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REVIEW ARTICLE

Unrevealing the potential of aquatic macrophytes for phytoremediation in heavy metal-polluted wastewater

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ABSTRACT

Aquatic macrophytes including *Eichhornia crassipes, Pistia stratiotes*, and *Lemna minor* have shown promise in phytoremediation for wastewater treatment and heavy metal removal. This approach may reduce pollution. However, plant metabolic rate and ability to break down organic compounds or tolerate heavy metals are limited. Biotechnology can significantly improve these issues. Scientists can alter plants to improve phytoremediation using genetic engineering. Direct gene transfer allows certain genes to be introduced into plants, giving them advantages. Introducing genes involved in metal absorption, transport, and detoxification into plants may increase their ability to store and survive heavy metals. Introducing genes that encode enzymes that break down organic pollutants can also improve decomposition. Transgenic plants with improved metabolic pathways, biomass yield, and stress tolerance have been created using biotechnology. These advances benefit phytoremediation. Selecting acceptable plant species for genetic modification is crucial. Aquatic macrophytes can produce a lot of biomasses, grow quickly, and absorb nutrients, making them excellent for genetic engineering in phytoremediation. In general, biotechnology can improve phytoremediation by overcoming plant capacity restrictions. The ecological and regulatory effects of introducing genetically modified organisms into the environment must be considered. The safe and sustainable use of genetically modified plants for phytoremediation requires collaboration between biotechnologists, environmental scientists, and legislators.

Keywords: heavy metal; phytoremediation; phytoextraction; phytochelatins

INTRODUCTION

Over the past few decades, urbanization and industrialization have become a significant contributor to the release of heavy metals into water and soil. These metals pose a hazardous pollutant and are released into water bodies such as rivers and ponds through the direct drainage of effluents, including municipal waste, fertilizers, pesticides, and sewage, without any prior treatment (Xin et al., 2020; Singh et al., 2022; Naeem et al., 2023; Eid, 2020). The effluents in question are found to contain heavy metals, including cadmium (Cd), arsenic (As), lead (Pb), and mercury (Hg). These metals have been found to have detrimental effects on the physiological and biochemical properties of plants (Macek, 2000; Suthindhiran, 2017), as well as posing potential health risks to humans (Sharma et al., 2015).

Heavy metals (HMs) are naturally occurring constituents that are often characterized by a density exceeding 5 g cm^{-3}. The presence of heavy metals (HMs) in water and soil leads to a wide range of ecological and environmental issues. Consequently, the introduction and transportation of heavy metals (HMs) into plants lead to a decrease in plant growth, reduced nutrient uptake, and decreased photosynthetic production. Numerous human-induced activities, including smelting, sludge production, mining, and dumping, have significant impacts on soil and water resources.

According to a study conducted by Ahmad et al. (2011), it has been observed that Cd can decrease the permeability of the plasma membrane, inhibit stem growth, and impede seed germination. As fruit yield declines, there is a corresponding decrease in leaf fresh weight and leaf area. Additionally, it reduces the productivity of dry matter. Stunted growth, wilting, and chlorosis are observed as consequences. Lead (Pb) is an extremely hazardous metal found in soil. It hinders the growth of seedlings. It has an impact on the structure, development, and process of photosynthesis in plants. Elevated levels of Pb trigger oxidative damage. Additionally, it reduces the generation of biomass.

Fontes et al. (1998) have documented that nickel (Ni) has been found to diminish the activity of protease and amylase enzymes, impede the growth of various crops, compromise membrane stability, and impact chlorophyll content. Similarly, Fontes et al. (1998) and Sompura et al. (2022) have investigated the impact of zinc (Zn) on plant biomass reduction and chlorosis, while copper (Cu) has been observed to decrease antioxidant activities and chlorophyll content. Furthermore, it results in a reduction in the length of the embryonic axis of developing seeds. Mercury decreases the elongation of the primary roots of *Zea mays*.

The magnitude of metal contamination in agricultural regions is exemplified by the subsequent instances: A survey conducted in Bulgaria has uncovered that a total of 19,360 hectares are affected by heavy metal contamination, while 1,913 hectares are contaminated by radionuclides. Poland has a contamination rate of 0.5% for heavy metals and/or other pollutants across its entire country area. France has an estimated 800,000 locations that may be potentially polluted, whereas the total number of sites in Western Europe might be up to 1,200,000 (NATO/CCMS 2002). As per the 2022 status of the environment report by the Centre for Science and Environment (CSE), an environmental NGO in India, the river Ganga exhibits elevated concentrations of Pb, Fe, Ni, Cd, and As. Out of the 33 monitoring stations along the Ganga, 10 were found to have excessive levels of these contaminants. Therefore, there is a requirement for a technologically efficient and cost-effective solution.

Aquatic plants possess the capacity to accumulate heavy metals, including iron (Fe), manganese (Mn), zinc (Zn), and others, which are crucial for their growth and development. In addition, these substances possess the capacity to collect non-essential heavy metals such as cadmium (Cd), chromium (Cr), selenium (Se), and others. Nevertheless, these heavy metals have the potential to be harmful to plants. Plants have evolved a way to endure the harmful effects of heavy metals. These heavy metals have the potential to cause ecotoxicity in both aquatic and terrestrial environments.

Given the detrimental impact of heavy metals (HMs) on plants, scientists should employ several strategies to mitigate this adverse effect. There are multiple methodologies for the removal of heavy metals (HMs) from water and soil, encompassing phytochemical, proteomics, genomics, metabolomics, and a more recent development known as transgenic plant-based techniques. Phytoremediation is a remediation technique that utilizes plants to mitigate heavy metals (HMs), offering efficacy, environmental friendliness, and cost-effectiveness. Phytoremediation has been identified as the most efficacious strategy for mitigating the adverse effects of heavy metal (HM) pollutants.

Phytoremediation refers to the process of eliminating contaminants from soil, wastewater, and sediment by the utilization of diverse green plant species, which can be found in both terrestrial and aquatic environments. This process involves the absorption, detoxification, and neutralization of the pollutants. This strategy is both environmentally beneficial and economically efficient, thereby safeguarding the environment. Numerous plant species have been identified in prior research as capable of absorbing and detoxifying soil and water contaminated with toxic metals, pharmaceutical waste, pesticides, and therapeutic waste, among other contaminants. The plant assimilates these perilous substances through its root system and then accumulates them within its body through the process of decomposition into less hazardous compounds.

Prior studies indicate that aquatic macrophytes have a crucial function in the purification of soil and water. The fast growth of aquatic macrophytes renders them highly capable of absorbing pollutants, including heavy metals (HMs). Several researchers examined the potential of HMs removal from wastewater such as Cd, Ni and Pb by *Eichhornia crassipes* (Mart.) Solms, *Ludwigia stolonifera* (Guill. & Perr.) P.H. Raven, *Echinochloa stagnina* (Retz.) P. Beauv, *Phragmites australis* (Cav.) Trin. ex Steud.) (Eid, 2020), Cd, Cu, Zn and Pb by *Ceratophyllum demersum* L., *Echinochloa pyramidalis* (Lam.) Hitchc. & Chase, *Eichhornia crassipes* (Mart.) Solms-Laub *Myriophyllum spicatum* L., *Phragmites australis* (Cav.) Trin. ex Steud, *Typha domingensis* (Pers.) Poir. ex Steud (Ahmed et al., 2012). Furthermore, *Pontederia cordata* (Xin et al., 2020) has been shown to employ other plant species for the detoxification of heavy metals (HMs). Similarly, *Glyceria grandis, Scirpus validus*, and *Spartina pectinate* have been utilized for the detoxification of Cu, Pb, and Zn (Weiss et al., 2006). However, the accumulation of heavy metals (HMs) can be influenced by factors such as plant species and element properties. The wise selection of plant species can significantly boost the removal of heavy metals (HMs) by plants. This review focuses on the process of phytoremediation of heavy metals (HMs) from wastewater through the utilization of aquatic macrophytes. In addition, we elucidate the methods by which we can augment the phytoremediation capacity using biotechnology means.

SOURCES OF HMs

HM pollution can originate from a range of sources, such as natural, agricultural, industrial, residential effluents, atmospheric, and other sources. These sources have a detrimental impact on plant processes. HM pollution can result from both anthropogenic and natural mechanisms. The pollutants encompassed in this study consist of Cd, Cu, and Zn, alongside Cr, Pb, Cu, Ni, Zn, and Cd. Heavy metals (HMs) are naturally present in soil due to the process of weathering, as they are a by-product of the Earth's crust. The primary natural causes of heavy metal (HM) contamination are volcanic eruptions and the process of rock weathering. The volcanic eruption emits heavy metals (HMs) such as lead (Pb), nickel (Ni), copper (Cu), and mercury (Hg), as well as harmful and depleting gasses. The weathering of rocks, which leads to the formation of soil and the release of heavy metals (HMs), is another natural source of HM contamination. For instance, the elements Cr, Mn, Ni, Cu, Co, Cd, and Hg can be absorbed by plants through the process of water uptake. Agricultural practices such as the use of fertilizers, liming, and irrigation fluids are significant contributors to the presence of heavy metals (HMs) in the agricultural sector. The utilization of fertilizers, which consist of both organic and inorganic components, exhibits diverse levels of heavy metals (HMs) including cadmium (Cd), chromium (Cr), nickel (Ni), lead (Pb), and zinc (Zn). These hazardous materials (HMs) have a detrimental effect on plant growth and development, leading to a reduction in photosynthetic production.

Human activity is a significant contributor to the release of heavy metals (HMs) into the environment, particularly through the release of industrial wastewater into rivers, ponds, and other water bodies. Heavy metals (HMs) are manufactured in an industrial setting by extracting, purifying, and recycling metals. Different heavy metals (HMs) are discharged depending on the mining method. Coal mines serve as significant sources of arsenic (As), cadmium (Cd), iron (Fe), and various other metals, which are released into the soil inside the coalfield, either through direct or indirect means. One notable contributor to this environmental pollutant is the utilization of mercury (Hg) in the gold mining procedure, as well as the release of exceedingly elevated concentrations of Hg from decommissioned mines (Lacerda, 1997). The pollution of heavy metals (HMs) can also be attributed to the utilization of domestic items or domestic effluents. Household detergent products might potentially affect water quality, as their use can represent a risk of pollution. According to a study conducted by Angino et al.

(1970), trace quantities of the elements Fe, Mn, Cr, Co, Zn, Sr, and B were identified in the majority of enzyme detergents. Urban runoff is becoming recognized as a significant issue of heavy metal contamination in connection to pollution originating from metropolitan areas. Based on a statistical analysis conducted by Bradford in 1997, it has been widely recognized that urban stormwater runoff is a substantial contributor to the presence of pollutants in surface water bodies. Based on the findings of Bolter et al. (1974), it has been observed that humic and other acids have the ability to leach lead, hence increasing its accessibility for runoff rather than seepage into the uppermost layer of soil. In addition to these factors, transportation (including automobiles, diesel-powered vehicles, and aeroplanes) and the combustion of garbage in landfills are further contributors to hazardous materials (HMs). Two significant anthropogenic sources of soil pollution are fly ash resulting from coal combustion and the corrosion of commercial waste products. These sources release chromium (Cr), copper (Cu), lead (Pb), and galvanized metals, particularly zinc (Zn), into the environment (Al-Hiyaly et al., 1988). Verkleji (1993) states that the combustion of coal results in the release of heavy metals (HMs) such as Cd, Hg, Mn, Ni, Al, and Fe into the soil. Valium (V), iron (Fe), lead (Pb), and nickel (Ni) are emitted into the environment through the combustion of oil. Zinc (Zn) is released from tires, aluminium (Al) from catalysts, cadmium (Cd) and copper (Cu) mostly from diesel engines, and nickel (Ni) and zinc (Zn) are released as aerosol emissions during the operation of automobiles.

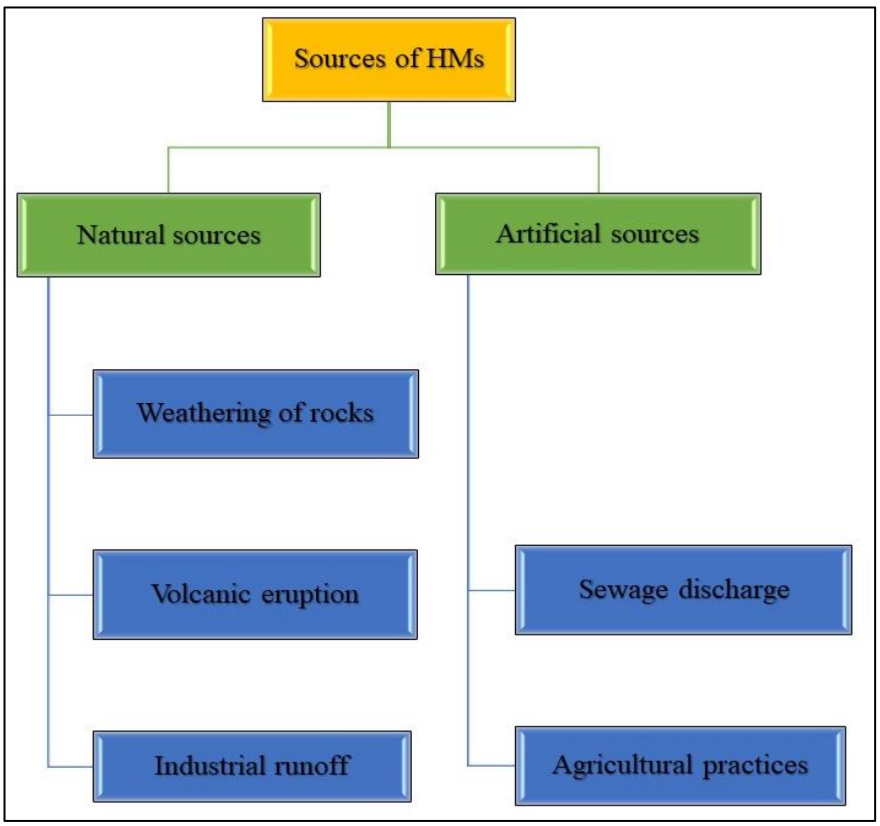

Figure 1. Flowchart depicting different origins of heavy metals.

PHYTOREMEDIATION

Phytoremediation can be defined as the removal (absorption, detoxification and neutralization) of any contaminated soil, wastewater and sediment by using various types of green plants which may be terrestrial or aquatic. This is an eco-friendly and cost-effective approach which protects the environment. Previous studies recognized many plant species which absorb and detoxify soil and water polluted with toxic metals, pharmaceutical waste, pesticides, medicinal waste etc. The plant absorbs these hazardous substances by their root and accumulates into the body by decomposing into less hazardous substances (Table 1).

Table 1. Classification of phytoremediation

Category of phytoremediation	Description	References
Phytovolatilization	Volatilization of pollutants by using plant	(Macek, 2000; Carvalho & Martin, 2001)
Phyto stabilization	Decrease the bioavailability of pollutants present in the environment by using plant	(Macek, 2000); Carvalho & Martin, 2001)
Phytodegradation	Degradation of organic pollutants in the presence of plants and microorganisms	(Macek, 2000); Carvalho & Martin, 2001)
Phytoextraction	Removal of heavy metals or effluents by use of plants	(Macek, 2000); Carvalho & Martin, 2001)
Rhizofiltration	Filtration with help of plant root by absorption and adsorption of pollutants	(Macek, 2000); Carvalho & Martin, 2001)

Mechanism of phytoremediation

Phytoextraction

Phyto sequestration, phytoaccumulation, or phyto-absorption are other terms for this process (Ali et al., 2013). Phytoextraction is commonly characterized as the process of eliminating heavy metals or effluents by the utilization of plants (Macek, 2000; Carvalho & Martin, 2001). Plant species are grown on polluted areas due to their ability to acquire heavy metals (HMs) and the accumulation of biomass enriched with HMs. Consequently, the elimination of soil contaminants. The utilization of phytoextraction has proven to be a highly effective method for the removal of heavy metals (HMs) and other toxins from contaminated soil. Phytoextraction efficiency is contingent upon the concentration of heavy metals (HMs), the capacity of the plant species, and the availability of HMs. During this process, plant species assimilate heavy metals (HMs), absorb them, and then move them to the aboveground portion of the plant. Transpiration and root pressure influence the movement of heavy metals (HMs). The use of chelating agents has been shown to enhance the phytoextraction potential. For instance, Suthar et al. (2014) found that the ability to extract phytochemicals from Zea mays L. is enhanced when EDTA, a chelating agent, is added. Introducing a chelating agent can improve the process of extracting heavy metals (HMs) from contaminated water. It is the optimal solution for eliminating non-biodegradable contaminants. Over 400 plant species have been recently identified as hyperaccumulators of pollution. The potential for hyperaccumulation is influenced by the efficiency of biomass and bioconcentration in plants. Hyperaccumulator plants exhibit significant potential for phytoremediation. Recent research has demonstrated that the application of genetic engineering techniques has enabled the transfer of certain genes to enhance the effectiveness of phytoextraction in plants. The incorporation of novel genes into plants has yielded favourable outcomes, including enhanced phytoextraction capacity and tolerance (Figure 2).

Phytofilteration

Phytofiltration is commonly characterized as a filtration process facilitated by the absorption and adsorption of contaminants through the utilization of plant roots. There are three types of filtration methods: rhizofiltration, which involves utilizing plant roots or hydraulic control; blastofiltration, which involves employing seedlings; and shoot filtration, as described by Ali et al. (2013). This approach enables the removal of heavy metals (HMs) such as Cd, Pb, Cu, Ni, and Cr. This method bears a resemblance to the process of phytoextraction. The occurrence of this phenomenon is contingent upon the effective establishment of a comprehensive fibrous root system. Plant species that thrive in contaminated environments can absorb water-containing contaminants through their root systems. Upon reaching the limit of pollutant saturation, the plant roots will be harvested.

According to Pang et al. (2023), plants possessing dense root systems are favoured for rhizofiltration due to their ability to concentrate impurities to a greater extent through the bigger adsorption surface of the roots. Numerous macrophytes exhibit notable efficacy and promise in the removal of heavy metals (HMs) such as Cd, Pb, and Zn from an aquatic environment. However, the success of this process relies on the metabolism of certain plant species and the sort of heavy metals present.

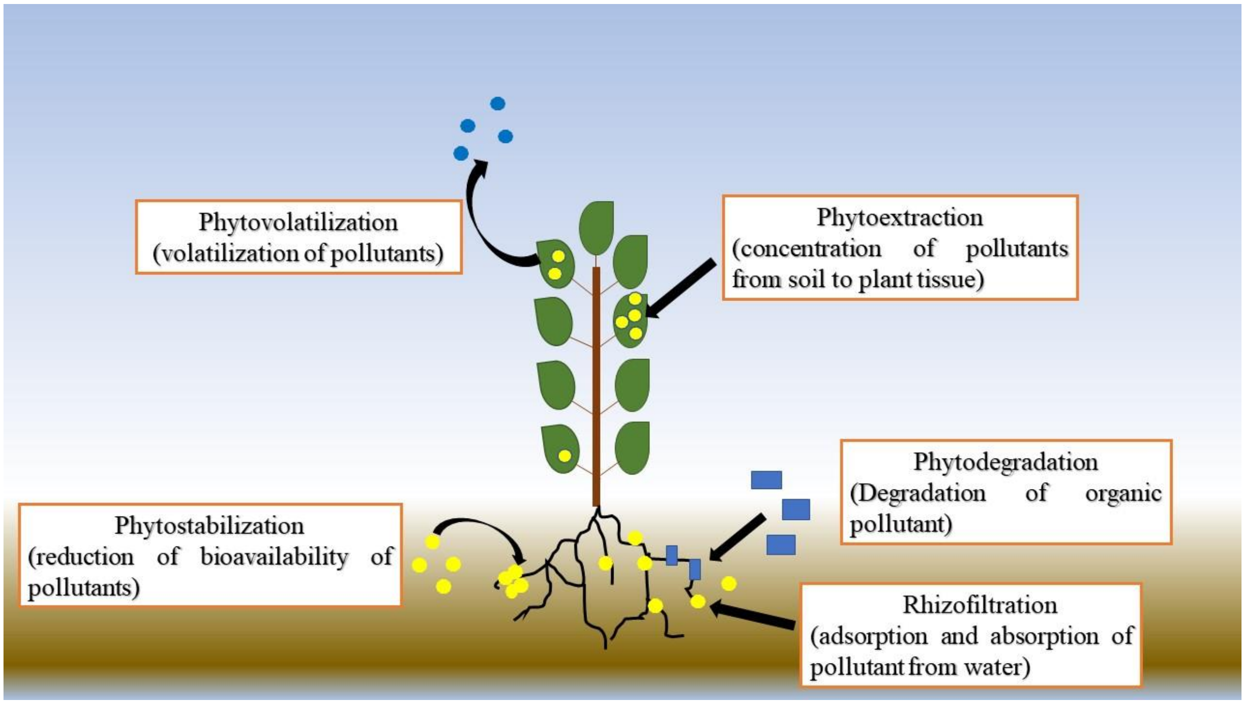

Figure 2. Mechanism of phytoremediation of heavy metals (HMs)

Phyto-stabilization or Phyto-immobilization

The concept of phyto-stabilization refers to the process of utilizing plants to stabilize heavy metal (HM) contaminants in soil that has been contaminated (Ali et al., 2013). This involves reducing the bioavailability of pollutants in the environment by employing plants (Macek, 2000; Carvalho & Martin, 2001). By doing so, phyto-stabilization effectively prevents the migration of contaminants into water bodies and the food chain within the ecological system. Plants possess the capacity to release heavy metal pollutants through processes such as absorption and precipitation. The toxicity of heavy metals (HMs) is dependent on their valency. Plants have been found to release a distinct class of enzymes that effectively mitigate the toxicity of heavy metals (Ali et al., 2013).

Phytovolatilization

The process under consideration involves the uptake of heavy metals (HMs) by plants, followed by their conversion into a volatile state and subsequent release into the environment (Ali et al., 2013). Alternatively, it utilizes plants to volatilize pollutants (Macek, 2000; Carvalho & Martin, 2001). This approach is employed in the analysis of organic pollutants and heavy metals. Nevertheless, it does not achieve total removal of contaminants.

Phytodegradation

This process is commonly referred to as Phytotransformation. The presence of plants and microbes has been found to facilitate the degradation of organic contaminants (Macek, 2000; Carvalho & Martin, 2001). Conversely, the enzymatic activity of associated microbes facilitates the reduction of heavy metal (HM) contaminants into simpler molecules that possess less toxicity. Degrading enzymes, such as oxygenase enzymes, are secreted by plants to facilitate the degradation of organic contaminants. Phytodegradation is restricted in its application as it solely eliminates organic contaminants and does not address heavy metals (HMs). Recent research has demonstrated that genetically engineered plants, often known as transgenic plants, can break down heavy metals (Ali et al., 2013). Phytoremediation is a significant process that involves the use of plants to remove organic contaminants including chloroacetanilide, TNT, TCE, and others by phytodegradation. Plants secrete many enzymes, including peroxidases, peroxygenases, laccases, phosphatases, nitro reductases, and dehalogenases, which play a crucial role in the breakdown of organic pollutants into inorganic molecules such as CO_2, water, and Cl_2.

ROLE OF AQUATIC MACROPHYTES IN PHYTOREMEDIATION OF HMs

Water-dwelling macrophytes, are alternatively referred to as aquatic plants or hydrophytes. Aquatic macrophytes can be classified into distinct types based on their growth patterns. These categories encompass emerging macrophytes, floating macrophytes, submerged macrophytes or plants, and free-floating macrophytes. Aquatic plants exhibit more efficacy in wastewater treatment as compared to terrestrial plants (Table 2). Aquatic plants possess distinct characteristics that set them apart from terrestrial plants, such as their ability to absorb pollution. The presence of these aquatic plants exerts a substantial influence on the aquatic ecology. These aquatic plants play a crucial role in the structure and operation of the aquatic ecosystem. Structurally, they serve as a food supply for aquatic organisms, offer refuge, and sustain the movement of water. In terms of functionality, these chemicals can gather heavy metals and hazardous compounds that are dissolved in water. These features are crucial to the study and treatment of wastewater.

Ricciocarpus natans

This is a floating aquatic plant species that can absorb heavy metals (HMs) from contaminated water. The plant is classified as a liverwort and lacks differentiation into root, stem, and leaf structures.

Lemna

It is also referred to as 'duckweed'. These are free-floating macrophytes that remain buoyant on the water's surface. This macrophyte is not only widely spread but also exhibits rapid growth in ponds, lakes, wetlands, and lagoons. It possesses a higher capacity for the accumulation of heavy metals (HMs) from polluted water and has been employed in the elimination of HMs for several decades. Lemna minor, Lemna gibba, and Lemna major are the three species present. Amare et al. (2017), demonstrated this with the administration of Mn. The researchers concluded that Lemna exhibits the capacity to acquire significant amounts of manganese (Mn) from both waste materials and water that has been contaminated. A separate investigation conducted by Jumbo in 2012 demonstrated that Lemna minor effectively eliminates Hg from polluted water through morphological alterations. However, it thrives exclusively in areas with minimal HM pollution. Furthermore, there have been reports indicating that L. minor possesses a highly efficient technique in countering HMs. Chlorophyll is a significant abiotic component that exhibits sensitivity to heavy metal (HM) stress. According to their research, a reduction in chlorophyll fluorescence indicated the hazardous properties of Cu Singh et al. (2022).

Spirodela intermedia

Duckweed, also referred to as a free-floating macrophyte, is a plant species. This organism exhibits a higher capacity to accumulate heavy metals (HMs) from water, including Cd, Cr, and Pb, and can thrive in many environmental circumstances. Nevertheless, it has the ability to disperse throughout the entire water column and reduce the growth of algae by obstructing the passage of light and the process of photosynthesis.

Hydrila verticilata

Hydrilla is a widely recognized weed. It creates a substantial coating throughout the entire water column. It efficiently sequesters Pb from water through its shoot.

Brassica juncea

Brassica species have been extensively researched for their effectiveness in phytoremediation of water bodies. Prior studies have documented the significant capacity of *Brassica juncea* to collect and repair soil and water. Research has demonstrated the efficacy of *Brassica juncea* in the remediation of Zn.

Eicchornia

The plant species *Eicchornia* is widely recognized as water hyacinth or water lily. The species in question is indigenous to tropical and subtropical landscapes. The species *Eicchornia cresipis* (Mart.) Solms is widely distributed and has quick growth compared to other species such as *Echinochloa stagnina* (Retz.) P. Beauv and *Echinochloa pyramidalis* (Lam.) Hitchc. & Chase. Compared to other species, it possesses a significant capacity for removing heavy metals (HMs) from contaminated wastewater. For instance, Mishra et al. (2008), found *that Eicchornia cresipis* (Mart.) Solms has a higher capacity to eliminate arsenic (As) buildup compared to other species of *Eicchornia*. The macrophyte in question has a notable growth rate and substantial biomass production, rendering it prevalent throughout the majority of the year. Moreover, it demonstrates exceptional efficacy in the removal of heavy metals (HMs) including Zn, Pb, Cu, Cd, Ni, and Mn. According to a study conducted by Eid (2020), it has been established that *Eichhornia crassipes* (Mart.) Solms and *Echinochloa stagnina* (Retz.) P. Beauv exhibit significant efficacy in the phytoremediation of Cd, Ni, and Pb. In addition, Irfan et al. (2012) conducted a study that also examined the phytoremediation capabilities of *Eicchornia cresipis* (Mart.) Solms in the elimination of chromium

(Cr) and copper (Cu), as well as cadmium (Cd), copper (Cu), zinc (Zn), and lead (Pb) from heavy metal (HM) contaminated water, as described by Ahmed et al. (2012). Water hyacinth is the optimal choice for phytoremediation of heavy metals (HMs) in contaminated water.

Ceratophyllum demersum

The organism in question is a submerged aquatic macrophyte that exhibits the potential to thrive in low light and murky water, displaying both oligotrophic and eutrophic growth patterns. Prior research has demonstrated the efficacy of *C. demersum* in the cleanup of heavy metals (HMs). In their study, Rai et al. (1995) documented that *C. demersum* exhibits a proficient mechanism for the accumulation of heavy metals (HMs) in wastewater.

Table 2. Role of aquatic macrophytes in phytoremediation of HMs.

Aquatic macrophyte	Heavy metal	References
Eichhornia crassipes (Mart.) Solms *Ludwigia stolonifera* (Guill. & Perr.) P.H. Raven *Echinochloa stagnina* (Retz.) P. Beauv *Phragmites australis* (Cav.) Trin. ex Steud.)	Cd, Ni and Pb	Eid, (2020)
Ceratophyllum demersum L. *Echinochloa pyramidalis* (Lam.) Hitchc. & Chase *Eichhornia crassipes* (Mart.) Solms-Laub *Myriophyllum spicatum* L. *Phragmites australis* (Cav.) Trin. ex Steud *Typha domingensis* (Pers.) Poir. ex Steud	Cd, Cu, Zn and Pb	Ahmed et al. (2012)
Pontederia cordata	Cd	Xin et al. (2020)
Glyceria grandis *Scirpus validus* *Spartina pectinata*	Cu, Pb and Zn	Weiss et al. (2006)
Typha latifolia L.	Cu, Ni, Zn, Fe, Mn and Ca	Taylor and crowder (1983)
Lemna minor L.	As, Pb, Cr, Hg, Co, Zn	Parra et al. (2012)
Lemna gibba L.	Pb, Cr, Co, Cd and Zn	Abdallah (2012)
Ceratophyllum demersum L. *Eichhornia crassipes* (Mart.) Solms-Laub *Myriophyllum spicatum* L. *Phragmites australis* (Cav.) Trin. ex Steud *Typha domingensis* (Pers.) Poir. ex Steud	Cd, Co, Cu, Ni, Zn and Pb	Kamel (2013)
Potamageton pectinatus	Cd, Cu	Salman et al. (2015)
Azolla pinnata L.	Cr, Hg, Cd	Rai (2010)
Wolfia globosa	As	Zhang et al. (2009)
Salvia natans	Cr	Dhir (2009)
Pistia stratiotes	Pb	Vesely et al. (2011)
Azolla pinnata	Ni, Cd, Cr, Hg, Pb	Arora et al. (2004); Rai & Tripathi (2009)
Salvia minima	Pb	Vesely et al. (2011)
Utricularia gibba	Cr	Augustinowycz et al. (2015)
Spirodela polyrhiza	As, Cd	Choudhary et al. (2014); Rahman et al. (2007)

BIOTECHNOLOGY FOR ENHANCEMENT OF PHYTOREMEDIATION POTENTIAL

There has been an increasing level of interest in the utilization of phytoremediation, a cost-effective and ecologically friendly approach for remediating soil or sediments contaminated with metals and other pollutants. It is a pragmatic method of soil remediation that harnesses the inherent capacity of plants to absorb, gather, and eliminate pollutants. It serves as a sustainable and alternative method for cleanup, but it may require a novel approach to enhance the plant's ability to tolerate and accumulate metals (Kastenhofer, 2007).

Biotechnological methodologies, such as genetic engineering, exhibit significant potential in modifying plants' capacity to withstand heavy metal (HM) stress. Recent advancements suggest that the field of plant genetic engineering has the potential to greatly contribute to the improvement of phytoremediation processes. The development of several transgenics with enhanced bioaccumulation, degradation capability, and tolerance to diverse pollutants has been facilitated by advancements in plant metabolism and molecular research (Sharma et al., 2021). Genetic engineering has the potential to enhance a plant's capacity to endure, accumulate, and metabolize pollutants, so rendering it an ideal candidate for environmental remediation (Smits & Pilon, 2002). In recent times, there has been a growing utilization of genetic engineering techniques to enhance the physiological and metabolic processes enabling plants to effectively remediate contaminated areas caused by metalloids and heavy metals (Terry et al., 2003).

Various techniques can be utilized to enhance the efficiency of metal phytoremediation. The imminent accessibility of emerging genomic technologies and computational analysis of the online database will facilitate the detection of hitherto unidentified genes, including regulatory factors and tissue-targeted transporters, which will be of particular interest for pollution cleanup. Initially, a screening study can be conducted to ascertain the most appropriate plant species or cultivars for remediating a certain metal. Additionally, to enhance biomass production and facilitate metal absorption for a certain species, it is possible to customize agronomic practices. One potential approach to enhancing plant productivity is the optimization of fertilization and planting density.

According to Horne (2000), it is possible to enhance the efficacy of phytoremediation by genetically modifying several plant species. Metal-hyperaccumulating plants and microorganisms possess unique abilities to endure, accumulate, and detoxify metals and metalloids, making them a crucial reservoir of uncommon genes. These plant species are commonly referred to as hyperaccumulators. The potential applicability of hyperaccumulators in phytoremediation is limited due to their low biomass and slow development rate (Brooks et al., 1993). Nevertheless, they serve as a great resource for studying and exploring the specific genetic and metabolic mechanisms that contribute to the excessive accumulation and detoxification of trace elements.

Additionally, these can serve as a reservoir of genes that enhance phytoremediation. Rapidly proliferating plant species could acquire these genes to enhance phytoremediation. To attain the desired characteristic, the selected species or variation can be enhanced by genetic engineering or traditional breeding methods (Fulekar et al., 2009). Plants that have been genetically modified to produce metal chelators will possess an improved ability to absorb metals (Eapen et al., 2005). The augmentation of phytoremediation could potentially be accomplished through the genetic modification of rapidly proliferating plant species, such as *Brassica juncea* (Indian mustard), with hyperaccumulator genes, particularly those associated with detoxification processes. According to Dhankher et al. (2013), the integration of the unique capabilities of a hyperaccumulator with those of high-biomass plants will be achieved. A multitude of genes are involved in many activities such as metal absorption, translocation, sequestration, chemical modification, and tolerance. One possible strategy in genetic engineering involves the upregulation of one or multiple genes (MacNair et al., 2000). The utilization of genetic engineering technologies offers two primary advantages in comparison to conventional breeding methods. The process enables the virtual replication of specific genes that encode desirable proteins from various living organisms, such as bacteria, higher plants, and animals. This expands the pool of available genes beyond the compatibility barriers imposed by genus. Additionally, it facilitates the genetic customization of specific commercial genotypes for a limited set of well-defined traits, while preserving the integrity of the genome.

Moreover, there is not always a direct relationship between the accumulation of metals, tolerance, and the productivity of plants. According to Chaney et al. (2000), it is imperative to develop a plant that possesses traits such as high productivity, high metal tolerance, and high metal accumulation by breeding or genetic engineering. According to a study conducted by Maurya et al. (2023), it has been demonstrated that genetically engineered plants, including *P. angustifolia, N. tabacum,* and *S. cucubalis*, exhibit a higher capacity to absorb heavy metal pollution compared to their wild counterparts. This can be attributed to the overexpression of glutamyl cysteine synthase. The utilization of these genetically modified plants for phytoremediation soil contaminated with metals has garnered increased interest in these biotechnological applications in recent times. Nevertheless, these technologies possess specific constraints. Currently, there exists a limited number of comprehensive applications, and the field is still in its nascent stages of advancement. To ensure the widespread use of this method in the future, it is imperative to raise awareness among the general population and provide them with accurate and comprehensible information. This will contribute to the increased acceptance of the technology as a globally sustainable solution.

FUTURE PROSPECTS

Phytoremediation indeed presents a promising approach for removing heavy metals (HMs) from wastewater in an eco-friendly and sustainable manner, with minimal impact on the flora and fauna of aquatic habitats compared to chemical and physical methods. As research progresses, there's a growing interest in understanding the genetic mechanisms behind phytoremediation. Identifying and studying the genes responsible for the phytoremediation process is crucial for advancing this technology. By pinpointing the specific genes involved, researchers can gain insights into the mechanisms plants use to absorb, transport, and detoxify heavy metals. This knowledge can then be leveraged to develop new plant species with enhanced phytoremediation capabilities. Membrane proteins play a significant role in the phytoremediation process as they are involved in transporting heavy metals across cell membranes. Research focused on identifying and characterizing these membrane proteins can provide valuable information for improving phytoremediation efficiency.

Biotechnological approaches offer exciting possibilities for enhancing phytoremediation potential. Gene editing techniques such as CRISPR/Cas9 can be used to modify the genes of aquatic macrophytes, thereby enhancing their ability to absorb, sequester, or detoxify heavy metals. By precisely manipulating the plant's genetic makeup, researchers can tailor plants to thrive in contaminated environments and effectively remediate heavy metal pollution. In summary, continued research into the genetic mechanisms underlying phytoremediation, particularly focusing on membrane proteins and biotechnological approaches, holds great promise for developing more effective and efficient phytoremediation strategies for addressing heavy metal contamination in aquatic ecosystems.

CONCLUSION

Indeed, heavy metal (HM) contamination in water is a significant environmental issue with far-reaching consequences. It not only adversely affects the environment but also poses threats to agricultural productivity and the health of aquatic organisms. In response to this challenge, various methods have been developed for the removal of heavy metals from wastewater. These methods range from conventional techniques like chemical precipitation and ion exchange to more advanced methods such as membrane filtration and electrochemical treatment. While these methods can be effective, many of them come with drawbacks such as high costs, generation of secondary pollutants, and intensive energy requirements. Phytoremediation stands out as a reliable and environmentally friendly approach for remediating wastewater contaminated with heavy metals. By harnessing the natural abilities of plants to absorb, accumulate, and detoxify pollutants, phytoremediation offers a sustainable alternative to traditional remediation methods. Moreover, phytoremediation can often be implemented at lower costs, especially in large-scale applications, and it can also help restore ecosystems and improve soil quality. However, despite its potential, there is still much to learn about optimizing and enhancing the phytoremediation process. Research efforts focused on understanding the mechanisms involved, identifying key genes and proteins, and exploring biotechnological interventions are essential for unlocking the full potential of phytoremediation. Additionally, ongoing studies on the selection of suitable plant species, optimization of growth conditions, and integration with other remediation techniques will further improve the efficiency and applicability of phytoremediation. In conclusion, while heavy metal contamination poses significant challenges, phytoremediation offers a promising solution that is both effective and sustainable. Continued research and development efforts are crucial for advancing phytoremediation as a preferred method for wastewater remediation and addressing the pressing environmental concerns associated with heavy metal pollution.

AUTHOR'S CONTRIBUTION

YS conceived the idea; YS, SB, G, SP and KA wrote the manuscript. The complete manuscript was revised and edited by YS, SP and KA.

DISCLOSURE STATEMENT

No potential conflict of interest was reported by the authors.

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