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Strategic management of contaminated water bodies: Omics, genome-editing and other recent advances in phytoremediation

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Authors dedicate this review article in the fond memory of our dear colleague late Ms. Sanchita Gupta, who left footprints of grace in our lives.

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ABSTRACT

Due to excessive industrialization, urbanization and rising level of pollutants, the water bodies are continuously exposed to diverse group of toxic pollutants whose concentration, chemical forms and bioavailability in the aqueous system are governed by natural geochemical cycles as well as human activities. Chemical form or species of these toxic compounds or heavy metals is governed by the prevailing specific physical and chemical parameters like pH, salinity, dissolved organics, hardness and sedimentary load which impacts their mobility, bio-availability and hence toxicity. To overcome the challenging situation, an increased emphasis has been given on *in-situ* innovative technologies like phytoremediation, capable of detoxification of contaminated water bodies. The use of inherent aquatic plants along with recent omics tools can improve their phytoremediation ability to a great extent. These plants have an extensive root system which can filter and immobilize sediments, contaminants, fertilizer and pesticide run-off thereby reducing water pollution. Thus, the assortment and improvement of plants phytoremediation ability using the modern biotechnological tools and techniques, can be a suitable approach for successful remediation of contaminated water bodies.

This review article focuses on the different biotechnological strategies like omics, proteomics, genomics, metabolomics and CRISPR in order to ameliorate the phytoremediation potential of the aquatic plants. The recent ground-breaking advancements to achieve desired genetic modification uses, CRISPR technology, to transfer a target set of genes to be assimilated into the plant genome of interest. Two variants of this technology, i.e. CRISPR-Cas9 as well as CRISPR-Cpf1 can be used for producing knock-outs, making gene substitutions, and targeting transcription and its regulation in the particular plant genome, to improve its phytoremediation efficiency.

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1. Introduction

Water bodies are considered as apposite places for disposing off the domestic and industrial wastes which drains ultimately into the sea (Jadia and Fulekar, 2009). Rapid expansion in urban areas and booming population worldwide have exacted a huge toll on the rivers and streams leading to their pollution. As stated by reports of Central Pollution Control Board (CPCB, 2016), India that 63% of the urban sewage discharged into rivers (approximately 62 billion liters a

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day) is untreated. However, the increase in noxious wastes and mistreatment of the water resources by agricultural fields, industrial and thermal power plants, in order to meet the requirements of the increasing population, has significantly reduced the assimilative ability of such contaminants in the environment (Bin-Dahman and Saleh, 2020; Saleh et al., 2019). Thus, the two-fold stress put forth on the aquatic systems is eventually confronted by the biota inhabiting them. Among other aquatic life forms, fish is the primary aquatic community concerning the health of human beings (Datta, 2015). Since the healthy aquatic ecosystem relies on a complex web of interaction among plants, animals, and other living organisms dwelling in the system and affecting each other directly or indirectly. So the harmful effect on one of the community can lead to chain-effect and jeopardizing entire ecosystem (Hoang et al., 2020).

The breeding, rearing and harvesting of aquatic organisms for food and other value products is referred as aquaculture, which is one of the fastest growing food economies in the world (Mohd Nizam et al., 2020). As in terms of value and production volume, the global production of aquaculture has approximately doubled in the past decade and Asia dominates this economy by producing 88.9% of the food volume by 2014 (Mohd Nizam et al., 2020; Nadarajah and Flaaten, 2017). And the major setback in the growth of this sector is the undue exploitation and pollution of the natural habitat (Tan et al., 2021). Thus in order to remove the toxic compounds from the aquatic ecosystems, various physical, chemical and biological processes are employed, but amongst all, phytotechnologies, being the eco-friendly, are the most suitable alternative to other conventional technologies (Saleh, 2021; Sbani et al., 2020). These conventional technologies which includes ion exchange, chemical precipitation, adsorption using carbon, membrane filtration etc., are too expensive as compared to phytotechnologies being employed for management of contaminated water bodies (Rajasulochana and Preethy, 2016).

Aquatic macrophytes that dwells in the contaminated environment owns a variety of machinery and mechanisms to cope with the contaminants especially inorganic heavy metals, organic-hydrocarbons and chemicals like – pesticides, fungicides, herbicides etc. (Kozmińska et al., 2017). Plants can sequester some cations and anions in their cell walls or store the pollutants inside the vacuoles. These plants have a range of mechanisms to uptake the pollutants and detoxify the aquatic environment (Ekperusi et al., 2020). Recent advancements in the molecular biology and genetic engineering enables the researchers to identify the critical genes in the plants of interest to improve the overall phytoremediation potential. Such genes can be exploited for the enhanced uptake of pollutants and can effectively manipulate the phytoremediation processes like phytoextraction, phytostabilisation, rhizofiltration, phytovolatilization, phytotransformation, rhizodegradation etc. But developing potent plants capable of withstanding high level of pollutants is a matter of interest in order to apply the recent ground breaking technologies effectively for field remediation.

In the preview, we have explored the current problems of pollution, challenges in the application of phytotechnologies and opportunities in this direction to achieve a sustainable solution towards reclamation of contaminated water bodies, using omics and genome editing tools in the plants of interest.

1.1. Problem of pollution in aquatic environment

The aquatic environment gets contaminated when the polluting substance is released into the water bodies and reaches a critical limit of accumulation in the system. Generally, the provenance of water pollution are categorized as point sources or non-point sources of pollution (Li and Wu, 2019). It is quite difficult to control the non-point sources of pollution as they indirectly delivers the pollutants to the nearby water bodies. Such sources includes pollutants which are carried away to the soil and groundwater system along with rain water. Thus at some places, it is quite common to find that groundwater system is contaminated with chemical fertilizers, pesticides, toxic heavy metals and their leachates (Chen et al., 2005, 2007).

One of the major non-point source of aquatic pollution includes acquittal of sewage and chemical fertilizers rich in phosphates and nitrates. In surplus levels, these nitrates and phosphates encourages the growth of algae and aquatic plants. Unrestrained growth of these algae and small plants consequently clogs the watercourses, uses up its dissolved oxygen (DO) in decomposing complex matter, and ultimately blocks sunlight to reach deeper waters. This process is called eutrophication (Khan, 2014). This process slowly increases the amount of organic matter and sediments in the water bodies. Thus it evidences the threatening effects to aquatic creatures as it distresses the breathing ability of fishes and other invertebrates dwelling in that water (Weber et al., 2011). Another cause of water pollution is the silt and suspended solids that are carried along with rainwater from ploughed fields, urban areas, construction sites, eroded lands and river banks. When these sediments enters aquatic environment, respiration ability of fishes becomes impaired, vegetal productivity is reduced and aquatic environment become suffocated (Kazlauskiene et al., 2012).

The nature of polluting substance can be varied including organic and inorganic pollutants. Organic pollutants may include polychlorinated biphenyls (PCBs), hexachlorobenzene (HCB), polybrominated diphenylethers (PBDEs), polycyclic aromatic hydrocarbons (PAHs), pesticides like lindane and Dichloro diphenyl trichloroethane (DDT) (Weber et al., 2011). These chemicals possessing high toxicity, highly persistent nature and bioaccumulation potential are often known as persistent organic pollutants (POPs), as they can remain in the water bodies for a longer time period even after the original source has been removed as they are resistant to degradation and have a tendency to bind strongly to sediments in water systems due to their hydrophobicity (Weber et al., 2011). They are not easily metabolized and removed from bodies of higher organisms and thus get accumulated in their lipid tissues. Such process is called as bioaccumulation (Jadia and Fulekar, 2009). Humans and marine mammals are mostly affected by the POPs being at the top most trophic

level of the food chain (Kazlauskienė et al., 2012; Bouwman, 2003). PCBs is also a category of compounds under persistent organic pollutants (POPs) (Larsen, 2006). According to EPA (2012), the ingestion of PCB contaminated water can result in an increased risk of reproductive and nervous disorders, immune-deficiency diseases and cancer (Scragg, 2005). The use of PCBs was prohibited in the year 1977.

While, Inorganic pollutants comprise of heavy metals, radionuclides, inorganic salts, trace metals and their compounds, metal-complex with organic compounds, cyanides and sulphates, etc. (Ali et al., 2020). These compounds are indestructible and can persevere in the environment for a longer period of time (Tchounwou et al., 2012). Commonly found heavy metal contaminants in soil and water bodies are chromium (Cr), arsenic (As), copper (Cu), lead (Pb), mercury (Hg), nickel (Ni) and zinc (Zn) (Ali et al., 2020). Some of them have biological functions in plant growth while others are toxic and responsible for various diseases in living organisms (Tchounwou et al., 2012; Wani et al., 2017). Based on their level of toxicity the heavy metals can be categorized as extremely poisonous including Cd, Se, Hg, As, Pb, Zn; moderately poisonous including Co, Ni, Cr, Cu, Mo; and least toxic includes Mn, Sr, Ba (Ashraf et al., 2019; Raikwar et al., 2008).

2. Phytoremediation approach to detoxify contaminated water bodies

Phytoremediation is a set of eco-friendly technologies (phytoremediation processes like phytoextraction, phytostabilisation, rhizofiltration, phytovolatilization, phytotransformation, rhizodegradation, phytodegradation) which aim to remove, reduce, degrade and immobilize the various organic and inorganic toxins accumulated in the environment, so the restored sites (soil or water) can become useable for public or private applications (Fig. 1). Plants own a variety of machinery and mechanisms to cope with the contaminations especially inorganic heavy metals, organic-hydrocarbons, and chemicals like – pesticides, fungicides, herbicides etc.

For organic contaminants, plants and their associated rhizospheric microbial communities can have multifaceted actions like pollutants can be immobilized, stored, transformed or degraded or mineralized into lesser toxic forms, but it is clearly dependent on the specific compounds and the environmental conditions in which plant is growing (Kushwaha et al., 2015). The phytotechnologies responsible for removing organic contaminants are rhizodegradation, phytodegradation, transformation, mineralization and phytostabilization (Agarwal et al., 2020; Rani et al., 2012).

On the other hand, in case of inorganic contaminants, the heavy metals, metalloids and radionuclides can get accumulated and extracted in the roots and shoots of the plant. The major phytotechnologies involved in amelioration of (Agarwal et al., 2020; Kushwaha et al., 2015) inorganic pollutants are phytoextraction, rhizofiltration, phytoaccumulation, phytovolatilization, enzymatic modification, etc. Plants can sequester some cations and anions in their cell walls or store the pollutants inside the vacuoles (Sheoran et al., 2010; Rascio and Izzo, 2011). Thus plants that can thrive in heavy metal contaminated environments are also called as metallophytes (Sheoran et al., 2010; Ashraf et al., 2019). Plants have phytochelatins, metallothioneins and cysteine rich peptides that help in the chelation of contaminants and complex them in the plant tissues (Hall, 2002). There are certain hyperaccumulator plants which can actively take up large amount of heavy metals, 100–1000 folds, from the soil and water environment in which they grow and translocate the absorbed heavy metal content to the shoots and accumulate it in the leaves of the plants without showing any symptoms of toxicity (Sheoran et al., 2010; Malik and Biswas, 2012; Rascio and Izzo, 2011; Rascio, 1977). In order to select the natural hyperaccumulators significant in phytoextraction, both bioconcentration factor (BCF) and translocation factor (TF) are important to be studied.

The two main characteristics of an ideal plant species useful in phytoremediation are:

- A. high metal accretion capacity, or
- B. Dense biomass production with greater metal uptake potential (root-to-shoot translocation) (Kozłmińska et al., 2017).

The paradigm for selection of plants for phytoremediation (Sarma, 2011; Fasani, 2012)

- (a) The level of tolerance for the type of contamination on the site should be high
- (b) The capability of the plant to uptake, accumulate and translocate the contaminants
- (c) Higher growth rate and biomass yield
- (d) Level of tolerance to drought and water logging
- (e) Level of tolerance to high pH and saline conditions
- (f) Characteristics of root zone

There are certain mechanisms which are attributable to contribute towards heavy metal tolerance level of plants, (refer Fig. 1) which includes:

- (a) Synthesis of metal ion binding chelating peptides like phytochelatin (PC) and metallothionein (MT), amino acids, and organic acids;
- (b) Alteration in membrane structures;
- (c) ROS species production and antioxidant enzymes production;

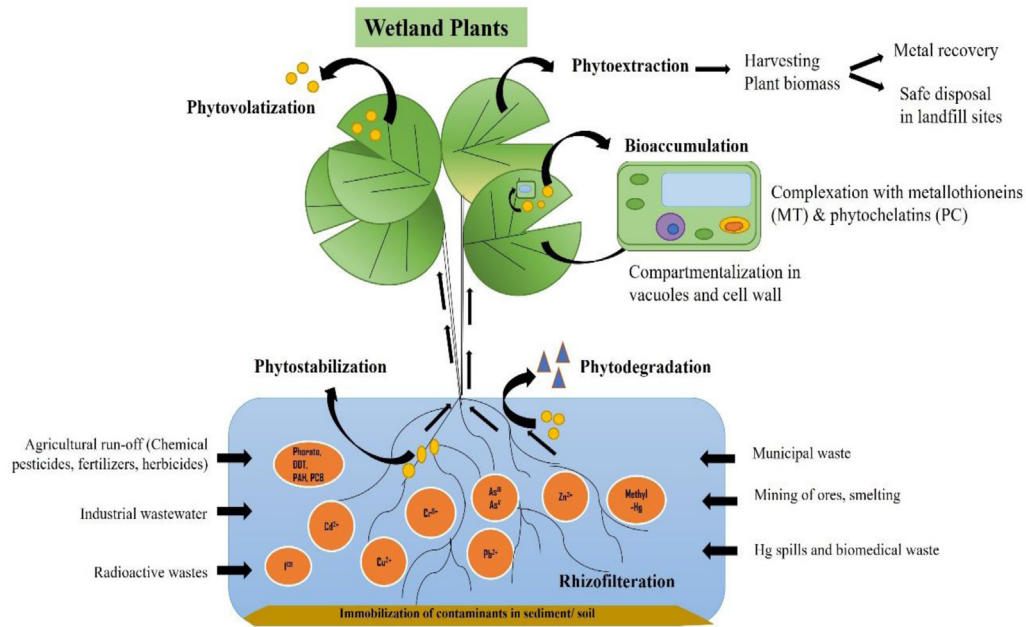


Fig. 1. Phytoremediation approaches in detoxification of contaminated water bodies.

- (d) Metal deposition in vacuoles (compartmentalization)
 (e) Transport proteins like ATPases, cation diffusor facilitator (CDF) proteins, zinc ion permeases (ZIP) plays role in metal uptake.

3. Aquatic macrophytes for organic pollutants removal

Aquatic plants are the potential phytoremediators as well as they serve to be bioindicators for monitoring of contaminated water bodies (Ekperusi et al., 2020). These plants have widespread root system and are useful in transforming organic and inorganic contaminants present in the water (Khataee et al., 2012). They have higher biomass production rate, sporadic growth pattern, widespread distribution in terms of habitat, and grows exponentially in the water systems for eg., natural and constructed wetlands. Free floating aquatic plants of *Lemnaceae* family, mostly *L. minor*, are able to transform and eliminate azo-dyes like Acid blue dye (AB92) and other textile dyes by converting it into its by-products (Ansari et al., 2020; Khataee et al., 2012). *Elodea* sp. are known to bioaccumulate and phytotransform the DDT into DDD (Ekperusi et al., 2020). *Azolla* and *H. verticillata* are reported to have the ability to remediate the toxic fly ash from the water body (Pandey, 2012; Srivastava et al., 2010).

Some of the macrophytes including *Lemna minor*, *Potamogeton perfoliatus*, *Chara vulgaris* and *Myriophyllum spicatum* have been reported to remove alkylbenzenesulfonate from contaminated waste water (Ansari et al., 2020). Similarly, Ekperusi et al. (2020) have assessed the removal of petroleum hydrocarbon by *Lemna paucicostata* plant, they have reported. They found the removal rate of petroleum hydrocarbons was significantly high within the initial 60 days of study which gradually decreased upto 120 days of study. Another study by Laet et al. (2019) has revealed that *E. crassipes* (water hyacinth) plant was able to bioaccumulate four organic pollutants from the water, while the dry biomass of its roots showed biosorption ability for di-n-hexyl phthalate and pentabromo-diphenyl ether (endocrine disruptors). Similarly, phytodegradation of bisphenol A (2,2-bis (4-hydroxyphenyl) propane) and ethion from natural habitat by *E. crassipes* (water hyacinth) was reported by Kang and Kondo (2006) and Xia and Ma (2006). Duckweed are popularly known for transforming the higher carbon chain hydrocarbons into lower carbon chain hydrocarbons (C30–C40), which are eventually conjugated and sequestered by the plants (Ekperusi et al., 2020; refer Table 1).

4. Aquatic macrophytes for inorganic pollutants removal

Plants often require some heavy metals in limited amount for their growth and development but certain plants have the capability to phytoaccumulate or transform these metals into some other forms. Excessive amount of these HMs can be toxic for plant and can impair physiological processes like ROS production, photosynthesis, respiration, cell growth and development and death of plants (Ansari et al., 2020; Zhang et al., 2017). Aquatic macrophytes that can survive

Table 1
Aquatic macrophytes for organic and inorganic pollutants removal.

Aquatic macrophytes/plants	Target contaminants	Phytoremediation/	References
Organic contaminants			
<i>Lemna minor</i> (Duckweed)	Azo-dyes like Acid blue dye (AB92) and other textile dyes and C30–C40 hydrocarbons	Transformation of dyes into by-products results into enhanced phytoremediation; Similarly, transformation of the higher carbon chain hydrocarbons into lower carbon chain hydrocarbons (C30–C40), which are eventually conjugated and sequestered by the plants.	Ansari et al. (2020); Khataee et al. (2012). Ekperusi et al. (2020)
<i>Elodea</i> sp. (American waterweed)	DDT (dichlorodiphenyltrichloroethane)	Bioaccumulate and phytotransform the DDT into DDD	Ekperusi et al. (2020)
<i>Myriophyllum spicatum</i> , <i>Lemna minor</i> , <i>Potamogeton perfoliatus</i> , <i>Chara vulgaris</i>	Alkylbenzenesulfonate		Ansari et al. (2020)
<i>Lemna paucicostata</i> (Duckweed)	Petroleum hydrocarbon	Removal rate of petroleum hydrocarbons was significantly high within the initial 60 days of study which gradually decreased upto 120 days of study.	Ekperusi et al. (2020)
<i>E. crassipes</i> (Water hyacinth)	Bisphenol A (2,2-bis (4-hydroxyphenyl) propane) and ethion, di-n-hexyl phthalate and pentabromo-diphenyl ether; Ethion, dicofol, pentachlorophenol present in pesticide contaminated water	Phytodegradation of target organic contaminants, and eventually uptake by the plants.	Kang and Kondo (2006), Xia and Ma (2006), Laet et al. (2019); Dhir (2013).
<i>B. juncea</i> (Indian Mustard)	Phorate {O,O-diethyl S-[(ethylthio) methyl]phosphorodi-thioate}	Absorption and uptake of phorate by plants resulted in 68.28% decrease in phorate concentration in the medium within 5 days of study.	(Rani et al., 2012)
<i>Eleocharis ochrostachys</i> (Spike Rush)	Water polluted with diesel (PAHs-contaminated water)	<i>E. ochrostachys</i> plants showed better results of phytoremediation of PAHs from 1% diesel contaminated wastewater.	(Sbani et al., 2020)
Inorganic contaminants/ heavy metals			
<i>Spirodela polyrhiza</i>	As	Removal of arsenic via sorption and the pathway involved is the phosphate uptake pathway from the As contaminated wastewater	(Rahman et al., 2007)
<i>Pteris vittata</i> (Brake ferns)	As	Phytovolatilization (90% efficiency)	Rascio and Izzo (2011)
<i>Utricularia gibba</i> (Floating bladderwort)	Cr (VI)	Efficient in removing Cr (VI) in conc. of 50µM over a short time	Augustynowicz et al. (2015)
<i>Oenothera picensis</i> , <i>Spirulina</i>	Cu and Cr	removal of copper and chromium by phytoextraction and chemisorption respectively	(Jasrotia et al., 2017)
<i>E. crassipes</i> (Water hyacinth)	palladium (Pd)	Phytoremediation of palladium by rhizofiltration	Laet et al. (2019) and Deyris and Grison (2018)
<i>Zinnia elegans</i>	Chromium [Cr (VI)]	Improved phytostabilisation and bioaccumulation of chromium into roots of this plant.	(Panda et al., 2020)

in contaminated environments and can detoxify the water bodies includes species of *Lemna*, *Eichornia*, *Pteris*, *Wolfia*, *Spirodela*, *Hydrilla*, *Pistia*, *Typha* and *Cryspogon* etc. (refer Table 1). Similarly species of *Thlaspi* are able to hyperaccumulate more than one metal and these plants are able to better survive in Ni contaminated environment. Also *T. caerulescens* is reported to accumulate Ni, Zn, Cd and Pb (Rascio and Izzo, 2011). *Spirodela polyrhiza* is a species of duckweed which is popularly known for removal of arsenic through sorption and phosphate uptake pathway (Rahman et al., 2007). *Eichornia crassipes* is a popular water hyacinth that can phytoextract the important metals like palladium (Pd) by rhizofiltration process. This is an effective remedial measure for recycling precious and expensive metals which can be recycled through

roots of the plant (Laet et al., 2019; Deyris and Grison, 2018). Researchers have reported the use of *Oenothera picensis* and *Spirulina* sp. for removal of copper and chromium from the contaminated water bodies by phytoextraction and chemisorption respectively (Jasrotia et al., 2017).

Islam et al. (2015) reported the removal of cadmium and arsenic from contaminated water using *Micranthemum umbrosum* through phytofiltration method. Brake ferns (*Pteris* sp.) can survive in arsenic rich water and are well studied for phytoextraction of arsenic from aquatic environment. As^V is reduced to As^{III} in the roots of these ferns due to the activity of glutathione dependent arsenate reductase and then translocated to shoots through the xylem sap in the As^{III} form (Ma et al., 2001; Rascio and Izzo, 2011). It is a reported fact that arsenic is stored in the hyperaccumulator ferns in the As^{III} form within the vacuoles although their transport mechanism located at the tonoplast which is not yet found (Rascio and Izzo, 2011).

Plants have metal chelating genes and their subsequent proteins including various metallothioneins (MT) and phytochelatin (PC) which are much needed by plant cells to maintain metal homeostasis and shows metal sequestration properties (Mustafa and Komatsu, 2016; Koźmińska et al., 2017). To sequester the heavy metals, these cysteine rich peptides, phytochelatin, forms complexes with metal ions and gets bioaccumulated in vacuoles of the plant cells (Mustafa and Komatsu, 2016). Similarly metallothioneins (MT) have unique ability to complex the metal ions by forming mercaptide bonds and they are known for their unique structural and redox properties (Koźmińska et al., 2017). Another study by Ahsan et al. (2012) revealed upon exposure to Cd, soybean plants showed more accumulation of glutathione and phytochelatin to cope up with the metal stress. The higher concentrations of glutathione in plant cells is often associated with an increased tolerance to metal stress and as a function in metal detoxification. The enzyme, glutathione S-transferases uses glutathione as a coenzyme, are expressed in increased amount in plants growing under heavy metal stress (Ahsan et al., 2012; Mustafa and Komatsu, 2016).

With the amendment of a chelating agent like EDTA, the efficiency of uptake of toxic contaminants by wetland plant species including *Typha* sp. and floating wetland macrophytes such as *Pistia* sp., *Azolla* sp., *Lemna* sp., *Salvinia* sp., and *Eichhornia* sp. are enhanced. It is a more pronounced phenomenon with the uptake of heavy metal like Pb and Cu. However, the pattern of uptake of heavy metals (HMs) by plants is similar as that without EDTA alterations (Mahar et al., 2016). This method is reliable and may continue for a long time with the recurrent application of chelating agents. In order to dissolve both Fe and Ca compounds simultaneously, an excess amount of the chelate forming agents are necessary for the transformation of heavy metals containing compounds (Mahar et al., 2016). The amendment of chelants to soil changes the main way of their absorption from the symplast to the apoplast by the plants as well as it upsurges the total concentration of the soluble metals (Mahar et al., 2016). But chemical amendment methods can have hazardous effects on physical and bio-chemical properties of soil and can cause contamination of groundwater (Aziz et al., 2015; Yang et al., 2016; Postigo et al., 2017). Thus, the environmentally sustainable approach to overcome this problem is the application of acidified manure which enhances heavy metal bioavailability by lowering the soil pH (Ashraf et al., 2019).

5. Genetic modification in plants species for enhanced phytoremediation

Genetic engineering tools are applied in bioremediation based studies in which the aim is to improve the biomass content and extensive root growth and hyperaccumulator genes of the target plant (Mosa et al., 2016). Certain model aquatic plants and their genomic sequences can be used to identify the critical genetic elements important for the phytoremediation potential of the particular plant species of interest. Such data sequences could be manipulated to be exploited for enhanced phytoremediation processes like phytoextraction, phytostabilisation, rhizofiltration, phyto-volatilization, phytotransformation, rhizodegradation etc. But in recent advances, more emphasis should be given on identification and development of genomic regions that correlate with the accumulation and survival of plant species with varying tolerance of the polluted environment, which is a major concern while developing potent plants for efficient on-site phytoremediation (Shriram et al., 2016; Mosa et al., 2016). Combination of the omics approaches can result in a more competent way to achieve desired results in terms of pollutant removal (Kaur et al., 2019) (refer Fig. 2).

5.1. Omics

Aquatic plants have great phytoremediation ability which can further be improved by modification at molecular levels, the omics approach has been of increasing interest for various researchers. Advanced approaches to improve the phytoremediation efficacy of aquatic macrophytes, different omics approaches like genomics, proteomics, transcriptomics, and metabolomics can be put to use (Fig. 2). Advancement in genomics research can comprise advances in application of molecular markers, comparative genomics, functional genomics and genome sequencing, using aquatic plants. The emerging omics approaches are revealing the genetic mechanisms behind the phytoremediation ability and molecular variability of the target plant (Rai et al., 2021). Genomic approaches have identified genes that play an important role in the tolerance mechanisms and phytoremediation ability of the plant towards the various contaminants. The high throughput

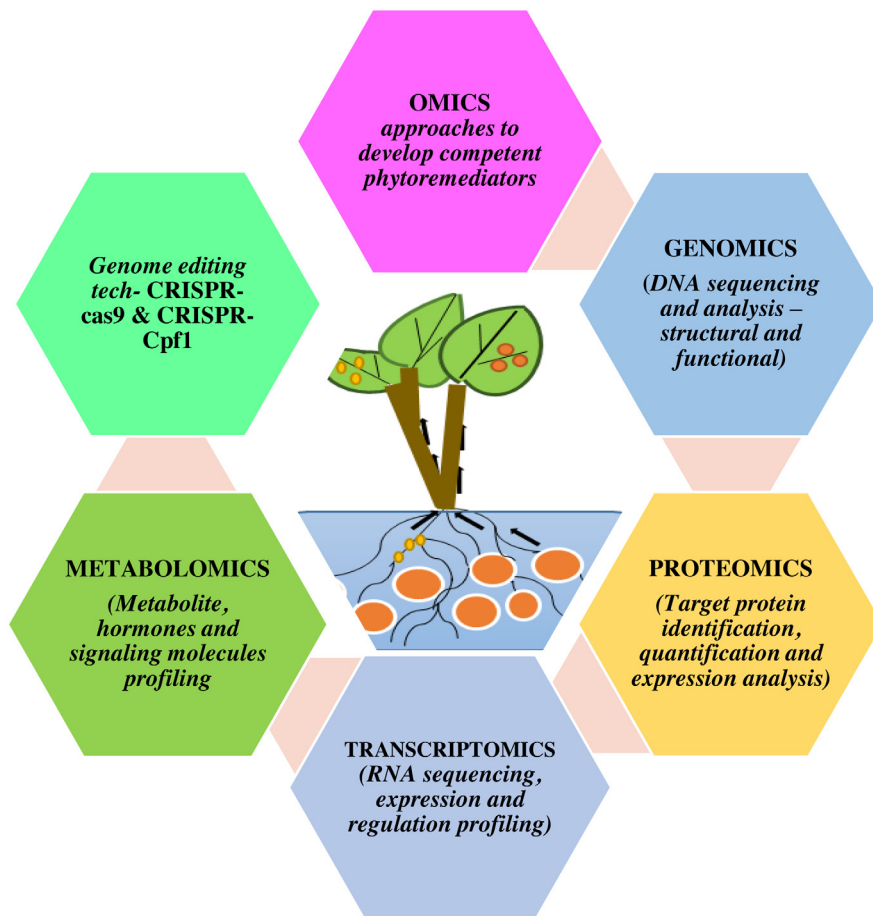


Fig. 2. Recent strategies which can enhance phytoremediation ability of the macrophytes/ plants.

molecular biology techniques are critically important for the identification of genes responsible for desired plant traits (Zheng et al., 2008).

5.2. Proteomics based regulation of heavy metal uptake

Altered protein function and structural changes are often depicted in the plant systems exposed to abiotic metal stress in the environment. These changes can occur at gene level or at translational protein level as well (Rai et al., 2021; Singh et al., 2016). HMs have the ability to obstruct the normal functioning at post-transcriptional level, protein–protein interactions and splicing mechanism, which can eventually leads to inhibition of protein expression (Hossain and Komatsu, 2013). Studies of proteomic changes in the hyperaccumulator plant *Phytolacca americana* upon exposure to Cd stress (0.4 mM), revealed that 11 genes that were found responsible for photosynthesis and glutathione metabolism pathway were downregulated (Zhao et al., 2011). Similarly, in Al stressed *Glycine max*, Researchers have identified and reported 40 differentially regulated spots and further characterization of these protein spots was performed by MALDI-TOF-MS. They reported that 21 of proteins were attributable for antioxidant defense system and were upregulated while 14 newly stored and 5 other proteins were downregulated (Zheng et al., 2008). These newly formed proteins were found to be concomitant with signal transduction, biosynthesis of cysteine synthase enzyme and sulphur metabolism in plant cells, which was confirmed with western blot.

Yannarelli et al. (2007) did proteome analysis of Cd stressed wheat roots by 2-D electrophoresis and revealed 46 protein spots. He reported that proteins belonging to antioxidant defense system were upregulated while downregulated proteins are found responsible for photosynthetic and ETS processes. Additionally, these proteomic studies had contributed to identification of prospective protein markers that can be used to differentiate between tolerant and susceptible genotypes. This can be achieved by studying the alteration in the specific physiological and biochemical processes of the plants system (Hossain and Komatsu, 2013).

It is critically important to understand the role of HMs in altering the expression of important proteins that are generally involved in signalling cascade, metal uptake, metal detoxification, enhanced phytochelatins and metallothioneins synthesis, or plant's growth under stressed environment through proteomics based studies. It would bring out the insight of the molecular knowledge and will give an enhanced idea of how this cascade could be dealt to manage the phytoremediation ability.

However, the major challenge in the application of proteomic studies that still persists is the limitation of genomic data of several plants, whose full-genome sequences are still not available. So, in order to study important physiological and molecular functions in the phytoremediators plants of same species or genus, complete proteomic reference map are an absolute necessity. However, substantial advancement in this direction, has been made that have expedited our knowledge of plant responses to heavy metals stress, which will be helpful in further screening of particular genes for preparing stress tolerant cultivars.

5.3. Metabolomics

Metabolomics studies are a powerful technique for finding the potential routes in improvement of phytoremediation potential of certain plants by reviewing the plant-metal interaction and identifying the metabolites synthesized by plant as the stress-response or detoxification mechanism. For instance, upon analysis of chromium treated *O. sativa* plants, key observations included extensive accretion of proline and ornithine which are known to protect the plants from Cr-induced oxidative damage. Additionally, an increase in the linoleic acid was also observed which is perceived to protect the cells from lipid peroxidation by strengthening the cell wall and membrane (Rai et al., 2021; Dubey et al., 2010).

Metabolomics study also provides knowledge about the novel metabolic pathways that are believed to be linked with the existing biochemical pathways or network, in order to find the lineage between them. This will help in filling gaps of valuable information related to detoxification pathways of such plants of interest whose genome are not fully sequenced and studied (Rai et al., 2021).

Various researchers have reported the studies related to alleviation Cd stress using the metabolomics approach in plants, as this heavy metal stress is commonly observed in the agricultural farmland as a result of certain industrial activities (Villiers et al., 2011). GC-MS analysis of *Arabidopsis* plants affected by cadmium toxicity, revealed an upregulation of particular metabolites such as proline, glycine, betaine, glycerol and trehalose along with glutathione and ascorbate to combat Cd-induced oxidative damages in the plant cells (Rai et al., 2021).

Sadi et al. (2008) revealed that LC-MS can also be used as a metabolomics tool for studying the Cd toxicity in *A. thaliana* plants and the analysis of peaks of LC-MS revealed the identification of several phytochelatins that were being synthesized by plants cells as a detoxification mechanism. Similarly, new metabolic pathways can be introduced into plants, for example, introduction of MerA and MerB genes which eventually lead to enhanced tolerance to Hg and improved phytovolatilization of mercury (Eapen and D'souza, 2005).

It is an important fact that metabolomics can serve as an implicit tool and holds a remarkable prospect to determine novel and distinctive information based on the assortment and selection of metabolic networks, or developing a metabolome survey that can eventually help in the elucidation of data, building metabolic networks, in order to find the novel stress-responsive metabolites. Such approach can be extremely beneficial for developing superior cultivars with enhanced heavy metal stress tolerance and phytoremediation ability.

5.4. Transgenic plants for phytoremediation of contaminated water bodies

Another important aspect in order to improve the potential of phytoextraction, is either through selective breeding or through development of transgenic plants that are engineered to express metal hyperaccumulation genes (Rascio and Izzo, 2011). Transgenic plants are produced with an intend to combine multiple desired traits like rapid growth rate, higher biomass production, better tolerance and accumulation of multiple contaminants, to achieve additional phytoremediation efficacy (Lu et al., 2017). Researchers have developed various transgenic plants by either transferring target genes in the plant of interest or by developing somatic hybrids for enhanced remediation of inorganic contaminants (Table 3) and organic contaminants (Table 4). For instance, somatic hybrid developed using *T. caerulea* and *B. napus* and *T. caerulea* and *B. juncea* shows enhanced biomass production and more Zn accumulation and Pb phytoextraction respectively (Rascio and Izzo, 2011). Wu et al. (2011) found and reported a transgenic *Petunia hybrid plant* with the *CAXcd* (an *Arabidopsis* CAX1 mutant) gene for enhanced Cd tolerance and accumulation. The transgenic plants were able to accumulate up to 2.5 times more Cd than the controls. Similarly, transgenic *B. juncea* is able to better remediate selenium from contaminated soil as well as hydroponically, by the expression of *APS* (ATP sulfurylase) & *SMT* genes. Moreover, it is critically important to understand the functions and regulations of genes involved in metal uptake, hyperaccumulation, translocation via xylem, detoxification and sequestration mechanisms in order to strategically manage the contaminated water systems without harming the wild species of the plants (see Table 2).

Similarly, on the other hand for the enhanced phytoremediation of organic or refractory pollutants, transgenic tobacco was used to express a number of genes comprising *Nfs1* (encodes for nitroreductase), *onr* gene that encodes

Table 2

Various strategies, their advantages and limitations which are useful to enhance phytoremediation ability.

S.No.	Strategy to enhance phytoremediation	Advantages	Limitations
1.	Genomics approach	1. An in-depth study of individual genes, proteins, or biological processes in aquatic plants can be helpful in achieving comprehensive development of aquatic plant genomics for enhanced phytoremediation.	1. The limitation of using this technology is that whole genome sequences of many aquatic plants of interest is not yet reported. Also this strategy cannot be employed as a stand-alone tool.
2.	Proteomics based regulation of heavy metal uptake	1. Proteomics approach focuses on the insight of the molecular knowledge and gives an enhanced idea of how this cascade could be dealt to manage the phytoremediation ability. 2. This technique is advantageous in screening of particular genes for preparing stress tolerant cultivars.	1. The major challenge in the application of proteomic studies, which still persists is the limitation of genomic data of several plants, whose full-genome sequences are still not available. 2. Complete proteomic reference map are an absolute necessity, which is a major limitation.
3.	Metabolomics approach	1. A powerful technique for finding the potential routes in improvement of phytoremediation potential of certain plants by studying the plant-metal interaction and identifying the metabolites synthesized by plant as the stress-response. 2. It provides knowledge about the novel metabolic pathways that are believed to be linked with the existing biochemical pathways or network, in order to find the lineage between them. 3. This technology is helpful in filling gaps of valuable information related to detoxification pathways of plants of interest whose genome are not fully sequenced and studied.	1. It is a tedious study to track the metabolism pathway and identify the potential targets.
4.	Transgenic plants for phytoremediation of contaminated water bodies	1. This method has been wide applicability and has been employed on field scale compared to omics based approaches. 2. Enhanced remediation ability in transgenic plants leads to low cost and reduces the use of chemicals or other costly conventional methods of remediation. 3. Low maintenance and eco-friendly.	1. It is a tedious task to produce transgenic plants using the identified hyperaccumulators. 2. Risk of invasion into the natural plant communities due to the high vigour and spread ability of transgenic plants.
5.	Genetically modified plants species for enhanced phytoremediation	1. GM plants can enhance phytoremediation to many folds due to higher accumulation of pollutants even for longer period of time. 2. Reduces treatment cost.	1. Risk of invasion into the natural plant communities.

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for pentaerythritol tetranitrate reductase enzyme, (PETN) for the enhanced removal of GNT and TNT (Abhilash et al., 2009). Dixit et al. (2008) reported the use of *CYP450 2E1* (Cytochrome P450 Monooxygenase enzyme) gene in *N. tabacum* for the enhanced degradation of TCE, ethylene dibromide, anthracene and chloropyrifos. Also, Up-regulation of ECS and GS in transgenic *B. juncea* could amplify tolerance of plants towards atrazine, 1-chloro-2, 4-dinitrobenzene, phenanthrene, metolachlor (Flocco et al., 2004). *XplA* and *XplB* genes isolated from soil bacterium, *Rhodococcus rhodochorus* (genus Nocardiaceae) was used to increase detoxification of RDX in *A. thaliana* plants as reported by Jackson et al. (2007). Uchida et al. (2005) also revealed the use of *A. thaliana* plants for enhanced rhizodegradation 2, 3-dihydroxybiphenyl (2, 3-DHB) by over-expression of *DbfB* gene isolated from *Terrabacter sp.* The genes *tpx1* and *tpx2* (that encodes for peroxidases)

Table 2 (continued).

S.No.	Strategy to enhance phytoremediation	Advantages	Limitations
6.	Genome editing tool like CRISPR technology	<p>1. CRISPR systems will enhance phytoremediation ability of plants via a break-through into bioaccumulation, complexation, volatilization and degradation processes.</p> <p>2. This technology is more inclined towards the monocotyledonous plants owing to their high G-C content but it is believed that it is a realistic approach in development of advanced phenotypes.</p>	<p>1. It cannot be ignored that increasing the accretion of a metal by expression of a desired gene or a set of genes could trigger hypersensitivity or toxicity in the plant that may affect its detoxification mechanisms and eventually cause the decay of plants.</p> <p>2. There are still few limitations associated with the implementation of CRISPR/Cas facilitated transformation in aquatic plants like the chances of higher incongruities among transformed colonies for desired genomic changes.</p> <p>3. Previous reports on plant transformations have indicated that only 20%–50% of the CRISPR-transformed colonies were possessing the desired mutation</p>

isolated from tomato plants were used to modify hairy cultures of transgenic tobacco which eventually showed better detoxification of phenol from contaminated water (Alderete et al., 2009). Also, Inui and Ohkawa (2005) reported *Solanum tuberosum* was modified using *CYP1A1*, *CYP2B6* and *CYP2C19* in order to improve resistance of transgenic plants towards sulfonyleurea and other herbicides (Refer Table 4).

5.5. CRISPR technology

Among certain genetic approaches in this direction, CRISPR (clustered regularly interspaced short palindromic repeats) is an incipient tool which can be exploited in order to enrich the directed traits in plants of interest (Kaur et al., 2019). CRISPR technology is based on transfer of a target set of desired instruction in order to get assimilated into the plant genome of interest (Agarwal et al., 2020; Basharat et al., 2018; Yin et al., 2017; Sander and Joung, 2014). Moreover, two variants of this cutting-edge genome editing tech., including CRISPR-Cpf1 and CRISPR-Cas9 that can facilitate removing a particular gene of interest for developing knock-outs plants, or else making gene substitutions, or doing transcriptional regulation in the particular plant's genome (Agarwal et al., 2020; Yin et al., 2017). CRISPR systems will enhance phytoremediation ability of plants via a break-through into bioaccumulation, complexation, volatilization and degradation processes (Rai et al., 2021; Sander and Joung, 2014). This technology can ameliorate the toxic effects of heavy metals, organic contaminants, radionuclides, etc. present in the contaminated sites. It can also enable the production of transgenic plants with specific genes being expressed (Rai et al., 2021).

CRISPR mediated strategy for enhanced phytoremediation focusses on scrutinizing and expressing the target genes to upsurge the synthesis of metal ligands (like phytochelatins and metallothioneins), metal transporters (HMA, CDF, MATE, YSL and ZIP families of transporter proteins), increased phytohormones and root exudates (Rai et al., 2021; Basharat et al., 2018). Also, it cannot be ignored that increasing the accretion of a metal by expression of a desired gene or a set of genes could trigger hypersensitivity or toxicity in the plant that may affect its detoxification mechanisms and eventually cause the decay of plants. A few researchers have also reported the use of CRISPR-Cas9 systems in integration with the genome of poplar and maize for their use in remediation (Fan et al., 2015; Miglani, 2017). CRISPR-Cas9 system is considered more efficient technology than other genome-editing tools like TALENs and ZFNs, in terms of developing a successful transgenic model plant with specific set of traits (Rai et al., 2021).

CRISPR-aided phytoremediation is a major breakthrough in this direction wherein the gene expression can be altered at specified site to gain new acumens into the plant functional genomics (Wolt et al., 2016; Kaur et al., 2019; Agarwal et al., 2020). According to Miao et al. (2013), this technology is more inclined towards the monocotyledonous plants owing to their high G-C content but it is believed that it is a realistic approach in development of advanced phenotypes. The focus of CRISPR mediated phytoremediation approach revolves around the expression of genes responsible for the increased synthesis of metal ligands like various phytochelatins and metallothioneins, metal transport proteins (CDF, HMA, ZIP families), or plant growth hormones (gibberellic acids and cytokinins), root exudates, siderophores and other such metabolites by the target plant (Saxena et al., 2020). CRISPR-based phytoremediation can successfully be used for refining the soil profile as well owing to its potential of removing toxic elements from the rhizosphere, which can be further used for agroforestry, social forestry or agricultural purposes (Thijs et al., 2016; Kaur et al., 2019).

Table 3
Transgenic plants for enhanced phyto remediation of inorganic contaminants.

S.No.	Transgenic plants	Genes used	Donor species	Target Contaminant and phyto remediation	References
1.	<i>Nicotiana tobaccum</i>	<i>AtACR2</i> (arsenic reductase 2)	<i>Arabidopsis thaliana</i>	Enhanced arsenic phytoaccumulation	(Nahar et al., 2017)
		<i>OsMTP1</i>	<i>Oryza sativa</i>	Cadmium accumulation increased	(Das et al., 2016)
		<i>PCS</i> (phytochelatin synthase) gene (<i>CdPCS1</i>)	<i>Ceratophyllum demersum</i>	Increase in Cadmium accumulation	(Shukla et al., 2012)
2.	<i>Nicotina glauca</i>	<i>TaPCS1</i> gene	<i>Wheat</i>	Increased tolerance to two HMs viz. Pb and Cd	
3.	<i>Arabidopsis</i>	<i>PCS</i> (phytochelatin synthase) gene (<i>CdPCS1</i>)	<i>Ceratophyllum demersum</i>	Arsenic accumulation	(Shukla et al., 2012)
		<i>NAS1</i> gene (NA synthase)		Higher tolerance against Cu, Fe, Ni, Mn, and Zn and enhanced phytoextraction of Zn and Mn.	Bashrat et al. (2018)
		<i>AtABCC3</i> (<i>abcc3</i>)	<i>Yeast</i>	Increased Cd tolerance	Brunetti et al. (2015)
		Jacalin-related lectin gene (<i>EcJRL-1</i>)	<i>Eichhornia crassipes</i>	Enhanced Sulphur tolerance	(Xiao et al., 2009)
		<i>OASTL</i> (cysteine synthase) gene	<i>A. thaliana</i>	9 fold increase in Cd tolerance and improved phyto remediation efficiency of transgenic plants is achieved	Domínguez-Solís et al. (2004)
		<i>AtNramp1</i> and <i>AtNramp3</i> (Fe transporter) genes		Increased accumulation of iron and improved plant growth	Domínguez-Solís et al. (2004)
4.	<i>Brassica juncea</i>	<i>SMT</i> (selenocysteine methyltransferase) gene	<i>A. bisulcatus</i>	60% more Se accumulation in the transgenic plants of <i>B. juncea</i> than wild types	(Zhao and McGrath, 2009)
		<i>APS</i> (ATP sulfurylase) & <i>SMT</i> genes		Increased tolerance and phytoaccumulation of selenium	Basharat et al. (2018)
5.	<i>Oryza sativa</i>	<i>PCS</i> (phytochelatin synthase) gene (<i>CdPCS1</i>)	<i>Ceratophyllum demersum</i>	Arsenic accumulation	(Shri et al., 2014)
		<i>OSHMA3</i>	<i>Oryza sativa</i>	Enhanced Cadmium sequestration and upregulation of Zinc transporters	(Sasaki et al., 2014)
		<i>OsTCTP</i>	<i>Oryza sativa</i>	Increased Hg tolerance in plants	Xu et al. (2015)
		<i>AmMT2</i>	<i>Avicennia marina</i> L.	Enhance tolerance to Pb, Zn, Cd & Cu	Huang and Wang (2010)
6.	<i>Hordeum vulgare</i>	<i>FRD3</i> , <i>SbMATE</i> genes	<i>Sorghum</i>	Increased tolerance towards Aluminium (Al)	(Zhou et al., 2014)

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Table 3 (continued).

S.No.	Transgenic plants	Genes used	Donor species	Target Contaminant and phytoremediation	References
7.	<i>Helianthus annuus</i>	<i>HaMT-1</i> & <i>HaMT-2</i>	<i>E. coli</i>	Cd phytoextraction	(Tomas et al., 2015)
8.	<i>Hirschfeldia incana</i> (formerly <i>B. geniculata</i>)	<i>MT2b</i> (metallothionein gene)		Improved tolerance and Increased phytoextraction of Pb (lead)	
9.	<i>Noccaea caerulescens</i>	<i>NcNramp1</i>	<i>A. thaliana</i>	Enhanced Cd tolerance	Milner et al. (2014)
10.	<i>Populus alba</i> L.	<i>PaMT1</i> , <i>PaMT2</i> & <i>PaMT3</i> , <i>PaSPDS1</i> , <i>PaSPDS2</i> and <i>PaADC</i> genes	<i>Populus alba</i> L.	Tolerance to Cd, Cu and Zn	Macovei et al. (2010)
11.	Somatic hybrids of <i>T. caerulescens</i> & <i>B. juncea</i>			Enhanced Pb accumulation	(Rascio and Izzo, 2011)
12.	Somatic hybrids of <i>T. caerulescens</i> and <i>Brassica napus</i>			Phytoremediation of Zinc	(Rascio and Izzo, 2011)

It is also a notable fact that when the bioaccumulation of a particular metal reaches the threshold in the object plant owing to the specific gene being introduced in the plant, it can consequently develop a hypersensitivity of that element in the particular plant and can eventually lead to decay of plant. This has been observed in few cases, for instance, a plasma membrane protein (*NtCBP4*) was introduced in *N. tabacum* plants for enhanced accumulation of lead in plant, but simultaneously it caused sensitivity towards Pb in the plant system. Similar results were found when *MerC* gene was transferred and expressed in two model plants, Arabidopsis and tobacco plants, causing sensitivity in these plants for mercury (Hg). Additionally, similar reports against the organic pollutants are also reported by researchers. Jaiswal et al. (2019) revealed that such sensitivity against RDX and TNT was observed in the transgenic plants being developed for enhanced phytoremediation of these organic contaminants.

Although CRISPR-Cas9 is an emergent tool for genome editing in plants system, various parameters like desired target sites, delivery vectors, and genotype of plants can play a key role in the success of this technology. Also, there are still few limitations associated with the implementation of CRISPR/Cas facilitated transformation in aquatic plants like the chances of higher incongruities among transformed colonies for desired genomic changes. Previous reports on plant transformations have indicated that only 20%–50% of the CRISPR-transformed colonies were possessing the desired mutation (Rai et al., 2021; Basu et al., 2018). Yang et al. (2019) has reported the use of CRISPR/ Cas9 system to produce the *OsNRAMP5* knockout plants for meliorating the tolerance of *O. sativa* exposed to Cd stress.

6. Conclusion

Aquatic plants holds a remarkable importance in phytoremediation of water bodies owing to their dense root system and higher biomass production rates. Also, these hyperaccumulator plants holds molecular variability, wide range of enzymes activity, expression of stress responsive genes, and protein profiling against a range of organic and inorganic pollutants. These hyperaccumulator plants which could be harnessed as a detoxification strategy for the contaminated water and soil systems. Apart from their natural potential, hyperaccumulator plants can substantially be improved over time with the use of genetic tools and other engineering technologies, in order to achieve productive results in phytoremediation and can be attributed in strategic management of such wastewater systems.

The various phytoremediation studies have revealed that the aquatic macrophytes possess an effective machinery to make them well adapted in the contaminated water bodies while simultaneously treating large volume of contaminated water. Research in the past decade has shown progress in the identification and successful transformation of plants of interest with the genes that code for specific metal accumulation or essentially function in the detoxification process of that contaminant. Such research can serve as a pillar for the integration of suitable traits in single plant species that can act as “superbug” plants for phytoremediation. However, gene expression profiling and the protein–protein interactions needs to be analysed and studied thoroughly in order to avoid any hassle and unintended changes in the plant genome of interest. It is also critical to understand the metal geochemistry within the plants tissues to help the researchers to finely tune the phytoremediation process. The use of these strategies involving omics, genome-editing, CRISPR, proteomics and metabolomics studies are only aiming at improved effectiveness of phytoremediators without affecting any other attributes so that it can open up new avenues for improving competence of phytoremediation, at the same time it is cost-effective, visually pleasing, managing the contaminated water bodies and sustainability of whole ecosystems.

Table 4
Transgenic plants for enhanced phytoremediation of organic or refractory contaminants.

S.No.	Transgenic plants	Genes used	Donor species	Target Contaminant and phytoremediation	References
1.	<i>Nicotiana tobaccum</i>	<i>Nfs1</i> (nitroreductase); <i>onr</i> (Pentaerythritol tetranitrate reductase enzyme, PETN)	<i>Enterobacter cloaceae</i>	Enhanced removal of TNT from medium	Abhilash et al. (2009)
		<i>CYP450 2E1</i> (Cytochrome P450 Monooxygenase enzyme)	Human	enhanced degradation of anthracene and chloropyriphos; degradation of ethylene dibromide and TCE	Dixit et al. (2008) and Doty et al. (2000)
		<i>ophc2</i> (Organophosphorus hydrolase enzyme)	<i>Pseudomonas pseudoalcaligenes</i>	Increase degradation of methyl parathion and other organophosphates	(Wang et al., 2008)
		<i>bphc</i> (2,3, dihydroxybiphenyl-1, 2-dioxygenase)	PCB degrading bacteria	Increased transformation and degradation of PCBs by transgenic plants	(Chrastilova et al., 2007)
		<i>tpx1</i> and <i>tpx2</i> (Peroxidases)	<i>Lycopersicon Esculentum</i> (Tomato)	Hairy root cultures of transgenic tobacco showed improved detoxification of phenol	(Alderete et al., 2009)
2.	<i>Brassica juncea</i>	γ -ECS (γ -Glutamylcysteine synthetase), GS (Glutathione synthetase) genes	<i>A. juncea</i>	Up regulation of ECS and GS could enhance tolerance of plants towards atrazine, 1-chloro-2, 4-dinitrobenzene, phenanthrene, metolachlor	(Flocco et al., 2004)
3.	<i>Solanum tuberosum</i>	<i>CYP1A1</i> , <i>CYP2B6</i> , and <i>CYP2C19</i> (Cytochrome P450 monooxygenase)	Human	Increased tolerance to sulfonyleurea and other herbicides develops in transgenic plants	Inui and Ohkawa (2005)
4.	<i>A. thaliana</i>	<i>XplA</i> and <i>XplB</i>	<i>Rhodococcus rhodochorus</i>	Enhanced detoxification of RDX	(Jackson et al., 2007)
		<i>DbfB</i> (Aromatic-cleaving extradiol dioxygenase)	<i>Terrabacter sp.</i>	Enhanced rhizodegradation 2, 3-dihydroxybiphenyl (2, 3-DHB)	(Uchida et al., 2005)

CRediT authorship contribution statement

Priyanka Agarwal: Conceptualization, Writing – original draft, Reviewing and editing, Visualization. **Radha Rani:** Conceptualization, Supervision in manuscript drafting, Reviewing and editing, Funding acquisition.

Declaration of competing interest

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

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