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Biochar; an effective factor in improving phytoremediation of metal(iod)s in polluted sites

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Phytoremediation is one of the cheapest and most widely used technologies for stabilizing and extracting pollutants from contaminated sites. Recently, a variety of solutions, such as the use of different elements, compost, nanoparticles, microorganisms, etc., have been explored for improving and accelerating the phytoremediation process. Biochar has also gained attention for its affordability, abundance, ability to improve soil structure and plant morpho-physiology and biochemistry, lack of environmental hazards, etc. As a first step, this study aimed to provide an overview of biochar's properties, and operation by identifying the method of production and examining the differences between different types of biochar. Following that, by examining various factors that pollute the environment, the influence of different types of biochar on phytoremediation efficiency was explored. Also, in this study, an attempt has been made to examine the effect of the combination of biochar with other factors in improving the phytoremediation of pollutants, as well as the use of the residues of phytoremediation for the production of biochar, so that future research can be planned based on the results obtained.

KEYWORDS

pollutants, biochar, phytoremediation, environment, stabilization, extraction

1 Introduction

Biochar is a stable carbon-rich product that is made by decomposing animal or plant residues under anaerobic (or low oxygen) and heat conditions (Lehmann and Joseph, 2015; Nguyen et al., 2017; Tomczyk et al., 2020). This porous material, which contains more than 60% carbon, along with oil and gas, is one of the products of biomass decomposition by heat (Lebrun et al., 2021a), and based on research, thousands-year-old biochar particles have been found in the soil of Amazon's tropical and humid regions (Narayanan and Ma, 2022). Since it has a special properties, this cheap material has a very high application and efficiency in improving different soil ecosystems (Ghosh and Maiti, 2021). In general, Biochar is obtained from the decomposition of various biomass such as urban waste, industrial waste, agricultural waste (sugarcane, sugar beet, corn, weeds, etc.), poultry litter, animal manure, forestry waste (waste of trees, wood, bamboo, etc.) and sludge (Khiari et al., 2019; Kiran and Prasad, 2019; Maroušek et al., 2019; Albalasmeh et al., 2020; Simiele et al., 2020) (Figure 1).

To prepare Biochar, biomass is heated to between 400°C and 700°C under limited oxygen conditions (decomposition by pyrolysis method) (Yavari et al., 2015; Simiele et al., 2020), and biochar's physicochemical properties are directly related to its raw material and pyrolysis conditions (Benavente et al., 2018; Albalasmeh et al., 2020) (Table 1), and to

the extent that biochar derived from animal manure has more nutrients than biochar derived from plants (Narayanan and Ma, 2022). Researchers also found that changing the temperature of pyrolysis from 400°C to 500°C increases the content (mg/kg) of magnesium (Mg), potassium (K), phosphate (P), Carbon (C), nitrogen (N), oxygen (O), hydrogen (H), and calcium (Ca) in the biochar produced, and this increase in temperature also increases the mineralization of raw materials (Ma et al., 2015; Brassard et al., 2017; Ferjani et al., 2019). Additionally, the cost of raw materials, labor costs, and production technologies affect the cost of biochar production, and the average cost varies from one hundred to five thousand US dollars per ton in different countries (Maroušek et al., 2019) (Figure 2).

Biochar can be widely used in agriculture and the environment due to its unique characteristics, which include its ability to improve soil fertility and adsorption of various pollutants (Xie et al., 2015; Zanganeh et al., 2022). The special characteristics of biochar include high porosity, high cation exchange capacity (CEC), alkaline pH,



Biochar type	N (%)	C (%)	H (%)	O (%)	рΗ	Ash content (%)	EC (dS/m)	Moisture content (%)
Bamboo	0.57	76.69	1.59	21.15	10.80	5.51	1.54	-
Wood apple fruit shell	0.12	81.18	2.12	16.58	-	3.41	-	4.17
Maize residue	0.93	42.94	-	-	8.54	36.7	3.34	-
Rice husk	0.51	47.13	-	-	8.75	31	-	-
Loblolly pine chips	0.65	72.6	0.77	21.3	-	4.7	-	-
Sewage sludge	4.9	33.1	4.1	-	6.7	46.9	0.91	-
Red spruce pellets	0.21	84.0	1.5	-	9.1	4.7	0.39	2.6
Grapevine pruning residues	0.53	75.5	1.3	-	9.9	9.9	2.23	4.5
Hydrochar from urban pruning residues	1.7	61.5	6.2	-	6.6	12.5	1.02	7.0
Paper mill waste	0.07	19.2	0.50	9.40	9.48	57.5	0.30	-
Bio-oil	5.6	27.7	1.2	5.9	-	60.3	-	-

TABLE 1 Characteristics of biochars produced from different raw materials.

The results are taken from the following references Wei et al. (2015), Kim et al. (2016), Kupryianchyk et al. (2016), Zhang et al. (2016), Li et al. (2019a), Regkouzas and Diamadopoulos (2019), Taskin et al. (2019), Kumar et al. (2021), and Liu et al. (2021).



low density, abundant functional groups (aromatic and heterocyclic carbons such as C=N, COOH, OH), high water holding capacity, high specific surface area and adsorption capacity (Czekała et al., 2016; Du et al., 2019a; Simiele et al., 2020; Tu et al., 2020; Li et al., 2021a; Gonzaga et al., 2022). According to Ahmad et al., biochar is an alkaline material with pH 8-11, a specific surface area of $10-1,400 \text{ m}^2 \cdot \text{g}^{-1}$, a porosity of $0-1.32 \text{ cm}^3 \cdot \text{g}^{-1}$, and CEC of 25–485 cmol (þ) kg⁻¹ (Ahmad et al., 2014). Furthermore, while biochar has a very low mineralization rate, its particle size affects its interactions with soil, changing its porosity and ions adsorption and exchange capacity (Williams et al., 2019; Li et al., 2021a).

Biochar is a low-cost and environmentally friendly amendment that can cause long-term positive effects in soil ecosystems by improving biogeochemical processes and physicochemical properties (Gul et al., 2015; Zhang et al., 2018). Biochar's higher content of polycyclic aromatic hydrocarbons (PAHs) than other organic carbons makes it more compatible with soils and contributes to climate change mitigation through improving soil ventilation, carbon sequestration, and reduction of greenhouse gases such as Nitrous oxide (N2O) (Rizwan et al., 2016; Kolton et al., 2017; Lian and Xing, 2017; Kiran and Prasad, 2019). Biochar increases soil fertility by improving water and nutrient availability and increasing the content of organic matter (Lebrun et al., 2018; Lebrun et al., 2021a; Ali et al., 2021), and by softening soil, it improves water retention capacity, reduces nutrient leaching, and improves nutrient cycling in soil (Zhang et al., 2018; Li et al., 2021a; Gholizadeh and Hu, 2021). Additionally, biochar in soil regulates pH, CEC, EC, and ash content as well as increasing catalase, urease, and dehydrogenase activity, and increasing microbial activities, especially indigenous microbes (Hossain et al., 2010; Binti Ab Aziz et al., 2015; Chen et al., 2017; Zhang et al., 2019b; Gong et al., 2021) (Figure 2).

Furthermore, biochar increases plant growth, biomass, and yield by improving soil conditions, improving water quality, providing nutrients, increasing root colonization by microbes, and improving the propagation and surface of roots (Paz-Ferreiro et al., 2014; Doan et al., 2015; Rees et al., 2016; Herman and Resigia, 2018; Zhang et al., 2019a; Zhou et al., 2020). Nonetheless, dissolved organic matter in biochar (aqueous biochar extract) contains a considerable amount of organic compounds, including organic acids with low molecular weight, humic organic molecules, and complex sugars, which have significant effects on plant growth (Lou et al., 2016; Bian et al., 2018). Enriching biochar with elements such as silicon, iron, selenium, calcium, etc., in addition to increasing biochar availability in soil, can improve the positive properties of biochar (Rajkovich et al., 2012; Simiele et al., 2020), further, other methods for modifying biochar have been considered, including ultraviolet (UV) radiation, which increases the specific surface area of biochar and can introduce oxygen-containing functional groups (Peng et al., 2019). Several studies have been conducted in recent years on the pyrolysis of lignocellulosic wastes to produce biochar, and if this technology becomes common, it can be seen as a promising method for reducing greenhouse gas emissions and other environmental pollution in addition to producing added value (Liu et al., 2015b; Du et al., 2019a; Du et al., 2019b). In this study, various pollutants in the soil ecosystem were investigated, as well as how phytoremediation reduces these pollutants, and how biochar affect in the process of phytoremediation.

2 Soil pollution problem

In general, HMs consist of elements with a high atomic mass and weight, and a specific density exceeding 5 g/cm³, and are classified into two groups, essentials, and non-essentials (Buha et al., 2014; Farooq et al., 2016). Essential elements (micronutrients) are necessary in very small amounts for plants and animals, such as

Heavy metals	Fish	Meat	Fruit	Vegetable	Cereals	Total intake
Cd	0.58	1.54	0.41	19.63	7.32	29.48
As	7.84	5.54	2.35	8.23	10.07	34.02
Pb	9.91	17.54	3.86	41.24	22.60	95.15
Hg	4.68	1.86	0.58	1.23	7.27	15.63
Cr	38.50	60.14	5.36	54.85	46.67	205.52

TABLE 2 Amount of heavy metals intake through food consumption (µg/day).

The results are taken from Liang et al. (2019).

copper (Cu), iron (Fe), manganese (Mn), cobalt (Co), zinc (Zn), and chromium (Cr), which in high concentrations can cause soil pollution and harm to organisms (Naja and Volesky, 2017; Awad et al., 2021; Rathika et al., 2021); However, non-essential elements [including cadmium (Cd), mercury, (Hg) arsenic (As), and lead (Pb)] are very dangerous and can lead to severe soil contamination (Huang et al., 2012; Rathika et al., 2021; Haider et al., 2022; Boorboori, 2023). In contrast to organic pollutants, HMs are resistant to degradation and remain in soil and sediment for a long time, for instance, it has been reported that HMs contamination can persist in soil for more than 5,000 years (Lu et al., 2015; Gong et al., 2018b; Li et al., 2021b), as a result, long-term persistence in polluted sites facilitates their entry into the food cycle and ultimately causes severe environmental damage (Xu et al., 2018b; Zhao et al., 2019; Zanganeh et al., 2022) (Table 2).

3 Phytoremediation of metal(iod)s

Considering the growing trend of combined pollution in soil, it seems necessary to find strategies for cleaning contaminated soil (Zhen et al., 2019; Zhang et al., 2021; Fernández-Braña et al., 2023). There have been several physical and chemical methods also are available for the remediation of soils contaminated with HMs, including encapsulation, soil washing, freezing, electrokinetic remediation, membrane filtration, reverse osmosis (RO), 3 electrodialytic remediation (EDR), landfilling, nanomaterials, immobilization, and surface capping (Barati et al., 2018; Liu et al., 2018; Kiran and Prasad, 2019; Shah and Daverey, 2020). However, all the aforementioned techniques have several limitations, including high economic costs, creating secondary pollution for the environment, inapplicability in wide areas, high energy consumption, and extensive chemical needs (Paz-Alberto and Sigua, 2013; Gkorezis et al., 2016; Lin et al., 2019; Yrjälä and Lopez-Echartea, 2021), therefore, finding cheaper and more effective methods can be a remedial to reduce pollution in soils contaminated with HMs.

During the past few decades, plants have been used to decompose, convert, stabilize, or remove pollutants in sediments, underground and surface water, soil, and the atmosphere (Reddy and Chirakkara, 2013; Ratnasari et al., 2020), and this green biological technique based on hyperaccumulators or non-hyperaccumulators plant species and their associated microorganisms is called phytoremediation (Ma et al., 2016; Jha et al., 2017; Rathika et al., 2021; Boorboori and Zhang, 2022a). The low cost of phytoremediation (about 25–100 US dollars per ton of

soil) makes it a cost-effective technology that, in addition to being environmentally friendly, is highly effective and simple to use (Wang et al., 2017a; Gao et al., 2018; Logeshwaran et al., 2018). As a result of its general acceptance, no disruption to soil fertility and biodiversity, prevention of soil erosion, carbon sequestration, extensive application, and use in sites with multiple types of pollutants, this method has become increasingly subject for potted and field studies (Lebrun et al., 2018; Cárdenas-Aguiar et al., 2020; Zanganeh et al., 2022).

Phytoremediation which can be used alone or in conjunction with other soil remediation methods improves soils contaminated with organic and inorganic compounds by various routes such as extraction, evaporation, transpiration, stabilization, Rhizofiltration, and transformation (Forján et al., 2018; Fancello et al., 2019; Shah and Daverey, 2020) (Table 3). Plant extraction and plant stabilization are the most important phytoremediation processes (Mahar et al., 2016), in the plant extraction method, environmental pollution is absorbed into the roots and transferred to the plant's aerial organs, which are then harvested and removed (Lebrun et al., 2021a). Although plant extraction offers many advantages, including safety and high efficiency, it faces a problem in disposing of contaminated biomass, which incurs additional costs (Boominathan et al., 2004; Lebrun et al., 2021a).

As the process of extracting plants from highly polluted lands takes tens of years, and because falling leaves and branches also are responsible for returning pollution to the ground, plant stabilization may be a suitable alternative in the contaminated environment (Lucchini et al., 2014; Laghlimi et al., 2015). In this method, which aims to reduce the mobility and bioavailability of pollutants, resistant plants with extensive root systems can be used along with soil amendment compounds, provided that the plants used do not transfer the pollutants from the roots to the aerial organs, but stabilize it on the surface and inside the roots through the complexation with root secretions (Kumpiene et al., 2008; Acosta et al., 2018; Lebrun et al., 2021a). Since HMs do not decompose, plant stabilization is more effective for them and prevents them from transferring to the surrounding environments, and the efficiency of this method depends on several factors, including the hydrological regime, pollution type, soil geochemistry, etc., (Chehregani et al., 2009; Forján et al., 2018; Kiran and Prasad, 2019). Despite all the benefits of phytoremediation, it also has weaknesses, including a long remediation period, non-implementation at high pollution sites, production of contaminated biomass, secondary pollution, slow growth of plants, limited plant resistance to unfavorable physical and chemical conditions found in polluted soils, and plants' limited

Heavy metals	Kinds of plant	Uptake of heavy metals (mg/kg)
Cd	Pteris vittata L.	20.67
	Thlaspi caerulescens J.Presl and C.Presl synonym of Noccaea caerulescens (J.Presl and C.Presl) F.K.Mey.	263
	Rorippa globosa (Turcz. ex Fisch. and C.A.Mey.)	>100
	Deschampsia cespitosa L. P.Beauv.	236.2
	Prosopis laevigata	8,176
	Phytolacca americana L.	10,700
	D. cespitosa L. P. Beauv.	966.5-3,614
As	Corrigiola telephiifolia Pourr.	2,110
	Pteris biaurita L.	2,000
	Pteris ryukyuensis Tagawa	3,647
	Pteris quadriaurita Retz.	2,900
	P. vittata L.	8,331
Hg	P. vittata L.	91.975
	Haumaniastrum katangense	8,356
	Aeollanthus biformifolius	13,700
	Eleocharis acicularis	20,200
	Cicer arietinum L.	0.2
	Poa pratensis L.	2.74
	Festuca rubra L.	3.17
	Silene vulgaris	4.25
	Rumex induratus s Boiss. and Reut.	6.45
	Marrubium vulgare L. (Moench) Garcke	13.8
	Achillea millefolium L.	18.275
РЬ	Euphorbia cheiradenia Boiss. and Hohen	1,138
	D. cespitosa P.Beauv.	966.5
	Medicago sativa L.	43,300
Ni	Isatis pinnatiloba P.H.Davis	1,441
	Alyssum serpyllifolium Desf.	10,000
	Alyssum heldreichii Hausskn	11,800
	Alyssum bertolonii Desv. synonym of Odontarrhena bertolonii (Desv.) Jord. and Fourr.	10,900
Cr	P. vittata L.	20,675

TABLE 3 The name of hyperaccumulator plants for remediating soils contaminated with heavy metals.

The results are taken from Kanwar et al. (2020).

capacity to accumulate pollution (Yang et al., 2017b; Cárdenas-Aguiar et al., 2020; Gu et al., 2020; Manori et al., 2021).

4 Phytoremediation efficiency, plant type, and other factors

In the phytoremediation process, plants are used to remove and stabilization of soil pollution without negatively affecting the structure, quality, and biological activities of the soil, so it is necessary to select plants that are not only tolerant of pollution but also fast-growing and provide maximum root surface (Claus et al., 2007; Barati et al., 2018; Li et al., 2021b). Also, phytoremediation can be used as a first step to creating wildlife habitats, which will only be possible if native plant species are used that are well adapted to the local climate and soil (Roohi et al., 2020), because native plants will have better growth, survival, and reproduction under environmental stress than non-native plants

(Barati et al., 2018) (Table 3). There has been a high degree of focus in recent years on woody plant species in phytoremediation because these plant species, in addition to concentrating soil pollution in their tissues, can be used for various types of pollution as well as be economically viable due to their efficiency in various industries (Pandey et al., 2009; Fancello et al., 2019). Plants belonging to the Salicaceae family (Salix and Picea) are among the most important woody species used in phytoremediation (Evlard et al., 2014), which are suitable for growing in abandoned agricultural lands and polluted marginal lands because of their rapid growth, extensive root system, high biomass production rate, ability to support microbial communities, high transpiration rate, capability to absorb nutrients, tolerance to a wide range of organic and inorganic pollutants, high antioxidant activities, and easy reproduction (Wu et al., 2010; Cloutier-Hurteau et al., 2014; Salam et al., 2019; Lebrun et al., 2021b; Li et al., 2021b).

Among other woody plants, Populus x euramericana clones could potentially be used for the phytoremediation of polluted lands due to their high growth, biomass, and ability to extract various types of HMs (Borghi et al., 2007; Redovniković et al., 2017; Simiele et al., 2020). In contrast, herbaceous species with extensive fibrous roots also are very beneficial for remediating polluted soils (APHA, 2003), especially those species known as hyperaccumulators, which are capable of absorbing large amounts of HMs and transferring them to their aerial organs, such as Sedum alfredii and Thlaspi caerulescens (Lebrun et al., 2021b). Symphytum officinale also is a perennial herbaceous plant that, due to its high biomass, rapid growth, and high accumulation of HMs, can be widely spread across large areas and improve phytoremediation (Wang et al., 2017b; Xiao et al., 2018). However, one of the most ideal plants for polluted environments is Solanum nigrum, which is relatively easy to adapt to different conditions and highly tolerant (Liang et al., 2017). This plant has a strong antioxidant system and the ability to produce macrophage proteins and various organic acids in different organs involved in the transport and accumulation of HMs, which makes it capable of extracting several types of HMs (such as Cr, As, Pb, Cd, and Ni) as well as carbon tetrachloride, polycyclic aromatic hydrocarbons, etc., (Liang et al., 2017; Ur Rehman et al., 2017). Among other herbaceous plants with a high ability to heavy metals accumulate and phytoremediation are Beta vulgaris var. cicla and Potentilla griffithii Hook. f. var. velutina Card (Ali et al., 2013; Gu et al., 2020).

The use of economic crops such as rice, cotton, sunflower, etc., which have high environmental compatibility and biomass production potential, has attracted the attention of researchers for the phytoremediation of contaminated land on a large scale (Bhardwaj et al., 2014; Lebrun et al., 2021b; Gusmini et al., 2021). Alfalfa (Medicago sativa L.) is another crop that can be used to remediate contaminated soils, as it has high yields, deep roots, and drought tolerance that distinguish it from other plants used in the phytoremediation process (Agegnehu et al., 2016). Another crop suitable for phytoremediation is corn, which, in addition to its economic importance, can provide a biological indicator of pollution (Khan et al., 2018). With its fibrous root system, global availability, ability to grow in diverse climates, and fast germination rate, corn has special advantages over other crops for phytoremediation (Khan et al., 2018; Lebrun et al., 2021b). In addition, there has been a recent focus on using a periodic system of oil crops to improve polluted soils, which has economic benefits as well as the effect on crop production (Yang et al., 2017b; Gong et al., 2018b).

On the other hand, in the stabilization process, certain plants are used in polluted soils that are not capable of transferring pollutants to their aerial organs, but in addition to reducing the bioavailability and mobility of pollutants in soil, they prevent them from entering underground water and the food chain (Fancello et al., 2019; Roohi et al., 2020). Therefore, plants should be selected that are capable of stabilizing pollutants on the epidermis of their roots or rhizosphere, Among the most important of these plants are Euphorbia Pithyulentum, Spartina anglica, Solanum lycopersicum, Juncus acutus, Lycopersicon esculentum, Phragmites australis, Vallisneria americana, and Bromus tomentellus (Fancello et al., 2019; Zhen et al., 2019; Roohi et al., 2020). In addition, plants of Salicaceae family are also potent stabilizers due to their extensive root systems, abundant biomass, high transpiration rates, ability to grow in polluted environments, high water-use efficiency, and high contaminant accumulation at the roots (Evlard et al., 2014; Redovniković et al., 2017; Lebrun et al., 2018).

In addition to the selection of suitable plants, phytoremediation's efficiency is highly influenced by the chemical and physical properties of soil, including the quantity and nature of organic matter and nutrients, mineralogy, texture, structure, and pH (Kayser et al., 2000; Zhao et al., 2019). Therefore, modifying polluted environments with mineral and organic modifiers improves phytoremediation efficiency (Cheng et al., 2020). Several studies have demonstrated that a wide range of microbial communities, including bacteria, algae, and fungi, enhance the effectiveness of phytoremediation by influencing the soil's physical and chemical properties (Koul and Taak, 2018; Cui et al., 2020). A microbial community produces different types of enzymes to regulate degradation pathways through various oxidation reactions that result in the production of CO2 and H₂O (Ali et al., 2021). Additionally, microbial communities can improve the morpho-physiological structures of plants through ACC-deaminase activity, phosphorus dissolution, siderophores production, and ethylene stress reduction, thereby increasing phytoremediation efficiency (Liu et al., 2015a; Fatima et al., 2018; Afzal et al., 2020). The microbes also play a major role in changing HMs' chemical behavior in the rhizosphere, and in addition to influencing plant growth during phytoremediation, that effect at improving HMs extraction, bioavailability, and accumulation in plants as well (Li et al., 2019b; Fancello et al., 2019; Farias et al., 2020).

The use of organic and inorganic modifiers in phytoremediation has gained popularity in recent years (Lebrun et al., 2021a; Manori et al., 2021). Several studies have shown that the addition of these soil modifiers through increasing the stabilization of HMs in the soil, reducing the toxicity of HMs in plants, increasing the cation exchange capacity (CEC) of the soil, improving soil structure, enhancing soil ventilation, increasing nutrient availability and content, and improving soil water holding capacity, increases the efficiency of phytoremediation in polluted environments (Bashir et al., 2018; Roohi et al., 2020; Lebrun et al., 2021a). Furthermore, these organic and inorganic modifiers increase phytoremediation efficiency by stimulating microorganisms' activities, increasing plant growth and biomass production (Clemente et al., 2012; Barati et al., 2018; Roohi et al., 2020; Boorboori and Zhang, 2022b). The



following are some of the most important modifiers used in recent years: poultry droppings, cow dung, biochar, rice husk, compost, technocell, steel slag, carbon materials (CMs), and nanomaterials (NMs) (Singh and Lee, 2016; Forján et al., 2018; Lebrun et al., 2021a; Deebika et al., 2021), and in the following, biochar's role in phytoremediation of HMs will be discussed in more detail.

5 Biochar and phytoremediation

Since contaminated soil is not an ideal environment for plant growth, phytoremediation is greatly reduced in efficiency (Fellet et al., 2014). Therefore, the use of modifiers like Biochar can, in addition to improving soil structure, promote the growth of plants used for phytoremediation (Simiele et al., 2020; Li et al., 2021b). Moreover, biochar has attracted researchers' attention due to its cost-effectiveness and ability to clean environments contaminated with organic and inorganic pollutants (Byrne et al., 2018; Ding et al., 2020). Phytoremediation can be improved with biochar due to its long-term stability compared to other organic amendments as well as its effective role in improving the soil's physical, chemical, and biological properties (Cárdenas-Aguiar et al., 2019; Ratnasari et al., 2020). By improving soil pH, carbon content, soil aeration, soil fertility, water retention capacity, microbial diversity, and plant growth, biochar is highly effective at cleaning environments contaminated with HMs (Forján et al., 2018; Gong et al., 2019; Deebika et al., 2021). Furthermore, Its high specific surface area, porous structure (macropores and micropores), cation exchange capacity, and active functional groups make biochar an effective tool for stabilizing soil pollution and reducing HMs bioavailability for organisms (Song et al., 2016; Yang et al., 2017a; Zhang et al., 2019a). It is important to note that all the benefits mentioned above depend on the particle size, intrinsic properties, application method, and the amount of biochar applied to the soil (Li et al., 2021a), and in the following, it will be discussed the details how biochar can improve the phytoremediation process of HMs (Figure 3).

Studies have shown that adding biochar to the culture medium can significantly increase the growth of plants, and this growth may be heterogeneous across different organs of the plant, but it generally results in a 10% increase in overall growth (Paz-Ferreiro et al., 2014; Burachevskaya et al., 2021). Biochar causes morphological changes in plant roots, as well as affects a plant's ability to absorb pollutants (Rees et al., 2016; Gu et al., 2020); however, the extent of reduction in pollutant absorption depends on the species of plants and the speciation characteristics of environmental pollutants (Gu et al., 2020). On the other hand, the cell walls of roots contain molecules such as pectin, proteins, and dissolved phosphates that stabilize pollutants in the root space and prevent their transfer to aerial organs, and biochar increases the efficiency of these physiological structures in plants' roots (Lebrun et al., 2017; Gu et al., 2020). Previous studies confirm that amended soils by biochar increase plant tolerance to environmental pollution by improving parameters such as chlorophyll pigment content, intercellular $\rm CO_2$ concentration, transpiration rate, photosynthetic rate, and stomatal conductance, as well as reducing the efficiency of HMs transport in plants (Farias et al., 2020; Li et al., 2021b; Lyu et al., 2021) (Figure 3).

According to a study conducted on *Brassica juncea* in a coppercontaminated environment, by increasing the rate of Holm oak biochar (BH), plant growth increased and Cu absorption decreased (Rodríguez-Vila et al., 2014). Furthermore, a study conducted on *Bidens pilosa* grown in Cd-contaminated soil found that pine needle biochar had a positive effect on root growth, and at the same time decreased chlorophyll concentrations and increased proline

Biochar feedstock and dose	Plant	Metal(iod)s	Effects on phytoremediation	References
Wood: 2.5%–5%	Lycopersicon esculentum	Cr, Mn, and Ni	Reduce exchangeable Cr, Ni, and Mn. Enhanced plant growth	Bandara et al. (2017)
Sewage sludge: 5% and 10%	Oryza sativa	As, Zn, Cd, Ni, Cr, Co, Pb, and Cu	Reduced pore water Pb, As, Ni, Cr, and Co owing to elevated soil pH. Mobilize Cd, Cu, and Zn	Khan et al. (2013)
Pruning residues and manure: 1.5%–3%	Anthyllis vulneraria	Ni, Cd, Ti, Zn, Cr, Pb, Cu, and Fe	Reduced water-extractable Zn, Cu, Cd, and Cr.Increased pH	Fellet et al. (2014)
Poultry manure and green waste	Brassica juncea	Cd, Pb, and Cu	Increased (353%) plant shoot dry biomass. Decreased Pb, Cd, and Cu accumulation in plants	Park et al. (2011)
Hardwoods: 20%	Miscanthus×giganteus	As	Improved pore water with As	Sun et al. (2018)
Miscanthus: 5% and 10%	Brassica napus	Cd, Zn, and Pb	Reduced metals bioavailability in shoot biomass	Bandara et al. (2017)
Oka, Ash, and Birch: 20%v/v	Lolium perenne L.	Pb and Cu	Reduced pore water-mediated Pb and Cu doses in shoots	Sun et al. (2018)
Hardwood	Solanum lycopersicum	As, Cd, Zn, and Cu	Raised pore water with Cu and As. Immobilize Zn and Cd owing to elevated DOC and pH	Beesley and Marmiroli (2011)

TABLE 4 Incorporating biochar into phytoremediation of polluted soils that contain metal(iod)s.

concentrations, which revealed an increase in Cd toxicity in plant tissues (Manori et al., 2021); therefore it can be concluded that pine needle biochar can be used as a soil amendment to increase plant HMs extraction. In a study conducted by Li et al. (2021b) on *Salix psammophila* grown in Cu, Zn, and Cd-contaminated environments, it was shown that bamboo biochar, in addition to a slight increase in plant biomass, improved Bioconcentration Factor (BCF) and Translocation Factor (TF), which indicate that bamboo biochar improves the efficiency of HMs extraction and phytoremediation. Additionally, the study on *Boehmeria nivea* demonstrated that tea waste biochar reduced Cd toxicity and increased its accumulation and displacement in seedlings by changing the intracellular distribution of Cd, reducing oxidative stress, and improving plant growth (Gong et al., 2019) (Table 4).

An investigation of the potential of Ipomoea reptans Poir for phytoremediation of Cd-contaminated soils revealed that adding corn biochar in addition to increasing Cd absorption by seedlings, increased the dry and fresh weight of shoots, the dry and fresh weight of roots, chlorophyll content, the leaf area, the number of leaves and plant height (Ratnasari et al., 2020). In the study conducted on coconut husk and orange bagasse biochars, it was demonstrated that their use for B. juncea under Cu stress with low concentration, increased root and shoot mass, photosynthetic activity, chlorophyll content, Cu absorption from the soil, and Cu concentration in the aerial parts of plants (Gonzaga et al., 2022), which showed that these biochars increased effectiveness of B. juncea in phytoremediation. Zhang et al. (2019b) found that adding rice straw biochar to alfalfa (M. sativa L.) culture medium improved the plant's ability to extract Cd from contaminated soils as well as produce the seedlings' biomass. Another study that was conducted on B. vulgaris grown in a Cdcontaminated environment found that cornstalk biochar, in addition to producing more root biomass, increased Cd extraction from the soil environment, as well as Cd absorption into the roots' cell walls (Gu et al., 2020), therefore, cornstalk biochar, in addition to helping the phytoremediation process, can also enhance B. vulgaris' resistance to pollutions. Similar results have been reported using straw biochar in improving Cd absorption and root morphology of Sedum plumbizincicola in acidic soils (Li et al., 2018) (Figure 3; Table 4).

As well as influencing the morpho-physiological structure of plants involved in phytoremediation, biochar also helps stabilize pollutants and reduce their bioavailability in the soil through its effects on soil structures (Ghosh and Maiti, 2021; Gonzaga et al., 2022). Since HMs are both cationic and anionic, they interact with the anionic and cationic ions on biochar and directly absorb on the biochar surface, therefore reducing their bioavailability in soil (Rees et al., 2014; Fijałkowska et al., 2021). Biochar Also contains functional groups on its surface that can form complexes with soil pollutants and stabilize them (Ali et al., 2021; Gupta et al., 2021). According to Gupta et al. (2021), when biochar is combined with the soil surface, the biochar particles with cation charged can absorb anionic HMs, such as arsenate and arsenite, while negatively charged particles of biochar stabilize soil positive components, including Pb and Cd (Figure 3).

Rodríguez-Vila et al. (2014) found that Holm oak biochar reduced the bioavailability of Cu in contaminated mine soils, and at the same time, Puga et al. (2015) showed that sugar cane biochar reduced Zn, Pb, and Cd bioavailability in contaminated mineral soils. A study conducted on soils contaminated with Pb and Cd showed that bamboo biochar could stabilize and reduce the bioavailability of these HMs, possibly due to the absorption of HMs on the surface of biochar (Xu et al., 2016). A greenhouse experiment showed that the addition of pig manure biochar reduced Pb leaching by 71% in soils with low organic carbon (Kiran and Prasad, 2019), while Fellet et al. (2014) demonstrated in their studies that garden pruning residues biochar reduces Pb, Cd, and Zn bioavailability in the soil. Moreover, biochar can convert Cr (VI) into less mobile Cr (III) by continuously transferring electrons, which connect with oxygen-containing functional groups on its surface (Dong et al., 2017). A study conducted on soils contaminated with Cd, Pb, Mn, Cu, and Fe showed that date palm waste biochar can significantly reduce the concentration of extractable HMs in the soil (Al-Wabel et al., 2015). Various studies have also shown that miscanthus, poplar wood, and rice straw biochars can significantly stabilize HMs in polluted soils, reducing their bioavailability to living organisms (Houben et al., 2013; Jones et al., 2016; Chen et al., 2018). According to Narayanan and Ma (2022) study, the type of



biochar used plays an important role in the amount of Ni and Cd stabilization.

In addition to being capable of stabilizing HMs, biochar can also cause the absorption of soil nutrients, reducing the competition between HMs and nutrients in the soil, which, in addition to the accumulation of soil nutrients, increases the absorption of HMs in plants used in the phytoremediation and extraction process (Rees et al., 2015; Li et al., 2021b). Studies have shown that adding biochar to the environment causes a deficiency of nitrogen, phosphorus, and calcium in plants, and in addition, biochar with different functional groups (carboxylic, lactonic, and organic nitrogen) and inorganic impurities (metal oxides and Ash) increases the capacity of soil to absorb carbon materials (Agrafioti et al., 2014; Rees et al., 2016; Roohi et al., 2020). Aside from competing for absorption sites on biochar, nutrients and HMs also compete for transporters and entry channels into plants (Farhangi-Abriz and Torabian, 2017). As a result of adding bamboo biochar to soil, Li et al. (2021b) found that the soil content of potassium and organic matter increased, and it decreased the accumulation of Zn, Cd, and Cu in the soil, while biochar increased the accumulation of these HMs in S. psammophila, which was used in the experiment (Figure 3).

The addition of biochar to soil changes soil pH, water holding capacity, and dissolved organic content (DOC) (Abdelaal et al., 2021; Narayanan and Ma, 2022). In general, biochar creates a liming effect and increases the pH of the soil, which is due to the increase of ash content in the soil (Simiele et al., 2020), and since pH and DOC have a significant effect on HMs removal and absorption in soil (Wang et al., 2020), thus, adding biochar to the soil increases the mobility of anionic HMs like Selenium (Se), As, Cu, Cr, and

Antimony (Sb), among which Cu mobility heavily depends on DOC content of biochar (Sun et al., 2018; Gupta et al., 2021; Narayanan and Ma, 2022). Furthermore, some studies have shown that increasing the pH of soil through biochar addition reduces the bioavailability of some heavy metals (such as Pb, Zn, and Cd), and reduces their concentration in soil pore water (Rees et al., 2015; Lebrun et al., 2017; Mehmood et al., 2018; Zhang et al., 2021) (Figures 3, 4).

Biochar treatment efficiency in the phytoremediation process is also influenced by several factors, such as pyrolysis temperature, heating rate, raw materials, aging, and particle size (Lu et al., 2014; Gasco et al., 2016; Rathnayake et al., 2021) (Figure 5). Although the addition of biochar improves the absorption and stabilization of various pollutants, in general, the temperature of the pyrolysis for the preparation of biochar plays an effective role in improving phytoremediation in addition to soil type, plant type, and target pollutant (Cárdenas-Aguiar et al., 2020). Biochar obtained from biomass at high temperatures is more stable in the environment and can be used for carbon sequestration (Gasco et al., 2016), while biochar made at low temperatures and from raw materials high in nutrients (such as sewage sludge or manure waste) is good for soil improvement (CEC and water retention capacity) and can be used to replace commercial fertilizers in soils contaminated with HMs (Cárdenas-Aguiar et al., 2020). Due to the risk of the re-release of pollutants stabilized by biochar over time (Gu et al., 2020), researchers have been exploring whether the aging of biochar increases the release of pollutants. Based on the limited research that has been done on biochar aging, it has been shown that during the aging process, a wide range of functional groups, including

Biochar properties	pyrolysis temperature	exposure time to soil	lignin content of the feedstock
рН	increase	decrease	decrease
carbon	increase	decrease	increase
cation exchange capacity	Increase	increase	decrease
Stability	increase	-	increase
Porosity	increase	-	increase
Aromaticity	increase	-	increase
specific surface area	increase	decrease	increase
negative surface change	decrease	increase	
nutrient availability and content	1741	-	decrease
anion exchange capacity		decrease	
pore clogging		increase	-

The effects of an increase in pyrolysis temperature, soil exposure time, and lignin content of the feedstock on the properties of biochar. The results are taken from Beusch (2021).

carboxylic, hydroxyl, and phenolic, are formed on the surface of biochar (Narayanan and Ma, 2022), and the aging process does not affect the ability of biochar to stabilize HMs, especially those with a positive charge (Ghosh and Maiti, 2021; Narayanan and Ma, 2022), In studies, aged biochar produced at high pyrolysis temperatures more effectively stabilized pollutants in contaminated soils than aged biochar produced at lower pyrolysis temperatures (Rathnayake et al., 2021), however, this process needs more studies.

On the other hand, studies conducted on the effect of biochar particle size on phytoremediation efficiency have indicated that biochar particle size plays a role in plants' response to polluted culture environments (Lebrun et al., 2021b). Biochar particle size, which is divided into large (>4 mm) and small (<20 µm) particles, is not expected to play a significant role in nutrient uptake, but it can alter biochar surface properties that affect contaminant stabilization (Lebrun et al., 2021a; Lebrun et al., 2021b). There have been limited studies on the effect of biochar particle size on the absorption and stabilization of HMs (Zhang et al., 2017; Li et al., 2021a; Lebrun et al., 2021b). In their study of S. psammophila treated with different particle sizes of bamboo biochar (P1<0.15 mm, 0.15 mm < P2 < 0.25 mm and 0.25 mm < P3 < 0.50 mm), Li et al. (2021a) found that treatment P2 had the greatest effect on Cd and Zn accumulation as well as plant growth. Furthermore, Zhang et al. (2017) showed that small particles of corn biochar increased Cd transfer from roots to shoots of Brassica chinensis L. Studies have also shown that treating rice and S. plumbizincicola with small particle size biochar reduces Zn concentrations in their aerial organs (Zheng et al., 2012; Lu et al., 2014).

The researchers found that biochar increases the activity and population of microbes (fungi and bacteria) in HMs-contaminated environments, whether there are plants present or not (Karppinen et al., 2017; Zhang et al., 2018; Gong et al., 2019). Additionally, biochar reduces the bioavailability of pollutants in soils by improving the physical and chemical properties of contaminated soils and creating conducive conditions for soil microorganisms, thus increasing the efficiency of phytoremediation in contaminated environments (Liu et al., 2017a; Kiran and Prasad, 2019; Arif et al., 2020). According to a study conducted on the effect of wheat straw biochar on cadmium remediation by M. sativa L, the addition of biochar improved soil enzyme productions (chitinase and protease), and fungal community growth (Zhang et al., 2018). In Gong et al. (2019)'s study on the effect of biochar derived from tea waste in reducing the toxicity caused by Cd in polluted sediments, it was shown that biochar improved the activity of microbes producing catalase, phosphatase, and urease and decreased the Cd toxicity to ramie and microbes. Mackie et al. (2015) also found in their study in copper-contaminated vineyards that biochar increases the activity and abundance of microbes and their ecosystem services. In another study, it was found that biochar derived from sugarcane bagasse, in addition to increasing the population of Actinomycetales and bacteria, increased the activity of soil enzymes in soils contaminated with Pb, Cu, and Cd (Nie et al., 2018) (Figure 3).

6 Phytoremediation with biochar in combination with other factors

Using biochar simultaneously with other compounds and amendment materials, in addition to overlapping their defects, enhances phytoremediation and pollution stabilization and removal (Forján et al., 2018; Gong et al., 2021), therefore, researchers have been focusing on how biochar can be combined with nanoparticles, compost, lime, sulfur, metal oxides, etc., to improve phytoremediation in recent decades (Xu et al., 2018a; Ding et al., 2020; Roohi et al., 2020; Gogoi et al., 2021; Narayanan and Ma, 2022). In a study conducted on *B. juncea* for



FIGURE 6

Mechanisms of immobilization of metal(iod)s (HMs) by soil microorganisms in biocharamended soil; EPS (Extracellular polymeric substances). The results are taken from Bandara et al. (2020)

lead extraction, it was found that the simultaneous application of Neem tree wood logs biochar and EDTA significantly increased the biomass and total chlorophyll content of plants and increased lead absorption by the seedlings compared to the application of each treatment separately (Rathika et al., 2021). Several studies have shown that the combination of iron sulfate and biochar can be an effective strategy for increasing the stabilization and reducing the bioavailability of As in polluted environments through absorption or surface precipitation mechanisms (Hartley and Lepp, 2008; Lebrun et al., 2020; Simiele et al., 2020), and besides improving soil fertility, this combination can also boost plant growth and survival (Hartley et al., 2009; Simiele et al., 2020).

A study conducted by Li et al. (2019b) on S. nigrum L. treated with biochar and attapulgite showed that the simultaneous application of these treatments improved the fresh weight and length of the seedlings, while also increasing the absorption of Zn, Mn, Hg, Cu, Cd, and Pb in the plant roots, which demonstrates the positive effect of this compound in improving the stabilization of HMs. Furthermore, adding lime and sulfur to biochar can reduce HM bioavailability and improve plant growth in the process of phytoremediation (Xu et al., 2018a; Narayanan and Ma, 2022). Studies have also been conducted on the effectiveness of compost and biochar combination in phytoremediation of soil contaminated with HMs (Roohi et al., 2020; Deebika et al., 2021), some of which are listed below. In a study that was conducted to evaluate the potential of B. tomentellus for phytoremediation of soils contaminated with Cr and Zn with the help of biochar treatment and urban waste compost, it was shown that by combining these treatments, HMs absorption by seedlings significantly increased (Roohi et al., 2020). In addition, Forján et al. (2018) showed that B. juncea L. + compost + biochar is the best

combination for reducing Zn, Pb, Ni, and Cu concentrations in polluted soils. In another study, Liu et al. (2017b) found that the combination of compost and biochar reduces the bioavailability of HMs such as Cd, Cr, Zn, As, Cu, Ni, and Pb due to the higher levels of extractable minerals (such as nitrogen, phosphorus, calcium, magnesium, and potassium).

In a study that was conducted to evaluate the effectiveness of rice husk ash and Prosopis biochar in phytoremediation of Pb by Ricinus communis, it was demonstrated that the simultaneous use of these treatments improves soil pH, seedling tolerance to Pb, antioxidant activity, and nutrient uptake, while it reduced the availability of Pb in the soil, and a detailed analysis of the infrared spectra confirmed that oxygenated (ester and ether), amide and phosphate functional groups absorbed Pb and reduced its toxicity for plants (Kiran and Prasad, 2019). Similarly, a study conducted with rice straw and bamboo biochar found that the combination of these treatments reduced the extractable concentration of Zn, Pb, Cu, and Cd in contaminated soils (Lu et al., 2017). A positive effect on plant growth factors was observed following the addition of the above-mentioned treatments to the cultivation environment due to the improvement of physical and biological properties and the enhancement of soil nutrients (Isitekhale et al., 2013; Barati et al., 2018).

It has also been shown that the simultaneous treatment of microbes and biochar can improve the phytoremediation of HMs and increase the resistance of plants to these pollutants (Farias et al., 2020). Alternatively, heavy metal toxicity could affect the microorganisms that are added to soil environments to improve phytoremediation, and their efficiency may decrease (Dzionek et al., 2016; Yao et al., 2017). Thus, cell stabilization is an effective way of increasing microorganism resistance to pollution and restoring native soil microflora, and biochar, as a good carrier material for

cell stabilization, can increase microorganisms' resistance to pollutants' toxicity (Dzionek et al., 2016; Chuaphasuk and Prapagdee, 2019), and It is clear that, in this case, a more indepth research is required. In an experiment conducted on Plant extraction of Cd by Chlorophytum laxum R.Br, it was shown that bacteria (Arthrobacter sp. and Micrococcus sp.) stabilized with biochar increased the accumulation of Cd in the roots and shoots of seedlings and the extraction of Cd from contaminated soils (Chuaphasuk and Prapagdee, 2019). Another study conducted on Portulaca oleracea L. under Zn, Al, Cr (III), Cr (VI), and Fe stress demonstrated that by using biochar combined with Oscillatoria sp, in addition to increasing root and shoot lengths, organic carbon, nitrogen content, chlorophyll content, and stabilizing HMs in the soil, the accumulation of HMs in plant organs decreased (Zanganeh et al., 2022). In a study on the role of Jacaranda mimosifolia in the phytoremediation of Zn, Mn, and Cu under the treatment of biochar and spore suspension of five fungal isolates (Metarhizium anisopliae, Trichoderma asperella, Beauveria bassiana, Purpureocillium lilacinum, and Pochonia chlamydosporia), it was found that the extraction rate of HMs and their concentration in plant tissues increased noticeably, reducing the risk of HMs leaching in the soil (Farias et al., 2020) (Figure 6).

7 Pyrolysis of phytoremediation residues

As phytoremediation plays an increasingly important role in cleaning polluted environments, disposing of plants used in this process has become an urgent issue (Gong et al., 2018b; Zhou et al., 2020). If contaminated biomass is returned directly to the soil after phytoremediation, it can cause secondary pollution in the soil and underground water, which can have irreparable effects on the ecosystem, animals, and humans (Huang et al., 2015; Özkan et al., 2016; Gong et al., 2017). Therefore, in recent years, several solutions have been proposed for recycling plants grown in polluted environments, including making furniture, burning, and composting, though each mentioned method has obvious disadvantages (Chalot et al., 2012; Li et al., 2020a; Ghosh and Maiti, 2021). For example, only wooden plants can be used to make household furniture, while herbaceous plants resulting from the phytoremediation process are not useful (Ghosh and Maiti, 2021); On the other hand, burning or composting contaminated biomass can release pollutants into the air, water, and soil (Zhang et al., 2021).

One of the best ways to utilize phytoremediation residues and produce biochar is through pyrolysis, which offers many advantages such as reducing contaminated biomass volume and mass, stabilizing (reducing bioavailability) and decomposing organic and inorganic pollutants, producing valuable pyrolytic products (such as coal, biofuel, etc.), and improving soil quality (carbon sequestration, stabilization of metals in polluted soil and increasing soil fertility) (Lievens et al., 2008; Xiao et al., 2015; Bert et al., 2017; Niu et al., 2017; Du et al., 2019b; Palansooriya et al., 2020; Zhang et al., 2020). A key factor in producing biochar from phytoremediation residues is the pyrolysis temperature, which determines the distribution of heavy metals in biochar (Huang et al., 2018). For instance, Cd evaporates at a temperature above 600°C, while Hg completely evaporates at 350°C (Gong et al., 2018a). Compared to high pyrolysis temperatures, low pyrolysis temperatures increase the accumulation of heavy metals and humic substances in biochar, while reducing the amount of low molecular weight acids (Bian et al., 2018). On the other hand, HMs species present in phytoremediation plants can influence the properties of biochar, for instance, Fe can enhance the graphicization of biochar, while Zn can increase the porosity of biochar (Ding et al., 2020).

In their studies on S. officinale L. used in phytoremediation, Du et al. (2019b) found that pyrolysis temperatures above 550°C stabilize HMs and greatly reduce their leaching into biochar, and improve biochar environmental safety. In another study by Zhang et al. (2020) on pyrolysis of Lolium perenne L., Pennisetum sinese, and Brassica napus L. residues obtained from phytoremediation, they found that a temperature higher than 600°C makes the Cd toxicity in biochar acceptable to the environment and its leaching rate in biochar is reduced to an acceptable level. A study on Silphium perfoliatum L. obtained from a phytoremediation process and containing HMs found that pyrolysis at 750°C reduces the long-term leaching of HMs, increases oxidation resistance, and prevents the release of HMs attached to organic materials (Du et al., 2019a). The study by Zhou et al. (2020) found that biochar obtained from Helianthus annuus L. containing Cd, Zn, Pb, and Cu can be used as a fertilizer to improve soil quality. Researchers found that converting cobalt-containing Siberian iris into biochar through one-step pyrolysis improved mercury phytoremediation of polluted soils and waters (Li et al., 2020b). The results of another study conducted on ramie after the phytoremediation process showed that pyrolysis can enhance the stabilization of Pb, Cu, Zn, Cd, and Cr in plant residues and increase the possibility of reusing phytoremediation residues (Gong et al., 2018b). Moreover, Zhang et al. (2021) found that biochar produced from cadmium-contaminated L. perenne L. and B. napus L. residues at high pyrolysis temperatures, due to the increase in Cd stabilization in biochar, had a greatly decreased risk of environmental contamination. However, to demonstrate the effectiveness of phytoremediation residues as biochar, more studies are necessary about environmental toxicity, washability, stability, environmental hazards, and its chemical forms (Han et al., 2018; Du et al., 2019a).

8 Conclusion

The results of the present study demonstrated that using biochar in the phytoremediation process can improve the extraction and stabilization of HMs pollution, as well as increase plant resistance under stress conditions. On the other hand, owing to the variety of pollutions, plants suitable for phytoremediation, biochars, and other factors that enhance the phytoremediation process, the positive effects of different types of biochar on reducing all pollution in the environment cannot be mentioned with absolute certainty. Therefore, in future studies, a complex investigation of the interplay between different factors (biochar, plants, pollutants, etc.) is required to improve the efficiency of phytoremediation. It is also suggested that future studies should focus on field experiments so that by obtaining more practical information, an effective step can be taken toward reducing pollution and creating a healthier environment for future generations.

Author contributions

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Conflict of interest

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