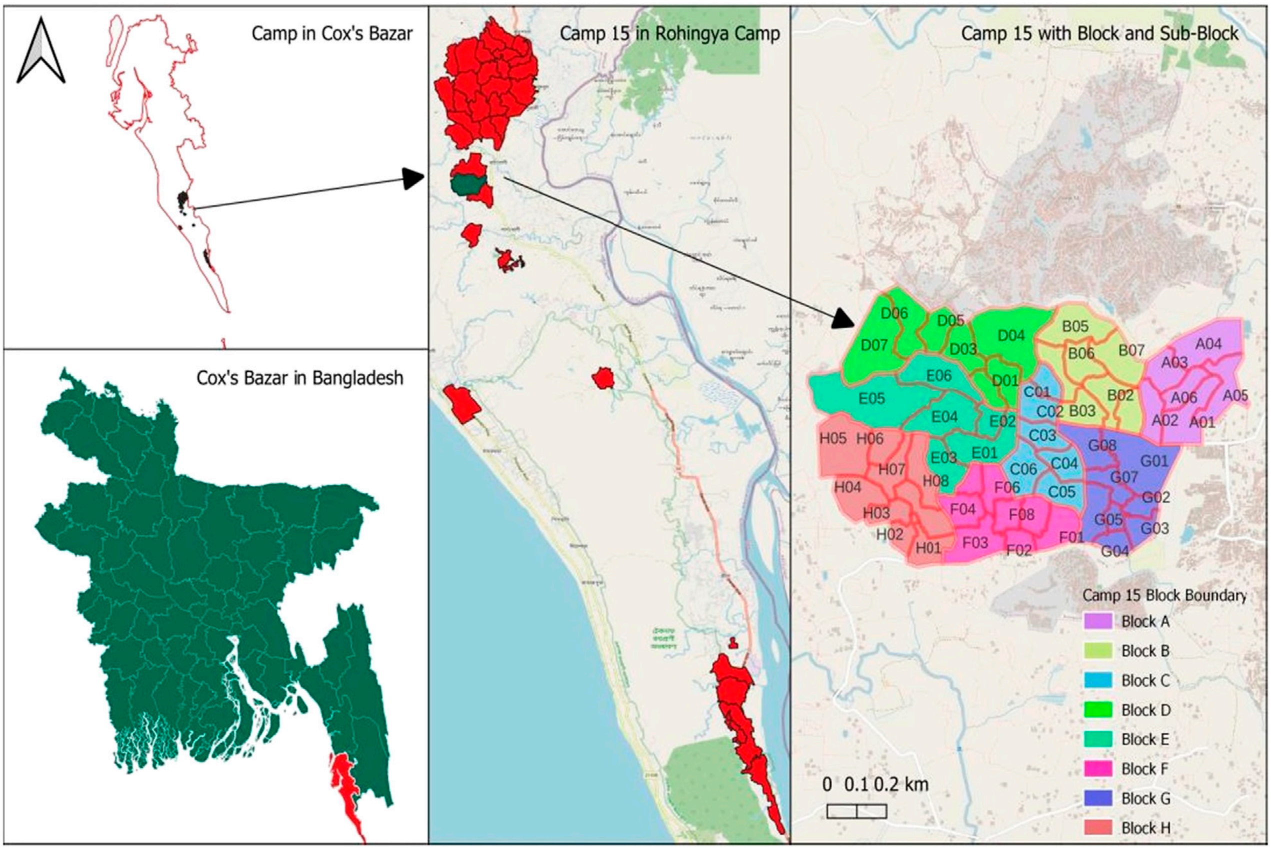

FIGURE 1
Study area of Camp-15 Ukhia, Cox's Bazar.

Bazar city of the Chattogram division. The massive influx of refugees into Bangladesh from violence in the neighbouring country Myanmar now stands at 33 crowded camps in Cox's Bazar district (Figure 1). The main source of quantitative data in this study was from the FDMNs, solid waste management personnel from Camp-15 (Ukhia). This camp houses refugees from the Rakhine state of the neighbouring country Myanmar. The camp and the respondents in the questionnaire survey were selected through a random sampling method, more specifically, the purposive random sampling method, which was done to ensure the most effective data collection in the study to paint out the complete scenario.

2.2 Study population

To do random sampling in this case, the sample size was calculated as follows (Krejcie and Morgan, 1970)

$$‘n’ = N^* \left[Z_{2^*} p^* \left(1 - p\right)/e^2\right] / \left[N - 1 + \left(Z_{2^*} p^* \left(1 - p\right)\right)/e^2\right]$$

Where N = Population Size; p = Population proportion; Z = Critical value of the normal distribution at the required confidence level; e = Margin of error. N is the sample size which is calculated and determined as 378 (=*n*) based on the following parameters. N is the population size, 21050; z value is 1.96 at 95% confidence level, e is the confidence interval or margin of error expressed as decimal (0.05), and population proportion, p is 0.5.

This study interviewed 499 households, 30 solid waste collectors, 30 HCF workers from 7 HCF, and 21 solid waste management (SWM) plant workers. Sample selection was made using random sampling. In addition to this, 15 KIIs (Key Informant Interviews), 5 IDI (In-Depth Interviews), and 10 FGDs (Focus Group Discussion) were conducted for collecting qualitative data from relevant stakeholders such as the Government of Bangladesh (GoB), WHO, NGOs, and International Non-governmental Organization (INGOs), hospital workers, doctors, nurses, local people, UNO, chairman, government workers.

2.3 Data collection procedure and its tools

The participants faced the questionnaire (qualitative and quantitative) physically and faced to face. An introductory briefing was given to them where the objectives of the study as well as ethical issues, were described. Then, informed verbal consent was taken from the participants before surveying. Field visits and household surveys were also conducted. Kobo Toolbox software (https://www.kobotoolbox.org/) was used in the continuous collection of data and

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as safe storage for the database. Interviews are done with the help of it. All of the questions were asked in Bengali and the answers were translated into English when the paper was written.

2.3.1 Interviews

Key informative interviews (KII), In-depth interviews (IDI), and semi-structured interviews are done to explore the experiences of targeted participants and the meanings they attribute to them. Researchers encouraged the participants to talk about issues or tropics they want from them by asking open-ended questions, in one-to-one interviews (Tong et al., 2007). All of the selected people were interviewed by the definite questionaries prepared by the authors, and the answers were collected for further data analysis.

2.3.2 Focus group discussion (FGD)

FGD is a helpful tool for finding specific scientific data from a community. It is often conducted with 4–10 people. However, the moderator has the option to moderate the talking or responding issue. In this part, the selected people answered the moderator's questions by interacting with each other (Tong et al., 2007). Two to five people from our team were present when this FGD was conducted. One was assigned to give them key point to talk and moderate the conversation. All of their conversations were recorded with their proper consent to ensure accuracy.

In addition, data from the MRF was also collected from their log book from February to June 2022.

2.4 Data variables

The questionnaire was designed to facilitate the assessment of the current pattern of medical waste management in the Rohingya camp, which adds to the challenges of SWM in Rohingya camps at Cox's Bazar. The information collected by this questionnaire attempted to accumulate information addressing the generation of different medical wastes amount and sources from other locations at the community level. All of the surveys started with demographic questions such as name, age, and sex, followed by questions about household-level practices to manage solid and medical waste. The interviewer asked about the respondents' knowledge of medical waste status as a hazard if they purchased medical items and the availability of medical items. If yes, where and what do they do after using the medical articles, and is any medical waste collection point available in their community? The solid waste management workers survey questionnaire included questions on their view of the impact of solid waste disposal on polluting the environment, the status of waste (existence of waste communal bin nearby, regular waste collection, waste left in communal bins, drains, roads/open space), where, by whom, the process of, at what time of the day, and how often household waste are disposed. The types of waste generated, segregation of waste into organic and inorganic, knowledge about waste processing, satisfaction with the collection level, and if any knowledge or message was provided to them were also among the questions asked.

Survey questions to waste collectors included how often they collected daily waste in the week, methods used to collect day-to-day waste, where they were collected from if they found medical waste during regular waste collection, and what they did with it.

Survey questions to SWM plant workers/volunteers/stuff contained, plant location, coverage block of solid waste management plant, population coverage from this plant, if any medical waste came to the plant from community level, how they are processed, and finally, how they are managed. In the case of HCF workers, questionaries contain the amounts of medical waste generated in HCF, what is the type of waste they generate, do they segregate them, how they manage their waste, do they satisfied with the existing waste management procedures, and what can be done to improve it.

For IDI, FGD the moderating conversation points were as follows: do they have knowledge about medical waste, is medical waste properly managed here, what is the procedure to manage them, is there any regulatory body for monitoring medical waste, are they satisfied with the management, and what should be done for the betterment of medical waste management.

2.5 Data analysis

Microsoft Excel was also used in the processing and analysis of the data. The collected data is analyzed with descriptive statistics in narrative form and through percentage analysis. Pearson Correlation Coefficient values were calculated, and a correlation matrix was produced for some datasets as required. Origin Pro-2022 was also used for creating graphs and statistical analysis. SWOT and DPSIR were performed based on the qualitative and quantitative data. The details are provided in the following subsection (SWOT analysis and DPSIR analysis).

2.5.1 SWOT analysis

Based on the KII, IDI, and FGD from the relevant stakeholders, SWOT analyses are performed. SWOT means strength, weakness, opportunity, and threat analysis of a definite organization, system, or guidelines (Büyüközkan and Ilıcak, 2019; Shammi et al., 2022). Among them, strengths and weaknesses are internal factors, and opportunities and threats are external factors. For the strength, weakness, opportunity, and threat part, the strengths, weaknesses, opportunities, and threats of existing waste management procedures are identified. This is done based on the KII, IDI, FGD, and literature surveys.

2.5.2 DPSIR analysis

A causal relationship is established for portraying societal and environmental interactions using the Driver-Pressure-State-Impact-Response (DPSIR) framework. It is a tactical and logical instrument for identifying, evaluating, and summarizing environmental issues at various spatial and temporal scales (Tscherning et al., 2012; Skondras and Karavitis, 2015; Vardopoulos et al., 2021). This analysis was also performed based on KII, IDI, and FGD information and a literature review. It is often used to link different parameters or factors with each other in qualitative data representation.

2.6 Literature review

For reviewing the literature, first of all the available literature, reports of different NGOs and governments in medical and solid

TABLE 1 Opinion of the household survey respondents on the relationship of waste disposal with the environment and the reason for pollution of the environment due to unplanned disposal of solid waste.

Relationship of waste disposal with the environment	Yes	No
Solid waste is not disposed of properly, which can pollute the environment	454 (91%)	45 (9%)
Medical waste is hazardous to the environment	439 (88%)	60 (12%)
Reason for Pollution of the environment due to unplanned disposal of solid waste	Frequency	Percentage (%)
Waste left in drains	292	52.33
Waste not collected regularly	235	42.11
Waste left on roads/open space	123	22.04
Waste left in communal bins	36	6.45
There is no communal waste bin nearby	329	58.96

waste management were collected. Then they were analyzed, and information related to our research question was segregated for use and compared with our analyzed data.

2.7 Ethics statement

All of the participants are first informed about the research objectives, and they were interviewed only if they agreed to participate. No interview was taken without their consent. In a continuous interviewing process, if anyone wished to terminate, they were able to do so.

3 Results

3.1 Analysis of the household survey respondents

The respondents belonged to Refugee Camp 15 (Blocks A, C, D, E, F, G, H). There were 279 (55.9%) male respondents and 220 (44.1%) female respondents. Their age varied from 19 to 60 years, with a mean age of 35. The current mechanism of waste disposal in this community location was documented since the medical waste was disposed of mixed with household waste. The respondents were asked questions to find their knowledge of the relationship between waste disposal and the environment. The refugees of the camp responded that unplanned dumping might have a serious effect on the environment and opined some causes or a combination of causes for this happening.

Most of the respondents (about 91% respondents) have an idea about the fact that inadequately disposed of solid waste can pollute the environment; 88% of respondents also concluded that medical waste could also pose threats to nature (Table 1). Moreover, most people opined that the disruption of nature was due to the absence of any communal bins nearby and the consequent existence of the waste being left in open drains. Many people also thought it was due to waste not being collected regularly and them being left on roadsides or open places. Many of them said that they came to know of this knowledge of disruption of the environment by waste through various WASH agencies and NGOs that helped build their awareness on the matter. However, at the community level, no system is yet established for medical waste management.

In the case of waste disposal practices of the respondents, it was found that disposal methods of their household wastes varied from person to person, as shown in Supplementary Table S1. Most people dump waste in an open place near households (48.92%) and into the drains (43.91%). Some people say that the waste collector collects their waste from their door; some also throw it in the communal bin. At the same time, a portion of the population dumps it by the side of the road (22.58%). The community's current practices of medical waste disposal systems were also found through the gender roles of household waste disposal. The HH's waste disposal activity was carried out majorly by women at 414 (74%), while men disposed of HH's waste 85 (15%) times. Most people were found to dispose of their waste on an everyday basis (70.79%), majorly in the morning and the evening. People used polythene (57.17%) and household bins (32.08%) to contain and throw waste outside. When asked about the type of waste produced in their household, they listed organic and inorganic materials, including vegetable waste, fish waste, polythene bags, papers, packets, clothes, leaves, etc. Almost 70% of the participants did not segregate their waste into organic and inorganic categories. Some people demonstrated that their waste materials were taken to their camp's solid waste management plant, some by CARE (Cooperative for Assistance and Relief Everywhere) and some by BDRC (Bangladesh Development Research Center). However, 32.62% of people had no idea about the processing of their disposed waste or where they were being taken. However, most people seemed satisfied with their locality's waste collection practices. Yet more than 70% of participants claimed they were made aware of waste disposal or the importance of segregation by different organizations.

The status of medical waste generation, disposal practices, and the respondents' knowledge level are demonstrated in Table 2. 83.87% of respondents said that they purchased medical items for their families. The medical item type they bought included syrup bottles, tablets, capsules, and syringes for injections. They bought vitamins, paracetamols, metero, omeprazole, diarrhea medicine, cough, fever, and gastric medicines such as the domperidone family of medicines. They purchased these medicines from markets (they named five such markets), hospitals (3 hospitals), pharmacies, and other small shops around

TABLE 2 Status of medical waste generation and disposal practices as well as the knowledge level of the respondents.

If they purchase medical items for their family	Frequency	Percentage (%)
Yes	468	83.87
No	31	5.56
Availability of medical Items	Frequency	Percentage (%)
Yes	484	86.74
No	15	2.69
What they did after using medical items	Frequency	Percentage (%)
Throw it in communal bins after using	131	23.48
Keep it to HH	222	39.78
Throw it open place after using	84	15.05
Throw it in drains	54	9.68
Throw into latrine Ring	2	0.36
Throw it on the ground	2	0.36
Bury into the ground	1	0.18
Give to waste collector	3	0.54
Availability of medical waste collection points in the community	Frequency	Percentage (%)
No	457	81.9
Yes	42	7.53

their locality. This gives the idea of the availability of many places to buy medicines from the locality. 86.74% said medical items were relatively available. On the other hand, 81.9% of respondents said that there were no medical waste collection points in their community. As a result, they mostly threw the medical items in communal bins after using them (23.48%), kept them in their household (39.78%), or threw them in an open place after using them (15.05%). Some people threw them in the drains. The rest of the respondents disposed of them in latrine rings, on the ground, or buried into the ground and given to waste collectors. From our observations and the respondents' statements, it was pretty clear that the camp has no establishments or infrastructure for hazardous medical waste.

3.2 Solid waste management workers survey findings and analysis

Solid waste collection is mainly done by the WASH actors in the camps. They are mostly government employees employed to collect waste. Camp local waste collectors collect waste from the community level, and by transportation, this waste is sent to MRF for further processing (Supplementary Table S2). Key findings from the respondents showed that predominantly 100% of waste collectors were male. The waste collection was done 5 days a week. As shown in Supplementary Table S2, solid waste vans and wheelbarrows are used for daily waste collection in camps. Wastes are mainly collected from household levels, roads, drains, shared spaces, and communal bins. This matches with the responses from the form of the household respondents where they expressed that they threw waste at these exact places. After the collection of waste, all waste was transferred to the solid waste management plant. They found medical waste mixed with general waste regularly. The current practice is to store medical waste in plants, and finally, the medical waste is dumped or open burned in most themes.

3.3 Health care facility (HCF) survey findings and analysis

The HCF is one of the major sources of medical waste generation. A total (of n = 7) healthcare facilities were surveyed. They included BDRCS (Bangladesh Red Crescent Society), Save the Children, BRAC, GK, IOM, and others. To identify the current practices at the camp level HCF survey was conducted. Different types of hazardous waste can be generated in all clinical institutions, including hospitals, health centers, dispensaries, and special clinics. The average waste generation rate for HCF was 2 kg per day. Types of waste generated in the healthcare facility as per our survey, were general waste, infectious waste, sharp objects, recyclables waste, chemicals (liquid and solid) waste, pharmaceutical waste, and anatomical waste. In terms of safe handling of medical waste, 57% of HCF said their safety levels were satisfactory, whereas 43% of HCF said it was insufficient. In this study, all the facilities used plastic bins for disposal and containing waste.

Medical waste has not received much attention in camps, and this study shows that it is disposed of together with domestic waste by the community. Figure 2 shows that 71% of HCF have their

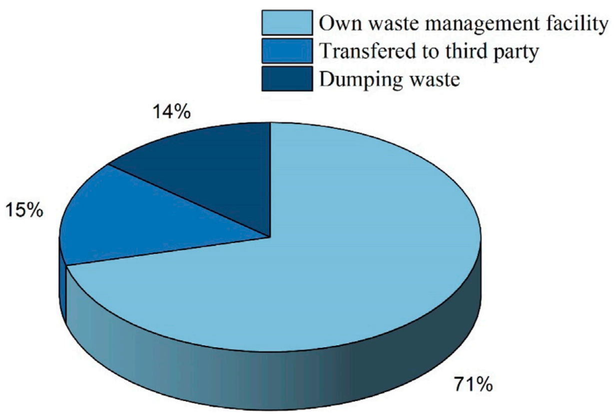

FIGURE 2
HCF's current method for the treatment and disposal of generated medical waste.

medical waste management system, 15% of HCF are transferred to a third party, and 14% dump their waste, which is threatening the environment. This is a clear indicator that through HCF, hazardous medical waste can be released in the camps as a wellbeing threat to public health. The authorities claimed they have some medical waste treatment facilities, but they cannot properly manage medical waste. They are suffering from a lack of equipment, training, and monitoring systems. 86% of the facilities did not recycle or put to recycle any of their wastes. All of this indicates less concern about the environment among the individuals in the refugee camp.

3.4 Medical waste found in the communal bin

A significant finding and drawback of the existing healthcare management of medical waste are being found regularly in the communal bin (Figure 3). Among 21 SWM workers, 73% of waste collectors said that while collecting waste, they found medical waste regularly during the collection of solid waste. Whereas 27% of respondents said that they did not find medical waste on a daily basis. Figure 3B shows the average medical waste found in a communal bin, and Figure 3A shows the waste in a segregated way.

After collecting waste from the communal places, waste collectors transport waste to SWM plants. Finally, medical waste is dumped or buried in the soil, posing a threat or creating a hazard to the environment.

3.4.1 Quantity and pattern of medical waste found in the MRF

In Camp-15 Block-E, an MRF survey and regular data collection were conducted to identify the amount of medical waste coming to MRFs from camps. These MRF facilities mainly collect solid waste, followed by segregation, storing, and recycling of solid waste. Data on waste coming to the plant was collected every day for 5 months to know the amount of medical waste coming from camps. Figure 4 shows the monthly amount of medical waste generated in the camp's communal places. The trend of generation of medical waste shows less in February (12.7 kg) and March (22.7 kg) at the end of the winter season in Bangladesh. After the winter season, the generation of waste increases. At the beginning of the summer season, medical waste generation is highest in April (65 kg) and then in May, which

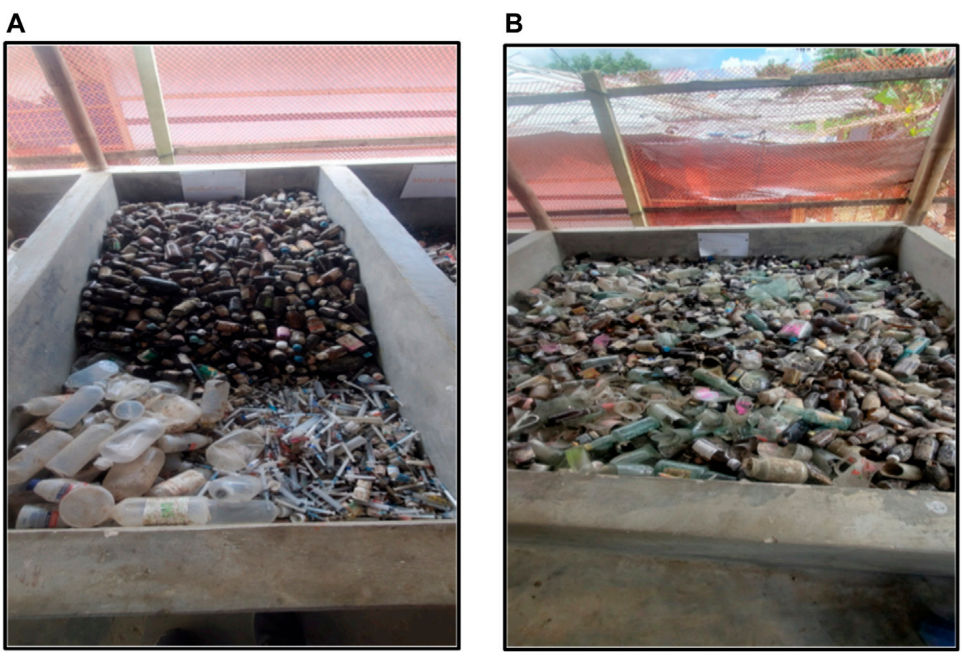

FIGURE 3
Current practice of storage of medical waste in MRF (Material Recovery Facility) In **(A)** (Medical waste in a segregated way), **(B)** (Medical waste is mixed with other Solid waste).

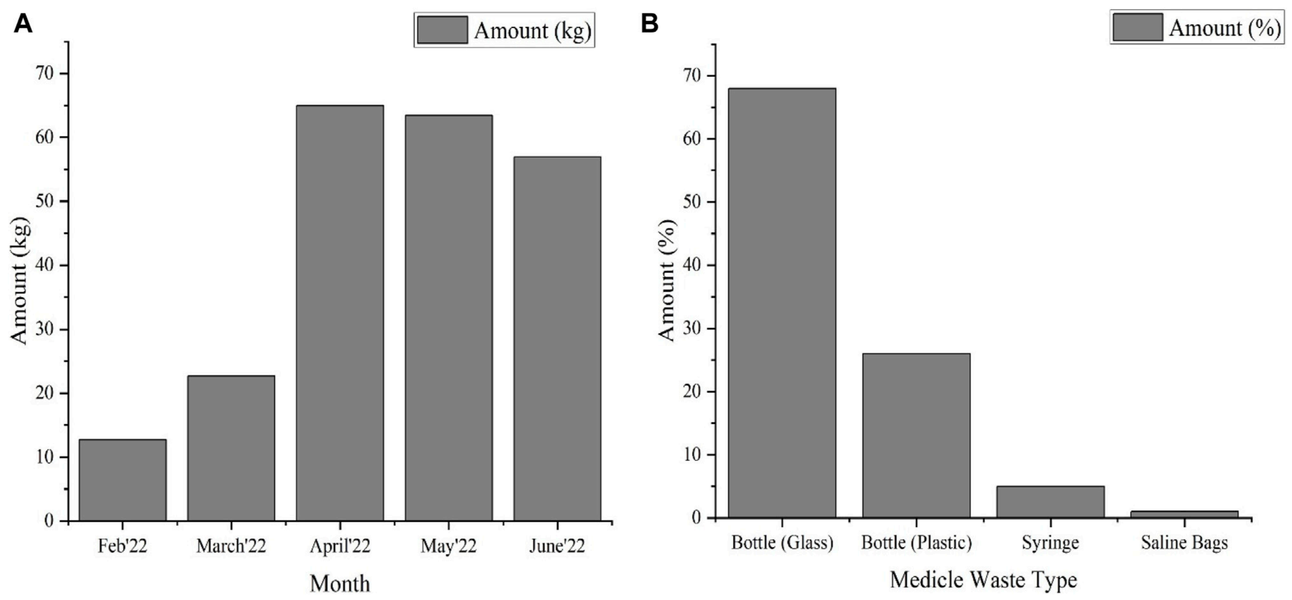

FIGURE 4
Medical waste generation in Rohingya camp. Here, **(A)** Monthly MW generation **(B)** Average waste types generation.

then somewhat maintains the high rate up to the rainy season in June.

During data collection, emphasis was put on the pattern of medical waste which is coming to MRF. This was done to understand the category of medical waste generated. Total data of 5 months were collected from MRF. Supplementary Figure S1 shows the breakdown of different categories of medical waste throughout the 5 months and an overall depiction. In February of 2022, medical waste was comprised of 64% bottles, 8% Plastic syrup bottles, 24% syringes and 4% saline bags. In March, there were 94% of bottles, 4% of Plastic syrup bottles, 2% of syringes and 0% saline bags generated in communal places. In April, the month of highest generated waste, 67% bottles, 26% Plastic syrup bottles, 5% syringes and 2% saline bags were collected. A somewhat similar trend was followed in May and June as well. A positive linear correlation exists between wastes generated every month (Supplementary Table S3). This shows a consistent trend of waste generation from month to month. An opposite trend is observed between different categories of waste, with only the exception of the use of glass bottles of medicines and syringes which have a positive linear correlation between them. Thus, the use of glass bottles of medicines has changed consistently with the use of syringes. The others had a negative linear correlation, as they did not increase or decrease usage with their counterparts. Overall, 94% of the medical waste was found to be general waste, including the daily use of medicines and non-infectious particles. Also, 5% sharp waste was found in the study, which is a threat to the community and also the environment because; it can cause injuries to humans and animals.

3.4.2 Ratio of solid waste and medical waste in the communal bin

During this study, the waste type of camp-15 was observed. The percentage and amount of medical waste found in the communal bin were measured. Monthly data collection from the camp's MRF showed that a total of 48,518 kg of solid waste was generated, containing 221 kg of medical waste in 5 months. The average medical waste in the communal bin is 44.2 kg/month. Moreover, the percentage shows that a total of 0.5% of medical waste is generated in camps while segregating from solid waste.

3.5 Medical waste management (MWM) rules and process: current scenario of the refugee camp

HCF inside the camp generally generates general wastes (such as packaging materials, food wastes, papers, etc.), sharps (such as syringes and needles, slides, cover slips, etc.), pharmaceutical waste (such as vials, expired vaccines, medicines, etc.), and infectious waste (PPE including used gloves and masks, band-aids, gauges, blood, placenta, etc.). These medical wastes were first segregated into different color-coded bins. Based on the field observation, only one of them had proper on-site treatment facilities (Autoclaving/Ash pit/Deep burial pit), they mostly handover to a third party (WASH collectors and sharps to the nearby Ukhiya Upazila health complex) without any disinfection processes (Figure 5). But they are suffering from a lack of infrastructure facilities, space, and electricity. In some cases, they also do open burning or open dumping of medical waste. Moreover, there was no liquid waste management facility in any HCF. According to them, liquid waste generation is very low in health camps. For this reason they don't treat it, and instead dump it in the drainage system. There were no specific guidelines or SOPs (standard operating procedures) for camp medical waste management in

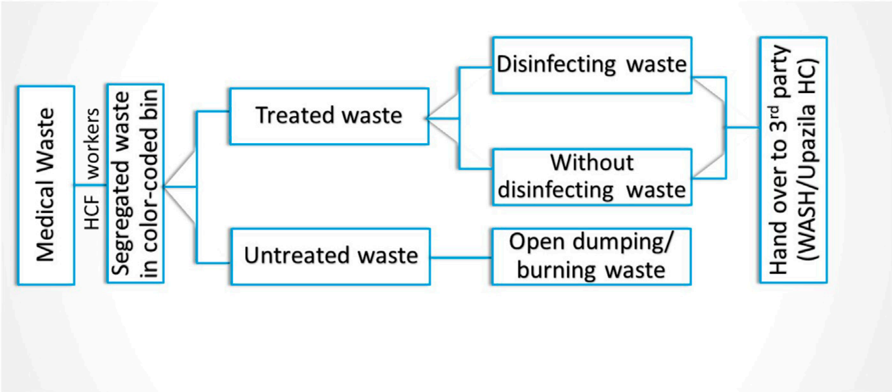

FIGURE 5
Medical waste treatment scenario in Rohingya camp.

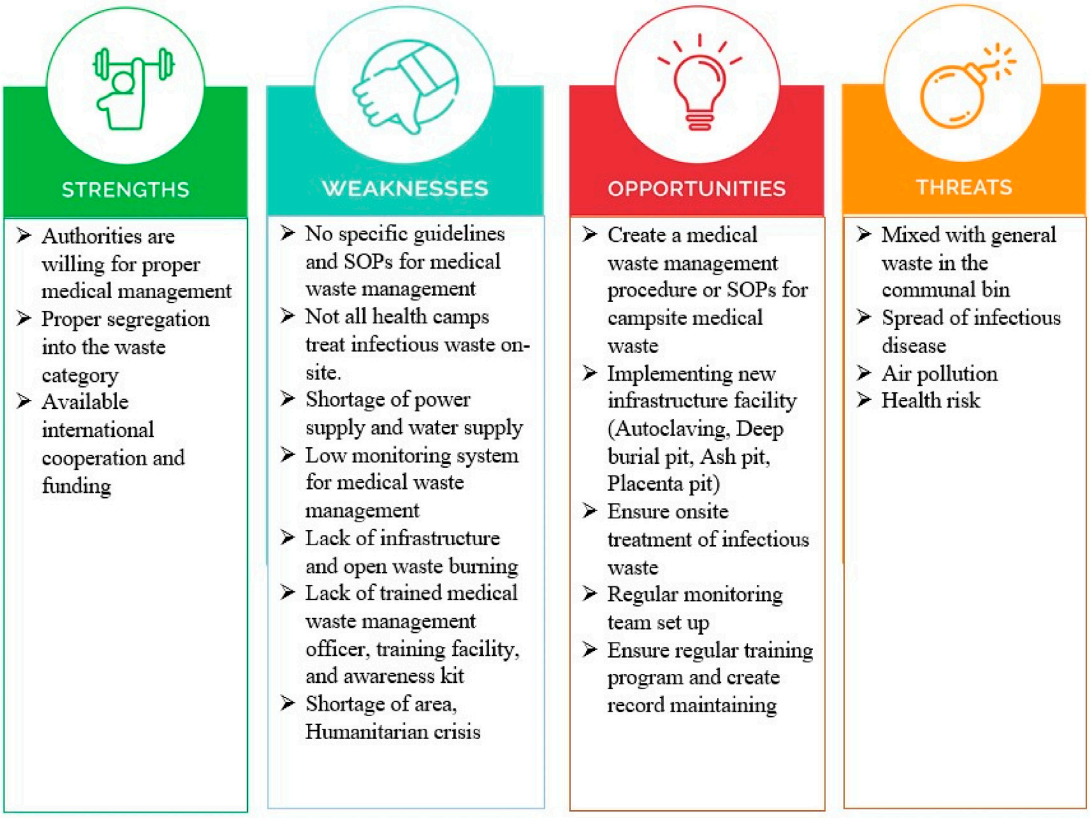

FIGURE 6
SWOT analysis of medical waste management (MWM) scenario in refugee camp Bangladesh.

the Rohingya camp. Moreover, they needed to correctly maintain occupational health safety protocols for medical waste management workers, and there is no training facility for medical waste management workers and authorities. A logbook and monitoring system to preserve the amount of medical waste generation and treatment are also unavailable.

3.6 SWOT analysis of medical waste management (MWM) scenario in the refugee camp

SWOT analysis is the most helpful tool to assess the efficiency of the existing facility or process. It also helps us to find out the weakness, opportunities, and threats. All of these characteristics make it more feasible in strategic management (Büyüközkan & Ilıcak, 2019; Shammi et al., 2022). Figure 6 represents the SWOT of the existing medical waste management scenario in the Rohingya camp.

Strength

The main strength of this MWM in the campsite is that authorities are willing to implement proper MWM procedures. Every HCF in the campsite contains a color-coded segregation waste bin. Moreover, international organizations are involved in funding and managing refugee campsites.

Weakness

Proper guidelines and SOPs for campsite MWM are the main weakness of existing campsite MWM. Because there are no specific guidelines and SOPs for medical waste management available now, for that reason, campsite medical waste is not managed correctly. It also suffers from the lack of monitoring systems, infrastructure, training systems, power supplies, and water supplies issues. The humanitarian crisis and shortage of area are other weaknesses in proper MWM.

Opportunities

Tremendous opportunities are available for better campsite MWM. One of the most significant opportunities is to create SOPs and specific guidelines for medical waste management in the campsite. Implementing new infrastructure facilities (Autoclaving, Deep burial pit, Ash pit, and Placenta pit) is another opportunity to make it more efficient. Furthermore, a robust monitoring system and training program on MWM can be implemented.

Threats

One of the most hazardous and concerning threats to the existing management of medical waste management MWM is untreated medical waste which has been found in the communal bins. It will spread the infectious disease among the contacted people. It will create a serious health threat to the entire community. Moreover, open waste burning helps spread disease and causes air pollution.

3.7 DPSIR framework on medical waste management (MWM) in Rohingya camp

The DPSIR model is the most often used method for establishing the connection between ecological causes and any problem's impacts (Vardopoulos et al., 2021). The DPSIR framework has some limitations, such as its inability to indicate non-linear links, account for natural drivers of environmental changes, and clearly define fresh indicators of progress or trends unless investigated at frequent intervals (Skondras and Karavitis, 2015). A total of 499 household surveys, 30 solid waste collectors, and 21 SWM plant workers KII, FGD, IDI, newspaper reports, research organizations report, and existing literature on MWM are the critical elements of the DPSIR framework. The summary of qualitative findings of the responses is shown using a DPSIR framework. The driving forces-pressure-state-impact-response (DPSIR) framework (Figure 7) was developed based on the stakeholder analysis and presented here to visualize the actual scenario of medical waste management in the Rohingya camp.

Environmental safeguard policy, international organizations, national and international guidelines, and international fundings are the main driving force of medical waste management in the Rohingya camps. They had the capability to improve or degrade pressure (congested area, over population, regulatory restriction) and states (unemployment policy, lack of MWM facility, lack of training, guidelines, and monitoring facility). In combination, these could be used to control the impact of the mismanagement of medical waste in the Rohingya camp. The adverse effects of MW mismanagement include communal waste, community transmission, environmental degradation, and human health risk.

This negative impact has also pressurized the community to respond in order to solve these issues. The response includes creating medical waste management guidelines and ensuring treatment facilities for campsite MWM, establishing SOPs, training an inspection team, ensuring record maintenance for MWM, and building up awareness among the stakeholders. All of this response will also modifies the state, pressure, and impact.

For the creation of an MWM procedure for the campsite, top-down and bottom-up management procedures should establish (Figure 8). Top-down and bottom-up approaches can be implemented to ensure sustainable MWM in Rohingya camps. In the top-down segment, a set of well-regulated stratified national bodies will exist, including the national advisory body, policy, stakeholders, technical body, capacity building, source of finance, and medical waste management system (MWMS). These bodies will be responsible for developing a campsite medical waste management policy and SOPs which are further directed towards forming an MWMS with the help of the technical committee. After that, sufficient stakeholder engagement will be done, followed by capacity building in terms of separations, collections, and final treatment, and the potential financial policy will also be settled by this top-down segment authority.

On the other hand, the bottom-up segment will be responsible for implementing the developed systems through capacity building of stakeholders, creating skilled manpower by ensuring training and education, awareness development, creating social enterprises, monitoring and evaluation, and performance review and evaluation of the system. This comprehensive approach (top-down and bottom-up) could ensure sustainable medical waste management in the Rohingya camp in Bangladesh.

3.8 Existing medical waste rules and policy review in Bangladesh

In 2008, Bangladesh's government established the country's first laws governing the handling of biomedical waste. That is entitled

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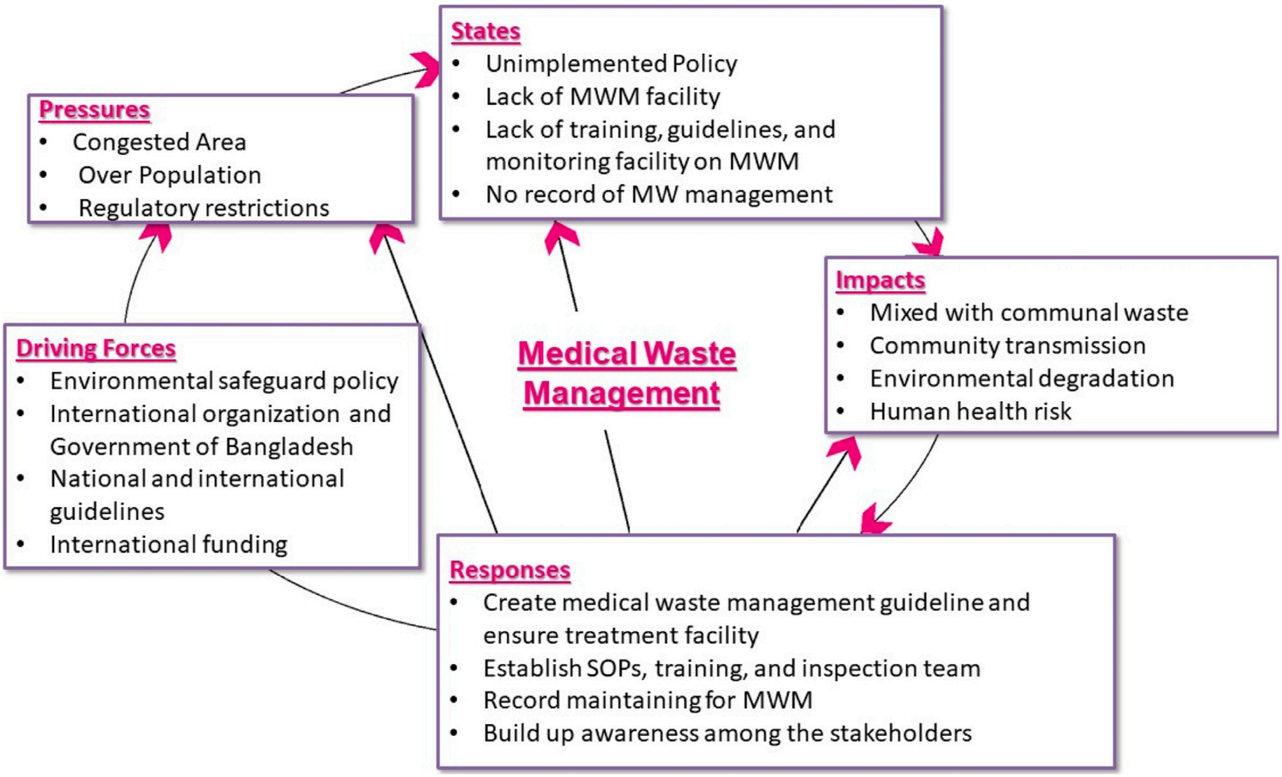

FIGURE 7
DPSIR framework for assessing the scenario of medical waste management in Bangladesh.

FIGURE 8
Top-down and bottom-up approach for proper management of medical waste.

"Bangladesh Medical Waste (Management and Processing) Rules 2008"(Bio-Medical Waste Management (Amendment) Rules (BMD), 2008) and governed by the Environment Conservation Rules, 1997 (ECR′97), the Bangladesh Environmental Conservation Act (1995) (Amended in 2010). According to medical waste management and processing rules 2008, waste should be segregated at the generation source in definite color-coded bins. Then this waste should be treated according to its category. Waste treatment methods are fixed for each type. There were also guidelines and standards fixed for treatment methods and safe disposal. A strong penalty is also mentioned for violation of these rules are also enacted. Above all the strengths, it also suffers from various weaknesses. Due to interministerial disagreements and rivalry among the authorities, the rules were not well implemented. Even after 13 years, the national advisory council was never established because of the rivalry between the authorities. The 2008 regulations did not specify the roles and responsibilities of government entities such as the Department of Environment (DoE), which is part of the Ministry of Environment, Forest, and Climate Change, the Directorate General of Health Services (DGHS), which is part of the Ministry of Health and Family Welfare, and the Dhaka North and South City Corporations (DNCC/DSCC), which are in charge of managing municipal solid waste and medical waste, respectively. A major weakness is that there is no strong monitoring team to ensure this management. The standard and treatment method should also improve with modern technology. There should be a provision for reporting system in every healthcare facility. Record of waste generation and treatment must be kept in a log book.

4 Discussion

Medical waste is any by-product of medical institutes, hospitals, health camps, pharmacies, pathologies, or other related organizations and establishments. In Rohingya camps, 88% of people are familiar with the hazardous effects of the mismanagement of waste. The knowledge rate about waste management is comparatively more satisfied than in other studies (Alomari et al., 2021). This is probably due to the benefit of different NGOs working in campsites.

A total of 91% of respondents said solid waste in Rohingya camps must be managed properly. They are found in roads, open places, and drains. This scenario is similar to the study of Nigeria (Orhorhoro and Oghoghorie, 2019) and the United States (Abdel-Shafy and Mansour, 2018). That indicates the mismanagement of solid waste worldwide and in the Rohingya camps.

Medical items are available to purchase from outside the healthcare camp. They can easily buy medical items from outside. But medical items sold and purchased in campsites are forbidden (De Montclos and Kagwanja, 2000; Werker, 2007). This medical item by-product medical waste needs to be properly handled. They generally mixed it with household waste or dumped it on roadsides or other open places. It converts general waste to infectious or hazardous waste (Jang et al., 2006; Oroei et al., 2014). This presents health risks to the surrounding community, waste management workers, and the environment.

Healthcare facilities generate medical waste of around 2 kg daily. The medical waste generation rate in healthcare camps is lower than in different hospitals (Cheng et al., 2009; Taghipour and Mosaferi, 2009). The medical waste is properly segregated in different color-coded plastic bins. Among different types of medical waste, liquid waste generation is very low in Rohingya camp healthcare centers. According to them, 71% have their own waste management facilities, 14% took help from a third party, and the rest open-dumped this waste. Generally, Wash and upazila health complex are the third party in Rohingya camp medical waste management.

Medical waste, such as sharp waste, may carry germs of diseases such as hepatitis B and AIDS (Henry et al., 1990; Almuneef and Memish, 2003)). This waste also increase the risk of exposure range from gastro-enteric, respiratory, Ocular infection, Anthrax, Meningitis, Acquired immunodeficiency syndrome (AIDS), Viral hepatitis A, B &C, Avian influenza, Haemorrhagic fevers, Septicaemia, Bacteraemia, and skin problems to more lethal diseases such as HIV/AIDS and Hepatitis (Rao, 2008; Babanyara and Ibrahim, 2013; New WHO Handbook on Healthcare Waste Management, 2013). Around 5.2 million people (including 4 million children) die annually from waste-related diseases globally (Akter, 2000). Despite this progress, in 2010, unsafe injections were still responsible for as many as 33 800 new HIV infections, 1.7 million hepatitis B infections, and 315 000 hepatitis C infections (Pépin et al., 2014). Furthermore, medical waste contains potentially hazardous microorganisms that might infect hospital patients, healthcare employees, and the general public. Additional potential infectious concerns include the transmission of drug-resistant microorganisms from healthcare facilities into the environment. It may also threaten doctors, patients, workers of HCF, workers of solid waste management workers, waste collectors, transporters, and visitors. Al-Khatib, (2013a) also mentioned the health risk of waste workers in his study. In another study, Jagger, (1999) reported that workers in support services linked to low-level health facilities (LLHFs) such as laundries, waste handling, and transportation service are often at risk.

In general, sharp waste management is a significant problem due to its ever-growing and endless generation. Though sharp waste constitutes a small fraction of solid medical waste, its potential environmental and health hazards could be deleterious if improperly handled. Syringes and needles are of particular concern because they constitute an essential part of the sharp waste and often are contaminated with patient body fluids.

The major concern of medical waste management in Rohingya camps is medical waste found in communal bins. This waste is mostly glass bottles, plastic bottles, syringes, and saline bags. Data from the MRF shows that 0.05% of the waste they collected is medical waste. This data also cleared that medical waste generation increased in the winter season. A study in Southern Gana also found medical waste in their daily communal bin. The probability of founding this waste was 89% (Udofia et al., 2017). Brazil (Da Silva et al., 2005) and Korea (Jang et al., 2006) also faced similar patterns of a problem in managing their waste. If medical waste is mixed with general waste in this process, it increases the volume of infectious waste, as it can contaminate general waste. For that reason, if this mixing process continues in Rohingya camps, it will create a considerable health risk for the camp community, the surrounding local community, SWM workers, and the

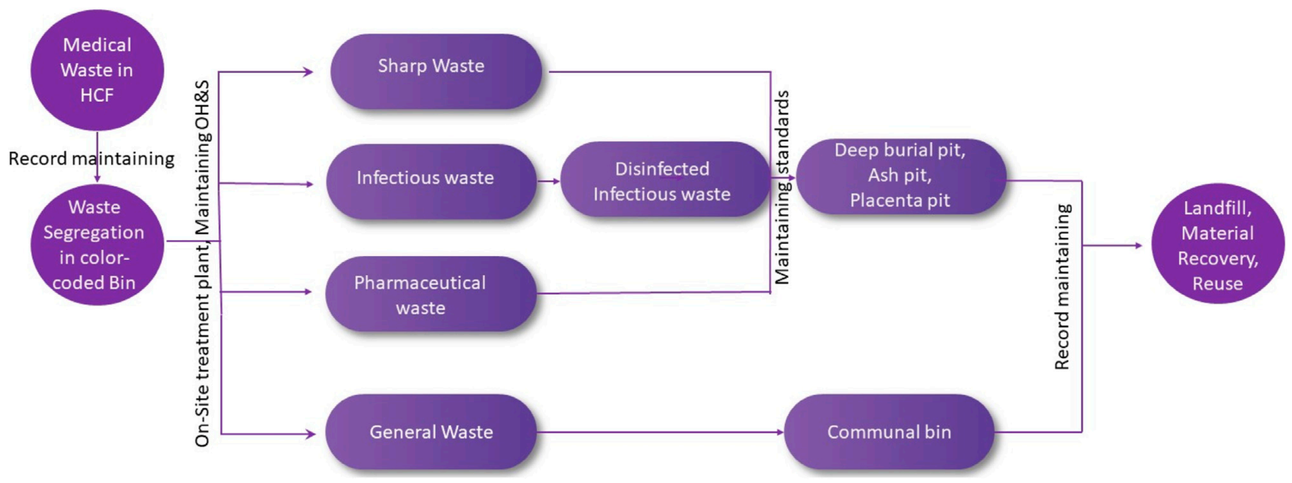

FIGURE 9
On-site Medical waste management procedures in Rohigya camp health camp.

environment (Al-Khatib, 2013b). It will also create a crucial burden for sustainably managing waste.

Another major concern is that the health camps of in the Rohingya camps also suffer from a lack of infrastructure facilities and water facilities, power shortage, area shortage, and inadequate training and proper monitoring systems. This causes a serious hamper in the medical waste management sector. Moreover, none of the health camps treated their liquid waste. According to them, the generation of this type of waste inside the health camp is very low. In addition, there is no provision for maintaining log books for medical waste generation and treatment entry. It creates a loop in proper medical waste management procedures. There is also no standard operating procedures (SOPs) or specific guideline maintaining rules for the health camps. That makes mismanagement in medical waste management. A lack of strong monitoring or regulating body and improper training makes it worst.

Overall medical waste management in a humanitarian context is challenging, as basic human needs are not adequately available. But the current situation of the Rohingya camp is comparatively well organized than Palestine (Al-Khatib, 2007; Al-Khatib, 2008). But adequate steps must be taken for proper waste management in Rohingya camps.

4.1 Proposed medical waste management guideline for Rohingya camp

Medical waste management guideline for Rohingya camp is created based on the field experience of health camp from KII, FGD, IDI, and the available literature. Medical waste from the health camp can be treated in two ways. One is being treated at an on-site waste management facility, and another is being handed over to a third party after disinfecting. HCF should record the amount of medical waste generation and treated waste amount. They must submit a report every month to the corresponding authorities. All of the processes must be regulated by a proper monitoring system and strong penalties must be fixed in case of violating guidelines.

4.1.1 On-site treatment plan

At first, camp medical waste must be segregated in color-coded bins like Figure 9. Infectious waste must be disinfected using an autoclave or chemical disinfection. If there is no lack of electricity issues, then they can use an autoclave. Otherwise, they will use a chemical disinfection process. Then, after disinfecting, sharp and pharmaceutical waste should be transferred to a deep burial pit, ash pit, or placenta pit. After this process, the end product can be disposed to a landfill site, or the material recovery site for reused and recycling. General waste can be transferred to a communal bin and handover to a third party (Figure 9). If liquid waste is produced in HCF then it will go through a chemical disinfection process. All medical waste management workers must wear proper PPE (personal protective equipment) to protect them from the hazardous effects of medical waste. The authority for medical waste management must adequately follow the occupational health and safety rules, as this profession has many occupational health risks (Al-Khatib, 2013a). Moreover, if it is needed to store medical waste in the on-site treatment plant or HCF, it must be stored by properly maintaining medical waste storage guidelines (WHO, 2005a; Al-Khatib, 2013b). In addition, medical waste management workers and authorities at HCF, On-site, and off-site treatment plants must be adequately trained. They should know the importance of medical waste management and its mismanagement effect. All of the processes must be monitored by a strong regulatory body. If any non-conformity occurs, then there must be rules and regulations for penalty. Moreover, all of the treatment quality standards must satisfy the Medical waste management rules 2008 and WHO guidelines.

4.1.2 Off-site HCF waste management

Off-site management can be done in a definite place, where HCF waste will be collected from every camp HCF facility and treated separately (Figure 10). This place can be the nearest

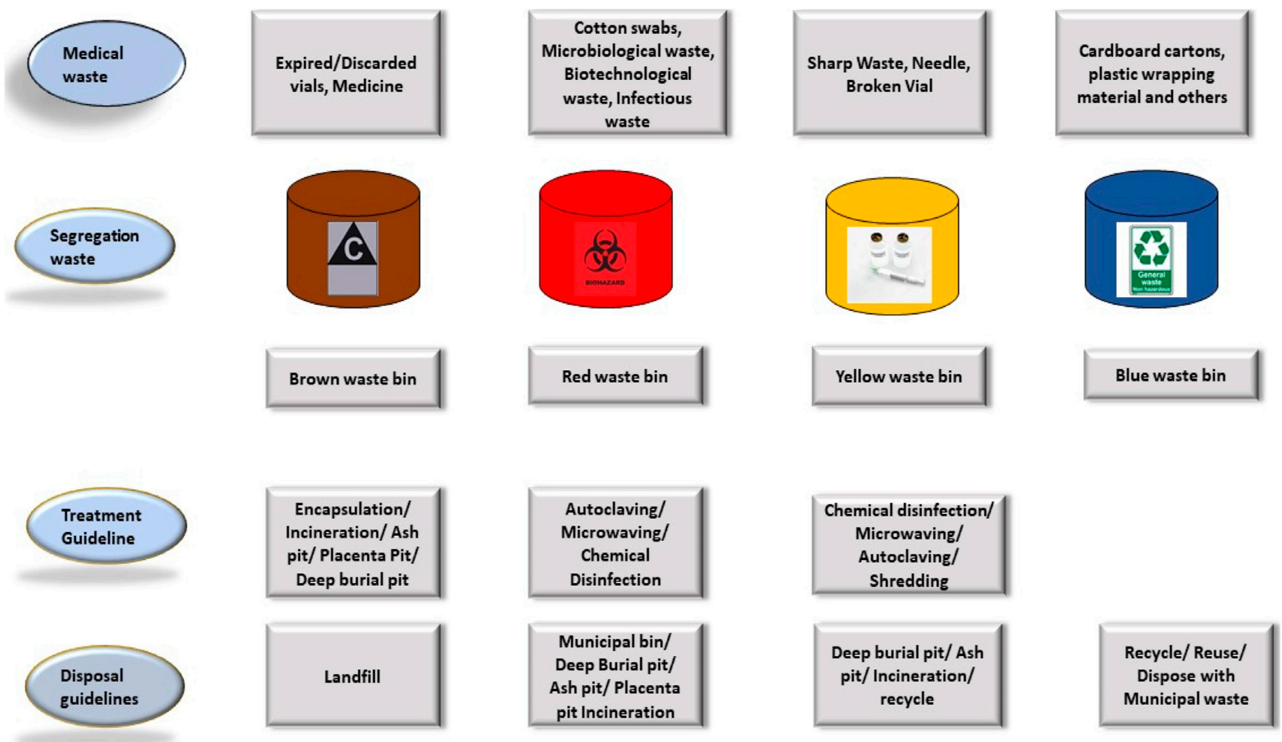

FIGURE 10
Off-site Medical waste management procedure.

Upazila healthcare establishment. As it already managed the HCF waste. But proper infrastructure, training, and logistic support must be ensured for the HCF workers of the Upazila complex. At first infectious waste must be disinfected by autoclaving or microwaving, or a chemical disinfection process depending on the availability of electricity. Then it can be further treated in a deep burial pit or ash pit or placenta pit or sharp pit. Pharmaceutical waste (expired medicine, saline, discarded vials) can be chemically disinfected and further treated by a deep burial pit, ash pit, or sharp pit. Sharp waste (needles, broken vials) must be decontaminated by autoclaving or shredding. Then it can be further treated by deep burial pit or ash pit or sharp pit or incineration. General waste can be reused, recycled, or handed over to a third party. In the case of liquid waste, it goes through a chemical disinfection process. Moreover, occupational health safety protocols for waste management workers must also be implemented appropriately (Al-Khatib, 2013a). If waste needs to keep stored for off-site treatment, then it should also follow the guideline of medical waste storage (WHO, 2005b; Al-Khatib, 2013b).

There were several medical waste guidelines for the safe handling and disposal of medical waste worldwide (WHO, 1999; UNEP, 2001; WHO, 2005a; UNEP, 2006; UNDP, 2010; DoE, 2012; Medical Waste US EPA, 2022; New WHO Handbook on Healthcare Waste Management, 2013). All of the rules and guidelines go through different amendments and improvements several times. Treatment methods for medical waste depend on the characteristics, quantification, capacity, financial capability, space, infrastructure, operation procedure, and skills to handle medical waste. The available technologies for MWM are thermal process, chemical process, radiation technology, biological process, and mechanical process. In Asian regions, the technology mostly used for medical waste treatment is the autoclave, incineration, chemical disinfection, microwaving, deep burial pit, ash pit, placenta pit, hydrolysis, encapsulation, and pyrolysis (Bio-Medical Waste Management (Amendment) Rules (BMD); Manekar et al., 2022; Medical Waste Management Rules in Pakistan, 2005; The Medical Waste Management Rules, 2008; Ye et al., 2022; Yong et al., 2009).

Waste treatment methods may also pose a threat to humankind. In the incineration process, flue gas is created. This flue gas has a destructive impact on the human body. This effect is also worst when the incineration process is poorly managed. If poorly managed, it also produces volatile metals, polycyclic aromatic hydrocarbons, particulates, dioxins, and furans (Fritsky et al., 2001; Lee et al., 2002; Segura-Muñoz et al., 2004; Al-Khatib, 2013a). This causes damage to our lungs, kidneys, immune system, and neurological system. Some of them are also carcinogenic, it also capable of creating cancer. Ash from incineration, placenta pits, and deep burial pits also poses a health threat to humankind. When sharp waste is handled, it also threatens to cut our bodies. Autoclaving procedures, chemical disinfection, and microwaving must be done carefully, because if temperature and other parameters are not properly maintained, it will create serious health threats for the physical properties of waste. Post-treatment water should also be properly maintained because it contains hazardous organic and inorganic compounds. Deep burial pits, ash pits, placenta pits, and landfill should also contain the threat of creating odour, smoke, and leachate. For that reason, following proper guidelines according to surrounding circumstances is essential when treating this waste.

5 Concluding remarks

The disposal of medical waste is a growing environmental problem in the Rohingya refugee camps, owing to the absence of proper management facilities, knowledge, and establishments. In these camps, the management of solid waste has gained attention, but the management of medical waste has received little attention. But medical waste is capable of imposing potential environmental hazards and public health risks on camp people and surroundings. The study has attempted to quantify different medical wastes generated in community places in the study area camp-15. Both non-infectious and infectious wastes were found to be generated in these places. Our field data show that community people do not segregate their generated wastes, and they dispose of their domestic waste at the same site as regular solid waste. Data analysis shows no specific handling process or collection system in camps for MWM. With the complete picture in view, the authors suggest a holistic management system for medical waste in the humanitarian context for the safeguarding of the environment and public health.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

Ethics statement

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent from the participants was not required to participate in this study in accordance with the national legislation and the institutional requirements.

Author contributions

MH: conceptualization, data acquisition, analysis, manuscript preparation FC: data acquisition, analysis, manuscript preparation AH: data acquisition, field survey, analysis RA: analysis, manuscript preparation MR: conceptualization, data acquisition, analysis, manuscript preparation, supervision. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fenvs.2023.1149314/full#supplementary-material

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frontiers | Frontiers in Environmental Science

TYPE Original Research
PUBLISHED 25 September 2023
DOI 10.3389/fenvs.2023.1252305

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OPEN ACCESS

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RECEIVED 03 July 2023
ACCEPTED 11 September 2023
PUBLISHED 25 September 2023

CITATION
Fianko DA, Nartey EK, Abekoe MK, Adjadeh TA, Lawson IYD, Amoatey CA, Sulemana N, Akumah AM, Baba ME and Asuming-Brempong S (2023), Sustainable P-enriched biochar-compost production: harnessing the prospects of maize stover and groundnut husk in Ghana's Guinea Savanna. *Front. Environ. Sci.* 11:1252305. doi: 10.3389/fenvs.2023.1252305

Sustainable P-enriched biochar-compost production: harnessing the prospects of maize stover and groundnut husk in Ghana's Guinea Savanna

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Farmers in resource-poor areas of the Guinea Savanna zone of Ghana often face declining soil fertility due to the continuous removal of nutrient-rich harvested produce from their fields. This study focuses on the Lawra Municipality in the Guinea Savanna zone of Ghana, where low soil fertility, specifically, limits phosphorus (P) bioavailability and hinders crop production. The objective of this research is to formulate P-enhanced biochar-compost from maize stover (MS) and groundnut husk, which abound in the area, to close the nutrient loop. MS was co-composted with groundnut husk biochar at varying rates of 0, 10, 20, 30, and 40% by volume. To facilitate decomposition using the windrow system, the composting heaps were inoculated with decomposing cow dung, and the moisture content was kept at 60% throughout the monitoring period. The addition of biochar shortened the lag phase of composting. However, rates above 20% resulted in reduced degradation of MS. Biochar incorporation enriched the available phosphorus content in the final compost from 286.7 mg kg^{-1} in the non-biochar-compost to 320, 370, 546, and 840.0 mg kg^{-1} in the 10, 20, 30, and 40% biochar-compost, respectively.

KEYWORDS

agroecology, farm residues, compost production, phosphorus availability, nutrient mining, carbon sequestration, pyrolysis

Introduction

Soil organic matter plays an important role in soil fertility and crop yield improvement via the geochemical cycling of plant nutrients such as P (Duarte and Duarte, 2019; Ch'Ng et al., 2014). Compost is an accepted form of organic matter applied to soils (Diaz and Bertoldi, 2007). However, in tropical regions where there is rapid mineralization, compost application has had limited long-term beneficial results (Gan et al., 2020; Oliveira et al., 2020). Consequently, desired benefits, including soil bulk density reduction, nutrient availability enhancement, and overall soil productivity improvements, have been minimal (Oorts et al., 2003; Ahad et al., 2015; Tsai and Chang, 2019). Considerable research attention has, therefore, been devoted to compost stabilization in recent years (Gabhane et al., 2012; Bazrafshan et al., 2016). One promising approach is the co-application of biochar and compost, which has shown the potential of synergistically enhancing soil quality and promoting plant growth (Fischer and Glaser, 2012; Gan et al., 2020; Antonangelo et al., 2021). Biochar as a feedstock in composting has had positive effects on compost stability, reducing composting time, and minimizing the leaching of exchangeable cations such as NH_4^+ (Gabhane et al., 2012; Vandecasteele et al., 2016; Akumah et al., 2021). The use of biochar in co-composting has mainly been in the context of managing municipal and market organic waste (Bazrafshan et al., 2016; Akumah et al., 2021; Sulemana et al., 2021). Limited studies have investigated the use of biochar as a co-composting feedstock to specifically harness nutrients from farm residues. Most farm residues in the Guinea Savanna zone of sub-Saharan Africa are either burnt or removed from farm sites to be fed to animals. Harnessing these residues into biochar-compost for application to the farms from which the produce is harvested will minimize nutrient mining and ultimately close the nutrient loop. Farm residues are classified as bulking agents (Batham et al., 2013), thereby limiting their prospects for composting. These residues have, however, sequestered nutrients that, upon composting, could be made available, especially in the Guinea Savanna zone, where soil organic carbon levels are very low.

Most compost plants are sited close to urban centers to primarily manage municipal waste and, thus, serve as a major boost to urban and peri-urban agriculture. However, the cost involved in transporting the amendment to the Guinea Savanna zone, where organic carbon contents of soils are very low, is a disincentive. In Ghana's Guinea Savanna zone, where low soil fertility continues to constrain crop production (Tetteh et al., 2016; IFDC, 2017; UNDP, 2018), the limited availability of household waste restricts the production of biochar-compost (Amoah and Kosoe, 2014). However, there is an opportunity to harness the potential of readily available farm residues such as maize (*Zea mais*) stover and groundnut (*Arachis hypogea*) husk, which have limited competing uses. Maize stover (MS) is abundantly generated in the zone due to the surge in maize production, surpassing that of millet and sorghum (Raheem et al., 2021). Approximately 10.47 tons/ha and 8.07 tons/ha of maize and groundnut, respectively, were produced in the Guinea Savanna zone of Ghana in the year 2020 (SRID, 2021). The cob and husk after shelling are used as feed for livestock and fuel for cooking, which creates competition for their use as composting feedstock. Similarly, groundnut husk offers potential as a composting material due to its large volume compared to waste from cowpea (*Vigna unguiculata*) (SRID, 2021). The transportation of tubers and vegetables outside of the Guinea Savanna zone results in limited quantities of waste available for compost production. Therefore, MS and groundnut husk are readily available feedstocks with limited competing uses for composting.

Groundnut is a legume and, thus, has a high demand for P. Being a pod, the husk is likely to sequester high levels of P. Groundnut husk has been found to contain, on average, 105.5 mg kg^{-1} of P (Mokolopi, 2022). Charred groundnut husk could, therefore, liberate P, making it a very good co-composting feedstock with MS, a readily available farm waste in the Guinea Savanna zone of Ghana. Organic acids produced from MS during composing would help minimize surface precipitation of P onto biochar, thereby making the nutrient readily available should the compost product be applied to the generally P-deficient soils of the Guinea Savanna zone of northern Ghana and sub-Saharan Africa in general. Such a biochar-compost should help recycle P in northern Ghana and decrease the import bill of Ghana for inorganic P fertilizer, especially in this era of high inorganic fertilizer costs. Carbon is highly recalcitrant in biochar-compost (Sulemana et al., 2019) and could, thus, be sequestered into the impoverished and fragile soils of the Guinea Savanna and invariably improve their productivity.

The ideal ratio of groundnut husk biochar (GHB) to MS and the protocol to be used in formulating an ideal P-rich biochar-compost are yet to be ascertained. It is in light of this that the present study seeks to exploit the potential of MS and groundnut husk as feedstocks to produce a P-rich organic amendment. This study also aims to provide practical solutions for closing the nutrient loop by harnessing farm waste, which would have otherwise been burnt in resource-limited farming systems.

Materials and methods

Characterization of feedstock

Groundnut husk and MS were collected from farmers' fields in Lawra, located in the Upper West Region of the Guinea Savanna agro-ecological zone of Ghana. Parts of these feedstocks were oven-dried at 70°C to attain constant weight. The samples were then ground and saved for chemical characterization.

Biochar production

Part of the groundnut husk was sun-dried, weighed (W1), and pyrolyzed using a locally fabricated retort stove (Kuntan kiln). Temperature readings were recorded at 10-min intervals using the Extech Dual Laser Infrared Thermometer (model 42,570) until pyrolysis was complete. The mean temperature of the readings (454.4 ± 19.8°C) was taken as the pyrolysis temperature. The product, GHB, was collected from the hot kiln after intermittent turning and then cooled with clean water. The cooled biochar was air-dried, weighed (W2), and then, bagged in jute sacks for storage. Biochar yield, which represents the quantity of biochar produced from a quantity of feedstock charred, was expressed as a percentage. Samples of the biochar produced were then ground to pass through a 2 mm sieve for characterization.

Biochar-compost production

The MS was chopped with machetes and then sieved to obtain fraction of length <5 cm. The sieved fraction of the MS was mixed with GHB on volume basis to attain varying MS-to-biochar ratios of 10:0, 9:1, 8:2, 7:3, and 6:4, representing 0, 10, 20, 30, and 40% biochar concentrations. Thus, five different composting heaps, each 2 m in diameter at the base and 1 m in height, were formulated, replicated three times, and arranged in a completely randomized design. To each compost heap, 1 kg of decomposing cow dung (nitrogen = 2.3%, organic carbon = 25.6%, available p = 0.38%, and pH_{water} = 8.2) was added as an inoculant to introduce decomposing microorganisms. The heaps were uniformly mixed on a cemented compost platform with a slope of about 0% and moistened to approximately 60% moisture content. Perforated polyvinyl chloride (PVC) pipes of 7 cm diameter with grooves at the top end were inserted into each of the heaps, making sure that the base of each of the PVC pipes touched the cemented platform. With the aid of ropes fastened onto twigs, thermometers were inserted into each of the compost heaps by hanging the twigs into the PVC grooves. Heat emanating from the heaps during the composting process passed through the perforations to be measured by the thermometers inside the PVC pipes. Daily temperature readings were recorded, and the compost moistened and turned when the temperature readings became stable. The maturity of the various heaps was determined when temperatures did not increase after compost moistening and turning.

Compost maturity determination

The maturity of the various compost types was assessed using the germination index (GI) method as described by Zucconi et al. (1981). Total humic substances were determined based on their relative solubility in acid and alkaline extractants according to the method of Serra-Wittling et al. (1996). The CEC of the compost samples was determined using the method described by Harada and Inoko (1980a).

TABLE 1 Some properties of the two feedstocks and the groundnut husk biochar derivative[a].

Property	Maize stover	Groundnut husk	Groundnut husk biochar
Yield (%)			35.1
Ash content (g/kg)			52.3 ± 2.6
pH (H_2O)	5.75 ± 0.12	7.06 ± 0.09	9.74 ±0.07
EC (dS/m)	nd	0.65±0.04	1.30 ±0.08
C (g kg^{-1})	333.1 ±3.7	373.65 ±3.8	671.3 ±7.3
N (g kg^{-1})	6.02 v0.03	7.36 ±0.03	7.13 ± 0.03
Available N (mg kg^{-1})	-	-	1.19 ± 0.05
C:N	55.7	50.8	94.2
Total P (m kg^{-1})	147.8 ± 11.8	921.7 ± 23.7	3400 ± 40
Available P (mg kg^{-1})	-	-	750 ± 30
Soluble Ca (g kg^{-1})	-	-	2.29 ± 0.02
Soluble Mg (g kg^{-1})	-	-	0.43 ± 0.09
Soluble K (g kg$^{-1)}$)	-	-	5.32 ± 0.04
Carboxylic acids (mmol kg^{-1})	-		1.05 ± 0.13
Phenolic groups (mmol g^{-1})	-	-	38.92 ± 1.7
Lactonic groups (mmol g^{-1})	-	-	37.95 ± 1.3
AFG_T (mmol g^{-1})	-	-	77.92 ± 1.1
CEC cmol kg-1)	-	-	49.63 ± 3.7

[a]nd, not determined; AFG_T, total acid functional groups.

Characterization of feedstock, biochar, and compost

The biochar was analyzed for its ash content according to the method prescribed by the ASTM D1762-84 (2007) for wood charcoal. The pH and EC of the milled groundnut husk and MS feedstocks, the GHB, and different compost types were determined electrometrically in a ratio of 1:10 (dried samples:deionized water). Total carbon and nitrogen in the samples were determined by dry combustion with a Leco Trumac version 1.3 Carbon–Nitrogen–Sulfur Analyzer. The hydrogen contents of the groundnut husk and its biochar derivative were determined using the Leco Trumac H analyzer, and their oxygen contents were determined by the difference method, i.e., [100% − (%H+%C+%N)] as sulfur was not detected by the analyzer. Soluble Ca, Mg, and K in the biochar were extracted with distilled water, and their concentrations were read on a PerkinElmer AAnalyst 800 spectrophotometer.

Total P, Ca, Mg, K, and Na in the matured compost types were determined by digesting 0.5 g of homogenized samples with 5 mL of concentrated H_2SO_4. The color development of phosphorus in the digest was carried out by the ascorbic acid method of Murphy and Riley (1962), and the concentrations of the element were read on a Cole–Palmer UV spectrophotometer. Calcium, Mg, K, and Na concentrations were read on a PerkinElmer AAnalyst 800 atomic absorption spectrophotometer. The available phosphorus contents of biochar and compost were determined according to the method of Watanabe and Olsen (1965). Available N was determined by Kjeldahl distillation after extraction with 2 M KCl. The cation exchange capacity of biochar and compost types was determined using a $BaCl_2$/triethanolamine (TEA) solution buffered at pH 8.2. The presence of surface functional groups was determined using the PerkinElmer Spectrum Two FT-IR Spectrometer fitted with MIRacle Accessory. The concentrations of carboxylic, lactonic, and phenolic functional groups in biochar and compost types were determined by Boehm titration (Boehm, 1994).

Statistical analysis

All the data were subjected to normality tests and thereafter subjected to both univariate ANOVA and multivariate analyses using GenStat Edition 12. Treatment means were compared using Fischer's protected LSD test at $p < 0.05$. Graphs from FT-IR analyses were redrawn using the Origin 2021 software.

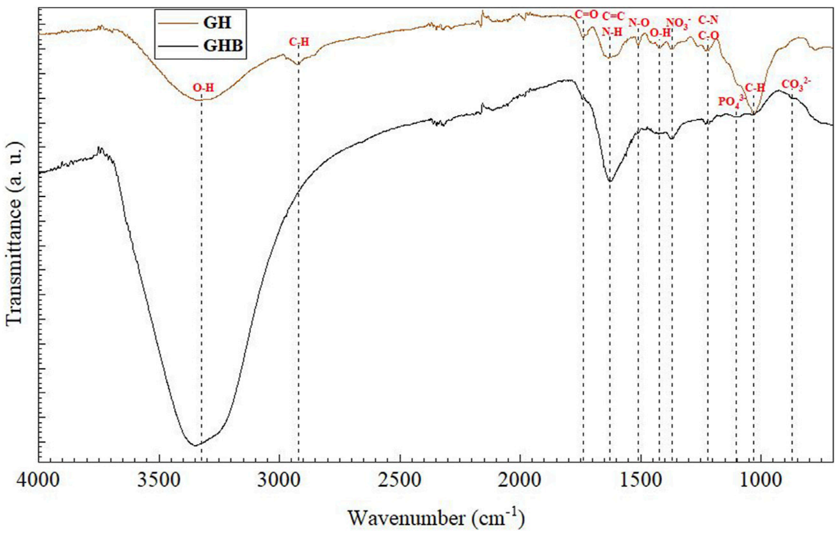

FIGURE 1
FT-IR of the groundnut husk feedstock and its biochar derivative.

Results

Characteristics of MS and groundnut husk feedstocks and GHB

Table 1 shows selected properties of the MS, groundnut husk, and GHB used for the study. The yield of biochar from groundnut husk using the Kuntan kiln was 35.1%. The biochar had an ash content of 52.35 g/kg via proximate analysis. The pH in water of the groundnut husk was 7.06, 1.31 pH units higher than that of the MS (5.75). The total P content of the groundnut husk (921.7 mg P kg^{-1}) was 6.2-folds that of the MS (147.8 mg P kg^{-1}). The total C and N contents of the groundnut husk were 373.65 g kg^{-1} and 7.36 g kg^{-1}, respectively, whereas in MS, they were 333.1 g kg^{-1} and 6.02 g kg^{-1}, respectively. These results yielded a C:N ratio of 50.8 for groundnut husk, which was lower than that of MS, which was 55.2. The superior concentrations of total P and C in the legume justified its choice for use as feedstock for biochar production for enhanced P availability and C sequestration.

Pyrolysis liberated bases from the groundnut husk to levels at which the concentrations of water-soluble Ca, Mg, and K in the biochar were 2.29 g kg^{-1}, 0.43 g kg^{-1}, and 5.32 g kg^{-1}, respectively (Table 1). Consequently, the pH of the groundnut husk, which was neutral (7.06), increased to strongly alkaline (9.74) in the biochar derivative. Electrical conductivity also increased to 1.3 dS m^{-1} in the resultant biochar. The CEC of the GHB was 49.63 $cmol_c$ kg^{-1}. Total phosphorus, which was 921 mg kg^{-1} in the feedstock, increased 3.7-folds to 3,400 mg P kg^{-1} upon charring, with approximately 22% of that being in the available form. Pyrolysis of the groundnut husk feedstock did not have any significant reduction in total N content, as the feedstock N, which was 7.36 g kg^{-1}, was similar to the 7.13 g kg^{-1} ($p < 0.05$) of the biochar derivative. Less than 0.02% of the total N in the biochar was in the available form. Pyrolysis of the groundnut husk increased the total carbon content from 373.7 g kg^{-1} in the feedstock to 671.3 g kg^{-1}, which culminated in an increased C:N ratio of 94.2 from the 50.8 of the feedstock.

The concentration of elemental O decreased from 422.7 g kg^{-1} to 291.7 g kg^{-1}. Elemental H also decreased from 52.9 g kg^{-1} to 24.7 g kg^{-1}. Thus, aromaticity (H/C) and stability (O/C) in the feedstock were 0.14 and 1.32, respectively. Aromaticity and stability were also 0.04 and 0.43, respectively, for the biochar. The total acidic functional groups contained in a gram of biochar were 77.91 mmol. The concentration of carboxylic group was very low (1.05 mmol g^{-1}), lower than that of phenolic and lactone groups, which were 38.92 and 37.95 mmol g^{-1}, respectively.

Effect of pyrolysis on the FT-IR spectra of groundnut husk

Functional groups present on the surface of the groundnut husk and the resultant biochar, as revealed by ATR-FTIR spectra, in the range of 4,000–500 cm^{-1} are shown in Figure 1. For the groundnut husk, the presence of a broad band around 3,320 cm^{-1} assigned to intermolecular -OH stretching vibration was indicative of alcoholic, phenolic, and carboxylic or N–H stretching of amino functional groups (Ossman et al., 2014; Eder et al., 2021). The intensity of this peak increased upon pyrolysis and re-centered around 3,350 cm^{-1}. The spectra further revealed a band at 2,920 cm^{-1} for the groundnut husk, which was ascribed to C–H asymmetrical stretching of methyl, methylene, and amine groups (Chun et al., 2004; Schwanninger et al., 2004). This band was lost upon pyrolysis. Similarly, the presence of strongly bonded C=O stretching vibration at 1739.40 cm^{-1} representing ester, aldehyde, and δ-lactone was also lost upon pyrolysis (Smith, 2011). The transmission band around 1,630 cm^{-1}, which indicates the presence of C=C stretching in alkenes and N–H bending in amines, was intensified upon pyrolysis. Strong N–O stretching for a nitro compound assigned

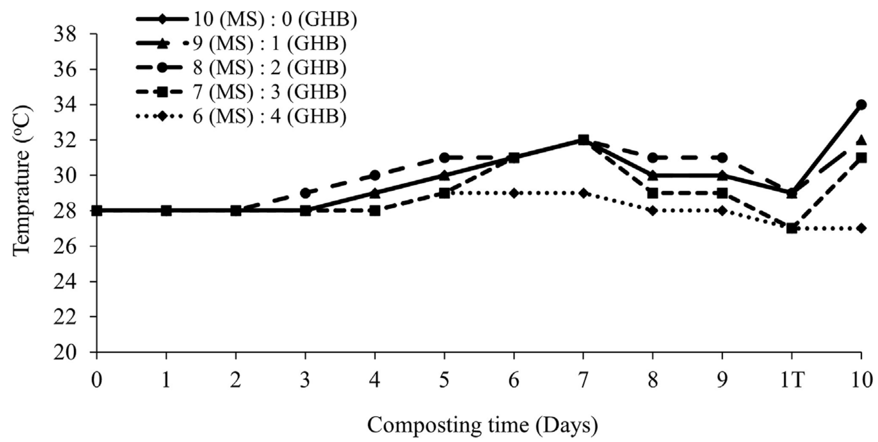

FIGURE 2
Temperature dynamics during first 10 days of composting.

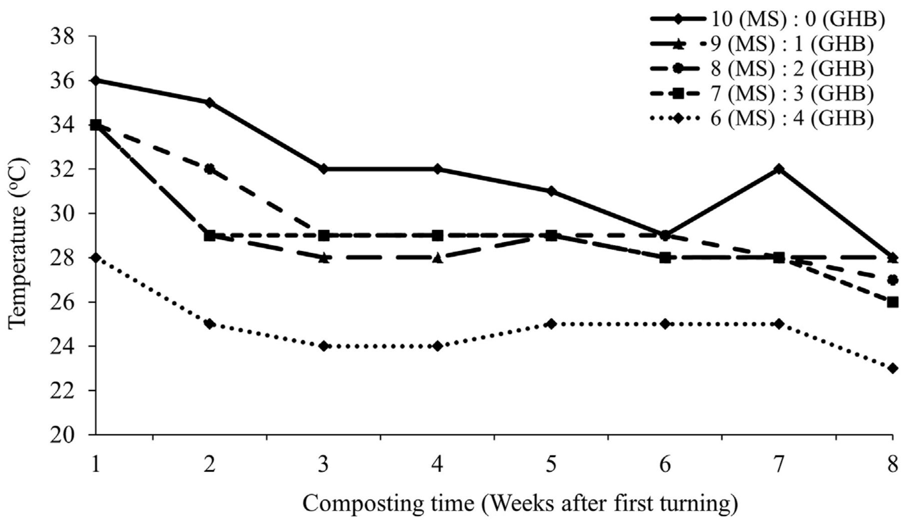

FIGURE 3
Weekly compost temperature dynamics after first turning (10 days).

to the peak around 1,508 cm^{-1} was lost in the biochar. The band at 1419.49 cm^{-1}, indicative of the medium O–H bend of carboxylic groups (Yang et al., 2006; Smith, 2011), was also lost upon pyrolysis. The band at 1379.64 cm^{-1} was retained upon pyrolysis and was assigned to nitrate ions. Strong C-O stretch and medium C-N stretch bonds were assigned to the band at 1215.65 cm^{-1}. This band was retained in the biochar. The bands at 1096.85 and 873.18 are indicative of phosphate ions and out-of-plane bending for carbonate ions, respectively (Smith, 2023).

Influence of GHB on temperature dynamics during composting

The composting lag phase varied for the various decomposing heaps. The initial increase in temperature occurred after 3 days in the compost without any biochar and in the 10% biochar heaps (Figure 2). Increasing the biochar rate to 20% shortened the lag phase to 2 days, while further increasing the biochar rate to 30% and 40% prolonged the lag phase to 4 days. The temperature in the various compost heaps had risen from 28°C to 32°C by day 7, except for the 40% biochar heap, which saw a marginal increase to 29°C (Figure 2). The temperature remained constant between days 8 and 9, and the heaps were turned on day 9. Except for the 40% biochar heap, which recorded no increase in temperature, the heaps showed an increase in temperature a day after turning. The temperature in the 0% biochar and 10% biochar heaps rose to 34°C, while that of the 20% biochar heap increased to 32°C. Temperatures in the 30% and 40% biochar heaps were 31°C and 27°C, respectively.

After the first turning (10 days), a decline in maximum weekly temperature was observed up to maturity (Figure 3). The weekly temperature was highest in the 0% biochar decomposing heap

TABLE 2 Maturity indices of the biochar-compost types[a].

	GI	Humin	Humic acid	Fulvic acid	HA/FA	CEC
Compost		(%)	(cmol kg-1)			
10 (MS):0 (GHB)	103.53	71.8 ± 3.0a	15.6 ± 3.6b	12.6 ± 0.6c	1.24	62.5 ± 2.9a
9 (MS):1 (GHB)	104.70	74.1 ± 2.9ab	14.9 ± 3.1b	11.0 ± 0.2b	1.36	65.0 ± 10.0a
8 (MS):2 (GHB)	106.67	74.4 ± 0.6ab	14.8 ± 1.0b	10.9 ± 0.4b	1.36	71.7 ± 7.6a
7 (MS):3 (GHB)	106.69	78.5 ± 4.3b	12.0 ± 4.0ab	9.5 ± 0.2a	1.26	65.8 ± 3.8a
6 (MS):4 (GHB)	108.59	80.5 ± 0.5b	7.4 ± 0.6a	12.1 ± 0.4c	0.61	66.7 ± 3.8a
Lsd (0.05)	4.81	4.88	4.60	0.68		11.32
Cv (%)	2.5	3.5	19.6	3.3		9.4
p-value	<0.235	<0.016	<0.014	<0.001		<0.511

[a]GI, germination index; HA, humic acid; FA, fulvic acid.
Means with the same letter are not significantly different. Values are expressed as mean ± SD.

(28°C–36°C) and lowest in the 40% biochar heap (23°C–28°C), with that of the 10, 20, and 30% biochar heaps being similar (26°C–34°C). By week 8, the temperature in the 40% biochar compost heap (23°C) was not different from that of the ambient air and was lower than the temperature at the start of the composting. The 0% biochar and 10% biochar compost heap temperatures were 28°C, while the temperatures for the 20% and 30% biochar compost heaps were 27°C and 26°C, respectively, by the end of the eighth week. In general, the temperatures recorded in the heaps were not thermophilic.

Biochar effect on the maturity, humification, and CEC of compost types

The indices used to assess the maturity of the composts produced are summarized in Table 2. The germination index is a measure of the effect of phytotoxic substances from compost on the germination rate and radicle elongation of seedlings. The germination index increased as the biochar percentage increased and ranged from 103.5 to 108.6. Biochar addition significantly increased the humin content with values ranging from 71.8% in the 0% biochar compost type to 78.5% in the 30% biochar compost. The humin content of the biochar compost types with biochar contents of 10% and 20% was similar ($p < 0.05$) to the non-biochar compost type. However, the compost types with higher biochar contents (30% and 40%) had statistically higher humin content compared to their non-biochar counterparts, albeit with similar content as the lower biochar (10% and 20%) types. Compost types with high biochar content (30% and 40%) had the least humic acid (HA) content with the acid content in the 40% biochar compost type being less than half of that in the non-biochar compost type. Similarly, fulvic acid (FA) concentration was highest in the 0% biochar compost and decreased to 9.5 in the 30% biochar compost. The ratio of HA:FA, which indicates the degree of polymerization of humification products, was 1.24 in the 0% biochar compost and increased slightly to 1.36 in the 10% and 20% biochar compost types. A further increase in the biochar content decreased the ratio to 1.26 in the 30% biochar type. The ratio of HA:FA dropped to as low as 0.61 with a further increase in the biochar rate to 40%. There seems to be no influence on the concentration of biochar on CEC as the various compost types had statistically similar CECs that ranged from 62.5 to 71.7 $cmol_c\ kg^{-1}$.

Effect of biochar on the quality of mature compost

Table 3 shows the electrochemical properties and total basic cations of the compost types. Compost pH tended to increase with increasing biochar addition from 6.5 in the 0% biochar compost to 7.5 in the 40% biochar compost. The addition of at least 20% biochar resulted in at least a significant 0.5 pH unit increase over the non-biochar compost type. Electrical conductivity of the 0% biochar compost was 3.69 dS m^{-1} and decreased with an increase in biochar percentage to as low as 1.36 dS m^{-1} in the 40% biochar compost. Total Ca and Mg contents of the compost types increased with an increase in biochar additions and, consequently, the sum of these two basic cations. Total Na and total K, on the contrary, decreased with an increase in biochar percentage. The concentration of K was the highest among the basic cations and ranged from 65.7 g kg^{-1} in the 0% biochar compost to 44.37 g kg^{-1} in the 40% biochar compost.

Total and available phosphorus contents of the compost types increased with an increase in biochar percentage (Table 4). Total P was lowest (6,083 mg kg^{-1}) in the 0% biochar compost and increased with an increase in biochar percentage up to 7,530 mg kg^{-1} in the 40% biochar compost. The available phosphorus content of the compost types was high, ranging from 286.67 mg kg^{-1} in the 0% biochar compost to 840 mg kg^{-1} in the 40% biochar compost. The percentage of available phosphorus in total phosphorus was 4.2% in the 0% biochar compost and increased with an increase in biochar percentage up to 11.2% in the 40% biochar compost. It is worthy of note that 30% and 40% addition of GHB to the MS improved P availability by 1.9- and 2.9-folds, respectively, over the non-biochar compost type.

The 0% biochar compost had the lowest concentration of total organic carbon (TOC) (128.1 g kg^{-1}). Total C increased with increasing biochar addition with an approximately 3.2-fold

TABLE 3 Electrochemical properties and total basic cation contents of the compost types.

Compost	pH	EC (dS m-1)	Ca (g kg-1)	Mg	Na	K	Ca + Mg
10 (MS):0 (GHB)	6.8 ± 0.1ab	3.69 ± 0.03e	2.23 ± 0.2a	2.16 ± 0.2a	3.10 ± 0.2c	65.7 ± 0.2d	4.39 ± 0.3a
9 (MS):1 (GHB)	6.7 ± 0.2a	3.54 ± 0.03d	5.00 ± 0.3b	3.09 ± 0.4b	2.49 ± 0.2bc	62.2 ± 0.3c	8.09 ± 0.3b
8 (MS):2 (GHB)	7.4 ± 0.2c	2.72 ± 0.09c	6.05 ± 0.2c	5.40 ± 0.2b	2.16 ± 0.2b	50.0 ± 0.3b	11.46 ± 0.4c
7 (MS):3 (GHB)	7.3 ± 0.2bc	1.74 ± 0.07b	6.99 ± 0.2d	6.27 ± 0.2c	1.99 ± 0.2b	44.9 ± 0.3a	13.2c ± 0.4d
6 (MS):4 (GHB)	7.5 ± 0.1c	1.36 ± 0.03a	10.07 ± 0.2e	6.64 ± 0.2c	1.23 ± 0.7a	44.4 ± 0.3a	16.71 ± 0.4e
Lsd (0.05)	5.00	0.08	0.4	0.44	0.62	0.42	0.70
Cv (%)	1.6	1.8	4.80	4.70	15.5	0.4	4.0
p-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Means with the same alphabet are not significantly different. Values are expressed as mean ± SD.

TABLE 4 Carbon, nitrogen, and phosphorus availability in the compost types.

Compost	Total P	Available P (mg kg^{-1})	Available P/ total P (mg kg^{-1})	TC (g kg^{-1})	TN	C/N	NH4+ (mg kg^{-1})	NO3-	NH4+/NO3-	Available N
10 (MS): 0 (GHB)	6,830 ± 0.03a	286.67 ± 8.4a	4.2	128.1 ± 8.8a	10.0 ± 0.8a	12.8	27.2 ± 2.0c	88.0 ± 3.0e	0.31	115.2 ± 3.0e
9 (MS):1 (GHB)	6,670 ± 0.03a	320.00 ± 12.1b	4.8	315.6 ± 9.2b	15.2 ± 0.4b	20.8	21.0 ± 1.0b	82.8 ± 0.0d	0.25	103.8 ± 1.0d
8 (MS):2 (GHB)	7,037 ± 0.03b	370.00 ± 17.0c	5.0	325.5 ± 22.9b	13.2 ± 0.3b	24.7	18.2 ± 1.0b	75.0 ± 2.0c	0.24	93.2 ± 3.0c
7 (MS):3 (GHB)	7,270 ± 0.03b	546.67 ± 20.9d	7.5	320.4 ± 6.4 b	14.3 ± 0.1 b	22.4	17.3 ± 1.0a	63.2 ± 1.0b	0.27	80.5 ± 1.0b
6 (MS):4 (GHB)	7,530 ± 0.02b	840.00 ± 22.3e	11.2	415.3 ± 12.5c	24.2 ± 0.5c	17.2	15.5 ± 0.0a	48.7 ± 4.0a	0.32	64.2 ± 4.0a
Lsd (0.05)	400.1	30.0		17.1	3.1		2.11	3.88		4.89
Cv (%)	3.1	0.5		3.0	10.9		5.9	3.0		2.9
p-value	<0.001	<0.001		<0.001	<0.001		<0.001	<0.001		<0.001

Means with the same alphabet are not significantly different. Values are expressed as mean ± SD.

increment in the 40% biochar compost type over the non-biochar type. The total nitrogen contents of the biochar compost types were higher than those of their non-biochar counterparts, with the 40% biochar compost type being almost 2.4 times higher in total N than in the non-biochar compost. Notably, there was no significant difference in total C and N content among the 10, 20, and 30% biochar composts. The carbon:nitrogen (C:N) ratio was lowest in the 0% biochar compost and increased with an increase in biochar percentage up to 24.7 in the 20% biochar compost. Further addition of biochar lowered the C:N ratios. It is worthy of note that among the compost types, only the non-biochar compost and the 40% biochar compost had C:N ratios below 20.

Nitrate-N and ammonium-N and, hence, the available N were highest in the non-biochar compost and decreased significantly with an increase in biochar percentage. The concentrations of NO_3^--N in the various composts were higher than those of NH_4^+-N. The difference in the concentration of the N forms resulted in NH_4^+/NO_3^- ratios being below 0.5.

Functional groups present in the diagnostic region of the FT-IR spectra of the compost types

The FT-IR spectra of the various compost types are shown in Figure 4. In the non-biochar compost, two sharp bands were observed at 3695.65 cm^{-1} and 3621.80 cm^{-1} of medium-bonded O–H stretch of free alcohols (Hagemann et al., 2018). These bands were absent in the biochar-containing compost types. The band at 3310.74 cm^{-1}, which was present in all the compost types, was assigned to strong broad, intermolecularly bonded O–H stretching of carboxylic, phenolic, and alcohol groups and N–H

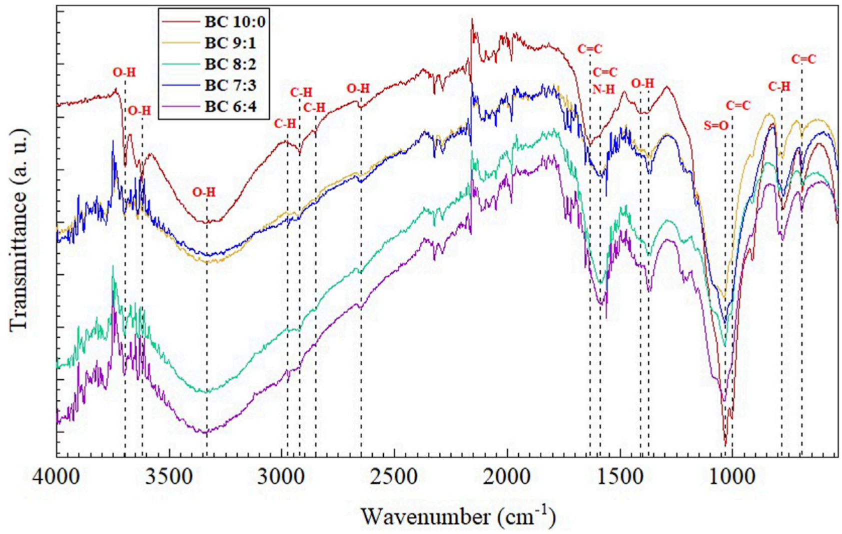

FIGURE 4
FT-IR spectra of the compost type.

stretching of aliphatic amines (Yang et al., 2006; Hagemann et al., 2018; Bong et al., 2020). The intensity of this band was highest in the non-biochar compost and lowest in the 10% and 20% biochar composts. The bands at 2917.47 cm^{-1} and 2851.40 cm^{-1} were assigned to the C–H stretch of aliphatic methyl and methylene groups (Carballo et al., 2008; Hagemann et al., 2018). The band at 2917.47 cm^{-1} was present in all the composts, albeit with reduced intensities in the biochar compost types. The band at 2851.40 cm^{-1} in the non-biochar compost was lost upon biochar addition. On the other hand, a band at 2968.29 cm^{-1} in biochar compost types, which was also assigned to aliphatic methylene groups (Carballo et al., 2008), appeared. A band at 2,650 cm^{-1} was present in all the compost types with similar intensity and was assigned to strong intermolecular O–H bonding of carboxylic acids (Sahoo et al., 2012). A medium-bond C=C stretch, indicative of conjugated and unsubstituted alkene, and C=O stretch of carboxylic acids, esters, ketones, and quinones (Carballo et al., 2008), was assigned to the band at 1634.59 cm^{-1} for the non-biochar compost. In the biochar compost types, this band (1634.59 cm^{-1}) re-centered at 1588.44 cm^{-1} and was assigned to the medium N–H bend of amine and the medium C=C stretch for cyclic alkene. The band at 1402.80 cm^{-1} was assigned to O–H deformation and C=O vibration of phenols and COO^- vibration of aliphatic deformation (Abouelwafa et al., 2008; Carballo et al., 2008). This band (1402.80 cm^{-1}) shifted and re-centered at 1372.44 cm^{-1} in the biochar composts. The band at 1034.47 cm^{-1} in the non-biochar compost was assigned to the strong S=O stretch of sulfoxide. The band re-centered at 1039.32, 1037.08, 1036.67, and 1036.67 cm^{-1} for the 10, 20, 30, and 40% biochar composts, respectively, indicating a chemical shift. The band at 1005.75 cm^{-1} in the non-biochar compost could be a strongly bonded C=C for mono-substituted alkene. This band disappeared in the biochar compost types. In all the compost types, the presence of strongly bonded C–H bends, suspected to be mono-, 1-3-di-, and 1,2,3-tri-substituted, was assigned to the bands at 779.73 cm^{-1} and 690.22 cm^{-1}. Moreover, the band at 690.22 cm^{-1} was again assigned to a strong C=C band indicative of a benzene derivative. This band (690.22 cm^{-1}) re-centered at higher wavenumbers with the addition of biochar.

Discussion

Feedstock properties and pyrolysis conditions can influence the properties of biochar and, subsequently, biochar application (Kloss et al., 2012; Spokas et al., 2012). Legumes accumulate high concentrations of N and P because they have a high demand for these nutrients compared to non-leguminous crops under similar cropping systems (Adamu et al., 2014; Anguria et al., 2017; Romanyà and Casals, 2020). Nitrogen and P absorbed by plants are translocated to reproductive regions (Logah et al., 2013; Bender et al., 2015). It, therefore, stands to reason that groundnut husk, which is part of the legume reproductive organ, accumulated higher N and P than the MS. The higher C, N, and P levels in the groundnut husk compared to the MS justified the former's choice as an ideal feedstock for pyrolysis. With higher C and P, groundnut husk upon charring should give elevated concentrations of these elements under similar pyrolysis conditions. The elevated P could be a source for organic fertilizer formulation. The lower N content of the MS may have contributed to its higher C:N ratio, as noted elsewhere by Ruan et al. (2019) and Kumar and Singh (2018).

Loss of acid functional groups such as the carboxylic groups after charring of groundnut husk as depicted by the FT-IR, which is consistent with the very low content of 1.05 mmol/g carboxylic functional group from the Boehm titration with a concomitant release of soluble basic cations such as K^+, Ca^{2+}, and Mg^{2+}, accounted for a higher biochar pH compared to the groundnut feedstock, as noted elsewhere by Novak et al. (2009) and Bourke et al., 2007. With most soils in humid sub-Saharan Africa being acidic, any amendment with a liming potential and high P levels should improve the availability of the macronutrient. The higher pH of the biochar than its feedstock is certainly attributable to the release of approximately 8.04 g/kg soluble K^+, Ca^{2+}, and Mg^{2+} upon charring.

The biochar yield in this study is indicative of biomass reduction, which can be attributed to the loss of volatile constituents such as CO, CO_2, H_2O, HCN, and NH_3, from the thermal degradation of cellulose, hemicellulose, and lignin (Lee et al., 2016; Pawar and Panwar, 2020). The loss of volatile constituents is evident in the decreased O and H contents of the biochar derivative compared with the groundnut husk feedstock. This finding is consistent with the biochar yield from slow pyrolysis reported by Tomczyk et al. (2020) and Fidel et al. (2017). Alkali salts such as carbonates, oxides, and hydroxides of Ca, Mg, Na, and K, together with nutrients such as P, S, N, Fe, and Zn, released upon separation of mineral matter from the organic matrix, constitute the mineral ash content of biochar (Fungai and Sanjai, 2016). Hence, the mineral ash content of biochar is closely correlated with pH, EC, and nutrient status (Lehmann et al., 2011; Uras et al., 2012; Fungai and Sanjai, 2016). The high concentrations of Ca, Mg, and K observed in the present study and the presence of carbonate ions revealed by FT-IR may account for the high pH of the biochar and suggest that the biochar could be used as a liming material in acidic soils (Eduah et al., 2019; Frimpong-Manso et al., 2019). The high pH of the GHB, coupled with its high soluble base content, is a positive attribute that could be exploited in composting. When co-composted with MS, the high pH and soluble bases would mitigate the inhibitory effect of low initial pH on decomposing organisms and promote a shorter composting duration.

High salt concentrations that reflect a high EC can inhibit seed germination and plant growth through water stress, salt stress, and nutrient imbalances (Al-Wabel et al., 2013; Tomczyk et al., 2020). The release of Ca, Mg, and K from the organic matrix may have accounted for the higher GHB EC, which was twice that of the feedstock. The EC of the biochar, being below 2 dS m^{-1}, is considered non-saline (Hazelton and Murphy, 2016). Thus, the biochar produced can be used as a growth medium, soil amendment, or feedstock to produce non-saline compost.

Oxygen-containing surface functional groups account for the CEC of biochar (Sohi et al., 2010; Briggs et al., 2012). Carboxylic acids (COOH) have pKa values lower than 5.0, above which deprotonation occurs to yield carboxylate (COO^-) ions, one of the main sources of CEC. Similarly, phenolic groups deprotonate at pH around 9.0 and contribute to negative charges for increased CEC (Banik et al., 2018; Eduah et al., 2019; Tomczyk et al., 2020). Considering the fact that the concentration of the carboxylic functional groups (1.05 mmol g^{-1}) in the biochar was just a paltry 2.7% of the phenolic group, the contribution toward negative charge development (CEC) is more attributable to the phenolic groups. At the biochar pH of 9.74, more than half of the phenolic group will deprotonate for negative charge development. When GHB is applied to acidic soils, the equilibrium pH would be lower than the pKa (9.8) of the phenolic group (Evangelou, 1998), and this may not favor the deprotonation of the phenolic group. As such, the CEC of the amended soils will only be marginally improved owing to the low content of carboxylic functional groups.

The soluble bases, 52% ash content, and high pH of the biochar would promote surface precipitation of liberated P, and that may, in part, account for the very high total P of 3,400 mg kg^{-1}, of which only 22% is available. Nevertheless, the available P content of the biochar is high enough to sustain crop cultivation should the amendment be applied in the appropriate form. The MS with its inherent lower pH (5.75) during co-composting with GHB will release more organic acids to minimize surface precipitation of P onto biochar by complexing with the cations in the ash of the biochar. The decomposing MS will, in addition, provide more carboxylate ions to repel phosphate anions into the soil solution to increase the availability of P when the soil is amended with the biochar compost. Co-composting GHB with MS should, therefore, provide a better P amendment than sole biochar or sole MS compost.

The increase in C concentration can be attributed to carbonization, whereas losses of H and O atoms may be due to the dehydration of groundnut husk. These two processes subsequently concentrate carbon in the biochar (Guo and Rockstraw, 2007). The ratios of H:C and O:C have been used to predict the stability of biochar in soil (Spokas, 2010; Budai et al., 2013; 2016). The reduction in the H:C ratio is an indication of the loss of alkyl C groups and the formation of aromatic C compounds in the biochar. According to Budai et al. (2013), approximately 70% of biochar would persist for more than 100 years in soil if the H:C ratio is at most 0.4. The H:C ratio of 0.04 reported in this study implies that the biochar is stable and that more than 70% would remain for at least 100 years when applied to soil. The O:C ratio of 0.43 suggests that the biochar produced has a half-life of between 100 and 1,000 years (Spokas, 2010), which will promote carbon sequestration in the fragile soils of sub-Saharan Africa.

Pyrolysis of biomass results in the conversion of organically bound phosphorus in feedstocks into plant-available forms at temperatures below 400°C (Xu et al., 2016). The plant-available forms of P are, however, converted to unavailable forms at temperatures between 400°C and 600°C (Figueiredo et al., 2021; Jiang et al., 2019; G; Xu et al., 2016). Phosphorus in biochar may become completely unavailable when pyrolyzed at temperatures above 700°C (Figueiredo et al., 2021; Jiang et al., 2019; G; Xu et al., 2016). In the present study, biochar was produced at a temperature of approximately 450°C, accounting for the very high total P, of which only 22% is in the available form.

The high C:N ratio of the biochar can be attributed to a high increase in C because of carbonization. Application of biochar with such a high C:N ratio would result in immobilization of N and possibly N starvation of plants, especially in N-deficient soils (Fungai and Sanjai, 2016). In the present study, nitrogen from decomposing cow dung was, thus, added to the farm residue composting heaps, with or without biochar, to facilitate decomposition.

According to Keiluweit et al. (2010) and Rutherford et al. (2012), decomposition of aliphatic carboxylic groups and aromatization of carbon lead to the formation of phenolic and/or amine groups of higher bond energy. Thus, the increased intensity and chemical shift of the broad OH-stretching vibration can be attributed to increased phenolic and/or amine groups formed from dehydration and decarboxylation reaction products of C–H, C=O, and O–H groups (Angın, 2013). This assertion was confirmed by the results of the Boehm titration, which revealed a relatively higher concentration of the phenolic and lactonic groups than the carboxylic group. The intensification of 1,630 cm^{-1} upon pyrolysis suggests an enrichment in aromatic groups containing amines. Thus, inorganic N released may have reacted with C=O groups to form more stable products (Chen et al., 2017; 2018). The loss of the bands at 2,920 cm^{-1} and 1739.40 cm^{-1} upon pyrolysis can be attributed to the decomposition of aliphatic carboxylic groups. Similar results have been reported by Rutherford et al. (2012) and Keiluweit et al. (2010). The retention of C–O and C–N groups can be attributed to the lignin component as biochar was produced at a temperature lower than the ceiling for lignin decomposition (Babu, 2008). Inorganic phosphate, carbonate, and nitrate ions identified are attributed to the presence of these anions in the mineral ash of the biochar produced (Montes-Morán et al., 2004; Carrier et al., 2012). Inorganic phosphates have been reported to be completely absent at temperatures above 700°C (Christel et al., 2014). Thus, considering the temperature (454 °C) at which the biochar was produced in this study, the presence of inorganic phosphate and carbonate was expected.

The initial increase in temperature during composting is indicative of active microbial decomposition in the various heaps. Consequently, the early initial increase in temperature of the heap with 20% biochar can be attributed to the neutralization of organic acids produced at the initial decomposition stage by the alkalinity of biochar (Akumah et al., 2021). The 1-day delay of the 0% and 10% biochar heaps suggests that the alkalinity of the 10% was not enough to neutralize the organic acids produced during MS decomposition and that similar microbial activities prevailed in both heaps. The delayed increase in temperature of the 30% and 40% biochar heaps can be attributed to the higher proportions of unavailable N associated with these higher biochar loading rates. The high N concentration in the 30% and 40% biochar heaps may have lowered the C:N ratio. However, these N forms are recalcitrant to microbial attack, culminating in lowered microbial activity in these heaps. Moreover, the absorption of moisture at these high biochar rates may have lowered the moisture at the interface between decomposing microbes and the MS, thereby reducing microbial activity (Antonangelo et al., 2021). Thus, in the composting of stover–biochar mix, moistening of heaps to higher contents (>60%) may be required. The temperature profiles of the compost heaps in this study (Figure 2; Figure 3) suggest that the composting process occurred in the mesophilic range. The dry form of the maize stover may have suppressed microbial activity and, consequently, the composting process.

GI, a measure of compost phytotoxicity, is mainly used to determine compost maturity (Luo et al., 2018). Mature composts must have very little or no phytotoxic effect on germinating seeds, in addition to their ability to supply nutrients. When cress or tomato seeds are used, values of 100 and above are accepted for commercially produced composts intended for use as growth media. Lower values are acceptable for composts to be used as soil amendments (Hase and Kawamura, 2012; Warman, 2013; Luo et al., 2018). In the present study, all the composts scored more than 100 for germination index, which indicates that the various compost types were non-phytotoxic and, hence, matured.

The humification process during composting is often used to ascertain compost maturity. Humic acid-to-fulvic acid ratio (HA: FA) is an index of humification that describes the progress of HA-to-FA transformation during composting. A ratio ranging from 3.6 to 6.2 has been recommended for mature composts (Hase and Kawamura, 2012; Zhou et al., 2014). The ratio in the present study (0.61–1.36) suggests that the composts contain nearly equal amounts of fulvic and humic acids and that they would undergo further humification when applied to soils. The results also indicate that the 40% biochar-compost was the least humified. Perhaps, the predominant recalcitrant biochar N and C in the 40% biochar heap accounted for low humification. This compost type, when applied to the degraded soils of the Guinea Savanna zone, is likely to improve their carbon status as it will persist in the soil relatively longer.

Humic acid and fulvic acid contain surface functional groups, of which carboxylic groups dominate in the latter. The complete dissociation of protons in carboxyl and phenolic groups results in the formation of negatively charged surfaces. The negative charges developed constitute the CEC of the compost. Thus, the high CEC (62.5–66.7 cmol kg^{-1}) of the compost types can be attributed to deprotonation of the abundant carboxyl group as the pH was below the pKa of phenolic functional groups. According to the CEC rating by Harada and Inoko (1980b), mature composts have CEC >60 cmol kg^{-1}. Thus, the composts from this study with CEC >60 cmol kg^{-1} can be considered mature. The statistically similar CEC of the five compost types despite the varying proportion of biochar applied implies that the contribution of biochar to charge development in the compost was negligible. Perhaps with the aging of the biochar in soils, the positive effects on CEC would manifest. The fact that the compost types are still undergoing humification implies that, with time, the CEC would increase to improve upon cationic nutrient storage. The use of these biochar compost types holds promise as P-enriched organic amendments in sub-Saharan Africa. With humification, the availability of P may increase with time, implying that residual P may be higher in subsequent seasons. Therefore, there may not be a need for annual applications, which is the main drawback of organic farming in the tropical environment. It is, however, imperative for trials to be carried out to determine when to reapply the amendment for sustainable crop growth. Again, with the high available P contents but very low available N, these biochar compost types could be exploited for use in legume production in the P-deficient ferruginous soils of the Guinea Savanna zone of Ghana. Phosphorus would be made more available, and the legumes would experience N deficiency, which could induce N fixation.

However, co-application of these compost types with cationic micronutrients such as Zn, Cu, and Mn into soils is not encouraged, as the readily available P may promote precipitation of the metals. The high CEC of these compost types will repel $H_2PO_4^-$ and HPO_4^{2-} in the compost. These anions will then persist in the soil solution to precipitate any metal that may be available. Co-application will rather promote metallic micronutrients and P deficiency. Preferably, in the use of biochar compost amendments, micronutrients should be applied as foliar fertilizers.

The pH of compost types in the present study increased with an increase in biochar percentage and was within the acceptable range (6.5–7.5) for crop production (Anthonis, 1994). The increase in pH with an increase in biochar percentage can be attributed to the release of alkaline compounds present in the biochar, as was revealed by a strong positive correlation between biochar pH and the sum of Ca and Mg (Supplementary Figure S1). According to the regression analysis, the sum of Ca and Mg accounted for 75% of the increase in compost pH (Supplementary Figure S1).

The aeration of the heaps in this study may have contributed to increased nitrification and consequently reduced NH_4^+ accumulation in the final composts. Although the composts produced in the present study were not alkaline, they can be applied to extremely acidic soils, such as Oxisols and Ultisols, to serve as liming materials. Application of these compost types to neutral soils would increase their pH buffering capacity due to their high CEC, as noted elsewhere by Antonangelo et al. (2021). The neutral nature of the compost types will minimize the volatilization of ammonium to preserve the already low N in the various amendments. However, it is expected that with further humification, the available N contents will increase. The neutral pH of the compost types is a result of the high nitrification rates of the amendments, as reflected in the very low NH_4^+:NO_3^- ratios of less than 0.4. High nitrification will culminate in the release of protons, which will nullify alkalinity.

Another parameter used to assess compost maturity is the nitrification index (NH_4^+–N:NO_3^{-1}–N ratio) (Rashad et al., 2010). Composts with NH_4^+:NO_3^- lower than 1 are free of the phytotoxic effect of NH_4^+ (Abouelwafa et al., 2008; Tumuhairwe et al., 2009; Vergnoux et al., 2009). Final compost ratings of NH_4^+–N:NO_3^-–N ratio of <0.5, between 0.5 and 3.0, and >3.0 are indicative of a very mature, mature, and immature compost, respectively (Das et al., 2011). All the composts had NH_4^+–N/NO_3^{-1}–N ratios <0.5, confirming that all the composts were very mature.

A significant positive correlation existed between EC and K (0.96**) and EC and Na (0.92**) in the compost types, as evident in Supplementary Figure S2, S3, respectively, indicating that the EC of the amendments was largely controlled by their K and Na contents. Approximately 92% and 85% of the EC can be attributed to K and Na, respectively. Thus, the Na and K contents of the MS contributed to the high EC of the non-biochar compost. The lower EC of the biochar-compost types could be attributed to the dilution effect of the GHB. Compost EC is one of the important properties that determines its quality and, therefore, class. An EC value of at most 4.0 dS m^{-1} is recommended for general-purpose composts and for landscaping (Lasaridi et al., 2006; Gondek et al., 2020). The various composts had EC values lower than the critical value of 4.0 dS m^{-1}, suggesting that they can be applied as soil amendments. The non-biochar-compost and the 10% biochar compost types, which have ECs of 3.69 dS m^{-1} and 3.54 dS m^{-1}, respectively, close to the critical limit, could be used with moderation for landscaping and not for the cultivation of crops to avoid salt injury and destruction of soil structure. The other biochar-compost types, particularly those with 30% and 40% biochar loading rates, could be used for crop production without fear of salt injury or salinity problems. The similarity in the EC values for the non-biochar compost and the 10% biochar compost and the fact that their P contents are marginally different implies that in the preparation of the latter, cost must be factored in. Would the additional cost incurred in charring groundnut husk to co-compost with MS compensate for the additional available P compared to the non-biochar compost type?

Increasing the biochar loading rate resulted in a high total organic C content of the compost due to the high levels of C in the biochar. In addition, the reduced decomposition upon incorporation of biochar into the compost was evident in the higher C:N ratios of the biochar-compost as compared with the non-biochar counterparts.

Total N retention in the groundnut husk biochar vis-à-vis the increasing loading rate resulted in the high total N content of the biochar-compost. The ratio of carbon to nitrogen is used as an indicator of the stability of mature composts (Bernal et al., 2009). Values around 20 show a satisfactory maturity of composts (Li et al., 2015). The slightly higher values for the biochar-containing compost can be attributed to the recalcitrant C content of the biochar added. The differences in the C:N ratio further suggest that the various composts would be mineralized to varying extents when applied to soil. For early-maturing crops such as corchorus, which is a leafy vegetable popular in the diet of people in the Guinea Savanna zone of Ghana, the compost of choice should be the non-biochar types as they would mineralize faster to release N for plant uptake. The non-biochar compost type would be characterized by faster release of N because it had the highest available N and the least C:N ratio of 12.8. It should, however, be used with regular monitoring of the soil's EC to avoid destruction of the structure of the soil. For tree crops and long-duration crops, the biochar compost types should be preferred. The biochar-composts, particularly the 20% biochar type, would be more appropriate for carbon sequestration because of their relatively high C:N ratio. The decrease in the available N content of the compost types with an increase in biochar percentages can be attributed to the effect of biochar on reducing degradation (Khan et al., 2014). The high proportion of carbon in the biochar may have accounted for the reduced mineralization of the MS and the resultant lower available N with an increased biochar percentage.

Biochar addition resulted in the loss of free hydroxyl groups in the final compost, which can be attributed to the loss of water caused by aeration in the biochar-containing compost types. The loss of the aliphatic groups can be attributed to microbial degradation due to their low recalcitrance (Barje et al., 2012). The chemical shifts observed in the bands of the biochar-compost types suggest surface modifications, which can be attributed to the adsorption of NH_4^+ onto biochar, as mentioned by Barje et al. (2012).

Conclusion

This study showed that MS and groundnut husk, which abound in the Guinea Savanna zone of Ghana, could be harnessed into sustainable production of P-enriched organic amendments to reduce the import bill of fertilizers while closing the nutrient loop. This may also reduce nutrient miming to minimize soil fertility decline in the soils of the Guinea Savanna, particularly when amendments are applied to soils from which the feedstocks are harvested.

Biochar addition affected the composting process. Germination index, HA:FA, and CEC showed that all the compost types were non-phytotoxic after 10 weeks of composting. Excessive dissipation of heat associated with the bulky nature of the feedstocks favored mesophilic activities during composting. The addition of GHB to the MS improved P availability by 1.9- and 2.9-folds, respectively, over the non-biochar compost type.

This study indicates that composting MS alone or co-composting MS with 10% GHB is not ideal for producing good-quality compost. Good-quality compost with low EC, very high available P, and high CEC for crop cultivation and the ability to sequester carbon could be produced for use in the Guinea Savanna zone of northern Ghana by co-composting 40% groundnut husk charred in the Kuntan kiln with MS.

Data availability statement

The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation.

Author contributions

The first author, DF, carried out the investigation as part of his Doctor of Philosophy thesis and prepared the original draft. Conceptualization, grant acquisition, methodology, supervision, writing, and reviewing and editing were performed by the corresponding author, EN. MA, TA, and IL were also supervisors and assisted in the methodology, curation of data, and the original write-up. CA assisted with grant acquisition, data curation, review and editing of the manuscript, and project administration. SA-B, AA, NS, and MB assisted with review and editing of the manuscript. All authors contributed to the article and approved the submitted version.

Funding

This research work was funded by the USAID/University of Ghana Feed the Future project (grant number 641-002-FY14-IL#02).

Acknowledgments

The authors acknowledge the assistance of the Director and staff of the Ministry of Food and Agriculture office in the Lawra Municipality, Ghana.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fenvs.2023.1252305/full#supplementary-material

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frontiers | Frontiers in Environmental Science

TYPE Review
PUBLISHED 09 October 2023
DOI 10.3389/fenvs.2023.1215317

OPEN ACCESS

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RECEIVED 01 May 2023
ACCEPTED 27 September 2023
PUBLISHED 09 October 2023

CITATION
Lou X, Sui Y, Fang C, Tang Y, Huang D and Guo Y (2023), Bibliometric analysis for global marine microplastic pollution control from 2013 to 2022.
Front. Environ. Sci. 11:1215317.
doi: 10.3389/fenvs.2023.1215317

Bibliometric analysis for global marine microplastic pollution control from 2013 to 2022

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The control of microplastic pollution in the marine environment has become a growing public concern in recent years. To better grasp the trends and development of microplastic pollution control in the marine environment, the published literature in Science Citation Index Expanded (SCIE) database of Web of Science Core Collection from 2013 to 2022, up to a total of 2,357 articles or reviews was analyzed through CiteSpace and VOSviewer tools. The results show an exponential growth in the number of papers related to the control of microplastic pollution in the marine environment, with China, United States, India, and Australia providing the main drivers, while China being the most active country, with *Science of the Total Environment*, *Marine Pollution Bulletin*, *Environmental Pollution* and *Chemosphere* being the most important sources for publishing relevant research. A relatively complete theoretical framework has been developed for the control of marine microplastic pollution, focusing on the quantification, traceability and collectability of microplastics. However, few papers have focused on policy implications and technological innovations in this area. The research on marine microplastic pollution control has transitioned from traceability and hazard analysis of microplastics to the impact of economic activities and synthetic fibre on microplastic pollution. Microplastics in wastewater discharged from municipal wastewater treatment plants, human consumption, man-made fibers and synthetic polymers have become the frontier of research. The present study is of significance for better understanding and supporting further research on the control of microplastic pollution in the marine environment.

KEYWORDS

marine environment, microplastics, pollution control, bibliometric analysis, visualization software

1 Introduction

The largest and most persistent portion of marine litter is synthetic polymers and thermosets, collectively known as plastics. (Law, 2017) Marine plastic pollutants account for at least 85% of total marine waste. (Agamuthu et al., 2019) The United Nations Environment Programme (UNEP) has indicated that the amount of marine litter and plastic pollution has been growing rapidly. Under a business-as-usual scenario and in the absence of necessary interventions, the amount of plastic waste entering aquatic ecosystems could nearly triple from some 9–14 million tons per year in 2016 to a projected 23–37 million tons per year by

2040. Other researchers predicted the amount of plastic waste is approximately double with 19–23 million tons per year in 2016 and around 53 million tons per year by 2030. (Irene Samy Fahim and Landrigan, 2021).

The plastics can be broadly classified into four classes, i.e., nanoplastics, microplastics (MPs), mesoplastics, macroplastics, with dimensions <0.001 mm, <5 mm, <25 mm, and >25 mm, respectively. (Shim et al., 2018). In recent years, MPs have become a growing concern because of the harm they cause to marine organisms and marine ecology. (Basili et al., 2020). Land-based sources are considered to be the main source of MPs entering the ocean, with two pathways, i.e., primary and secondary MPs. The former come from engineered MP beads and industrially produced plastic powders widely used in cosmetic formulations (Castaeda et al., 2014; Napper et al., 2017) and abrasives, and also include fibers released during the manufacture of synthetic textiles and clothing (Salvador Cesa et al., 2017). These MPs are barely visible to the naked eye, and are likely to flow directly from bathroom drains or industrial discharges into the drainage system, easily escaping capture by sewage treatment plants and eventually entering the aquatic environment. The latter comes primarily from large plastic debris that enters the marine environment directly from shorelines, rivers and sewage pipes through a combination of physical (mechanical), chemical (photolytic) and biological processes when exposed to high levels of solar UV radiation and mechanical wear and tear. (Jiajia et al., 2018) MPs in the ocean not only damage the health of marine species, but also affect the carbon cycle in the ocean by altering the transfer of carbon to the deep sea, (Wieczorek et al., 2019) which further affects marine ecosystems.

MPs have a profound impact on marine ecology (Lusher et al., 2013; Tanaka et al., 2013; Seltenrich, 2015) and even on the mainland environment (Lönnstedt and Eklöv, 2016). They affect marine organisms through uptake and ingestion, resulting in physiological and behavioral effects. The transformation of MPs through the food chain leads to bioaccumulation, which affects the stability of the entire ecosystem. In addition, the accumulation and deposition of MPs can lead to changes in the seabed and the destruction of benthic habitats. Taken together, these ecological problems have widespread and long-term effects on the marine ecosystem, threatening marine biodiversity, ecological balance and the sustainable use of marine resources. Analyzing the current state of this area is therefore crucial. Bibliometrics is an interdisciplinary discipline that uses mathematical and statistical methods to analyse literature data and is a powerful tool for studying the current state of a field and future research trends. Firstly, it identifies research hotspots, enabling targeted resource allocation for more effective problem solving. Secondly, it highlights emerging frontiers for potentially high-impact research, promoting innovative technologies and strategies. It also informs the development of policy and management strategies, helping governments and environmental agencies to develop effective regulations and practices. Finally, it identifies knowledge gaps and directs researchers to areas that need further investigation, thereby contributing to the advancement of knowledge in the field. Overall, bibliometric analysis enhances research direction, policy formulation and knowledge expansion in the field of marine MP pollution control.

This study quantitatively analyzes the relevant literature in the scope by using statistical and mathematical analysis through software packages such as VOSviewer. With the advantage of its objective and quantitative macroscopic research, literature data such as titles, authors, institutions, journals, keywords, and references are processed to generate citation networks, co-occurrence networks, and coupling networks. The following is a comprehensive analysis of publication trends, source journal analysis, author contributions and collaborations, keyword co-occurrence analysis, co-citation analysis, and research frontiers and hotspots. Further visual analysis of the evolution of research hotspots and frontiers of marine MP pollution treatment in the last decade provides new insights into marine MP pollution control.

2 Data and methods

2.1 Data retrieval

The Web of Science Core Collection (WoSCC) was selected as the data source for this study. Valuable scientific information and a comprehensive understanding of this field can be acquired conveniently using the powerful search function of WoSCC. (Ying et al., 2023).Scope of research selection with "topic" (TS) as the search field and refined in the Advanced Search module of WoSCC database with the search formula: TS = (microplastic OR microplastics OR "plastic debris" OR micro-plastic OR nanoplastics) AND TS = (marine OR sea OR ocean OR beach OR bay OR gulf OR estuary OR coastline OR shoreline) AND TS = (contamination OR pollution OR contaminate OR pollute OR stain OR filth OR contaminant OR foul) AND TS = (removal OR removal OR removed OR remove OR exenterate OR dispose OR expulsion OR erasing OR eliminate OR degradation OR degrade OR decomposition OR decompose OR degeneration OR hydrolysis OR degradable OR dissipation OR harness OR governance OR treatment OR control OR management OR government OR govern OR administration OR regulation). In addition, the language is further limited to English, and the document type is restricted to "article" and "review". To collect relevant papers from the last decade, the search was set to span from 01/01/2013 to 12/31/2022. Finally, the research directions and topics of all publications were reviewed to remove irrelevant content, such as "Food Science & Technology, Political Philosophy," resulting in a total of 2,357 relevant papers written by 10,074 authors from 408 countries, spanning 6,840 institutions and published in 394 journals.

2.2 Methods

Bibliometrics is an interdisciplinary discipline that uses mathematical and statistical methods to analyze data from the literature collected from the WoS core repository, including number of publications, authors, institutions, countries/regions, citations, etc., through tabulation and visual mapping. (Shiffrin and Boerner, 2004) CiteSpace and VOSviewer are the most commonly used bibliometric tools among various software. Using VOSviewer, visual collaboration graphs based on the collaboration between elements such as countries, institutions, authors, etc., can be displayed in three dimensions. (van Eck and Waltman, 2010; Pan et al., 2018) CiteSpace is a data analysis and visualization software based on set theory with a special focus on the temporal dimension, allowing the analysis of time slices through timeline analysis to further explore trends in the field. Reference co-citation knowledge domain maps and keyword timelines were created using the CiteSpace software package (Chen, 2006).

VOSviewer and CiteSpace assign each node in the network to a class cluster—a class cluster is a set of closely related nodes assigned according to a specific connectivity metric (e.g., co-citation, bibliographic coupling, etc.). These clusters are classified by the software based on the clustering algorithms built into the software, e.g., Likelihood Ratio (LLR) clustering algorithm and Latent Semantic Indexing (LSI), where the nodes are knowledge bases and the labels are taken from the literature at the forefront of research. Unfortunately, we are not fully aware of the logic behind the software's clustering algorithms, and have only analysed and discussed the results based on those derived from the software, and therefore have not discussed the division of the clusters further. VOSviewer and CiteSpace allow researchers to apply filters. We can zoom in on a certain part of the data, e.g., focusing on authors or organisations with at least 5 articles. In order to visualise the main contributions to the literature on the control of marine microplastic pollution, we have used filters, as detailed in the information below. In the course of this study, authors with 5 or more publications were selected for analysis in the author analysis, generating a total of 146 nodes. In the organizational analysis, organizations with 5 or more articles were selected for analysis, generating a total of 276 nodes. For the country analysis, countries with at least five articles were selected for analysis based on the first author assigning the article to a country, where Northern Ireland, Wales and Scotland were merged to form the United Kingdom and China included Taiwan, resulting in a total of 66 nodes. For the literature co-citation analysis, literature with a minimum of 200 citations was selected, resulting in a total of 66 nodes. Among the 394 journals obtained, those with no less than 100 citations to cited journals were selected for co-citation analysis, resulting in a total of 154 nodes. A total of 3,160 keywords were extracted from the Web of Science core database. Keywords with more than 20 occurrences were selected for visual analysis, resulting in a total of 153 keyword nodes. In addition, the "Top 22 Keywords with the Strongest Citation Bursts" and "Keyword timeline" were analyzed by the software Citespace.

3 Results and discussion

3.1 Yearly quantitative distribution of the literature

The statistical analysis of the number of publications in the decade 2013 to 2022 provides a clear picture of trends in scientific output and the ongoing development and maturation of marine MP research field. The distribution of research outputs on the control of MP pollution in the ocean based on time series is shown in Figure 1. From 2013 to 2017, the number of articles related to marine MP pollution control grew slowly, with less than 100 articles published in 2017. However, there was a large increase in 2018 and 2019, with annual publication volumes of 198 and 245, respectively, and the research related to marine MP pollution control gradually became a hot spot, which cannot be separated from the UNEP guidance and related policies in several countries. (Xanthos and Walker, 2017) From 2020 onwards, there is a sudden increase in published articles, with 1,686 articles related to the topic published in the last 3 years (2020–2022), accounting for 71.53% of the overall number of articles published in the last decade. This indicates that the MP issue is gaining dramatic attention from researchers, especially in the environmental and marine sciences.

The inset of Figure 1 shows the number of articles published each year by the top 10 countries or regions in terms of the total number of articles published that year. In 2013 and 2014, United Kingdom, Australia and the United States almost all ranked in the top three, contributing significantly to the research on marine MP pollution control. Also, it is worth noting that the number of articles from China has increased tremendously after 2016. After 2019, the number of articles from Chinese researchers has steadily exceeded 28%, which indicates that Chinese researchers have been actively involved in research on marine MP pollution control for several years and have achieved considerable results.

3.2 Source journal analysis

Journals have vital importance in scholarly communication and the dissemination of scientific discoveries. By conducting a journal analysis, we were able to identify influential journals within the field. Table 1 lists the top 10 prolific journals that published more than 30 articles related to the control of MP pollution in the ocean, and they are all included in SCI/SCIE.

Science of the Total Environment (Sci. Total Environ.) is an international journal for publication of original research on the total environment, which includes the atmosphere, hydrosphere, biosphere, lithosphere, and anthroposphere. With 303 papers, Sci. Total Environ. has published the largest number of papers on marine MP pollution control studies, accounting for about 25.72%. The second-ranked journal is *Marine Pollution Bulletin* (Mar. Pollut. Bull., 293 papers, accounting for 24.87%), which focuses on the rational use of marine resources in estuaries, oceans and seas, as well as the documentation of marine pollution and the introduction of new forms of measurement and analysis. It deals not only with sewage treatment and pollution control but also with the management, economic aspects, and protection of the marine environment in general. The third-ranked one is *Environmental Pollution* (Environ. Pollut., 215 papers, accounting for 18.25%), which covers all aspects of environmental pollution and mitigation measures related to ecosystems and human health. Other core journals have also focused on the sources, distribution, biological effects and toxicity of marine MP pollution. These journals provide scholars with an important scientific basis for in-depth studies on the effects and control of marine MP pollution.

3.3 Author contribution and collaboration

3.3.1 Author characteristics

The bibliometric analysis identified authors who contributed to specific areas of research and their collaborative relationships. (Kholidah

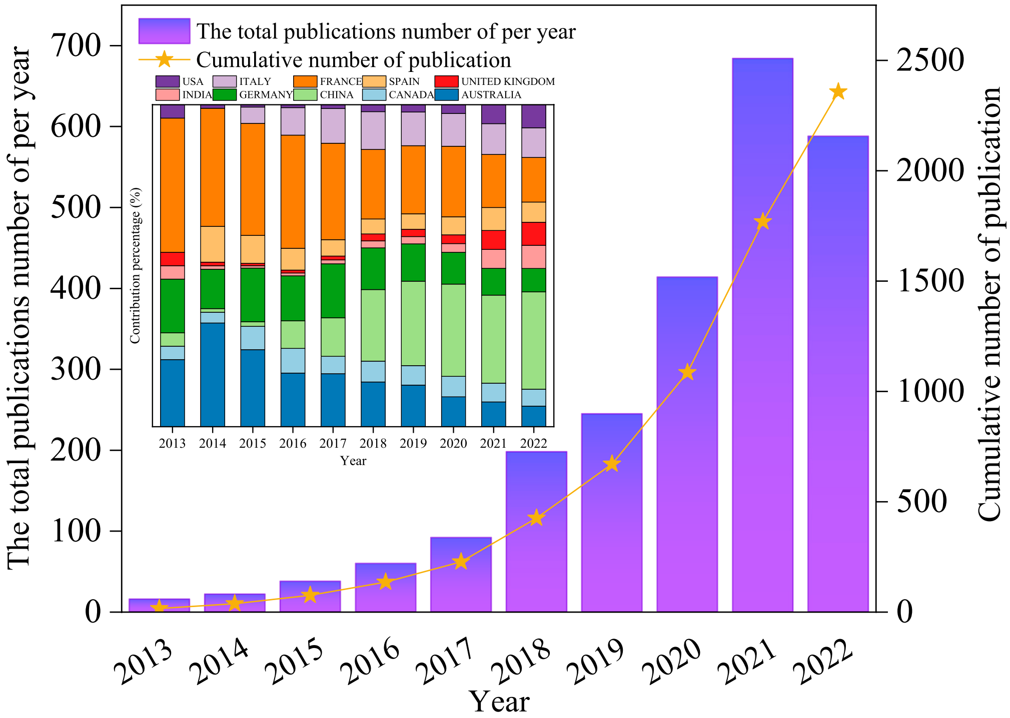

FIGURE 1
Number of publications per year and cumulative number of publications (Inset: Annual contribution of the top ten countries in terms of total number of publications between 2013 and 2022).

TABLE 1 Top 10 source journals ranked by the number of publications, 2013–2022.

Rank	Source journal	NP①	H②	TC③	CPP④	IF⑤
1	Sci. Total Environ.	303	59	14,226	47.0	10.753
2	Mar. Pollut. Bull.	293	59	12,137	41.4	7.001
3	Environ. Pollu.	215	66	15,602	72.6	9.988
4	Chemosphere	99	32	3,878	39.2	8.943
5	Environ. Sci. Pollu. Res.	80	26	3,401	42.5	5.190
6	J. Hazard. Mater.	76	30	2,729	35.9	14.224
7	Front. Mar. Sci.	73	18	1,033	14.2	5.247
8	Water Res.	58	33	8,067	139.1	13.400
9	Environ. Sci. Technol.	47	34	9,159	194.9	11.357
10	Sustainability	33	9	348	10.5	3.889

① Number of papers on marine MPs; ② h-index; ③ Total Citation; ④ Citations per paper (the number of citations per paper on average); ⑤ 2021 impact factor as of 12/31/2022.

et al., 2022). A total of 10,074 authors were involved in marine MP pollution control studies during the period 2013–2022, according to the conditions in “2.1 Data collection.” As shown in Figure 2A, the size of the circles indicates the number of publications, with Chelsea M. Rochman from the University of Toronto, Canada, publishing the highest, with a number of 18 papers. Richard C. Thompson from the University of Plymouth (United Kingdom), Huahong Shi from East China Normal University (China), and Teresa Rocha-Santos from Universidade de Aveiro (Portugal) ranked second, each one with 17 articles. The total link strength (TLS) obtained from VOSviewer can effectively reveal the relationship between the number and frequency of co-authors. Different colors represent different clusters of author collaborations. The radiation intensity of the author clusters represents the collaboration between them, as shown in Figure 2B, the redder the color, the closer the collaboration between the authors. The results in Figure 2 show that several clusters of authors with close collaboration emerge, such as Teresa Rocha-Santos, Chelsea M. Rochman and Richard C. Thompson. International cooperation in

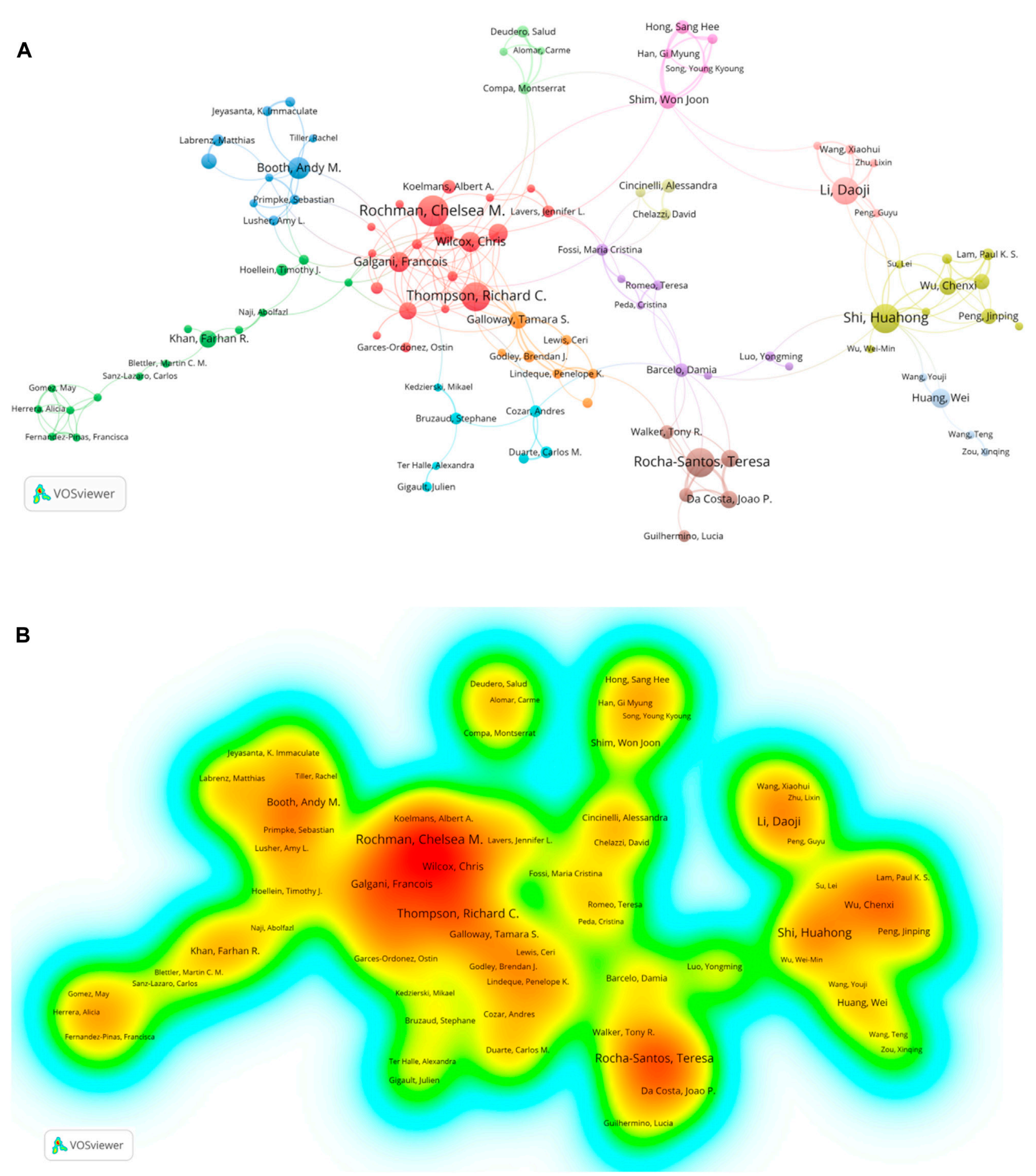

FIGURE 2
Author's cooperation network **(A)** and intensity of cooperation **(B)**.

this area is currently not strong enough, given the links between the different clusters.

3.3.2 The most productive and influential institutions

An analysis of organizational collaboration reveals information on the most influential and productive institutions. To further identify the leading institutions, Table 2 lists the top 10 institutions with the highest number of publications. Among these institutions, the top three are from China, while the fourth and fifth are from Portugal and the United Kingdom, respectively. Figure 3 shows the knowledge domain graph of the collaborating institutions using VOSviewer, where each node represents an institution and the size of the node indicates the number of

TABLE 2 Top 10 institutions with the most publications in the field of marine MP pollution control research.

Rank	Institution	Country	NP①	P②	TLS③	TC④	CPP⑤
1	Chinese Academy of Sciences	China	89	3.78	128	5,221	58.66
2	East China Normal University	China	43	1.82	52	3,256	75.72
3	University of Chinese Academy of Sciences	China	43	1.82	82	3,020	70.23
4	University of Aveiro	Portugal	36	1.53	23	2,916	81.00
5	University of Exeterlfremer	United Kingdom	34	1.44	66	4,102	120.65
6	University of Plymouth	United Kingdom	27	1.15	49	3,956	146.52
7	University of Porto	Portugal	26	1.10	32	1,284	49.38
8	Ocean University of China	China	25	1.06	20	562	22.48
9	Ministry Of Natural Resources	China	24	1.02	25	657	27.38
10	University of Cadiz	Spain	22	0.93	28	2,219	100.86

① Number of papers; ② Proportion of total publications %; ③ Total Link Strength; ④ Total Citation; ⑤ Citations per paper.

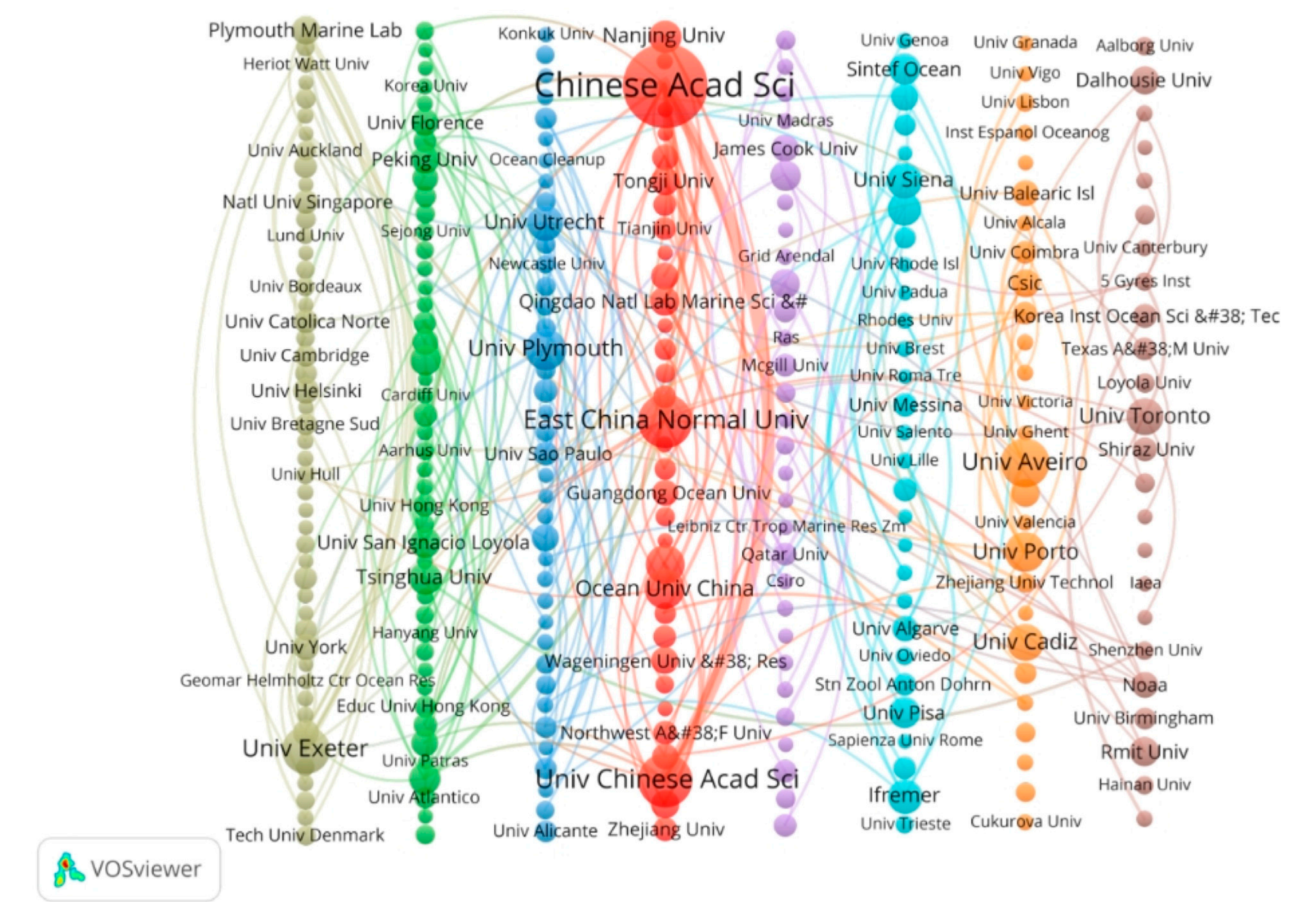

FIGURE 3
Mapping of the knowledge areas of the collaborative on marine MPs Pollution control.

published papers. The link between two nodes indicates the collaboration between institutions, and the larger the link width, the larger the link width means the closer the collaboration between institutions. Different colors represent different clusters of institutional collaboration. Chinese Academy of Science has the highest production of publications and the top-ranked TLS, indicating a broader collaboration and greater academic influence. In addition, the results in Figure 3 show that the two

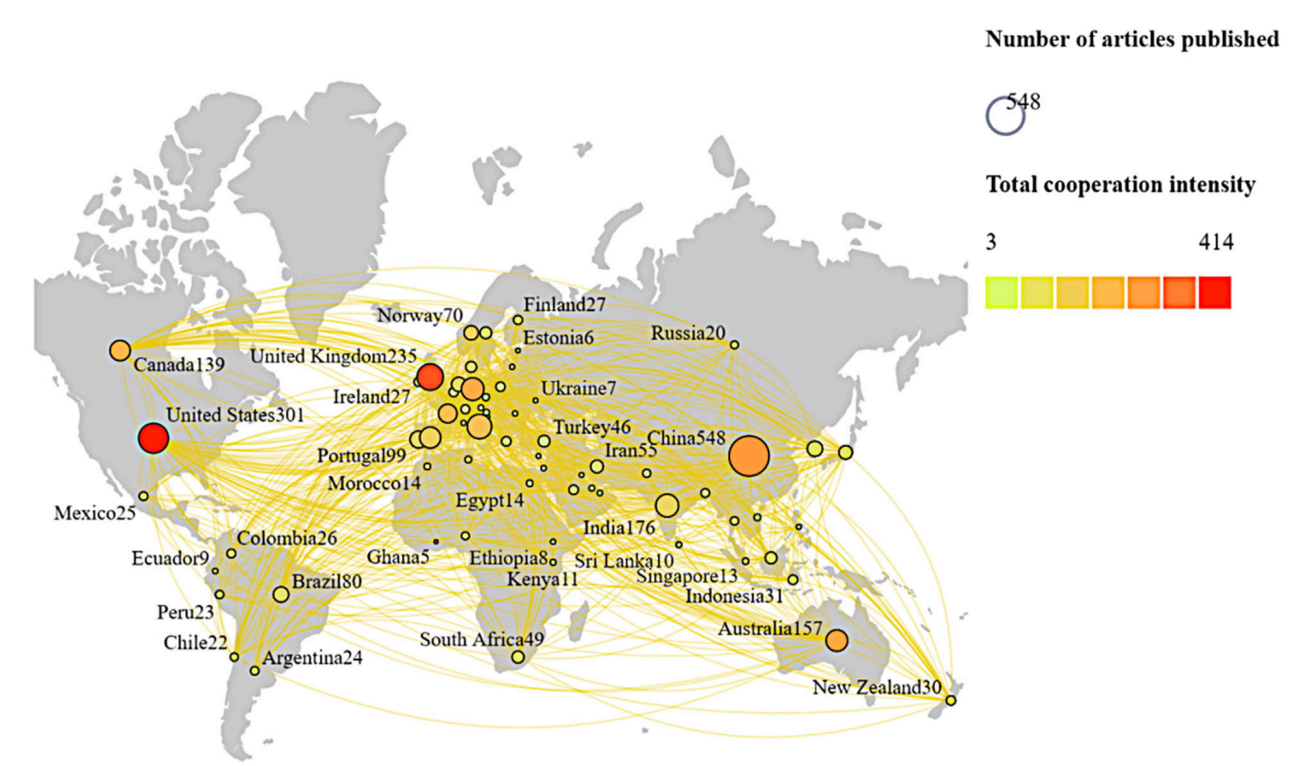

FIGURE 4
Country/region distribution of the literature.

institutions with the closest collaboration are the Chinese Academy of Sciences and the University of Chinese Academy of Sciences, and the second are the University of Exeter Fremer and Plymouth Marine Laboratory.

3.3.3 The most productive and influential countries/territories

In order to analyze the cooperation among the countries/regions involved in the study of this theme, the distribution of countries/regions was analyzed. As many as 116 countries/regions were involved in this theme study, and 66 countries/regions published more than five articles. The top five countries/regions were China, the United States, the United Kingdom, Italy, and India, with 548 (23.25%), 301 (12.77%), 235 (9.97%), 202 (8.57%), and 176 (7.47%) publications, respectively. The map of knowledge areas for co-authored countries/regions is shown in Figure 4. The nodes on the map represent different countries/regions, and their sizes represent the number of publications. The link between two nodes implies that they have a collaborative relationship and the denser the link line, the closer the collaboration between the two countries/regions. The United States, UK, China, Australia, Germany, Canada, France and Italy are the countries with closer cooperation with the other countries. China and the United States have the closest cooperation, followed by Australia-UK, and United States-Canada.

3.4 Co-citation analysis

The co-citation analysis is based on an understanding of the relationship between reference lists and co-citation aspects, and can be used effectively to disclose relationships between scholarly publications or works in various fields of study. (Osareh, 1996) Since scientific literature is generated by citing earlier scholarly work, citation networks provide evidence of the knowledge base of a knowledge domain. Journal co-citation and article co-citation analyses were used in this study because they help explore structure, dynamics, and paradigm development. (Liu et al., 2015).

3.4.1 Journal co-citation analysis

The knowledge domain of journal co-citation mapping in the field of marine MP pollution control research is shown in Figure 5. Two different journals are connected by connecting lines, indicating that two articles published in different journals are cited in the same article (later published). The denser the connecting lines, the higher the co-citation intensity of these two journals.

The blue color in Figure 5 is mainly focused on the engineering and technology area of environmental pollution and mitigation measures related to ecosystems and human health, represented by Environ. Sci. Technol., Environ. Pollut. and Sci. Total Environ. journals. In terms of co-citation intensity, they have a close co-citation relationship with other journals. The green cluster mainly covers journals related to materials chemistry science, including J. Hazard. Mater., *Angewandte Chemie-Internationa Edition* (Angew. Chem. Int. Edit.), and *Polym Degrad Stabil* (Polym. Degrad. Stab.). The purple cluster, led by *Chemosphere* focuses on the scientific knowledge of environmental chemistry and toxicology.

From Figure 5, Mar. Pollut. Bull. is the largest node among all journals, indicating that it is the most cited journal along with other

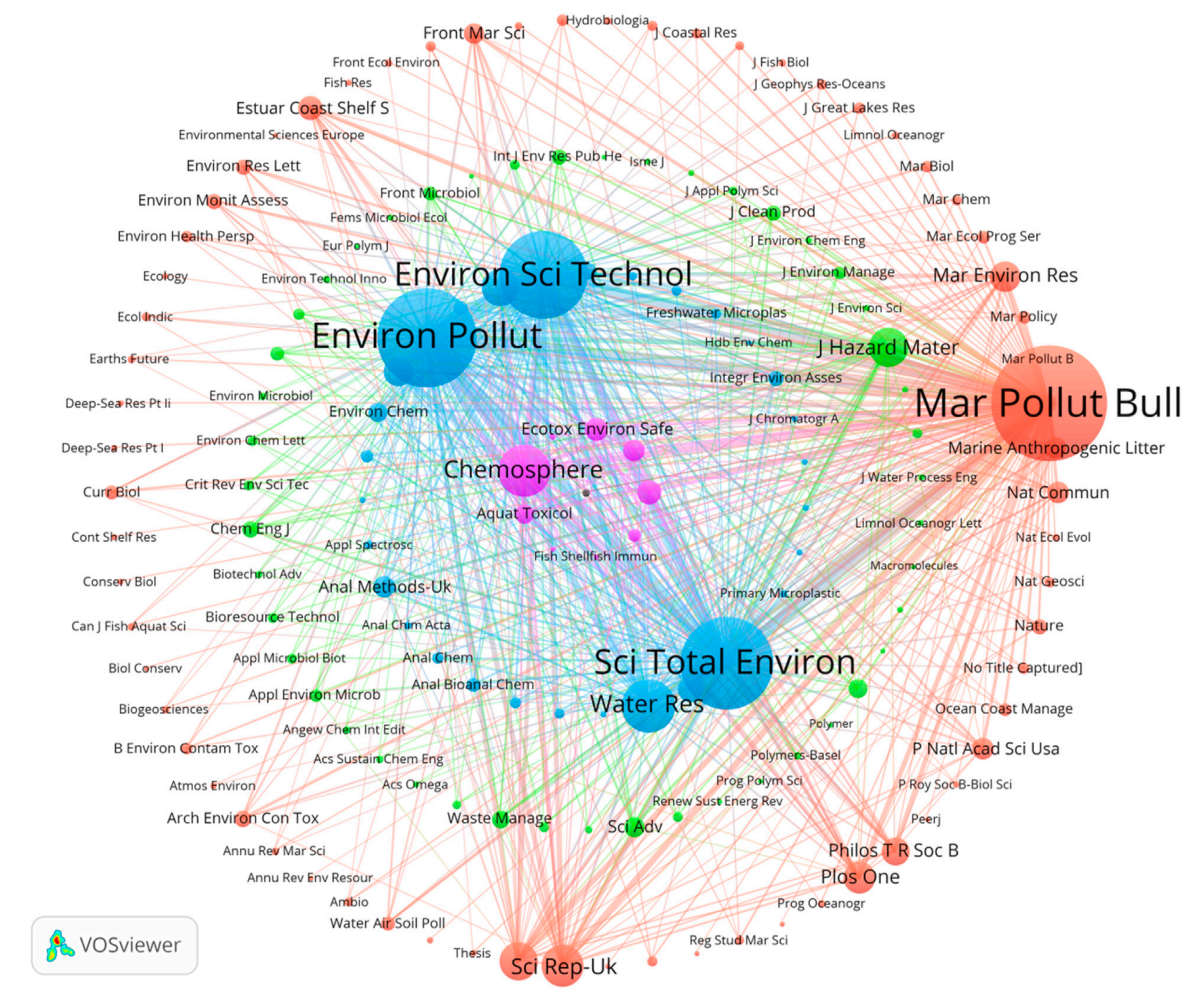

FIGURE 5
Graphical representation of journal co-citation knowledge domains in the field of marine MP pollution control.

journals. Mar. Pollut. Bull. has a higher influence in the MP marine pollution research, which is not only related to the influence of the journal but also to the number of articles published in the journal; Mar. Pollut. Bull. has only 10 publications (293) less than Sci. Total Environ., the one with the largest number of publications on the MP topic. In the blue cluster, Environ. Pollut., *Environmental Science & Technology* (Environ. Sci. Technol.) and Sci. Total Environ. were also widely cited, indicating that journals related to environmental governance research generally have a broader reach. In addition, the connecting lines between the four journals mentioned above are thicker than the other connecting lines, indicating a higher frequency of co-citations between these four journals.

In summary, the main research aspects of marine microplastic pollution control include environmental pollution engineering and technology, materials chemistry, environmental chemistry and toxicology. These research streams work together to provide the scientific basis and technical support for the comprehension, monitoring and control of marine microplastic pollution.

3.4.2 Literature co-citation analysis

Top 20 most influential papers in the field of marine MP pollution control research, in terms of co-citations are listed in Table 3. The top 20 highly cited papers, 16 of which were published after 2010, indicate that the field has evolved rapidly in the past decade or so, which is highly compatible with the scope of this paper (2013–2022). In terms of topics, four of the 20 papers are reviews of marine MP pollution, 10 are related to sources of marine MPs, three more are about the effects of marine MPs on marine organisms, and two are studies on the full life cycle of plastics and potential solutions to marine MP pollution. In addition, a paper on MP pollution in surface water of lakes is of interest.

The most cited paper was "Plastic waste inputs from land into the ocean" by Jenna R. Jambeck et al. (Jambeck et al., 2015) with 790 citations. This study estimated the mass of land-based plastic waste entering the ocean by linking data on global solid waste production, population density, and economic conditions. The size of the population and the quality of the waste control system largely determine which countries provide the most unrecovered waste that

TABLE 3 Top 20 most cited papers in the field of marine MP pollution control.

Rank	Title	Journal	Author	Year	Citations	If (2021)	References
1	Plastic waste inputs from land into the ocean	Science	Jenna R. Jambeck, et al.	2015	790	63.714	Jambeck et al. (2015)
2	Microplastics in the marine environment	Mar. Pollut. Bull.	Anthony L. Andrady	2011	786	7.001	Andrady (2011)
3	Microplastics as contaminants in the marine environment: a review	Mar. Pollut. Bull.	Cole M, et al.	2011	692	7.001	Cole et al. (2011)
4	Microplastics in the Marine Environment: A Review of the Methods Used for Identification and Quantification	Environ. Sci. Technol.	Valeria Hidalgo-Ruz, et al.	2012	606	11.357	Hidalgo-Ruz et al. (2012)
5	Accumulation of Microplastic on Shorelines Woldwide: Sources and Sinks	Environ. Sci. Technol.	Mark Anthony Browne, et al.	2011	600	11.357	Browne et al. (2011)
6	Lost at Sea: Where Is All the Plastic?	Science	Richard C. Thompson, et al.	2004	576	63.714	Thompson et al. (2004)
7	Accumulation and fragmentation of plastic debris in global environments	Mar. Pollut. Bull.	David K. A. Barnes, et al.	2009	543	7.001	Barnes et al. (2009)
8	Production, use, and fate of all plastics ever made	Science Adv.	ROLAND GEYER, et al.	2017	491	14.957	Geyer et al. (2017)
9	The physical impacts of microplastics on marine organisms: A review	Environ. Pollu.	Stephanie L. Wright, et al.	2013	487	9.998	Wright et al. (2013)
10	Plastic Pollution in the World's Oceans: More than 5 Trillion Plastic Pieces Weighing over 250,000 Tons Afloat at Sea	PLoS One	Marcus Eriksen, et al.	2014	445	3.752	Eriksen et al. (2014)
11	The Pollution of the Marine Environment by Plastic Debris: A Review	Mar. Pollut. Bull.	José G B Derraik	2002	380	7.001	Derraik (2002)
12	River plastic emissions to the world's oceans	Nat. Commun.	Lebreton, L. , et al.	2017	334	17.694	Lebreton et al. (2017)
13	Microplastics in freshwater systems: A review of the emerging threats, identification of knowledge gaps and prioritisation of research needs	Water Res.	DafneEerkes-Medrano, et al.	2015	324	13.400	Eerkes-Medrano et al. (2015)
14	Wastewater Treatment Works (WwTW) as a Source of Microplastics in the Aquatic Environmen	Environ. Sci. Technol.	Fionn Murphy, et al.	2016	315	11.357	Murphy et al. (2016)
15	Plastic debris in the open ocean	PNAS	Andrés Cózar, et al.	2014	311	12.779	Cozar et al. (2014)
16	Microplastic Ingestion by Zooplankton	Environ. Sci. Technol.	Matthew Cole, et al.	2013	304	11.357	Cole et al. (2013)
17	Transport and Release of Chemicals from Plastics to the Environment and to Wildlife	Philos. T. R. Soc. B	Teuten, E. L. , et al.	2009	295	6.671	Teuten et al. (2009)
18	Microplastic pollution in the surface waters of the Laurentian Great Lakes	Mar. Pollut. Bull.	Eriksen, M. , et al.	2013	288	7.001	Eriksen et al. (2013)
19	Distribution and importance of microplastics in the marine environment: A review of the sources, fate, effects, and potential solutions	Environ. Int.	H. S. Auta, et al.	2017	284	13.352	Auta et al. (2017)
20	Tesoro. Transport and fate of microplastic particles in wastewater treatment plants	Water Res.	Steve A. Carr, et al.	2016	273	13.400	Carr et al. (2016)

could become marine plastic litter. The proposed model in the article aims to calculate the order of magnitude of mismanaged plastic waste likely to flow into the global ocean based on the best available data at the time, in order to assess the factors that determine the largest sources of mismanaged plastic waste. To curb the growth of plastic pollutants, it is critical to reduce waste and improve "downstream" waste management strategies, such as improving waste management infrastructure, expanding recycling systems, and expanding producer responsibility.

"Microplastics in the marine environment" was the second most cited paper with 786 citations. This review discusses the mechanisms of generation and the potential impacts of MPs in the ocean environment. The main mechanism of MP production is related to plastic breakage and surface embrittlement due to weathering in the beach environment. MPs and nanoplastics readily absorb and concentrate persistent organic pollutants (POPs), and MP particles containing POPs can be ingested by marine plankton, which cause toxic transfer and accumulation through the biological food chain,

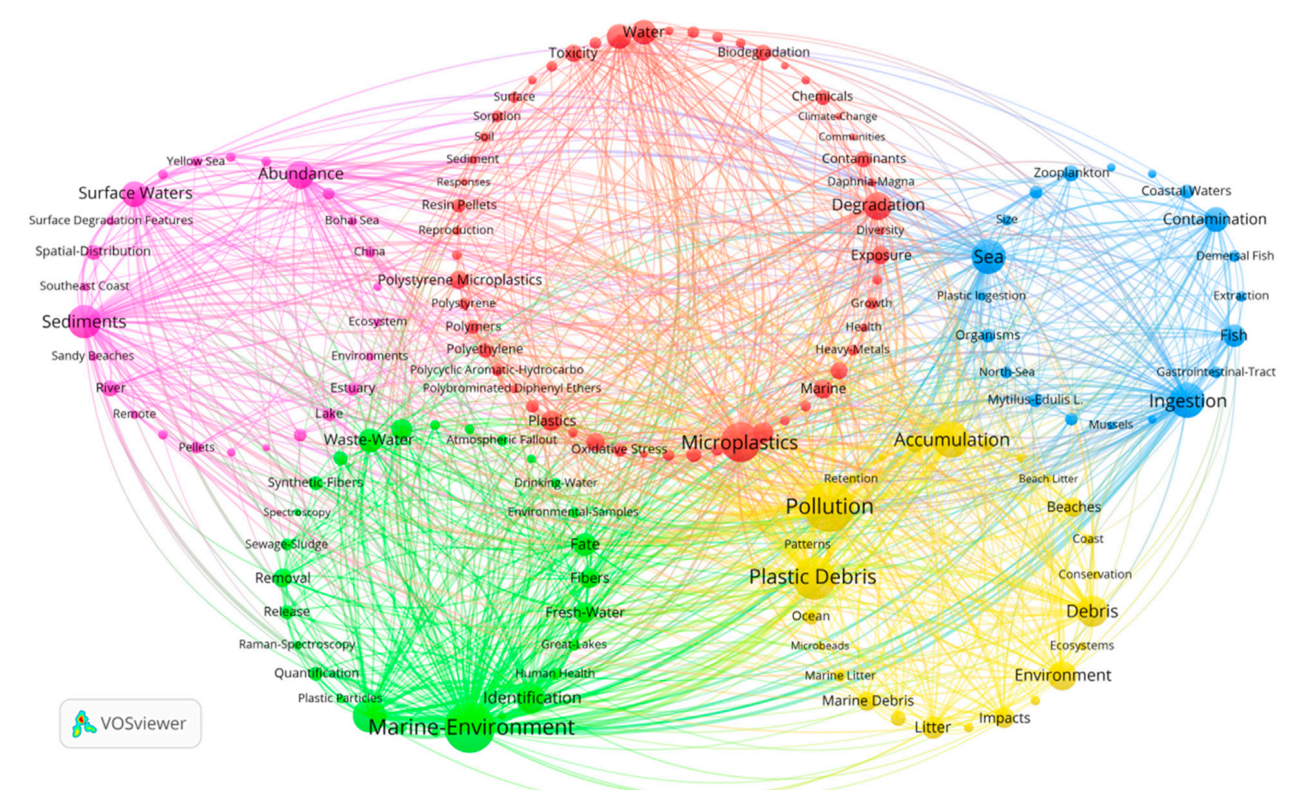

FIGURE 6
Keyword co-occurrence knowledge domain map in the field of marine MP pollution control.

ultimately having serious and far-reaching impacts on marine ecosystems. (Andrady, 2011). The third-ranked article is "Microplastics as contaminants in the marine environment: a review" (692 citations), which provides an overview of the nature, nomenclature, and sources of MPs; in addition to exploring the pathways by which MPs enter the marine environment, determining spatial and temporal trends in MP abundance, and identifying their environmental impacts. The article also briefly assesses methods for detecting MPs in the marine environment, which include 1) beach combing; 2) sediment sampling; 3) ocean trawling; 4) ocean observational measurements; and 5) biological sampling. (Cole et al., 2011) The fourth-ranked article "Microplastics in the Marine Environment: A Review of the Methods Used for Identification and Quantification" (606 citations) comparatively analyzed 68 research articles through five aspects: 1) research objectives, 2) sampling procedures, 3) laboratory processing of samples, 4) identification of microplastics, and 5) microplastic abundance. Three main sampling strategies were identified: selective sampling, volume reduction sampling, and batch sampling. Basic criteria and methods are recommended to ensure comparable quantitative estimates in the future, and this work contributes to the establishment of standardized sampling procedures that provide a more comprehensive understanding of the sources, sinks, and fluxes of MPs in the marine environment (Hidalgo-Ruz et al., 2012).

As can be seen in Table 3, marine MPs have attracted great attention in terms of sources and quantification approaches due to their specific environmental and pollution transmission capabilities. Among them, freshwater and municipal wastewater treatment plant discharges are marine sources, and MPs in freshwater systems, (Eerkes-Medrano et al., 2015) such as lakes (Eriksen et al., 2013), rivers (Lebreton et al., 2017) and wastewater treatment plants (Carr et al., 2016; Murphy et al., 2016), are also gradually attracting attention.

3.5 Keywords co-occurrence analysis

The purpose of keyword co-occurrence analysis is to study the core content and structure of a particular field and thus reveal the research frontiers in the field. As can be seen from the results in Figure 6, five clusters were obtained by the analysis software, where a node represents a keyword, the size of the node represents the frequency of that keyword, and the density of connecting lines between nodes represents the intensity of co-occurrence between keywords.

Cluster 1 (red): the most frequent keyword in the red cluster is "Microplastics," in Figure 6, which is connected to more than thirty percent of the nodes. Other major nodes in the red cluster are "Polystyrene Microplastics," "Polyethylene," "Toxicity," etc., An assessment of MP contamination around the island of Mauritius in the South West Indian Ocean (SWIO) region found that blue MP was the most common type of MP and polyethylene was the most common type of polymer. (Mattan-Moorgawa et al., 2021). The composition and pollutability of MPs and the harm caused to marine organisms by further formation of POPs through

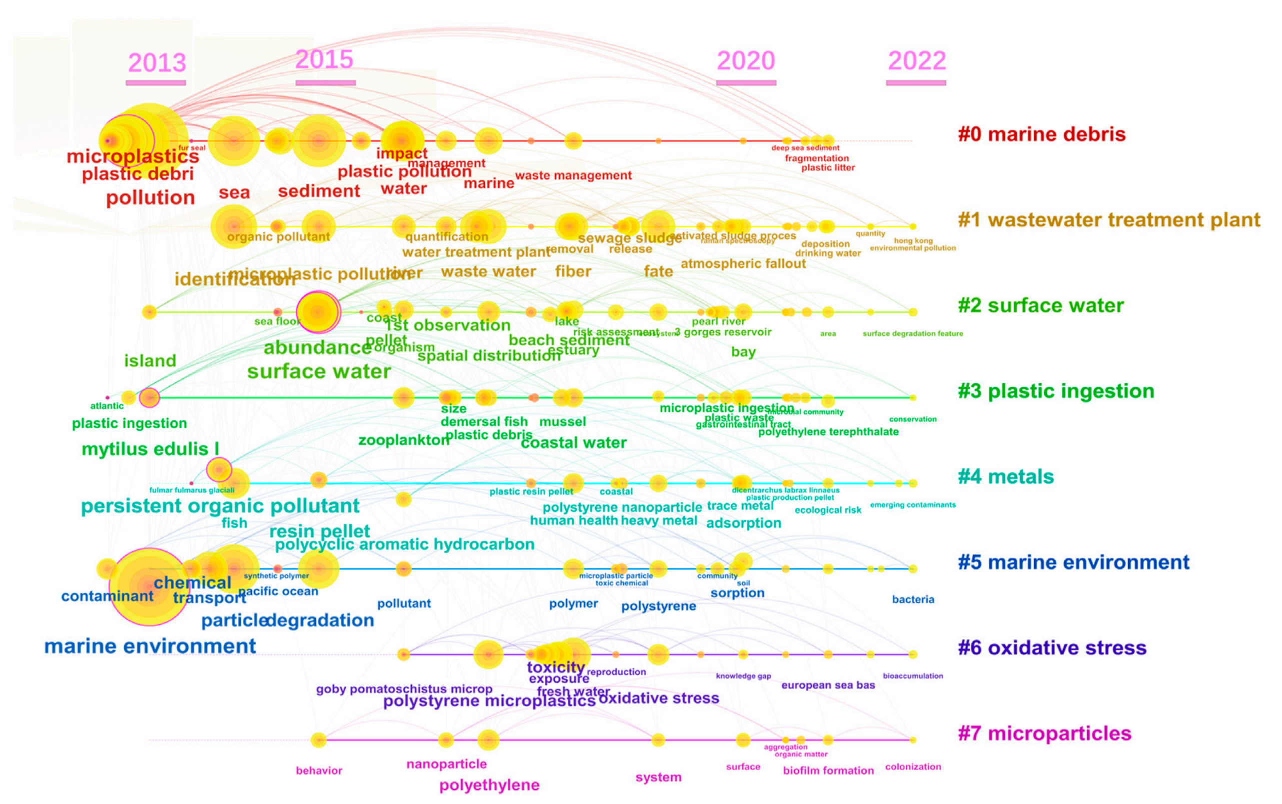

FIGURE 7
Timeline of keywords in the field of marine MP pollution control.

bioconcentration are the main studies. Ingestion of MPs by marine microorganisms can affect their movement, feeding, mating, and mechanical reception, which may limit their ability to detect prey, feed, reproduce, and evade predators. (Cole et al., 2013). Cluster 2 (yellow) has a good correlation between "Pollution", "Plastic Debris" and "Accumulation" among 153 keywords, which are also some of the largest nodes in the red cluster. This cluster concentrates on the study of plastic debris accumulation behavior on the seafloor, beaches, and bays. Cluster 3 (green) mainly includes "Marine-Environment," "Water Treatment Plants," "Waste-Water" and "Fibers," etc., A detailed understanding of the sources of marine MPs is an important prerequisite for mitigating marine MP pollution, and the discharge of sewage treatment plants in coastal cities is one of the important sources to seawater, and the MP content in discharged wastewater has an important impact on the marine environment. Not only wastewater treatment plant drainage but also freshwater systems in the mainland are important sources of seawater. (Jiajia et al., 2018). Cluster 4 (purple) includes "Sediments," "Abundance," and "Surface Waters." WANG et al. analyzed MPs in freshwater rivers, such as the Yellow River, Three Gorges Reservoir and Pearl River in China, to investigate the difference between the presence of MPs in freshwater, and the impact on the abundance of marine MPs. Improving the understanding of the sources and sinks of MPs in the marine environment through their pathways to the ocean is important for reducing MP pollution. (Browne et al., 2011). In addition, a regional understanding of the abundance, distribution, and composition of beach litter is fundamental for developing strategies to manage litter pollution in specific areas. (Nelms et al., 2020). The main nodes of cluster 5 (blue) are "Sea," "Ingestion," "Contamination," "Fish" and "Zooplankton." There is evidence that MPs can be ingested by fish, shellfish, etc., and absorbed and stored by their tissues and cells, leading to a negative impact on the health of marine organisms through bioconcentration, which in turn has a profound impact on marine ecology (Bowmer and Kershaw, 2013).

3.6 Research frontier identification

In Figure 7, the x-axis shows the publication years from 2013 to 2022 and the y-axis shows the clustering of different keywords. A node represents a keyword, i.e., a larger node represents a stronger keyword, while the links between keywords indicate co-occurrence relationships with each other. (van Eck and Waltman, 2010) The Citespace software analysis produced a total of eight clusters: "marine debris," "wastewater treatment plant," "surface water," "plastic ingestion," "metals," "marine environment," "oxidative stress," and "microparticles." The timeline view clearly reflected the evolution of keywords under each representative cluster in the marine MP research literature over this period. (Seltenrich, 2015).

From 2013, the keywords that appear on the timeline include "marine environment," "pollution," "plastic debris," etc., In 2014, the keywords include "sea," "identification," "water" and "fish." In

TABLE 4 Top 22 most cited keywords in the field of marine MP pollution control.

No.	Keywords	Strength	Begin	End	2013–2022
1	plastic debris	17.77	**2013**	2020	
2	*mytilus edulis*	17.76	**2013**	2018	
3	accumulation	17.73	**2013**	2017	
4	marine debris	9.51	**2013**	2017	
5	ocean	9.12	**2013**	2018	
6	environment	8.9	**2013**	2017	
7	ingestion	8.34	**2013**	2017	
8	marine debris	4.98	**2013**	2018	
9	beach	4.7	**2013**	2015	
10	island	4.69	**2013**	2017	
11	persistent organic pollutant	8.05	**2014**	2018	
12	chemical	4.23	**2014**	2019	
13	resin pellet	9.12	**2015**	2020	
14	Great Lake	6.76	**2015**	2018	
15	Pacific Ocean	4.87	**2015**	2018	
16	pollutant	8.11	**2016**	2020	
17	zooplankton	5.12	**2016**	2018	
19	north sea	8.9	**2017**	2018	
18	synthetic fiber	5.61	**2018**	2019	
20	toxic chemical	6.52	**2019**	2020	
21	Pearl River	4.49	**2020**	2022	
22	microplastic ingestion	4.27	**2020**	2022	

Bolded data in the "Begin"column highlights the start of the keyword outbreak, and bolded red lines indicate the time period in which the keyword was cited multiple times, corresponding to the "Begin" and "End" columns in the table.

2015, "degradation," "surface water" and "micro plastic pollution" appeared. In 2021, keywords involved "polyethylene degradation" and "plastic litter". In 2022, the keywords included "surface degradation feature," "conservation," "bacteria" and "bioaccumulation."

As the highlighted keywords may reflect a sudden spike in the number of citations for a keyword over a period of time, it is possible to use CiteSpace to obtain a keyword highlighting graph to study research trends in marine microplastic control over time. As shown in Table 4 below, "plastic debris" has been a research buzzword from 2013 to 2020; "*mytilus edulis*," "ingestion," and "accumulation" are buzzwords that accompanied research on marine microplastics from 2013 to 2018. "synthetic fibres" was a research buzzword from 2018 to 2019; "toxic chemical" is a research hotspot from 2019 to 2020; "Pearl River" and "microplastic ingestion" are research hotspots from 2020 to 2022, while "ingestion" was previously looked at from 2013 to 2017. The persistence of the terms "Pearl River" and "microplastic ingestion" up to the present indicates that they are the dominant trend in research on the control of marine MPs.

4 Conclusion

A bibliometric analysis of published papers related to marine MP pollution control in the WOS core database between 2013 and 2022 was conducted to obtain a knowledge map through information visualization analysis. So far, the characteristics of marine MP control research are as follows, with research in this area showing initial growth between 2015 and 2017 and rapid development after 2018. China has emerged as the most active country, with research spanning environmental science, marine science, engineering and materials. However, there is room for greater global collaboration, as research in some countries appears to be relatively independent. Key research hotspots include quantification, traceability, contaminability and control strategies for MP. In addition, surface water, municipal wastewater and freshwater with MP contamination were identified as important research topics. The oxidative stress response of marine organisms to MPs and the impact of surface waters are at the forefront of marine MP pollution control research, which is multi-faceted and focuses not only on marine pollution management but also on reducing MP production at source.

This study provides some guidance for the control of marine MP pollution. It identifies research hotspots and frontiers, allowing for more focused resource allocation and more effective problem-solving. It can facilitate international collaboration and knowledge sharing through partnerships between authors and countries. Collaboration facilitates the exchange of information, technology transfer and sharing of experiences, thereby enhancing global cooperation in the management of marine MP pollution. By analyzing the relevance and co-citation relationships of journals, influential research publishing platforms can be identified, increasing impact and visibility. It can also provide insights to guide the development of policy and management strategies for effective regulation.

Existing policies and measures focus on reducing plastic waste emissions and increasing plastic recycling rates, but this does not fully address the problem of marine litter. Future research needs to focus on policy implications and technological innovation. Scientific and technological innovation is needed to strengthen scientific research on marine MP pollution, to carry out ecological risk assessments of MP, to find efficient removal technologies, to overcome difficulties in switching to recyclable and degradable materials, etc., to develop and promote alternatives to plastics, and to change production and consumption patterns. Of course, the above is also relevant to guide the management of MP pollution in freshwater, which is becoming a popular research topic.

Author contributions

XL: investigation, data curation, formal analysis, writing-original draft. YS: data curation, investigation. CF: validation, data curation, supervision. YT: data curation. DH: investigation, data curation, resources. YG: funding acquisition, supervision. All authors contributed to the article and approved the submitted version.

Funding

This work was financially supported by the Central-Public interest Scientific Institution Basal Research Fund (2019T13, 2019T14). YG also appreciates Natural Science Foundation of China (52270129), Shanghai Natural Science Foundation (20ZR1421100) and Science and Technology Development Fund of Pudong New Area (PKJ2021-C01, PKJ2022-C07).

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Publisher's note

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frontiers | Frontiers in Environmental Science

TYPE Original Research
PUBLISHED 16 November 2023
DOI 10.3389/fenvs.2023.1296606

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OPEN ACCESS

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RECEIVED 18 September 2023
ACCEPTED 23 October 2023
PUBLISHED 16 November 2023

CITATION
Kadirvel G, Diengdoh J, Deori S, Dewry RK, Abedin SN and Moirangthem P (2023), Cytotoxic effects of heavy metals on functional attributes of boar sperm: an *in vitro* study.
Front. Environ. Sci. 11:1296606.
doi: 10.3389/fenvs.2023.1296606

Cytotoxic effects of heavy metals on functional attributes of boar sperm: an *in vitro* study

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Objective: Reproductive toxicology is a field that deals with the effects of heavy metals on various aspects of reproduction, including sperm count, motility, viability, spermatogenesis, follicular atresia, hormonal imbalance, and oocyte maturation, among others. The present study was carried out to examine the effects of heavy metals, viz., arsenic (As), lead (Pb), and fluoride (F), on boar sperm quality parameters *in vitro*.

Materials and Methods: Forty (40) ejaculates from six (6) boars, averaging eight ejaculates per boar, were collected with the gloved hand technique using a dummy sow. Six (6) different concentrations were selected for the *in vitro* study: 5, 10, 25, 50, 100, and 200 μM for As and Pb, and 5, 10, 25, 50, 100, and 200 mM for F. The ejaculates were co-incubated with heavy metals at these different concentrations and assessed after different incubation periods (0, 0.5, and 1 h) for sperm functional attributes, viz., sperm progressive motility, viability and membrane integrity, and sperm mitochondrial membrane potential (MMP). The combined effects of heavy metals on sperm functional attributes were also evaluated at different doses (5, 10, 25, 50, 100, and 200 μM/μM for As–Pb; 5, 10**,** 25**,** 50, 100, and 200 μM/mM for As–F; and 5, 10**,** 25, 50, 100, and 200 μM/mM for Pb–F).

Results: The present study revealed a highly significant (p <0.001) decrease in sperm progressive motility, viable sperm, membrane integrity, and sperm MMP in samples treated with heavy metals under different incubation periods; furthermore, the longer the incubation time, the greater the toxicity. There was also a significant (p <0.05) decrease in sperm motility, membrane integrity, and MMP in the samples treated with combined heavy metals (As–Pb, As–F, and Pb–F), as compared to the control, after different incubation periods. A significant (p <0.05) reduction in sperm quality attributes was recorded even at the lowest concentrations in the case of heavy metal combinations.

Conclusion: It can be concluded that As, Pb, and F are toxic to boar spermatozoa *in vitro*, causing reductions in sperm functional attributes in a dose- and time-dependent manner.

KEYWORDS

cytotoxicity, arsenic, lead, fluoride, sperm attributes, boar semen, heavy metals

1 Introduction

Heavy metals alter several reproductive functions in both males and females, causing infertility; the outcomes include a decrease in sperm count, motility, viability, and spermatogenesis; hormonal imbalance; follicular atresia; and delay in oocyte maturation, and these effects form an important aspect of reproductive toxicology. Heavy metals are regarded as one of the oldest environmental issues across the globe, and are toxic even at the micro level (Carvalho et al., 2011). The main sources of heavy metals in the environment are either natural or man-made, and these sources contribute to exposure to human and animal health hazards. Anthropogenic activities such as rapid industrialization, overcrowding, and environmental manipulation can release heavy metals into the environment, in turn infiltrating the food chain (Maartens et al., 2015). Exposure to heavy metals produces either acute or chronic poisoning cases; their accumulation in different organs of an individual has the potential to harm particular organ systems or possibly the entire organism (Kumar and Singh, 2015). Gastrointestinal and kidney dysfunction, vascular damage, birth defects, skin lesions, nervous system disorders, immune system dysfunction, and cancer are a few examples of such harm, and simultaneous exposure to two or more metals may have cumulative effects, each potentiating the toxicity of the other (Gazwi et al., 2020; Balali-Mood et al., 2021). The amounts of components that accumulate in body organs is determined by exposure interval, the amount swallowed, animals' production and reproduction periods, age and breed, the method of consumption, and the interplay between necessary and harmful elements, which determines the toxicity of the element in the animal's biological systems (Mendiola et al., 2011). During grazing or feeding, fodder contaminated with toxic metals or toxic compounds enters the animal's body through the respiratory and digestive systems or through dermal contact and affects physiological functions (Alam and Shilpa, 2020). Among the heavy metals, arsenic (As), lead (Pb), and fluoride (F) are among those that most significantly endanger human and animal health even at low levels of exposure. Arsenic is one of the most toxic substances that organisms can be exposed to through food and drinking water; it produces toxicity in livestock, humans, poultry, and aquatic animals due to its use in herbicides, rodenticides, and fungicides, and is particularly harmful to the male reproductive system (Knazicka et al., 2012; Shankar et al., 2014). It has been reported that protein responses are dysregulated in the male reproductive organs in poultry and animals as a result of arsenic toxicity, in turn lowering the sperm count per ejaculate, reducing sperm motility and viability, and causing abnormal sperm morphology (Zhao et al., 2017; Renu et al., 2018; Verma

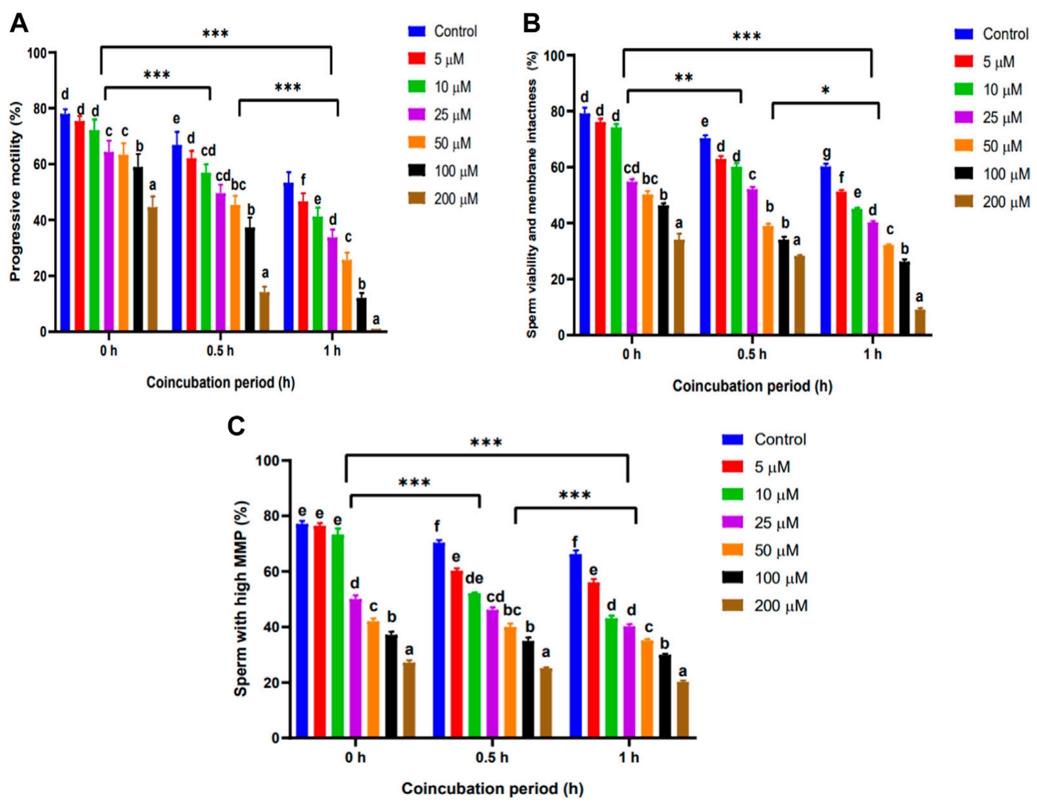

FIGURE 1
Effect of different concentrations of arsenic on sperm functional attributes after different co-incubation periods. **(A)** Effect of As on sperm progressive motility (%). **(B)** Effect of As on sperm viability and membrane integrity (%). **(C)** Effect of As on sperm MMP (%). Different superscripts indicate significant differences in means between the different treatment groups (p <0.05). * p <0.05; ** p <0.01; *** p <0.001 (indicating significant differences under different incubation periods). Abbreviations: As, arsenic; MMP, mitochondrial membrane potential.

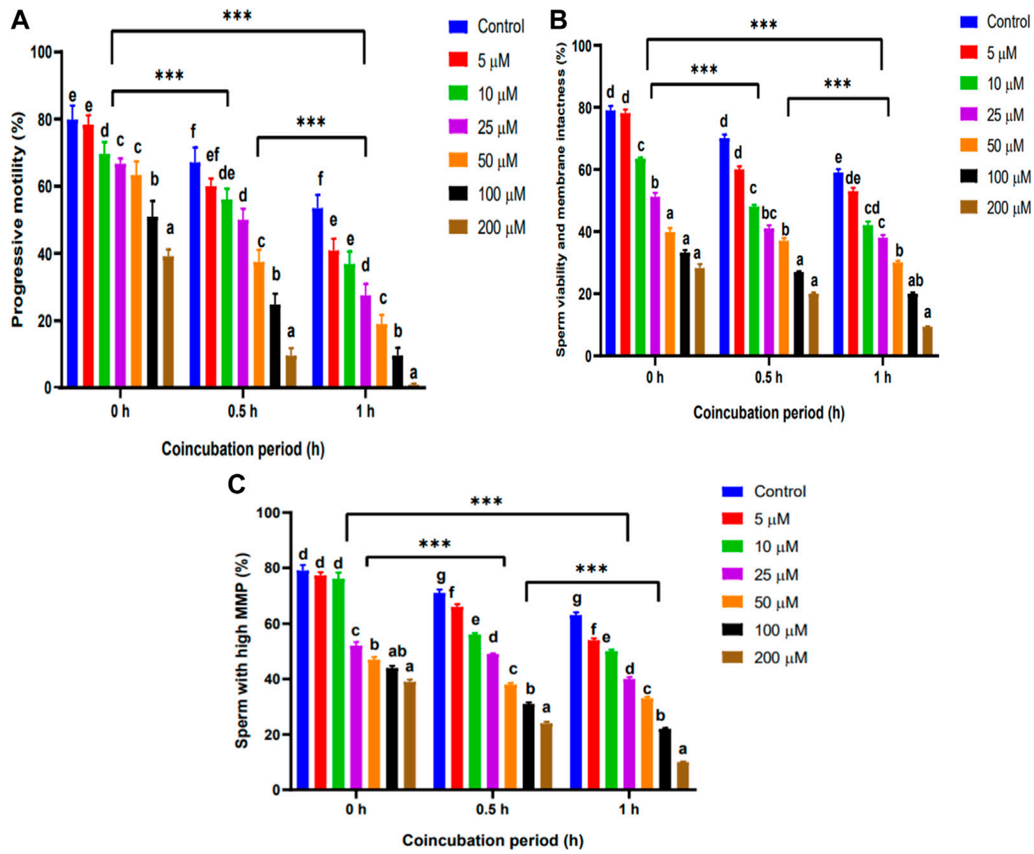

FIGURE 2
Effect of different concentrations of lead on sperm functional attributes after different co-incubation periods. **(A)** Effect of Pb on sperm progressive motility (%). **(B)** Effect of Pb on sperm viability and membrane integrity (%). **(C)** Effect of Pb on sperm MMP (%). Different superscripts indicate significant differences in means between the different treatment groups (p <0.05). ***p <0.001 (indicating significant differences under different incubation periods). Abbreviations: Pb, lead; MMP, mitochondrial membrane potential.

et al., 2018). Lead is widely used in acid battery plant refineries, smelters, the fuel combustion industry, printing presses, and automobile exhausts, where tetraethyl Pb acts as an anti-knocking agent; this results in exposure of humans and animals to lead, and its toxicity in the male reproductive system is manifested by Pb deposition in the testes, epididymis, vas deferens, seminal vesicles, and seminal ejaculate, leading to negative effects on sperm count and motility (Chowdhury, 2009); a decrease in sperm quality is also seen in mice (Li et al., 2018). Finally, the third heavy metal, fluoride, usually exists widely in the environment as an inorganic or organic compound; due to its greater reactivity toward the reproductive system, it causes decreased sperm count and abnormal sperm ratios, disrupted spermatogenesis, and a significant decrease in testosterone levels (Nickson et al., 2005). The toxicity of heavy metals has been reported in laboratory animals, but no studies have documented the *in vitro* effect of heavy metals on boar spermatozoa, either separately or in combination. Therefore, the present study was designed to investigate the *in vitro* toxic effects of As, Pb, and F separately and in combination, at different concentrations and with different incubation periods, on sperm functional attributes in a boar model.

2 Materials and methods

2.1 Chemicals and reagents

Standard solutions of arsenic (CAS No. 119773; H_3AsO_4 in HNO_3 0.5 mol/L; 1,000 mg/l As Centipur®) and lead (CAS No. 119773; $Pb(NO_3)_2$ in HNO_3 0.5 mol/L; 1,000 mg/l Pb Centipur®), stabilized in 0.1 mM PBS (phosphate-buffered saline), were purchased from Sigma-Aldrich (St. Louis, MO, United States), and fluoride (CAS No. 7681-49-4) was purchased from HiMedia™ (Thane West, Maharashtra, Pin-400 604, India). The stock solutions of the heavy metals were diluted with distilled water to prepare the working solutions for the experiment with the desired concentration, as per the formula M1V1 = M2V2. Sperm Tyrode's albumin lactate pyruvate (TALP-100 mM NaCl, 3.1 mM KCl, 25 mM $NaHCO_3$, 0.29 mM NaH_2PO_4, 21.6 mM $C_3H_5NaO_3$, 2.0 mM $CaCl_2$, 1.5 mM $MgCl_2$, and 10 mM HEPES) medium was prepared using chemical components purchased from HiMedia™.

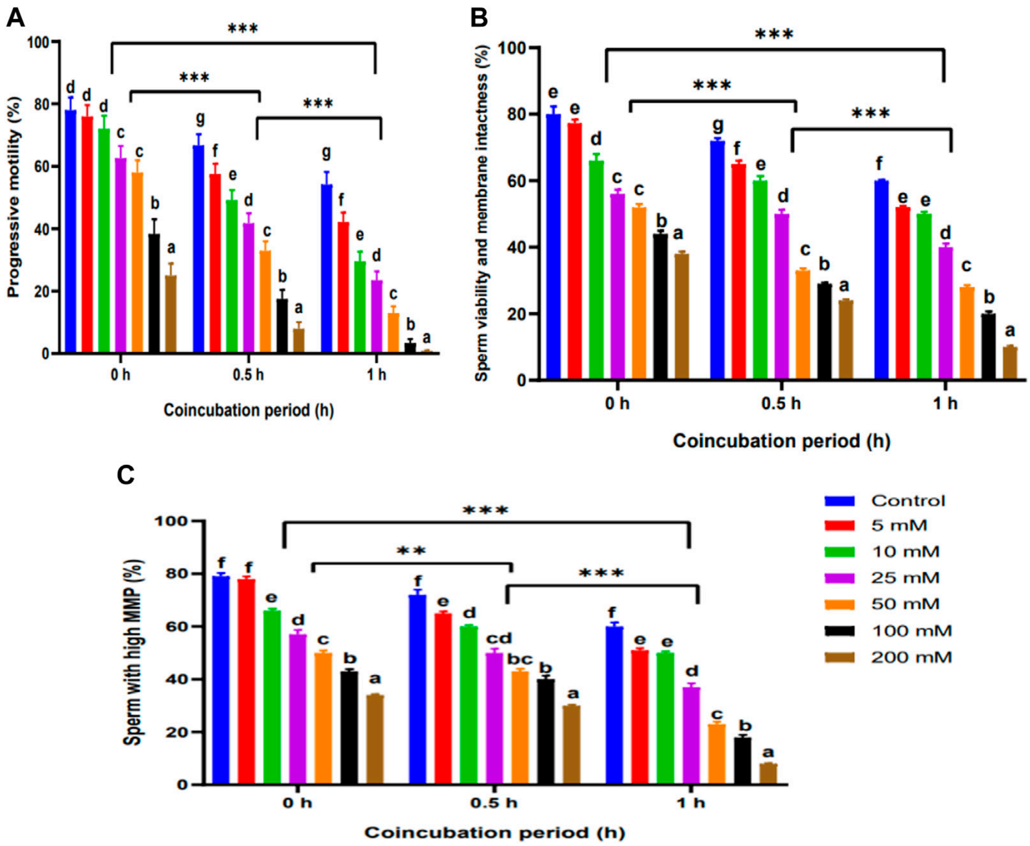

FIGURE 3
Effect of different concentrations of fluoride on sperm functional attributes after different co-incubation periods. **(A)** Effect of F on sperm progressive motility (%). **(B)** Effect of F on sperm viability and membrane integrity (%). **(C)** Effect of F on sperm MMP (%). Different superscripts indicate significant differences in means between the different treatment groups (p <0.05). **p <0.01; ***p <0.001 (indicating significant differences under different incubation periods). Abbreviations: F, fluoride; MMP, mitochondrial membrane potential.

All other chemicals and reagents were procured from Sigma-Aldrich, unless otherwise stated.

2.2 Semen collection and evaluation

Six superior quality Hampshire crossbred boars (75%) with sexually proven fertility were used for the experiment. A semen sample was collected twice weekly from each boar using the gloved hand technique with a dummy sow. Following the collection of semen, ejaculates were transferred into a Minitube container pre-heated to 37°C in an incubator and transported to the laboratory within 30 min. A total of 40 ejaculates were collected and analyzed immediately for progressive motility. Semen samples with 70% progressive motility or higher were used for further processing and assessment of sperm functional attributes.

2.3 Sperm co-incubation with arsenic, lead, and fluoride

In the first experiment, the ejaculated semen sample was aliquoted in a 1.5-mL Eppendorf tube with different concentrations of As (5, 10, 25, 50, 100, and 200 μM), Pb (5, 10, 25, 50, 100, and 200 μM), and F (5, 10, 25, 50, 100, and 200 mM) and co-incubated for 0, 0.5, or 1 h in a CO_2 incubator (Thermo Fisher Scientific, United States) with 5% CO_2 and 95% humidity at 37°C. A semen sample without the addition of heavy metals was incubated as a control. The *in vitro* characteristics of sperm (viz., progressive motility, viability and membrane integrity, and MMP) from samples incubated in different concentrations of heavy metals were assessed after each incubation period. Similarly, in the second experiment, semen samples were co-incubated with different combined concentrations of heavy metals, viz., As–Pb (20, 50, 100, and 200 μM/μM), As–F (20, 50, 100, and 200 μM/mM), and Pb–F (20, 50, 100, and 200 μM/mM), and evaluated for sperm functional attributes, namely, progressive motility, viability and membrane integrity, and MMP.

2.4 Assessment of sperm functional attributes

Sperm progressive motility was evaluated by placing 10 μL of sperm suspension on a glass slide and then covering it with a coverslip. Sperm motility was observed under a phase-contrast

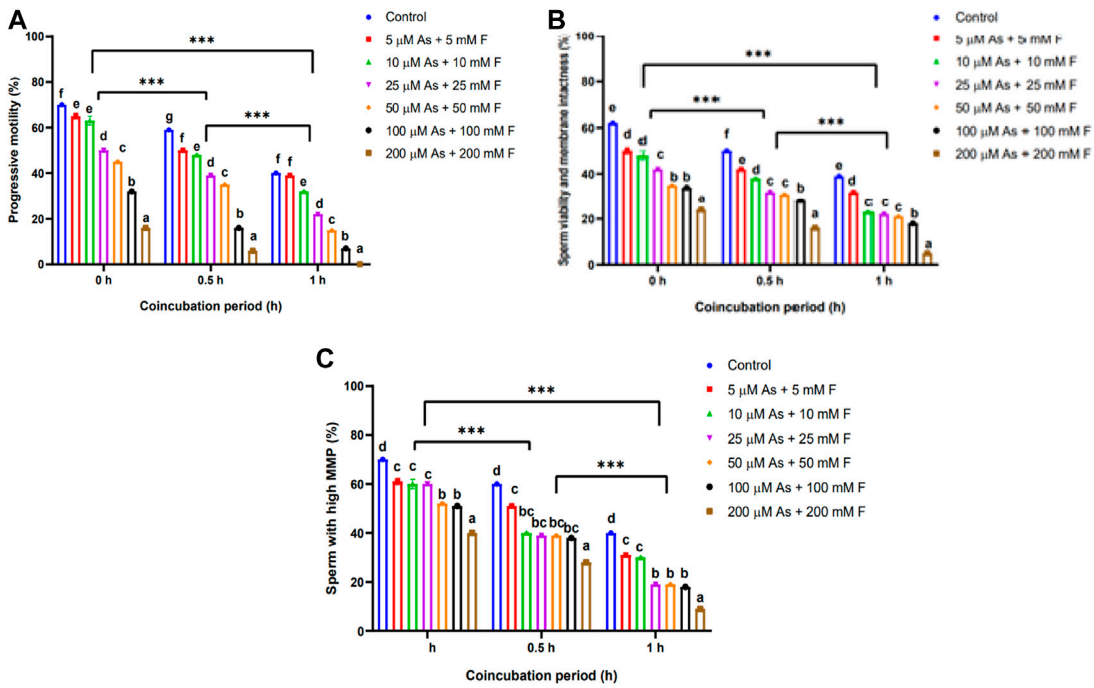

FIGURE 4
Effect of arsenic and fluoride in combination at different concentrations on sperm functional attributes after different co-incubation periods. **(A)** Combined effect of As–F on sperm progressive motility (%). **(B)** Combined effect of As–F on sperm viability and membrane integrity (%). **(C)** Combined effect of As–F on sperm MMP (%). Different superscripts indicate significant differences in means between the different treatment groups (p <0.05). ***p <0.001 (indicating significant differences under different incubation periods). Abbreviations: As, arsenic; F, fluoride; MMP, mitochondrial membrane potential.

microscope equipped with a 37°C microscope stage warmer at 400× magnification (Olympus, BX51 FT, Japan). The percentage of spermatozoa with normal, vigorous, and forward linear motion was subjectively assessed to the nearest 5% in different areas of the sample on each slide.

Sperm membrane integrity was assessed using carboxyfluorescein diacetate succinimidyl ester (CFDA) fluorescent dye as per the method described by Kukov et al. (2009), with minor modifications. Briefly, stock solutions of CFDA and propidium iodide (PI) were prepared at a concentration of 1 mg/mL in DMSO (dimethyl sulfoxide) and 1 mg/mL in PBS, respectively. The stock CFDA was added to 250-μL semen aliquots containing different concentrations of As, Pb, and F at a final concentration of 20 μM in an Eppendorf tube and incubated at 37°C for 5 min. Subsequently, 5 μL of PI was added at a final concentration of 15 μM and the solution was incubated for 5 min. After incubation, the tubes were centrifuged at 800 rpm for 5 min, and the supernatant was removed. Then, 200 μL of PBS was added to the sperm pellet in each Eppendorf tube and mixed gently. A drop of 10–15 μL from the stained sperm suspension was placed on a clean, dry glass slide covered with a coverslip, and 200 spermatozoa were observed under a fluorescent microscope (Nikon, Eclipse 80i, Japan). Sperm cells were classified as having an intact plasma membrane (stained with green fluorescence), having a damaged plasma membrane (stained with red fluorescence), or morbid spermatozoa (stained with both green and red fluorescence).

For the assessment of mitochondrial membrane potential, a stock solution of 1.53 mM JC-1 (5,50,6,60-tetrachloro-1,10,3,30-tetraethylbenzimidazolyl carbocyanine iodide) stain was prepared in DMSO. Next, 250 μL of each of the semen aliquots containing different concentrations of As, Pb, and F was stained with 1 μL of the JC-1 stock solution (final concentration of 2 μM) for 30 min at 37°C. Counterstaining of nuclei DNA was performed with 5 μL of the PI stock solution (final concentration of 0.27 mg/mL) and solutions were then incubated for 5 min. At least 200 spermatozoa were counted under a fluorescent microscope at 400× magnification; spermatozoa were observed using FITC and TRITC filters, and the images obtained from the two filters were merged to obtain the final image. When MMP levels are high, the mitochondrial protein JC-1 forms J-aggregates and exhibits orange/red fluorescence, while at a low MMP, it remains in the monomer form and emits green fluorescence (Selvaraju et al., 2008).

2.5 Statistical analysis

All the data obtained were analyzed using SPSS software (version 16.0 for Windows; SPSS Inc., Chicago, IL, United States). Data on various parameters are expressed in the form mean ± SE and analyzed via multivariate analysis of variance (ANOVA), with the specific sperm treatment as the main variable. Post-hoc testing for significant differences was carried out using Tukey's test; p values <0.05 were considered to represent significance.

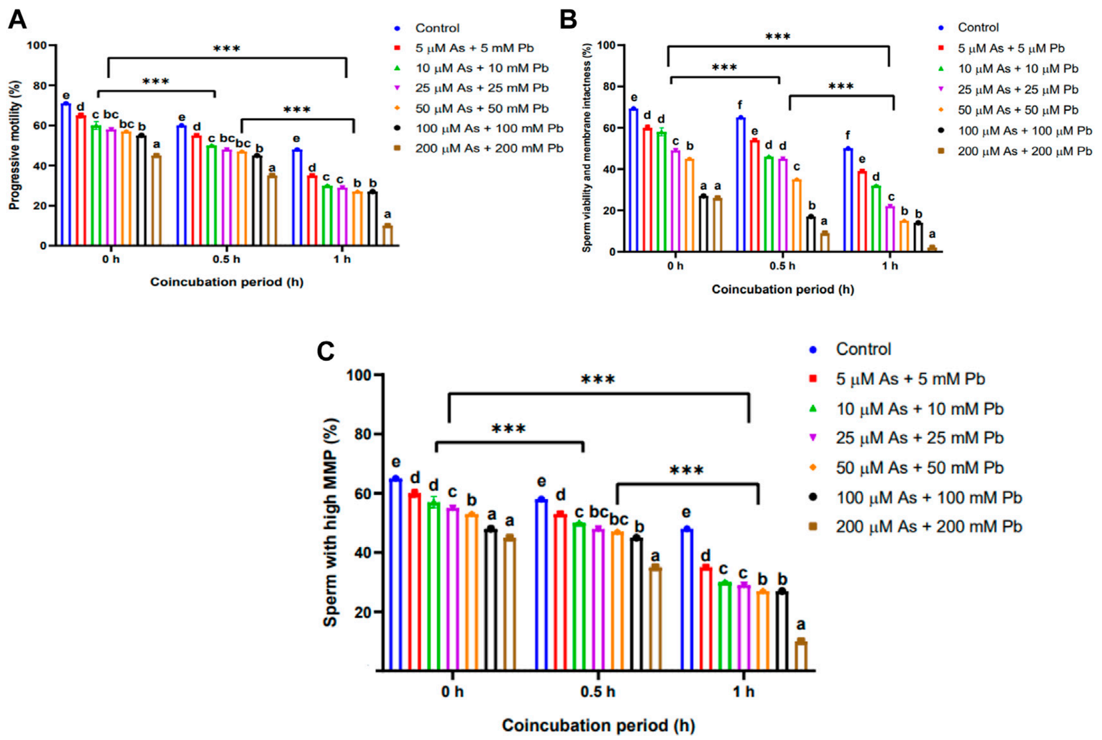

FIGURE 5
Effect of arsenic and lead in combination at different concentrations on sperm functional attributes after different co-incubation periods. **(A)** Combined effect of As–Pb on sperm progressive motility (%). **(B)** Combined effect of As–Pb on sperm viability and membrane integrity (%). **(C)** Combined effect of As–Pb on sperm MMP (%). Different superscripts indicate significant differences in means between the different treatment groups ($p < 0.05$). ***$p < 0.001$ (indicating significant differences under different incubation periods). Abbreviations: As, arsenic; Pb, lead; MMP, mitochondrial membrane potential.

3 Results

3.1 Effect of heavy metals on sperm functional attributes

After 0 h of incubation, a significant reduction ($p < 0.05$) in sperm motility was recorded at 25 µM As concentration in comparison to the control (Figure 1A). After 0.5 h of incubation, sperm motility at a lower As concentration (5 µM) did not differ significantly ($p > 0.05$) from that of the control. However, a highly significant ($p < 0.05$) decrease was recorded when the concentration was increased to 10 µM, signifying that a lower As concentration became toxic with increasing incubation time. After 1 h of incubation, a significant ($p < 0.05$) reduction in sperm motility in comparison to the control was recorded as the concentration was increased beyond 10 µM. Furthermore, when sperm motility was compared between different incubation periods, a significant decrease was observed ($p < 0.001$) after 0.5 and 1 h of incubation in comparison to 0 h for different As concentrations. Regarding sperm viability, after 0 h of incubation, no significant reduction ($p > 0.05$) was recorded at a 10-µM As concentration in comparison to the control (Figure 1B). However, a highly significant ($p < 0.05$) decrease in sperm viability and membrane integrity was observed with an increase in As concentration to 25 µM and above. After 0.5 h of incubation, even at a lower As concentration (10 µM), a significant ($p < 0.05$) reduction in sperm viability was recorded in comparison to the control; and after 1 h of incubation, a significant ($p < 0.05$) reduction was recorded for a 5-µM As concentration in comparison to the control. In comparisons between the incubation periods, sperm viability and membrane integrity were found to be reduced significantly ($p < 0.01$) between 0 and 0.5 h, and a significant reduction ($p < 0.05$) was also recorded between 0.5 and 1 h. Regarding sperm MMP, after 0 h of incubation, the number of sperm with high MMP was not significantly reduced ($p > 0.05$) at 10 µM (Figure 1C), but a significant reduction ($p < 0.05$) was seen at concentrations from 25 µM. Similar trends were observed after 0.5 and 1 h of incubation, whereby the 5 µM As concentration resulted in significantly ($p < 0.05$) lower numbers of spermatozoa exhibiting high MMP, while in comparisons between the incubation periods, sperm MMP showed a significantly ($p < 0.001$) declining trend with increased time of incubation.

After 0 h of incubation, no significant ($p > 0.05$) decline in sperm motility was recorded at 5 µM Pb concentration in comparison to the control (Figure 2A). However, as the concentration was increased beyond 25 µM, a significant ($p < 0.05$) reduction was observed. After 0.5 h of incubation, even 5 µM Pb concentration caused a significant ($p < 0.05$) reduction in sperm motility. Similarly, after a longer incubation period (1 h), a significant ($p < 0.05$) reduction in sperm motility was recorded even at 5 µM Pb concentration. Furthermore, in comparisons between the different incubation periods, it was observed that sperm motility significantly ($p < 0.001$) decreased at 0.5 and 1 h of incubation

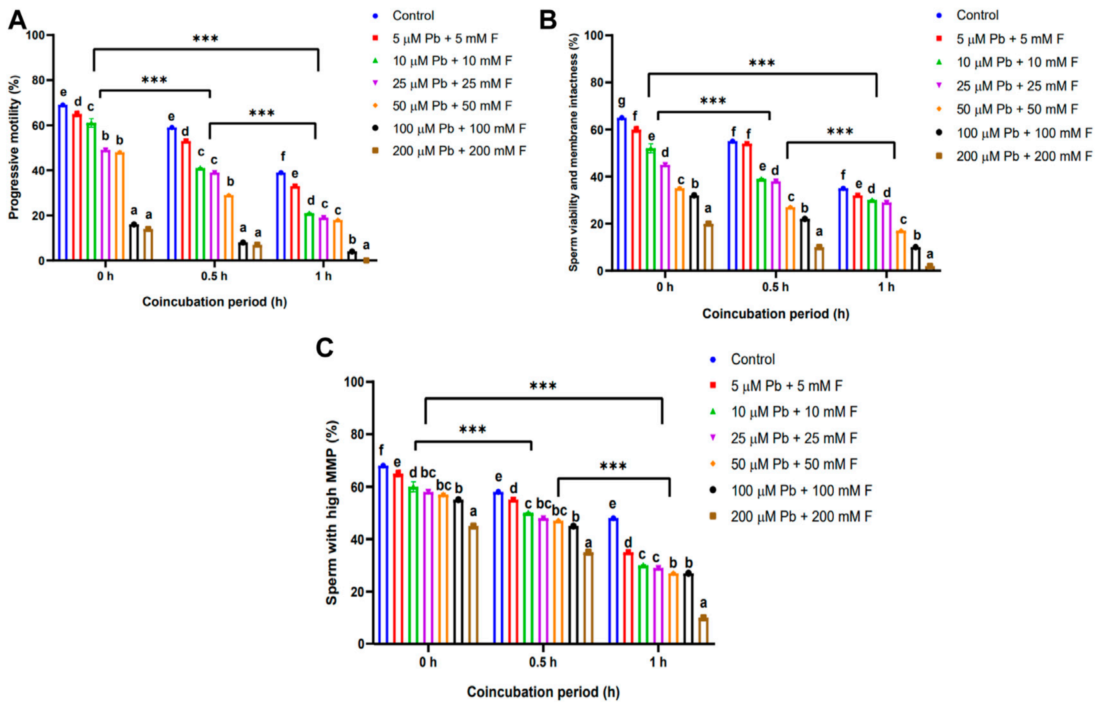

FIGURE 6
Effect of lead and fluoride in combination at different concentrations on sperm functional attributes after different co-incubation periods. **(A)** Combined effect of Pb–F on sperm progressive motility (%). **(B)** Combined effect of Pb–F on sperm viability and membrane integrity (%). **(C)** Combined effect of Pb–F on sperm MMP (%). Different superscripts indicate significant differences in means between the different treatment groups (p <0.05). ***p <0.001 (indicating significant differences under different incubation periods). Abbreviations: Pb, lead; F, fluoride; MMP, mitochondrial membrane potential.

relative to motility at 0 h for different Pb concentrations. Regarding sperm viability and membrane integrity, no significant (p >0.05) differences in comparison to the control were recorded at 5 μM Pb concentration after 0, 0.5, or 1 h of incubation (Figure 2B). However, there was a significant reduction (p <0.05) after the different incubation periods as the concentration was increased beyond 10 μM Pb. Furthermore, in comparisons between the different incubation periods, it was observed that sperm viability and membrane integrity significantly (p <0.001) decreased after 0.5 and 1 h of incubation relative to measures at 0 h for different Pb concentrations. Regarding sperm MMP, no significant (p >0.05) reduction was seen at 10 μM Pb concentration in comparison to the control at 0 h of incubation (Figure 2C). However, sperm MMP significantly (p <0.05) declined when the concentration was increased to 25 μM and beyond. Similarly, a significantly (p <0.05) declining trend was observed at 10 μM Pb concentration after 0.5 and 1 h of incubation. Furthermore, in comparisons between different incubation periods, it was observed that sperm MMP significantly (p <0.001) decreased at 0.5 and 1 h of incubation relative to MMP at 0 h for different Pb concentrations.

We observed a significant (p <0.05) decline in sperm motility at 10 mM F concentration in comparison to the control after 0 h of incubation (Figure 3A). A similar trend was seen after 0.5 and 1 h of incubation for a lower concentration of F (5 mM). Furthermore, in comparisons between different incubation periods, it was observed that sperm motility significantly (p <0.001) decreased after 0.5 and 1 h of incubation in comparison to motility at 0 h for different F concentrations. Regarding sperm viability and membrane integrity, after 0 h of incubation, a significant (p <0.05) reduction was seen higher concentrations (Figure 3B). After 0.5 h of incubation, a significant (p <0.01) decline was recorded even at lower doses of F (5 mM concentration and beyond). Furthermore, in comparisons between different incubation periods, it was observed that sperm viability significantly (p <0.001) decreased after 0.5 and 1 h of incubation. Regarding sperm MMP, no significant reduction was observed (p >0.05) at 5 mM F concentration after 0 h (Figure 3C). However, as the incubation period was increased to 0.5 h, even 5 mM F caused a significant reduction in sperm MMP.

3.2 Effects of combined heavy metals on sperm functional attributes

In the experiment examining the As–F combination, even at 0 and 0.5 h of incubation, sperm motility significantly reduced at 5 μM As + 5 mM F in comparison to the control. (Figure 4A). A similar trend was observed for sperm viability (Figure 4B) and sperm MMP (Figure 4C), whereby 5 μM As + 5 mM F caused a significant (p <0.05) reduction in these parameters in comparison to the control. Similarly, in the case of the As–Pb combination, at 0 h, with the lower concentration of 5 μM As + 5 mM Pb, a significant (p < 0.05) reduction in sperm motility was observed (Figure 5A). Similar results were obtained regarding sperm viability (Figure 5B) and sperm MMP (Figure 5C), with a significant (p <0.05) decrease observed after each incubation period when sperm was incubated with a combination of 5 µM As + 5 mM Pb. Overall, a highly significant (p <0.001) decrease in sperm MMP was recorded if sperm was incubated for longer. Similarly, in the case of the Pb + F combination, we observed a significant (p <0.05) decrease in sperm motility even at 5 µM Pb + 5 mM F in comparison to the control after each incubation period (Figure 6A). Furthermore, a highly significant (p <0.001) reduction in sperm motility was recorded after longer periods of incubation. We also observed a significant (p <0.05) decrease in sperm viability after 0 and 1 h of incubation if sperm was incubated with 5 µM Pb + 5 mM F, although this was not the case after 0.5 h (Figure 6B). As the concentration was increased, a significant (p <0.05) decrease in the parameters was observed for all incubation periods (0, 0.5, and 1 h). Similarly, sperm MMP also showed a significantly (p <0.05) declining trend even at the lowest concentration of this heavy metal combination (5 µM Pb + 5 mM F) in comparison to the control (Figure 6C).

4 Discussion

Among all toxic pollutants, humans and animals have been increasingly exposed in particular to heavy metals (particularly As, Pb, and F) due to their industrial uses, biomedical applications, and use in other diagnostic tools in recent years. Toxic metals may have adverse effects on the reproductive system, either directly if they target specific reproductive organs or indirectly when they act on the neuroendocrine system (Pandey and Jain, 2013). The current study focused on the cytotoxic effects of different concentrations of As, Pb, and F, and combinations of these, on sperm functional attributes *in vitro*. The sperm functional attributes analyzed were observed to deteriorate in a dose- and time-dependent manner under incubation with different concentrations of As, Pb, and F, separately and in combination.

Sperm motility is crucial for male fertility, and lower sperm motility can cause male infertility, as reported in Han Chinese men (Tang et al., 2017). Many recent *in vitro* studies have shown that As exposure can reduce sperm motility and male fertility (Lima et al., 2018; Zeng et al., 2018). In this study, sperm motility was evidently reduced in a dose-dependent manner when sperm were incubated in different concentrations of As, a finding that is in agreement with previous reports in different laboratory animals (Pant et al., 2004; Zeng et al., 2019). A possible mechanism underlying the decrease in sperm motility might be associated with direct binding of As to sperm (Uckun et al., 2002). A recent study has reported on the partial impairment of spermatogenesis through disorganization of the elongation of spermatids due to As toxicity (Han et al., 2020).

Our findings on sperm membrane integrity corroborated those of earlier reports, whereby exposure to As has been found to cause reduction in sperm membrane integrity in mice (Reddy et al. (2011) and rabbit bucks (Seadawy et al., 2014). Similarly, another study documented a significant reduction in sperm viability when sodium arsenite was fed orally to experimental rats (Momeni and Eskandari, 2012). Exposure to As may induce cell death or apoptosis in the testicular germ cells or somatic Sertoli cells *in vitro* (Kim et al., 2011). Therefore, it might be possible that apoptosis-related cellular events were more prominent when sperm cells were co-incubated with increased concentrations of As. In this regard, it has also been reported that exposure of cells to As generates reactive oxygen species (ROS), in turn inducing cell death via both caspase-dependent and caspase-independent pathways; this is concomitant with downregulation of p53 and arrest of the cell cycle (Kim et al., 2011). Mitochondria play a crucial role in ATP synthesis and are the major energy producers for sperm motility. In this study, we have reported a significant reduction in sperm MMP upon incubation of sperm with various concentrations of As. Similarly, previous studies have shown that As causes mitochondrial dysfunction, resulting in damage to the structural and functional activity of the spermatozoa (Ulloa-Rodriguez et al., 2017; Keshavarz Bahaghighat et al., 2018; Losano et al., 2018). There are also reports relating As to a rapid decrease in MMP, thus changing the activities of mitochondrial enzymes, resulting in notable changes in morphology and destruction of the internal integrity of the organ, consequently disrupting cell morphology and sperm viability (De Vizcaya-Ruiz et al., 2009). In addition, toxicity caused by sodium arsenite due to generation of oxidative stress and apoptosis of the mitochondria has been reported in rat sperm (Das et al., 2009).

The current study also assessed the possible consequences of Pb exposure with varying concentrations on *in vitro* sperm functional attributes in boar semen. Pb, as a heavy metal, is widely used in the manufacture of products for daily use, including paints, lead acids, pigments, and varnishes, and human and animal exposure to Pb causes detrimental effects for male reproductive function, including decreased semen quality, thereby causing male infertility (Abadin et al., 2007). We have reported a significant reduction in boar sperm quality in terms of sperm motility, sperm membrane integrity, and sperm MMP after co-incubation with Pb, with effects occurring in a dose- and time-dependent manner. Our findings were congruent with the reports of Li et al. (2018), who reported a significant reduction in sperm quality parameters and DNA integrity in mice on exposure to Pb. The cellular pathways relating to Ca^{++} control sperm motility and act as an indirect measure for sperm mitochondrial function. Pb, being a divalent metal, can mimic Ca^{++} entry and its functions, thereby affecting the sperm capacitation process by inhibiting or inducing tyrosine phosphorylation (Kushwaha et al., 2021). The reduction in sperm quality observed in this study may be due to Pb exposure, which is thought to generate ROS in sperm cells, and an increase in ROS can decrease sperm membrane fluidity (Barbier et al., 2010). These findings support our hypothesis regarding the potential toxicity of Pb for sperm function even at micro levels in a dose- and time-dependent manner.

We have also reported a significant reduction in sperm quality attributes after incubation with F, with effects occurring in a dose- and time-dependent manner. Similarly, previous studies have also documented reduced sperm count and increased sperm abnormalities in mice exposed to F-contaminated drinking water (Wei et al., 2016; Sun et al., 2017). Toxic effects of NaF and F on male reproduction have been reported, taking the form of reduced sperm motility, testicular steroidogenesis, increased sperm abnormalities, histological alterations in the testis, and the loss of different stages of spermatozoa and spermatogenesis in the lumen of the seminiferous tubules of the testis (Chaithra et al., 2020). Furthermore, recent studies have documented the finding that F exposure can lead to mitochondrial damage and ROS accumulation, leading to mitochondrial fission/fusion imbalances (Zhou et al., 2020). F toxicity has been found to cause mitochondrial damage by decreasing the mitochondrial membrane potential, which promotes an increase in the production of ROS, and a higher level of ROS results in an increase in abnormal spermatozoa and decreased sperm motility, which in turn affects fertility (Wang et al., 2003). Therefore, we speculate that co-incubation of sperm with F might have resulted in increased ROS generation, which might have interacted with purine and pyrimidine bases to increase the content of messenger RNA, destroying single or double strands of DNA and DNA-dependent proteases, resulting in sperm cell apoptosis.

The combined effects of different concentrations of heavy metals on sperm functional attributes in spermatozoa are poorly documented in human and animal models as of now. In the present study, co-incubation with combinations of different concentrations of As, Pb, and F was found to significantly reduce sperm motility, viability, and MMP in a dose- and time-dependent manner. It was also evident from our findings that combinations of heavy metals were far more toxic, even at the lowest concentration and irrespective of the incubation period, than their individual counterparts, suggesting that heavy metals in combination might act synergistically in mediating the cytotoxic effects on the sperm membrane, thereby damaging sperm fluidity. One previous report on human subjects supports our current findings, whereby heavy metals were found to interact either additively, synergistically, or antagonistically (ATSDR, 1999). The toxicity of heavy metals is dose-dependent, and high-dose exposure leads to severe responses in animals and humans, causing more DNA damage (Gorini et al., 2014). The toxic mechanisms of heavy metals function in similar pathways, usually via ROS generation, enzyme inactivation, and suppression of the antioxidant defense (Balali-Mood et al., 2021). However, some of them cause toxicity in a particular pattern and bind selectively to specific macromolecules. Therefore, more well-designed *in vitro* investigations should be conducted in order to validate the complicated relationship between these metals and male fertility factors.

5 Conclusion

The present study is the first of its kind to have demonstrated the cytotoxicity of As, Pb, and F separately and in combination in a dose- and time-dependent manner in terms of boar semen quality *in vitro*. Moreover, the environment is frequently contaminated with various heavy metals, so it is imperative that *in vivo* studies on large animal populations are conducted to validate the toxicity of As, Pb, and F, individually and in combination, on sperm functional attributes in a broader sense. The current findings may be of practical significance in the field of livestock and other wild animal species that are frequently exposed to a wide range of environmental contaminants through their food chain.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material; further inquiries can be directed to the corresponding author.

Ethics statement

The animal study was approved by the Institutional Animal Ethics Committee of ICAR Research Complex for the NEH Region, Umiam, Meghalaya. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

GK: conceptualization, methodology, project administration, writing–original draft, and writing–review and editing. JD: investigation and writing–original draft. SD: conceptualization, methodology, validation, and writing–review and editing. RD: data curation, formal analysis, and writing–original draft. SA: data curation, formal analysis, investigation, writing–original draft, and writing–review and editing. PM: data curation and writing–review and editing.

Funding

The author(s) declare that financial support was received for the research, authorship, and/or publication of this article. The research was funded by the Indian Council of Agricultural Research (ICAR) Complex for the North Eastern Hill Region through an in-house project.

Acknowledgments

The authors thankfully acknowledge the Director, ICAR Research Complex for the North Eastern Hill Region, for granting necessary permissions and providing the facilities to carry out the work.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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frontiers | Frontiers in Environmental Science

TYPE Original Research
PUBLISHED 30 November 2023
DOI 10.3389/fenvs.2023.1304033

OPEN ACCESS

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RECEIVED 28 September 2023
ACCEPTED 13 November 2023
PUBLISHED 30 November 2023

CITATION
Saibu S, Adebusoye SA, Oyetibo GO and Rodrigues DF (2023), Geochemistry and metagenomics analyses of bacterial community structure in selected waste dumpsites in Lagos Metropolis, Nigeria. *Front. Environ. Sci.* 11:1304033.
doi: 10.3389/fenvs.2023.1304033

Geochemistry and metagenomics analyses of bacterial community structure in selected waste dumpsites in Lagos Metropolis, Nigeria

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Dumpsites are reservoirs of persistent organic pollutants (POPs) and heavy metals (HMs), constituting environmental hazards to humanity. Autochthonous microorganisms in dumpsites exhibit various degrees of responses to contaminants. Unfortunately, there is a dearth of information on the types and concentration of pollutants and the array of microorganisms in these dumpsites which may play important roles in the metabolism of such pollutants or other community processes. Therefore, determining the microbial community structure in such contaminated sites across a municipality is essential for profiling the taxa that would serve as consensus degraders of the pollutants. In this study, soil samples from three dumpsites (Cele, CS; Solous, SS; and Computer Village, CVS) were characterized for geochemical properties using GC-MS, MP-AES, and other analytical protocols, while the dynamics of bacterial communities were evaluated based on their 16S rRNA gene barcodes. A significant difference in the bacterial communities was observed among the dumpsites in relation to the extent of pollution caused by POPs and HMs. CVS, with the highest HM contamination, was rich in Actinobacteria (41.7%) and Acidobacteria (10.2%), in contrast to CS and SS. Proteobacteria (34.1%) and Firmicutes (20%) were the dominant phyla in CS (highest POP contamination), while Bacteroidetes (45.5%) and Proteobacteria (39.9%) were dominant in SS soil. *Bacillus* was the dominant genus in the most polluted dumpsite. Canonical correspondence analysis revealed that polycyclic aromatic hydrocarbons (PAHs) and HMs shaped the structure of the bacterial operational taxonomic units (OTUs) in the most polluted dumpsite. Out of a total of 706 OTUs, 628 OTUs exhibited a significant correlation (>50%) with benzo(b) fluoranthene, azobenzene, dibenzofurans, pyrene, dibenzo(a,l)pyrene, Cu, and Zn. In particular, Proteobacteria (*Achromobacter* sp. and *Serratia* sp.), Bacteroidetes (*Zhouia* sp.), and Firmicutes (*Bacillus* sp.) were suggested to be pivotal to the ecophysiology of dumpsite soils contaminated with POPs and HMs. The results generally underscored the importance of metagenomic and physicochemical analyses of polluted systems in enabling correlations for useful prediction of drivers of such ecosystems. This will further improve our understanding of the metabolic potential and adaptation of organisms in such systems.

KEYWORDS

soil, dumpsite, bacterial diversity, persistent organic pollutants, heavy metals

1 Introduction

Soil is a highly complex environment that provides a habitat for a diversity of plants, animals, and microorganisms. Soil has a mean prokaryotic density of roughly 10^8 organisms per gram, with Proteobacteria, Acidobacteria, and Actinobacteria making up the majority of the biomass within the bacteria domain (Janssen, 2006; Delmont et al., 2011; Xavier and Naoise, 2014; Xu et al., 2014). The type and number of microorganisms in the soil are influenced by soil characteristics and wastes that flow into the soil, which either inhibits or stimulates their growth (Zhang et al., 2010; Tang et al., 2013). Microorganisms are an essential part of terrestrial ecosystems, playing important roles in soil biogeochemical cycles as well as in the fate of organic pollutants (Basu et al., 2021). In addition, there is enough evidence confirming the important role played by soil microorganisms in several ecosystem services such as erosion control, soil formation, nutrient cycling, and plant health (Nannipieri et al., 2003; Gardi et al., 2009; Ravi et al., 2019). However, microbes are potentially one of the most sensitive organisms to anthropogenic activities. Soil impacted by different pollutants would consequently have a significant detrimental effect on its quality and will also have a deleterious effect on the flora, fauna, and microorganisms. Soil microorganisms are generally considered the best indicators of soil pollution, as they are very responsive and provide important information about the changes occurring in soil (Sumampouw and Risjani, 2014). Studies have shown the alteration of soil microbial composition and diversity of dumpsites, revealing the dominance and loss of different microorganisms (Wang et al., 2017; Salam and Varma, 2019). Therefore, gaining in-depth knowledge of the impacts of pollution on the microbial communities and potential degraders in these environments is important.

Globally, waste generation rates are increasing because of urbanization and rapid population growth. Approximately 4.3 billion urban residents are projected to generate approximately 1.42 kg of solid waste per person, totaling 6.1 million metric tons per day by 2025, resulting in faster generation of waste than of other pollutants such as greenhouse gases (Hoornweg et al., 2013). In developing countries, waste management strategies include recycling, landfill dumping, and incineration (Igbinomwanhia, 2011). In recent years, attention has been drawn to open dumping sites where large amounts of municipal solid wastes have been dumped, and improper disposal of e-waste leads to high concentrations of heavy metals (HMs) leaching into the surrounding environments and bioaccumulation in humans (Longe and Balogun, 2010). HMs are non-biodegradable and bioaccumulate in the environment, and they can severely affect the microbial diversity and abundance because of the toxicity induced (Zhao et al., 2019; Hu et al., 2021). However, some microorganisms are tolerant to HMs and may convert them to less-toxic forms or remove them from the polluted soil (Qiao et al., 2019). Unfortunately, most of such open dump areas, incineration sites, and landfills are located near residential areas and as such, exposure to various toxic chemicals originating from the sites is of serious concern because of the effects on human health, wildlife, and environmental quality. Indiscriminate burning of solid waste, generation of methane gas, lack of advanced waste incineration technology, and natural low-temperature burning are major problems in dumpsites. These are favorable factors for the formation of polycyclic aromatic hydrocarbons (PAHs), dioxins, furans, and their chlorinated analogs which can persist in the environment undegraded for years (Duan et al., 2012). These dioxins can permeate the soil and contaminate groundwater and nearby vegetation; not only does ecological contamination negatively affect overall ecosystem functions, but it also affects the health and functioning of living organisms.

Microbial activities in dumpsite soil play a crucial role in greenhouse gas emissions (Bajar et al., 2021). However, only a few studies have examined the metagenomic microbial profiles in landfills, e-waste, and incinerators simultaneously, especially in African contaminated systems and particularly in Nigeria, where no such investigations have ever been applied to dumpsites or contaminated soils. Nevertheless, quantification of pollutants alone does not provide cogent information about the potential biological impact; a combination of pollutant speciation with an in-depth evaluation of the microbial community structure will give a more informative and definitive assessment. Therefore, this study aims to gain a better understanding of the structure of microbial communities in three dumpsites that are exposed to different types of pollutants and at varying concentrations, with a view to identify the microorganisms that are important in natural biogeochemical cycles that may be adversely affected by pollution. The study also intends to determine whether they may serve as degraders of environmental contaminants. The objective was to establish the specific pollutants or abiotic factors that are the main drivers of the community structure and function. To this end, the correlation between soil physiochemistry and microbial community structure was deduced, as was the level of similarity among the dumpsites. The information obtained would be key to revealing the possible impact of the pollutants and their effects on microbial succession and metabolic mechanism, which overall would be valuable in designing effective bioremediation schemes for the polluted sites.

2 Materials and methods

2.1 Study sites and soil sample collection

Three major dumpsites were mapped out in the Lagos metropolitan area of Lagos State, Nigeria. Lagos metropolis is

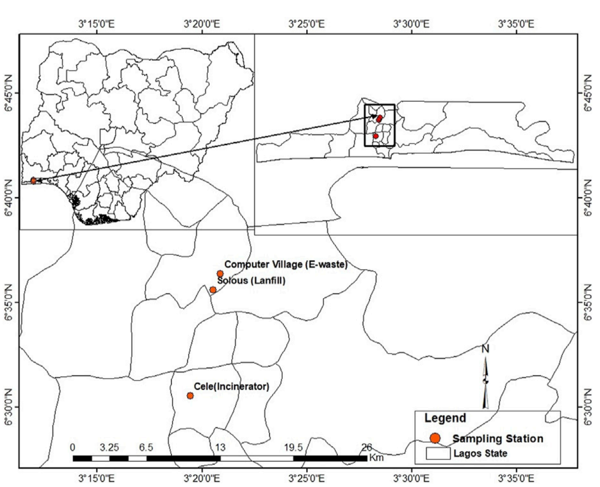

FIGURE 1
Geographical map of Lagos state showing the sampling dumpsites. Sampling sites are given in red dots: Cele, Computer village, and Solous.

Africa's model megacity, which contends with waste generated by its residents. The study areas were 'Cele' dumpsite (CS) at Itire, where open burning occurs consistently; 'Computer Village' dumpsite (CVS) at Ikeja, which is an electronic recycling site that largely contained electronic wastes (e-wastes); and 'Solous' dumpsite (SS) located at Igando, which is an open landfill comprising varieties of domestic and industrial wastes. The GPS coordinates of the dumpsites are delineated in the map presented in Figure 1. Soil samples (10 g apiece) were collected randomly from a depth of 0–20 cm at six different points at each site, pooled to form a composite of each dumpsite in sterile ziplock bags, and transported on ice to the laboratory for further analysis. One part of each soil sample was stored at −80°C for microbial community analysis, while another part was stored at 4°C for physicochemical analysis.

2.2 Physicochemical analysis

The physicochemical parameters of the samples were determined using standard protocols. Soil particle size was determined using the hydrometer method according to the protocol described by Day (1965). The pH was determined *in situ* using a pH meter (British Standard, 1995), and the moisture content was determined by using the drying method (Chopra and Kanwar, 1998). The total organic carbon and total organic matter were determined by using the Walkley–Black chromic acid wet oxidation method. Other physicochemical parameters were determined as follows: total nitrogen using the Kjeldahl method, phosphate using the spectrophotometric method, and oil and grease using the solvent extraction method. All these methods were applied according to protocols previously described by Nelson and Sommers (1983), Storer (1984), AOAC (1990), and Chopra and Kanwar (1998).

For the HM analysis, a sieved air-dried soil sample (1 g) was first digested with $HCl/HNO_3/H_2SO_4$ (1:2:2 v/v) for 15–20 min on a heated platform. The sample was cooled, after which 20 mL of distilled water was added and again heated to boiling point to bring the metals into solution. The sample was filtered (Whatman 42 filter paper and <0.45 µm Millipore filter paper) and stored in plastic bottles. The contents of metals in the soil samples were evaluated through the microplasma atomic emission spectrophotometer (MP-AES 4200) coupled to a computing system and operated on the MP-AES software interface which enabled elemental analysis at mg/kg for a wide range of metals. Standard solutions used in the calibration of the instrument prior to analysis were prepared by diluting multi-element standard solutions to a concentration of 100 mg/L (AOAC, 1990). For quality control measures, all standards, replicates, and blanks were prepared at the same time and used immediately. The resulting calibrated curve was verified by analyzing initial calibrated curve verification (ICV) and initial calibrated blank samples. The ICV standard was ±20% of its true value.

Persistent organic pollutants (POPs) present in the soil samples were evaluated based on the USEPA method 8270D (USEPA, 2007). Ten gram of homogenized soil was sonically extracted with 20 mL of a 1:1 acetone:dichloromethane solvent complex at 70°C for 30 min. The extracts were dried with sodium sulfate, concentrated, exchanged into cyclohexane, and finally cleaned up via passage over silica gel. The purified extracts were subsequently converted back to acetone: dichloromethane and analyzed via gas chromatography mass spectrometry (GC-MS). The extract composition was examined by total ion scans, and the USEPA 16 priority pollutant PAHs were identified and quantified by using primary and secondary ions. Polychlorinated biphenyls (PCBs), nitroaromatic compounds, dioxins, and furans in the soil were also determined using GC-MS equipped with a HP-5 capillary column coated with 5% phenylmethylsiloxane (30 m length × 0.32 mm diameter × 0.25 µm film thickness) (Agilent Technologies). The injector and detector temperatures were kept at 250°C and 300°C, respectively. The carrier gas (helium) was maintained at a flow rate of 1 mL/min (initial pressure, 1.715 psi; average velocity, 37.758 cm/s). The chromatograph was programmed at 130°C for 2 min initially, then ramped to 200°C at 10°C min^{-1} for 16 min, then to 265°C at 5°C min^{-1} for 7 min, and finally to 300°C at 5°C min^{-1} and held for 3 min. The MS was operated in electron-impact ionization mode at 70 eV with an ion source temperature of 230°C, a quadrupole temperature of 150°C, and a transfer line temperature of 300°C. Acquisition of ions was carried out via scan mode (scanning from m/z 50–500 amu at 2.0 s/scan rate) and selective ion mode (SIM). In all preparations, octachloronaphthalene solution was added to the soil solutions as an internal standard prior to extraction. This was necessary to assess the recovery of the organic pollutants from the soil. Prior to analysis, the instrument was properly calibrated by injecting a series of calibration standards. The typical coefficient of correlation r for the calibrated curve was ≥0.995. The efficiency and accuracy of GC were constantly ascertained by injecting the standards prior to sample analysis.

2.3 Soil DNA extraction, 16S rRNA gene amplification, and sequencing

Total DNA of the soil samples (0.25 g) was extracted using a MOBIO PowerSoil DNA Extraction Kit (MO BIO, Carlsbad, CA, USA) following the manufacturer's protocol. DNA was quantified using a NanoDrop spectrophotometer (Thermo Scientific, Wilmington, DE, United States), the quality was visualized through 1% agarose gel electrophoresis, and the DNA was stored at −20°C until further use. The V4–V5 hypervariable regions of the bacterial 16S rRNA gene were amplified (98°C, 30 s; 12 cycles: 98°C, 45 s; 62°C , 30 s, and 72°C, 30 s; the final primer extension at 72°C, 5 min) using primers Hyb515F (5′-GTGYCAGCMGCCGCGGTA -3′) and Hyb909R (3′-TGARTTTMCTTAACYGCCCC-5′) as previously described (Nguyen and Rodrigues, 2018). PCR products were purified using the AMPure XP bead purification system and subsequently barcoded with Hyb primers. Sample libraries were generated from the purified amplicons. The quantity and quality of the libraries were assessed using a Bioanalyzer DNA chip (Agilent). Sequencing was performed using an Illumina MiSeq system (Illumina, San Diego, CA, USA) using primers Hyb515F and Hyb909R with Illumina adapters and barcodes. Pair-end sequencing was employed using 600-cycle MiSeq® Reagent Kit V3 (Illumina, U.S.A.) to generate 2 bp × 300 bp reads at the Genomic Sequencing and Analysis Facility, University of Texas, Austin, United States.

2.4 Processing of sequencing data and taxonomic classification

Sequences were obtained in FASTQ format and analyzed using the Quantitative Insights into Microbial Ecology (QIIME) V. 1.9.1 software pipeline to demultiplex and filter out low-quality and chimera sequences (Caporaso et al., 2010). High-quality sequences were assigned using the ribosomal database project (RDP) classifier at a 97% similarity, and α-diversity indices (Shannon and Chao1) were calculated. The clustering and classification of OTUs at several taxonomic levels (kingdom, phylum, class, order, family, genus, and species) were deduced from the processed dataset. The Illumina sequence data reported here have been deposited in the NCBI Sequence Read Archive database under accession number: **PRJNA540227**.

2.5 Statistical data analysis

The relative abundances of the phylum and class levels for the three sites were plotted as a bar graph using the Prism 9.0 software program (GraphPad Software, San Diego, CA, USA). The R language platform (XLSTAT) was used to generate a heatmap based on the data summary for the three sites. Principal component analysis (PCA), a multivariate data dimensionality reduction method, was used to further analyze the environmental factors affecting the microbial community. The biological dataset was square root-transformed in order to downweight the effects of the highly abundant OTUs, and the Bray–Curtis similarity index was used to construct the resemblance matrix. Transformation and normalization of environmental data were used in calculating the resemblance matrix using Euclidean distance. Beta diversity patterns of the soil communities were examined by PCA ordination developed on Cluster routine using PRIMER v 7 software (PRIMER-E Ltd., Lutton, UK) (Clarke and Gorley, 2015). Canonical correspondence analysis (CCA) was performed using paleontological statistics software (PAST), which was used to test the relationship between physicochemical parameters and bacterial diversity. Pearson's correlation was used to test the strength of the association between OTU abundance and soil physicochemistry. Statistical significance was determined at the 95% level ($p < 0.05$).

TABLE 1 Dumpsite physicochemical properties.

Soil description	CS	CVS	SS
Gravel (%)	3	8	1
Sand (%)	84	86	95
Silt (%)	13	6	4
pH	8.4	8.7	8.5
Moisture content	8.7	8.8	3.5
TOC (%)	20.2	5.3	11.2
TOM (%)	34.8	9.2	19.2
Total nitrogen (mg/kg)	0.18	1.27	0.49
Phosphorus (mg/kg)	1.07	1.35	1.22
Oil and grease (mg/kg)	17,550	12,042	14,688

CS, cele dumpsite; CVS, computer village dumpsite; SS, solous dumpsite.

3 Results

3.1 Environmental conditions across dumpsites

The dumpsite soils lacked clay particles and were mostly made up of sand (84%–95%), with trace amounts of gravel and silt particles (Table 1). Further investigation of the soil physicochemistry revealed that the pH in all samples was slightly basic and did not differ significantly. The moisture content recorded in CS and CVS samples was more than twice the value obtained for SS. The highest proportion of oil and grease was recorded in CS, followed by SS and CVS in that order. A similar trend was also reported for the TOC and TOM (Table 1). The geochemistry with respect to HM composition in the dumpsites is presented in Table 2. The dumpsites exhibited high concentrations of HMs, with Fe (246.85 mg/L) and Zn (38.01 mg/L) having the highest concentrations, especially in CS soil, which, respectively, correspond to 1.7–3.2 and 25 times more than the levels encountered in other sites. In contrast to Fe and Zn, the highest concentration of Al was found in the CVS site at a threshold that was over ten times the level recorded for CS. Relative concentrations of the HMs in the three sites were of the order Fe > Al > Zn > Cd > Cu > Ni > Pb > Cr. It is noteworthy that the concentrations of Ba, Co, and V were all below the detection limit in the three study sites (Table 2). With the exception of Cu, the concentration of which was within the Federal Ministry of Environment (FMEnv) regulatory limit (except for CS soil), the concentrations of other HMs were above the recommended limit.

Among the 23 PAHs determined in the soil samples collected, dibenz(a,h)anthracene, benzo(b)fluoranthene, 3-methylcholanthrene,

TABLE 2 Heavy metal composition of the dumpsite soils.

Heavy metal (mg/kg)	CS	CVS	SS	FMEnv
Al	25.33	255.67	102.00	0.20
Ba	<0.0001	<0.0001	<0.0001	NA
Cd	4.8594	3.1688	1.4360	NA
Co	<0.0001	<0.0001	<0.0001	NA
Cr	0.2283	0.2219	0.1795	0.0500
Cu	8.1288	0.4591	0.2057	2.0000
Fe	246.85	142.45	76.14	3.00
Mn	4.5254	1.3974	1.0232	0.1000
Ni	2.4682	2.4624	2.6496	0.0700
Pb	4.5235	1.5696	0.6428	0.0500
V	<0.0001	<0.0001	<0.0001	NA
Zn	38.0098	1.5604	1.4781	0.3000

CS, cele dumpsite; CVS, computer village dumpsite; SS, solous dumpsite; FMEnv, Federal Ministry of Environment; NA, not applicable; <0.0001 implies that the composition is less than the detection limit.

benzo(g,h,i)perylene, and acenaphthene constituted the major POPs associated with all the dumpsites (Table 3). Total PAHs in the dumpsites were in the order: CS (8.04 mg/kg) > SS (6.46 mg/kg) > CVS (5.29 mg/kg), which were not significantly different among the sites ($p < 0.05$). Dibenzofuran, a model compound of dioxins, was detected only in CS (7.61 mg/kg), unlike in the other two dumpsites, where it was below the detection limit. As presented in Table 3, halogenated organic compounds (PCBs and chlorinated dioxin congeners) were not detected in any of the three soils. However, concentrations of other compounds including aniline, nitrobenzene, diphenylamine, carbazole, benzidine, and pentachlorophenol were all below detection limits across the dumpsites. The highest concentration of the total POPs detected was in CS (23.76 mg/kg), with azobenzene (7.95 mg/kg) predominating other POPs. In addition, there was a significant difference in the total POPs detected in the three dumpsites ($p > 0.5$).

3.2 Alpha-diversity characteristics of the three dumpsites

A total of 19,567, 17,173, and 22,641 high-quality sequence reads were recovered, respectively, from CS, CVS, and SS following quality filtering and subsequently assigned to corresponding 1,008, 3,293, and 2,653 OTUs. The metagenomics analysis and characteristics of alpha-diversity indices are summarized in Table 4. These alpha diversity parameters (richness and evenness of OTUs) differed greatly among the dumpsites. The lowest OTU richness and evenness were observed in the CS, while CVS exhibited the highest OTU richness and evenness. The Choa1 and Shannon indices showed that the bacterial community in the latter region (CVS) displayed higher abundance and was more diverse than the other two dumpsites (SS and CS). It is noteworthy that the level of richness observed in CVS was more than twice the level observed in the other sites. Whittaker plots, otherwise called rank abundance plots, display species OTU evenness as line slopes and OTU richness as line lengths (Figure 2). The figure revealed relatively evenly distributed OTUs in CVS, unlike a single dominant OTU that accounted for 18% of reads in the libraries as evidence of a less uniform distribution in SS, where the curve was initially rather steep. OTU abundance was highest in SS, while dominant OTU was absent in CVS, where OTU abundance was comparatively evenly distributed. The CVS site also recorded the greatest OTU richness, as evidenced by the longest sample line, while the least was found in CS (Figure 2).

3.3 Interactions between the microbial communities and environmental factors

PCA based on Euclidean distance revealed the transformation of environmental data with the OTUs as monitored with principal coordinate ordination analysis (PCO) (Figure 3). As summarized in Figure 3, the first PCO accounts for a large proportion of the data, 63.9% of the variation in the samples, separating CS from the rest of the sites. Most of the environmental variables (POPs) had more effect on the CS bacterial community, and most of the PAHs had a correlation with dibenzofurans and a few of the HMs (Mn, Zn, and Cu). However, organic pollutants such as (1,1′-biphenyl)-4-amine, 7,12-dimethylbenz(a)anthracene, 2-methyl-4,6-dinitrophenol, and 2,4-dinitro phenol exhibited more impacts and were the drivers of the SS ecosystem compared to other sites. Total nitrogen, phosphate, chrysene, 2-nitro-phenol, and Al had more effect in CVS, which, interestingly, was the least polluted site (Figure 3). Furthermore, CCA demonstrated that environmental factors were strongly correlated with the bacterial communities ($p < 0.05$) (Figure 4). Moreover, it was established that the three different dumpsites ordinated

TABLE 3 Concentration of organic compound pollutants in the dumpsite soils.

Organic compound (mg/kg)	CS	CVS	SS
Naphthalene	0.137	0.114	<0.0001
Acenaphthylene	<0.0001	<0.0001	0.299
Acenaphthene	0.446	0.353	0.472
Fluorene	0.160	0.276	0.243
Anthracene	0.297	0.216	0.296
Phenanthrene	0.099	0.239	0.296
Fluoranthene	0.084	0.085	0.091
Pyrene	0.091	<0.0001	<0.0001
Benzo(c)phenanthrene	0.062	0.022	0.013
Benz(a)anthracene	0.327	0.196	0.206
Chrysene	0.285	0.505	0.272
Benzo(e)pyrene	0.315	0.066	0.283
Benzo(b)fluoranthene	0.690	0.448	0.426
Benzo(k)fluoranthene	0.305	0.199	0.202
Benzo(a)pyrene	0.385	0.352	0.356
7,12-Dimethyl benz(a)anthracene	0.254	0.203	0.365
3-Methylcholanthrene	0.844	0.361	0.311
Indo(1.2,3-cd)pyrene	0.332	0.247	0.341
Dibenz(a,h)anthracene	0.624	0.567	0.835
Dibenzo(a,l)pyrene	0.594	0.352	0.351
Benzo(g,h,i)perylene	0.586	0.486	0.393
Dibenzo(a,i)pyrene	0.604	<0.0001	0.073
Dibenzo(a,h)pyrene	0.516	<0.0001	0.333
Total PAHs	8.039	5.285	6.458
2-Nitro-phenol	*ND*	1.863	*ND*
2,4-Dinitro phenol	2.482	*ND*	*ND*
Dibenzofuran	7.609	*BDL*	*BDL*
4-Nitro-phenol	0.023	0.014	0.020
Azobenzene	7.952	0.288	*BDL*
2-Methyl-4,6-dinitro phenol	3.373	*ND*	10.666
p-Nitroaniline	0.702	*ND*	0.706
[1,1′-Biphenyl]-4-amine	1.614	1.148	3.773
Total organic compound (excluding PAHs)	23.289	3.313	15.165

CS, cele dumpsite; CVS, computer village dumpsite; SS, solous dumpsite; BDL, below detection limit; ND, not detected.

differently, whereby most of the POPs and HMs shaped the bacterial community of the CS.

The CCA bi-plot resolved the geographic differences of the CS and CVS by 58.77% variance along the *x*-axis. The bacterial communities in relation to environmental variations were subdivided across a slope gradient through 41.2% variability. A critical scrutiny of the data summarized in Figure 4 readily suggested a positive effect of 2-nitrophenol and chrysene on the abundance of the dominant OTUs; Acidobacteria, Actinobacteria, Chloroflexi, Planctomycetes, and α-Proteobacteria. On the other hand, γ-Proteobacteria seemed to be negatively correlated with 2-nitrophenol and chrysene and positively correlated with indo(1,2,3-cd)pyrene and acenaphthene. Organic compounds such as benzo(a)pyrene, benzo(c)phenanthrene, and benzo(g,h.i)

TABLE 4 Characteristics of Illumina libraries and alpha diversity metrics of soil communities.

	Soil		
	CS	CVS	SS
Number of reads	21,891	18,640	24,307
Number of quality filtered amplicons	19,567	17,173	22,641
Median amplicon length (bp)	413	415	413
Number of OTUs	1,008	3,293	2,603
Chao1	1855.21	5,745.66	2,653
Shannon	6.16	9.85	6.60

CS, cele dumpsite; CVS, computer village dumpsite; SS, solous dumpsite.

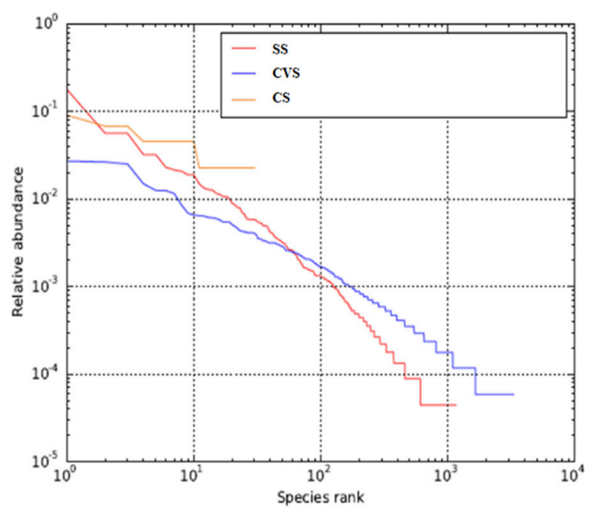

FIGURE 2
Rank abundance curve of average OTU abundance in CS, CVS, and SS dumpsite soil communities.

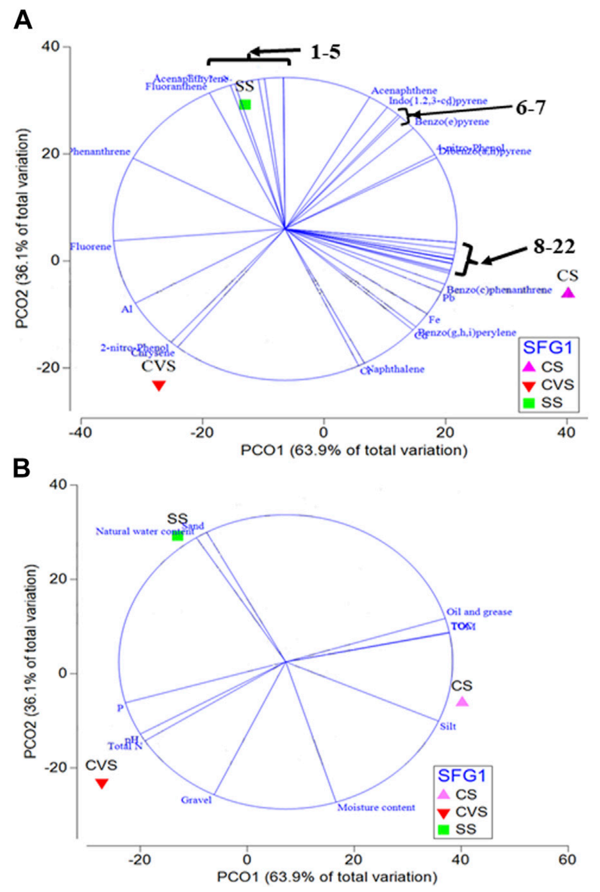

FIGURE 3
Principal component ordinations of bacterial communities in CS (Cele), CVS (Computer village) and SS (Solous) soils depicting levels of similarity and environmental variables [**(A)** POPs; **(B)** physicochemical] associated with the segregation of communities along the PCO axes. 1-5: Ni; [1,1′-biphenyl]-4-amine; 7,12-dimethylbenz(a)anthracene; 2-methyl-4,6-dinitrophenol; 2,4-ninitro phenol; 6–7: p-nitroaniline; Anthracene; 8–22: Mn; Zn; Cu; Azobenzene; Dibenzofuran; Dibenzo(a,i) pyrene; Dibenzo(a,l)pyrene; Dibenzo(a,h)anthracene; 3-methylcholanthrene; Benzo(a)pyrene; Benzo(k)fluoranthene; Benzo(b) fluoranthene; Benzo(e)pyrene; Benz(a)anthracene; Pyrene.

perylene as well as HMs Cd, Pb, and Fe were strongly associated with the phylum Firmicutes. Similarly, the same environmental variables that were positively associated with the Firmicutes were negatively correlated with the phyla Verrucomicrobia and Bacteroidetes. However, both phyla shared a positive correlation with phenanthrene, fluoranthene, and acenaphthylene.

Out of a total of 706 OTUs, 628 OTUs had a significant correlation with environmental variables such as benz(a) anthracene, benzo(b)fluoranthene, azobenzene, dibenzofurans, pyrene, dibenzo(a,l)pyrene, 2,4-dinitrophenol, Cu, and Zn, with each variable accounting for over 50% of the OTU correlation (Table 5). A dendrogram displaying the groupings of environmental characteristics that frequently co-occurred as variables significantly correlated with OTU abundance had three main branches, as shown in Figure 5. Branches IA and IB contained dibenzofurans, some PAHs, Zn, and Cu, whereas branch II was predominantly represented by PAHs and other organic pollutants. Within branch III, there were three sub-clusters (III A–C) of frequently co-occurring PAHs and/or metals. In addition, 4-nitrophenol and acenaphthylene significantly correlated with the abundance of Proteobacteria and Firmicutes, respectively, in the soils. Anthracene and *p*-nitroaniline had a significant negative correlation with Acidobacteria, Cyanobacteria, Chloroflexi, and Nitrospirae, while in contrast, there was a positive correlation between some of the HMs (i.e., Cu, Zn, and Ni), dibenzofurans, azobenzene, and pyrene, with an abundance of Bacteroidetes and Firmicutes (Supplementary Table S1). Heatmap analysis of both the sequence and environmental data further reinforces the microbial community composition divergence of the CVS from the rest of the sites. Having revealed the patterns of correlation between environmental variables and OTUs (phyla) and correlations among the three sites vis-à-vis the taxonomic composition of the bacterial community, it was established that CVS and SS had relatively similar responses (Figure 6A). As seen in Figure 6B, the side dendrogram consists of two main clusters, where the lower

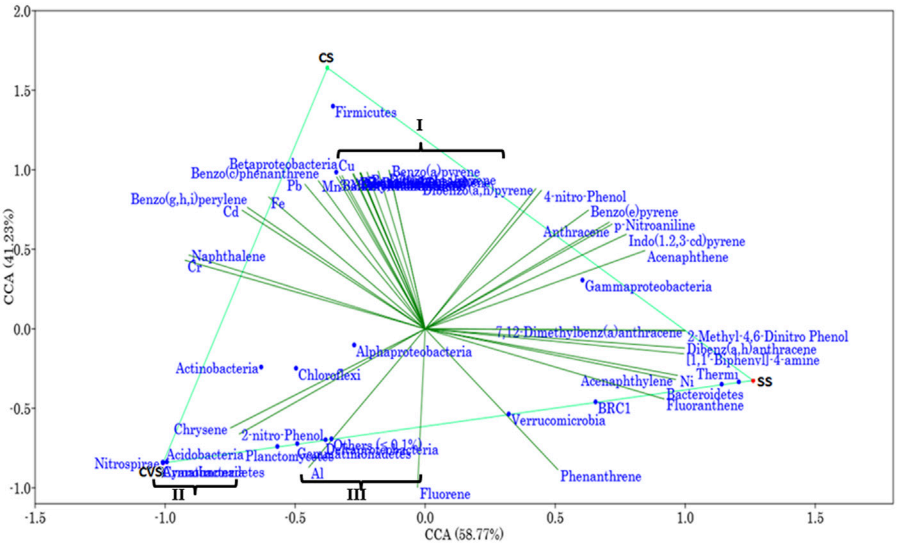

FIGURE 4
Canonical correspondence analysis, two-axis plot of variation partitioning of bacterial communities and environmental variable in CS, CVS, and SS dumpsites. The environmental variables clustered in I are Zn, azobenzene, dibenzofurans, pyrene, 2,4-dinitrophenol, dibenzo(a,i)pyrene, dibenzo(a,l) pyrene, 3-methylcholanthrene, benzo(a)anthracene, benzo(k)fluoranthrene, and benzo(b)fluoranthene; clustered OTUs in II are Armatimonadetes and Cyanobacteria; and those in III are Deltaproteobacteria and Gemmatimonadetes.

cluster had a strong correlation with OTU abundance in CS when compared with the other two sites, which exhibited a low correlation with OTU abundance.

3.4 Analysis of the microbial community composition and structure of the polluted sites

The bacterial community structures of the soil samples as determined by Illumina sequencing of the 16S rRNA gene generated across all libraries were a total of 706 bacterial and five archaeal OTUs (97% of sequence reads). Despite presenting the least diversity among the three sites, the relative abundance of unclassified archaea at the CVS site was greater than that of either SS or CVS soil. The archaeal community profiles in CS comprised 95% of total archaeal OTUs in all three sites, whereas CVS and SS accounted for 4% and 1%, respectively (Figure 7). Irrespective of the extremely low profile in CVS, this site was more diverse in the archaeal community than the other two. Whereas three phyla, namely, Euryarchaeota, Crenarchaeota, and Parvarchaeota, were encountered in the CVS, the archaeal community, in both CS and SS, primarily contained only unclassified archaea (Supplementary Table S2).

In terms of the bacterial community structure, soil originating from CVS had the highest unique OTU (34.7%) when compared with others, while the lowest unique OTU was observed in CS (Figure 8). Although the highest similarity index (23.5%) was recorded between SS and CS, the bacterial diversity pattern of the three dumpsites clearly separated CS from the two sites along PCO1, which accounted for 63.9% of the total variation, showing a 40% similarity index for CVS and SS sites (Figure 8). Proteobacteria, Actinobacteria, and Bacteroidetes were the core phyla, accounting for approximately 77.22% of the bacterial taxa in all the sites combined. The phyla Actinobacteria, Chloroflexi, Firmicutes, and Proteobacteria were detected in the three soil communities, though to varying degrees, as depicted in Figure 9. While Proteobacteria (48.32%) was the dominant phylum in CS, Bacteroidetes (45.40%) and Actinobacteria (42.21%) unequivocally dominated the communities in SS and CVS, respectively. Among the Proteobacteria group in the three dumpsites, the class α-Proteobacteria, led by the genus *Balneimonas*, in the order Rhizobiales and family Bradyrhizobiaceae, predominated in CVS; while in CS, the dominant class was γ-Proteobacteria, led by the family Enterobacteriaceae and genus *Serratia* (Figure 9). In the case of the SS dumpsite, approximately 75% of the members of Proteobacteria encountered belonged to the class γ-Proteobacteria, which was mainly dominated by the families Alteromonadaceae, Xanthomonadaceae, and Pseudomonadaceae. The community structure of the CS site presented an interesting observation. Unlike the CVS and SS soils, the bacterial community was practically made of four phyla consisting of Proteobacteria in addition to Firmicutes, Actinobacteria, and Chloroflexi. The latter was merely 3.26% of the population. By implication, other phyla, including Acidobacteria, Bacteroidetes, Gemmatimonadetes, and Planctomycetes, which were found in other sites, were absent in CS. It is also noteworthy that Firmicutes, which constituted nearly 30% of the communities in CS, contributed less than 2% to the bacterial flora in both CVS and SS systems (Figure 9). Conversely, the δ-proteobacteria class, which was absent in CS, was found in SS and CVS, but its relative abundance was higher in the latter than in the former. With the exception of Bacteroidetes, Proteobacteria, and Actinobacteria, all other representative phyla in SS constituted generally less than 3% of the soil microflora. Bacteroidetes that was the dominant phylum in the SS dumpsite constituted barely 2.5% of the community in CVS; in contrast, Actinobacteria, which dominated the bacterial community in CVS, constituted less than 7% of the composition in the SS soil.

TABLE 5 Environmental variables that have correlations with different OTUs (genus level).

Environmental variable	OTU correlation	%OTU correlation
Naphthalene	5	0.70922
Acenaphthylene	22	3.120567
Acenaphthene	14	1.985816
Fluorene	1	0.141844
Anthracene	134	19.00709
Phenanthrene	9	1.276596
Fluoranthene	4	0.567376
Pyrene	389	55.1773
Benzo(c)phenanthrene	18	2.553191
Benz(a)anthracene	365	51.77305
Chrysene	142	20.14184
Benzo(e)pyrene	34	4.822695
Benzo(b)fluoranthene	397	56.31206
Benzo(k)fluoranthene	352	49.92908
Benzo(a)pyrene	1	0.141844
7,12-Dimethylbenz(a) anthracene	5	0.70922
3-Methylcholanthrene	40	5.673759
Indo(1.2,3-cd)pyrene	30	4.255319
Dibenz(a,h)anthracene	4	0.567376
Dibenzo(a,l)pyrene	390	55.31915
Benzo(g,h,i)perylene	11	1.560284
2-Nitro-phenol	137	19.43262
2,4-Dinitro phenol	389	55.1773
Dibenzofuran	389	55.1773
Azobenzene	396	56.17021
2-Methyl-4,6-dinitro phenol	17	2.411348
p-Nitroaniline	125	17.7305
[1,1′-Biphenyl]-4-amine	4	0.567376
Cd	13	1.843972
Cr	3	0.425532
Cu	394	55.88652
Fe	6	0.851064
Mn	36	5.106383
Ni	17	2.411348
Pb	36	5.106383
Zn	372	52.76596
Moisture content	22	3.120567

Further interrogation of the obtained sequence data revealed *Zhouia* (18.29%) belonging to the family Flavoacteriaceae as the most abundant genus, and it was only found in the SS soil, thus making it the dominant genus in that site. This genus is more than three orders of magnitude higher than *Marinobacter*, the next dominant genus in the same dumpsite (Figure 9B). Like *Zhouia,* the genus *Marinobacter* was not detected in the other dumpsites. In the case of CS and CVS, *Bacillus* (11.36%) and an unclassified genus in the family Norcardioidaceae (9.8%) were the respective dominant genera.

The type and distribution of bacterial genera in the soils were somewhat unique and obviously different from site to site, an inference that further reinforces the data pattern shown in Figure 8. For instance, out of the 32 genera represented in Figure 9B, only 10 were found across the dumpsites, accounting for less than 32% of captured genera, and eight of these had less than 0.1% abundance in either or two of the soil's microflora. In addition, only two of the ten genera had a relative abundance of >0.1% across the sites. These genera included *Bacillus* and an uncultured genus in the family Norcardioidaceae.

4 Discussion

Lagos, the commercial hub of Nigeria, is the second fastest-growing city in Africa and seventh in the world and is one of the largest producers of solid waste (if not the largest) in Africa. The latest reports estimated its population to be more than 21 million, making it the largest city in entire Africa. In Lagos, over 12,000 metric tons of waste are generated daily at a generation *per capita* (GPC) of 0.72 kg/person/day (Olukanni and Oresanya, 2018). Over 4.5 million tons of waste are generated annually in Lagos, and 30% of the waste is recycled to reduce the amount going to landfills (Bakare, 2016). The climate of Lagos State is tropical with alternate dry and wet seasons, with a temperature range of 28°C–33°C.

Dumpsites are the final repository for most of the discarded materials from human society. It is generally known that polluted sites exert environmental pressure on fundamental ecological parameters such as abundance, diversity, and nutrient cycling. Therefore, an in-depth knowledge of the microbial community structure is essential to gain insights into processes that may affect the fate of pollutants specifically and biogeochemical cycling more broadly. The three dumpsites examined in this study were extensively polluted, which may have paved the way for selective pressure and evolution of competent pollutant degraders (Saibu et al., 2020). In Lagos dumpsites, PAHs, oil and grease, HMs, and other organic pollutants were widespread in soils, but the concentrations depended on waste-type input and other activities occurring on such sites (e.g., incineration and open burning). Indiscriminate and extreme incineration activities, which the CS site is constantly subjected to, may have accounted for the highest burden of PAHs, oil, and grease encountered (Ferronato and Torretta, 2019). In the case of dibenzofuran, since its concentration was below the detection limit in both CVS and SS and dioxins are relatively ubiquitous in nature, it is plausible that aerial transport and deposition from air

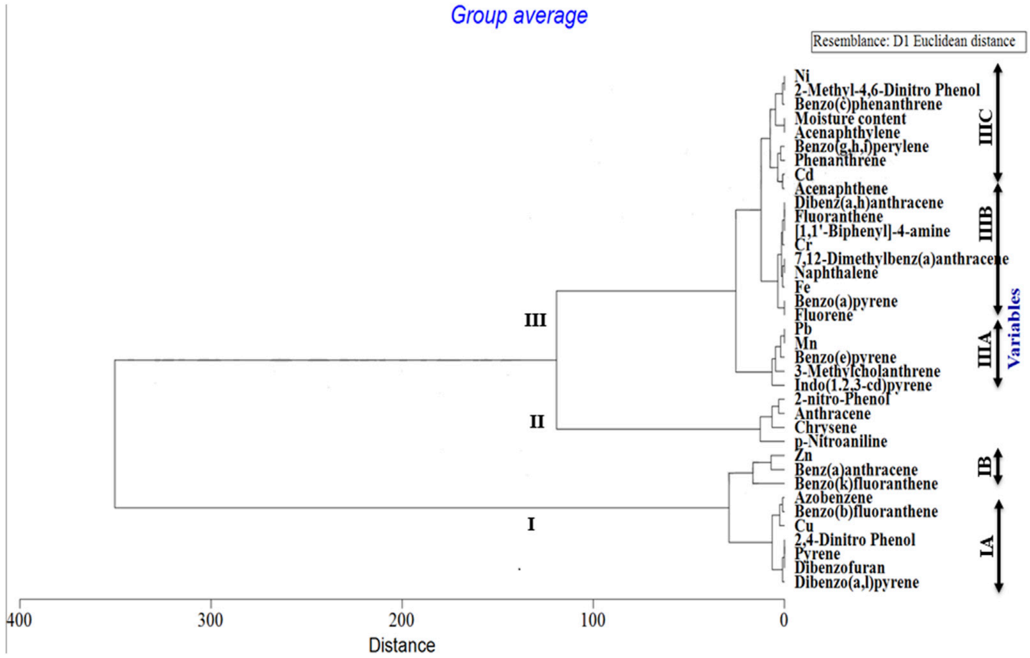

FIGURE 5
Dendrogram illustrating groupings of environmental properties that frequently co-occurred as variables that significantly correlated with OTU abundance. Three major clusters are represented with sub-clusters within groups I and III.

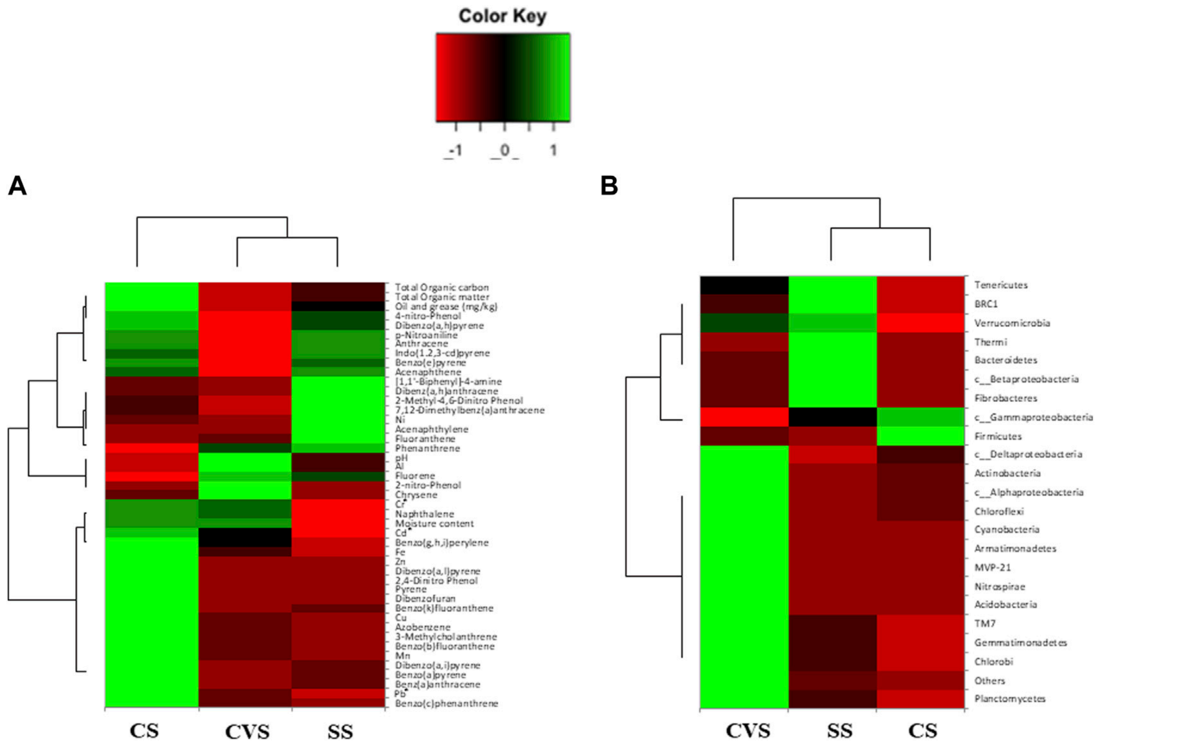

FIGURE 6
Heatmap comparison of three dumpsite soil environmental variables **(A)** and OTU abundance at the phylum level **(B)**. Red and green denote negative and positive correlation, respectively, while black represents no correlation. Euclidean distance is computed to evaluate the similarity, and clustering is conducted using the complete linkage method. CS, CVS, and SS dumpsites.

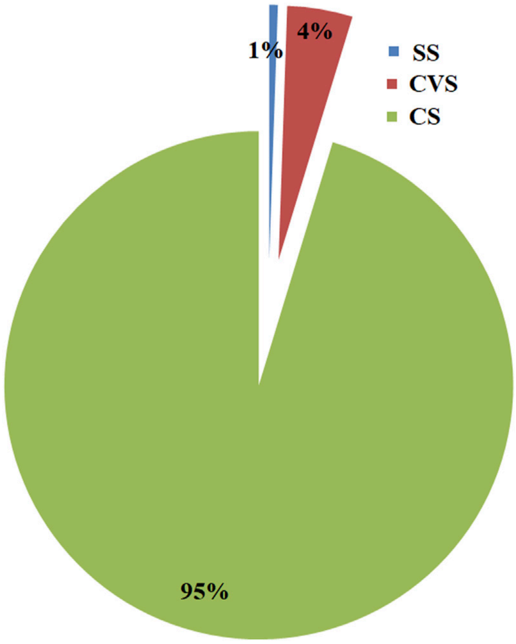

FIGURE 7
Relative abundance of archaeal taxa in CS, CVS, and SS dumpsites. SS dumpsite soils. Although 95% of the total archaeal OTUs were found in CS soil, CVS had more diversity than other soils.

to soil may have occurred, thus reducing concentration at one spot and increasing it at another location (Mahfouz et al., 2020). This remobilization, distribution, and volatilization may also be responsible for the absence of PCBs and polychlorinated dibenzo-*p*-dioxins/furans (PCDD/Fs) across the sites. Interestingly, this phenomenon has been observed in several environmental matrices by several researchers (Šrédlová and Cajthaml, 2022). Chlorinated organics, particularly PCBs and PCDD/Fs, can travel long distances via air and get deposited in areas remote from where they were released.

Cumulatively, CS may account for the highest proportion of POPs; the highest cumulative concentration of HMs was found in CVS soil, where both Al and Fe occurred at significantly high concentrations. Although Al and Fe may not pose a serious environmental threat compared to other metals, the level of occurrence can, nonetheless, be very dangerous to plants, weakening and eventually killing them (Marzorati et al., 2022). Among the list of priority pollutant metals, Cu, Cr, Ni, Pb, and Zn were detected in all three sites at concentrations above the permissible limit, with the exception of Cu at SS and CVS. The environmental hazards of these metals, particularly on fragile ecosystems, cannot be overemphasized. Sensitive fauna and flora can be wiped out, thus giving rise to the evolution of some HM-resistant microorganisms (Ayangbenro and Babalola, 2017).

Generally, the level of pollutants detected across the sites is not unexpected. What is frightening is the significant threat posed to other ecosystems within their vicinity. In Nigeria, the widely used three main solid waste disposal processes are landfilling, open dumping, and open burning, which are not satisfactory technologies. Landfills, for instance, are poorly designed and poorly managed, creating several adverse environmental impacts such as wind-blown litter, attraction of vermin, and formation of leachate (Ishaq et al., 2022). Leachate could percolate through the waste, contaminating ground and surface water bodies and causing damage to the ecosystem by entering into the food chain (Kamboj et al., 2020; Ishaq et al., 2022). Therefore, the level of pollutants in the dumpsites under investigation is a latent poison to water bodies and soils within their vicinity.

The microbial community structures in the dumpsites were shaped by inherent environmental factors. The impact of organic wastes on CS and SS soils would also be consistent with the much higher levels of PAHs, TOM, and other organic compounds (total)

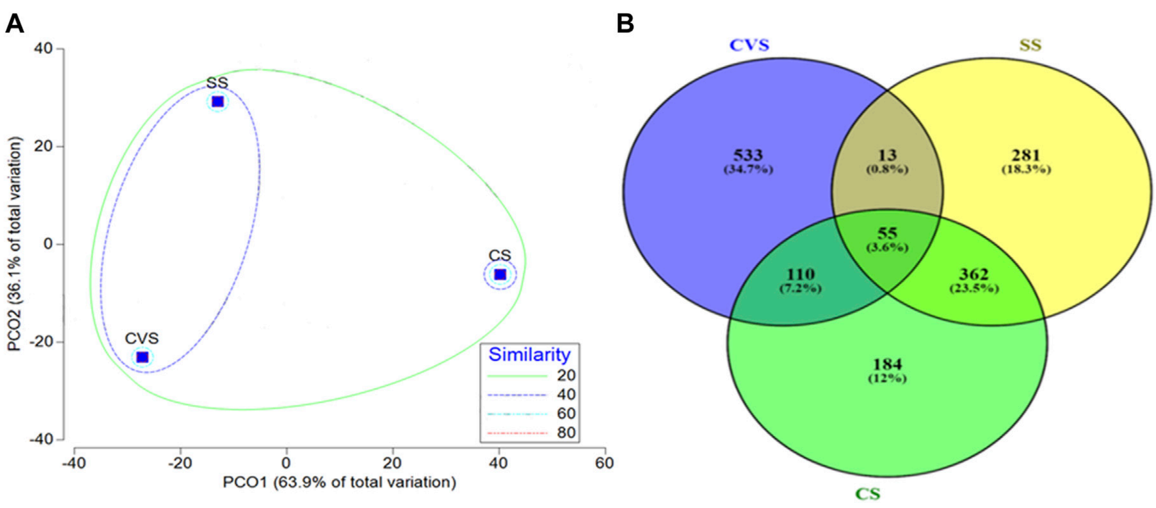

FIGURE 8
Principal component ordination of bacterial communities in CS, CVS, and SS dumpsite soils, with contours indicating the percent resemblance between samples **(A)** and Venn diagram displaying distribution of common and unique OTUs (genus) among the dumpsites **(B)**.

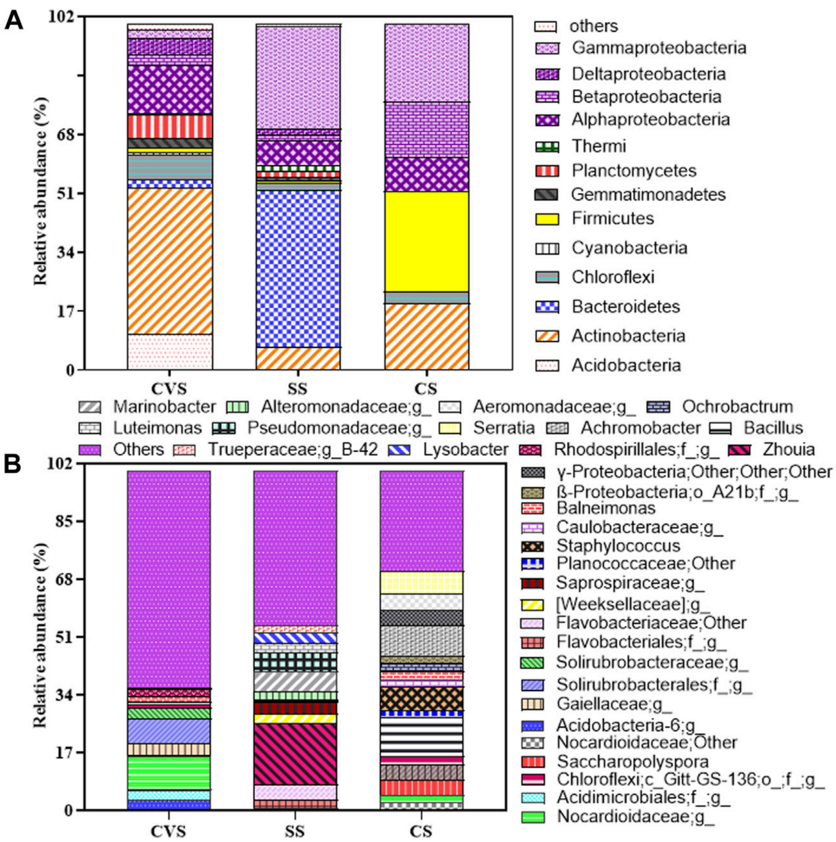

FIGURE 9
Relative abundance of major bacterial genera **(A)** and phyla (>0.5% of a given library; **(B)** in CS, CVS, and SS dumpsite soils. Proteobacteria is subdivided by class unlike other phyla and are all color-coded purple. Taxonomic abundance <0.5% at the phylum level was classified as others. Abundance is the fraction of the total sequences in a given library represented by a single OTU.

at these two sites compared to CVS. The potential impacts of anthropogenic pollutants on microbial communities in the dumpsites were reflected in variations in alpha diversity. In comparison to CVS, CS and SS microbial communities had significantly low species richness as well as species evenness. For instance, Shannon's diversity index was highest in CVS, where POP levels were significantly low. Therefore, it follows that the reduction in diversity observed in both CS and SS is a direct function of the amounts of POPs and other organic wastes inputted into the environment. Data obtained from β-diversity suggest that neither HMs nor POPs alone were the major limiting factors for bacterial activities. However, the combination of both variables had a profound impact on bacterial communities. Multiple stressors, such as HMs and POPs, elicit a more complex response from microbial community depending on the type of stressor, soil properties, and other factors (Azarbad et al., 2015). While PCO analysis could not resolve the abiotic factors driving the microbial community, CCA readily did. For example, most of the POPs and HMs were the environmental factors driving the population of Firmicutes and β-Proteobacteria in CS, in which the former was the dominant taxon in that community. Therefore, CCA determined the correlation between the environmental factors and the bacterial community in each of these sites. Such information is necessary for providing baseline knowledge of contaminated sites, which is essential for designing bioremediation strategies in polluted systems (Saibu et al., 2023).

Metagenomics data emanating from several laboratories have often declared Bacteroidetes and Proteobacteria as predominant bacterial taxonomic groups in landfill sites (Wang et al., 2017; Zainun and Samarani, 2018; Sandhu et al., 2022), a trend that is consistent with the results obtained in this study, except for CVS. Generally, the three dumpsites investigated exhibited stark variations in terms of microbial community structure and dominance. However, the configuration of the microbial communities in CS and SS was similar in terms of the relatively high population of α-Proteobacteria, in contrast to CVS, where the α-Proteobacteria was a minor constituent. In the less polluted CVS site, β-Proteobacteria, next to γ-Proteobacteria among the Proteobacteria phylum, predominates, suggesting the vulnerability of this class to POPs and not necessarily HMs. The resilience of α-Proteobacteria in POP-burdened environmental systems noted in this study is consistent with our recent findings (Saibu et al., 2023) and those of Obi et al. (2016). Similar resilience is also observed among the δ-Proteobacteria, a taxon that was not found in the CVS soil. In addition, the organisms in this class are better adapted to POPs and perhaps are a major player in the degradation of these pollutants in both CS and SS since they were notably absent in CVS. It is somewhat amazing that the community conformation of the dumpsites is not only the dominance of Actinobacteria and Bacteroidetes in CVS and SS, respectively, as against the much-expected Proteobacteria taxon, but the decrease in the members of the γ-Proteobacteria class in the SS soil in spite of the huge input of organic pollutants. Organisms in this taxonomic group play fundamental roles in nutrient cycling and are widely known for their metabolic versatilities which are variously exploited for bioremediation of polluted systems (Vinas et al., 2005; Horel et al., 2015; Obi et al., 2016; Vikram et al., 2016). Therefore, it is expected that they would constitute a dominant population in SS at a threshold much higher than that of soil obtained from CVS. Notwithstanding the level of γ-Proteobacteria in SS, the dominance of the genus *Marinobacter* is a vindication of the existence of organisms with the capacity to metabolize an extensive spectrum of xenobiotic pollutants, cementing their roles in nutrient cycling in that ecosystem (Horel et al., 2015). This is more so because *Marinobacter* is an organism in the family Pseudomonadaceae which houses *Pseudomonas*, and both organisms are not represented in either CVS or CS. Just like *Pseudomonas, Marinobacter* is a key hydrocarbon degrader that has become recognized as obligate hydrocarbonoclastic bacteria and a prolific biosurfactant producer (Raddadi et al., 2017).

These observations further show that there were significant variations in microbial community structure associated with soil contamination that could lead to shifts in pathways of fundamental biogeochemical processes (Bissett et al., 2013). Therefore, speciation and the concentration of (in)organic pollutants notwithstanding, the primary populations in CVS including Acidobacteria, Chloroflexi, Planctomycetes, Gemmatimonadetes, and Cyanobacteria, which were either absent or present at very low abundance in other soils, are phyla that are typically abundant in healthy soils (Xu et al., 2014). Some of these bacteria, like Proteobacteria, also play considerable roles in global carbon and nitrogen cycles. Cyanobacteria, for instance, through their metabolic proficiency, embodied a potentially notable pathway for carbon turnover, aeration of the environment, and direct nitrogen uptake through non-symbiotic nitrogen fixation (Karlson et al., 2015; Adam et al., 2016). This important taxon, though a minority member of the CVS community, is conspicuously absent from the rest of the sites.

In the case of *Actinobacteria,* its predominance in CVS may be connected with elevated concentrations of Al and Fe and in the sum total of HMs, which was highest at this site. This phylum is dominated by organisms of the order Solirubrobacterales, which, according to Seki et al. (2015), consists of three families, three genera, and eleven species. These organisms showed a propensity for soils with reduced carbon/nutrient availability (Shange et al., 2012) and are also widespread with high abundance in HM-polluted soils (Navarro-Noya et al., 2010; Gołębiewski et al., 2014; Yan et al., 2016; Salam and Varma, 2019). Recently, Goswami et al. (2023) reported a positive correlation between this taxon and Zn and Pb concentration. Interestingly, the group has been identified as an indicative taxon in HM-contaminated mining and agricultural soils (Huang et al., 2021) and similarly demonstrated to associate with metal-accumulating plants and are easily enriched in HM-supplemented agricultural soils (Wu et al., 2021; Kuo et al., 2018). In addition to Actinobacteria, other phyla encountered in the CVS soil including Acidobacteria, Gemmatimonadetes, and Chloroflexi readily suggest their potential importance in metal transformation and sequestration. This finding is supported by those of previous studies, which revealed their strong tolerance to HMs (Macdonald et al., 2011; Li et al., 2015; Girardot et al., 2020). Unfortunately, little is known about the ecology and metabolism of *Gemmatimonadetes*. However, the presence and increased abundance, together with those taxonomic groups, can suggest their indispensability and essential functions in the contaminated soils.

Another interesting observation in the community structure of the dumpsites is the relative abundance of the Firmicutes in CS at a proportion that was nearly 20 times greater than that of the population found in other sites, a pattern similar to the archaeal domain. This phylum is essentially dominated by the genus *Bacillus*, and the unabated incineration activity at the site may have accounted for their overriding success. *Bacillus* are more resilient and better adapted to harsh environmental conditions due to their ability to produce spores that are impervious to heat, radiation, and chemicals, while in the case of archaea, the lipid bilayer cell membrane enables them to function at high temperatures (Koga, 2012; Bruns, 2021). In fact, *Bacillus* is among the most reported bacteria in soil possessing much higher tolerance to organic pollutants and HMs, which, in this study, exhibited a positive correlation with Ni. Incidentally, this genus emerged as the most dominant when bacterial communities of food waste co-composting degradation of PCDD/F-contaminated soil were investigated (Huang et al., 2019). A connection between dioxin degradation and the genus *Bacillus* was also established by Mahfouz et al. (2020). These authors reported that bacterial species belonging to *Bacillus* were randomly distributed in soils highly polluted with PCDD/Fs and unequivocally demonstrated the abilities of several species of *Bacillus* to degrade 2,3,7,8-tetrachlorodibenzo-*p*-dioxin.

Other genera encountered in CS at varying proportions that are either effectively absent or diminutive in other sites were *Serratia, Staphylococcus, Achromobacter*, and *Ochrobactrum*. Interestingly, two bacterial species belonging to the genera *Serratia* and *Bacillus* which were unambiguously demonstrated to utilize dibenzofuran, 2,8-dichlorodibenzofuran, and 2,7-dibenzo-*p*-dioxin as the sole sources of carbon and energy were obtained from this site (Saibu et al., 2020). In a related study, we also reported the successful adaptation of both taxa to dioxin-contaminated soil and touted their potential use as bioindicators of dioxin pollution (Saibu et al., 2023). Interestingly, CS was the only site where dioxin was detected, which may likely be responsible for the populations of the encountered genera. Therefore, there is no uncertainty regarding the dioxin metabolic capabilities of these taxa.

Among the genera of organisms identified in this study, the highest percentage was credited to *Zhouia*. In addition, this genus was the most dominant in the SS site, where Bacteroidetes also dominated. This relatively novel genus is yet to be explored for its environmental benefits. The organism in this taxon was first isolated from marine sediment and mangrove soil (Liu et al., 2006; Hong et al., 2023) and was among the tropical mangrove sediment communities demonstrated to readily degrade *Enteromorpha prolifera* (Zhao and Ruan, 2011). Similarly, with regard to the sharp increase in population of the genus observed in treated petroleum hydrocarbon-contaminated soil, Rahmeh et al. (2021) attributed this surge to the less complex ring structure derivatives resulting from the degradation of aromatic compounds with complex structures. A critical evaluation of the metagenome data clearly showed that among the population of bacterial phyla reported across the sites, none came close to the Bacteroidetes. The phylum is essentially made up of the family Flavobacteriaceae, of which *Zhouia* is a representative. The potential of Bacteroidetes in soil ecosystems has been accredited to their ability to degrade complex organic compounds (McBride et al., 2014) and the secretion of carbohydrate-active enzymes that specifically target the highly wide-ranging polysaccharides in soil (Larsbrink and Mckee, 2020). Members of this phylum have also been implicated in the degradation of chemical weapons (Thouin et al., 2019). According to these authors, soil historically contaminated by the burning of chemical ammunition was dominated by Bacteroidetes in addition to Proteobacteria and Acidobacteria. Therefore, the huge number of members of this group of organisms in SS may not be unconnected with these metabolic properties coupled with their capacity to fix nitrogen non-symbiotically. Perhaps, we are beginning to understand the functional roles of the genus Zhouia in polluted systems, where they play important, albeit unknown, roles in pollutant degradation and/or other community processes.

5 Conclusion

We have provided an in-depth insight into the potential impacts that POPs may exert on soil bacterial communities. The MiSeq methodology obtained a high resolution of the microbial community profile in dumpsites. The communities of two of the sites (CS and SS) strongly impacted by POPs were significantly different from the relatively less-impacted site (CVS), which is the obvious deletion of OTUs (Nitrospirae and Cyanobacteria) involved in key biogeochemical processes (nitrogen and carbon fixation). In addition, further understanding of the bacterial communities that may arbitrate PAHs and degradation of other POPs in Lagos dumpsites was given. There were strong indications in the types of potential degraders apparently enriched in the soil compared to those identified in other soil ecosystems impacted by POPs. For instance, γ- and α-Proteobacteria were indicated as key PAH/POP phylotypes responsive to the high concentration of pollutants inherent in the CS site, which, interestingly, also had the highest level of contamination. In contrast, the less PAH/POP-heavily impacted soil (SS), the organisms with the potential metabolism of these pollutants shifted to the α-Proteobacteria taxon. Thus, Proteobacteria were a significant group potentially associated with PAHs/POPs, but the classes varied depending upon the concentration of these pollutants and possibly other factors such as HMs and prevailing environmental parameters. Furthermore, the evident increase in populations of *Bacillus* and *Serratia* in CS, which were both absent in SS and CVS sites, highlighted their superior dioxin metabolic functionalities in addition to other POPs, consistent with findings from our previous study (Saibu et al., 2020). Since dioxin was only detected at the CS site, these organisms may have been particularly enriched by the presence of this pollutant.

Overall, our investigation provides a detailed report on the abundance and diversity of bacteria in Lagos municipal dumpsites, revealing the impacts of waste inputs on the soil microflora and the important environmental factors driving the microbial communities in the ecosystem. The bacterial species richness in this study shows the complexity of anthropogenic activities in these ecosystems and calls for further research on *archaea* and *eukarya* diversities as well as investigation of the functional dynamics of the microorganisms that have been hypothesized in this study.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: NCBI Sequence Read Archive, accession number PRJNA540227.

Author contributions

SS: data curation, formal analysis, investigation, methodology, validation, and writing–original draft. SA: conceptualization, data curation, project administration, supervision, and writing–review and editing. GO: supervision and writing–review and editing. DR: funding acquisition, project administration, supervision, and writing–review and editing.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

Acknowledgments

This study was financially supported partially by the Welch foundation award number (E-2011-20190330) and Biological and Environmental Research Science Focus Area grant: U.S. Department of Energy (Grant No. DE-AC52-06NA25396).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fenvs.2023.1304033/full#supplementary-material

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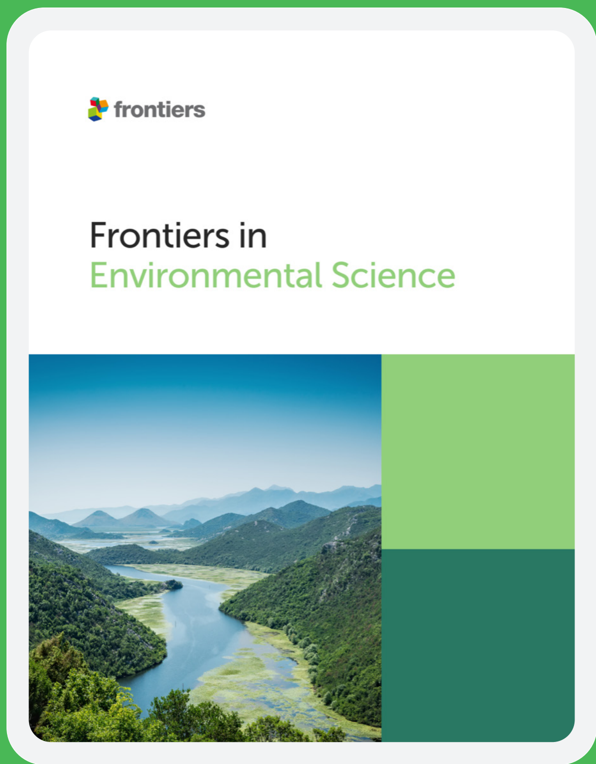

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