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# Phytoremediation of Arsenic Contaminated Water Using Aquatic, Semi-Aquatic and Submerged Weeds

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## Abstract

Arsenic (As) is the one the most toxic element present in earth which poses a serious threat to the environment and human health. Arsenic contamination of drinking water in South and Southeast Asia reported one of the most threatening problems that causes serious health hazard of millions of people of India and Bangladesh. Further, use of arsenic contaminated ground water for irrigation purpose causes entry of arsenic in food crops, especially in Rice and other vegetable crops. Currently various chemical technologies utilized for As removal from contaminated water like adsorption and co-precipitation using salts, activated charcoal, ion exchange, membrane filtration etc. are very costly and cannot be used for large scale for drinking and agriculture use. In contrast, phytoremediation utilizes green plants to remove pollutants from contaminated water using various mechanisms such as rhizofiltration, phytoextraction, phytostabilization, phytodegradation and phytovolatilization. A large numbers of terrestrial and aquatic weed flora have been identified so far having hyper metal, metalloid and organic pollutant removal capacity. Among the terrestrial weed flora *Arundo donax*, *Typha latifolia*, *Typha angustifolia*, *Vetivaria zizinioids* etc. are the hyper As accumulator. Similarly *Eichhornia crassipes* (Water hyacinth), *Pistia stratiotes* (water lettuce), *Lemna minor* (duck weed), *Hydrilla verticillata*, *Ceratophyllum demersum*, *Spirodella polyrhiza*, *Azola*, *Wolfia* spp., etc. are also capable to extract higher amount of arsenic from contaminated water. These weed flora having As tolerance mechanism in their system and thus remediate As contaminated water vis-à-vis continue their life cycle. In this chapter we will discuss about As extraction potential of various aquatic and semi aquatic weeds from contaminated water, their tolerance mechanism, future scope and their application in future world mitigating As contamination in water resources.

**Keywords:** Arsenic, Phytoremediation, Weed

## 1. Introduction

Arsenic (As) is the one the most toxic element present in earth which poses a serious health hazard to animal and human health. Generally arsenic is present

in the earth crust in the form minerals, especially associated with iron pyrite and zinc ores. Arsenic contamination occurs through both by natural as well as anthropogenic processes [1]. Unlike other toxic heavy metals (Cadmium, mercury and chromium) arsenic contamination in environment predominately occurs through natural biogeochemical process [2] and some manmade activities play important role (triggering the process) in that process. Anthropogenic activities such as coal mining and burning smelting of As containing metal ores and other industrial activities are also responsible for distribution of arsenic in the environment [3]. Arsenic contamination of drinking water in South and Southeast Asia reported one of the most threatening problems that causes serious health hazard of millions of people of India and Bangladesh [4]. The source of As contamination in water in those countries were due to two different natural processes; oxidation of arsenopyrite minerals lies below ground water table due to water mining process and reduction of As containing iron hydroxides [5]. Arsenic exists in the nature in  $-3$ ,  $0$ ,  $+3$  and  $+5$  oxidation states and environmental forms include arsenious acids, arsenic acids, arsenites, arsenates, methylarsenic acid, dimethylarsinic acid, arsine, etc. Two inorganic forms are very common in natural waters: arsenite ( $\text{AsO}_3^{3-}$ ) and arsenate ( $\text{AsO}_4^{3-}$ ), referred to as arsenic (III) and arsenic (V). Pentavalent ( $+5$ ) or arsenate species are  $\text{AsO}_4^{3-}$ ,  $\text{HAsO}_4^{2-}$ ,  $\text{H}_2\text{AsO}_4^-$  while trivalent ( $+3$ ) arsenites include  $\text{As}(\text{OH})_3$ ,  $\text{As}(\text{OH})_4^-$ ,  $\text{AsO}_2\text{OH}^{2-}$  and  $\text{AsO}_3^{3-}$ . The solubility of inorganic species depends on pH and redox potential of the environment and arsenite ( $\text{As}^{3+}$ ) is the most soluble form inorganic As. Pentavalent species or arsenate ( $\text{As}^{5+}$ ) predominate in oxygen rich aerobic environments, where as trivalent arsenites ( $\text{As}^{3+}$ ) dominant in moderately reducing anaerobic environments such as groundwater [4].

Arsenic concentration in drinking water reported more than  $50 \mu\text{g L}^{-1}$  in many areas in the world [6], whereas maximum permissible limit set by World Health Organization (WHO) is  $10 \mu\text{g L}^{-1}$ . The use of arsenic contaminated ground water for irrigation purpose causes build up of As in soil and leads to entry of As in food crops, especially in rice and vegetables [7, 8]. This causes serious health hazard, in those As containing areas. In Southeast Asian countries like Bangladesh, Eastern parts of India (West Bengal and Bihar) and Vietnam, rice is consumed as major staple food and is very efficient in As translocation in grains [9]. Thus rice crop play a major pathway for As entry in human body living in those contaminated areas apart from drinking water. Thus remediation of arsenic contaminated water is important for environmental point of view. Various technologies are for remediation of arsenic contaminated water like ion exchange, electro dialysis, membrane filtration, adsorption and coagulation-flocculation generates lot of arsenic enriched waste. That waste material generally dumped or disposed in nearby surroundings, from where arsenic can also come back to soil and water by leaching thus making system susceptible to arsenic contamination. Along with above mentioned problem, huge cost is involved in this existing arsenic remediation technology. That necessitates finding out an alternate low cost technology which can take care of arsenic contaminated water.

Phytoremediation is an alternate and low cost technology that utilizes green plant to extract arsenic from water and store it vegetative cells. Phytoremediation process includes phytoextraction, phytostabilization, phytovolatilization, phyto-transformation, and rhizofiltration [10]. Researchers find out that plants uptake arsenic by roots through phosphate uptake pathway and transfer it their above ground parts (shoot and leave). But how much amount of arsenic translocated from source (water) to sink (plant parts) depends on phytoremediation efficiency of the plant concern. However, more than 90% of total arsenic accumulated into the plant is stored in roots.

The plants utilized for phytoremediation have some criteria like (1) plant have higher specific growth rate under contaminated environment, (2) higher

translocation capability of the toxic element concerned [11]. Metal translocation capability depends on factors like (1) bio concentration factor (BCF) and (2) translocation factor (TF). Plants having BCF >1 are ideal for Phytoremediation. Chinese brake fern (*Pteris vittata*) is the most promising plant for phytoremediation of arsenic from contaminated soil [12]. For instance, plants species like water hyacinth (*Eichhornia crassipes*), duck weed (*Lemna minor*, *Spirodela polyrhiza* and *Wolffia globosa*), water lettuce (*Pistia stratiotes*) and fern (*Azolla pinnata*) have been successfully utilized for arsenic removal from water purpose by many researchers [13–15]. Among the semi aquatic weeds. Apart from these free floating aquatic weed flora such as *Arundo donax*, *Vetivaria* sp., and *Alternanthera philoxeroides* had been successfully utilized for remediation of As contaminated water [16, 17]. In this chapter we are going to discuss about arsenic removal potential of various aquatic and semi aquatic weeds along with their future use for phytoremediation purpose.

## 2. Phytoremediation pathways

The terminology “Phytoremediation” consists of two words, “Phyto” means “green plants” and “remediation” means “curative measures or restoration”. The word “phytoremediation” was first given by Chaney [18]. In phytoremediation process, generally green plants are used which uptake toxic chemical substances (such as heavy metals and metalloids, pesticide residues etc.) from contaminated sites (soil and water) by various mechanisms and remove them from environment. Various crop and weed plants are found to be suitable for phytoremediation purpose. But research results indicated that weed flora had higher phytoremediation potential than cultivated crops (Example- Brassica sp). There are various pathways of phytoremediation process such as, rhizofiltration, phytoaccumulation or phytoextraction, phytostabilization, phytodegradation or phytotransformation and phytovolatilization etc.

- Rhizofiltration: Plants uptake toxic substances by their roots through adsorption or absorption process and sequester in their root system. Aquatic plants mainly exhibited this process.
- Phytoaccumulation or phytoextraction: Plants uptake toxic substances by their root system and translocated to other plant parts such as stem and leaf or other modified plant parts. This mechanism mainly exhibited this process are suitable for remediation of contaminated soil.
- Phytostabilization: In this process, plants restrict movement of toxic substances in soil or water, thus reduced their availability to plants. In this method, plants do not uptake toxic substances from environment. Rather, plants secrete some root exudates or phytochemicals which form stable chemical bond with toxic substances and increases its stability in environments.
- Phytodegradation or Phytotransformation: In this process, plants uptake toxic substance from soil or water and degrade these primary toxic substances into nontoxic forms. A large number of metabolic and physiological factors are involved in this process.
- Phytovolatilization: Plant uptake toxic substances by their root system and translocated to their aerial plant parts especially in leaves; and release toxic substances in the form of vapor which may not be toxic as their primary source.



Apart from this there are some other terminologies often used in phytoremediation process are bioconcentration factor (BCF) and translocation factor (TF).

BCF = toxic substance uptake by plant/toxic substance present in environment (soil or water).

TF = toxic substance present in shoot or stem/toxic substance present in roots or.

Toxic substance present in leaves/Toxic present in shoot or stem.

For, Hyper accumulator plants both BCF and TF is  $>1$  is desired. In other words, plants suitable for phytoremediation, BCF  $>1$  is always desirable. But for aquatic weeds, as their dominant pathways is rhizofiltration; their toxic substances BCF  $>1$  but TF for root to shoot or shoot to leaves is  $<1$ .

### 3. Potential of various aquatic plants for phytoremediation

#### 3.1 Phytoremediation by free floating aquatic weeds

***Eichhornia crassipes*:** *Eichhornia crassipes* is commonly known as water hyacinth, a free-floating perennial aquatic plant native to tropical and sub-tropical South America, and is now wide spread in all tropic climates. The genus *Eichhornia* comprises seven species of water hyacinth among which *E. crassipes* is the most common and have been reported to grow very first. However, its enormous biomass production rate, high tolerance to pollution and absorption capacity of heavy-metal and nutrient qualify it for use in wastewater treatment [19].

The capability of removing arsenic from contaminated water was earlier observed by Misbahuddin and Fariduddin [20] and they observed that water hyacinth can removes arsenic from water within 3–6 hr. exposure time. Amount of arsenic removed depends on number of the plant used, exposure time, presence of air and sunlight. They concluded that whole plants were more effective than fibrous roots alone. It was observed that dried roots of water hyacinth can rapidly reduces As content in contaminated water within below WHO recommended critical level ( $<10 \mu\text{g Lg}^{-1}$ ) [21]. A fine powder was prepared from dried roots of water hyacinth plants (obtained from Dhaka, Bangladesh) removed more than 93% arsenite and 95% of arsenate from a solution containing As @  $200 \mu\text{g L}^{-1}$  within 1 hr. exposure time [21]. Higher biomass production ability of water hyacinth allow it to remove As at higher rate ( $600 \text{ mg As ha}^{-1} \text{ day}^{-1}$ ) and greater efficiency (17%) compared to lower biomass producing aquatic macrophytes such as lesser duck weed (*Lemna minor*) which removed As at lower rate ( $140 \text{ mg As ha}^{-1} \text{ day}^{-1}$ ) and lesser efficiency (5%); though there was no difference in bioaccumulation capacity [13]. Similarly better As extraction capacity of water hyacinth (80%) compared to *Lemna minor* and *Spirodella Polyrrhiza* from tropical coalmine effluent was also been reported [22] from India. Unlike lower biomass producing aquatic macrophytes, water hyacinth poses better As extraction ability compared to higher biomass producing vetivar grass [23]. Not only higher biomass, higher reproduction ability also plays an important role in As phytoremediation by water hyacinth. Water hyacinth was a suitable phytoremediation agent when As present in contaminated water at lower concentrations. When As was provided at lower concentrations @ 1 and  $2 \text{ mg L}^{-1}$ , water hyacinth removed 90 and 65% of total As from contaminated solutions ( $1$  and  $2 \text{ mg L}^{-1}$  respectively) provided respectively within 7 days [24] and maximum As stored in roots. Water hyacinth can extract higher amount As from contaminated water but their presence in water bodies reduces dissolved oxygen content (DOC), which makes its application for a larger water bodies a problematic pathway which needs to be taken care.

**Pistia stratiotes:** *Pistia stratiotes* is commonly called as water lettuce. There are many previous studies indicated that *Pistia stratiotes* capable of removing toxic heavy metals from contaminated water [25–27], but there were few studies was done on As uptake by water lettuce. Earlier a field study carried out using *P. stratiotes* and results showed that *Pistia stratiotes* can remove As from contaminated water, along with higher bioconcentration factor (BCF) for root (8632) vis-à-vis lower BCF for leaf (2342) [28]. In a laboratory study it was demonstrated that maximum As removal efficiency of *P. stratiotes* was found at pH 6.5 and *Pistia* removed 87.5% of the metalloid provided in the solution [29]. From Laboratory study it was revealed that *P. stratiotes* can accumulate As efficiently when As was provided at lower concentrations, though total As uptake was increased with increase in As concentration in the solution [30]. Arsenite accumulation in *P. Stratiotes* was found more in root and less in leaves like water hyacinth. Arsenic accumulation in roots and leaves were respectively 1120 and 31.60  $\mu\text{g g}^{-1}$  DW respectively when 10  $\mu\text{M}$  As ( $\text{As}^{3+}$ ) solutions are employed [31]. When higher concentration of As solutions used (>20  $\mu\text{M}$ ), As toxicity symptoms like chlorosis, suppressed growth, lower photosynthetic rate, suppressed enzymatic activities and increased cell damage were observed in *P. stratiotes* [30, 31].

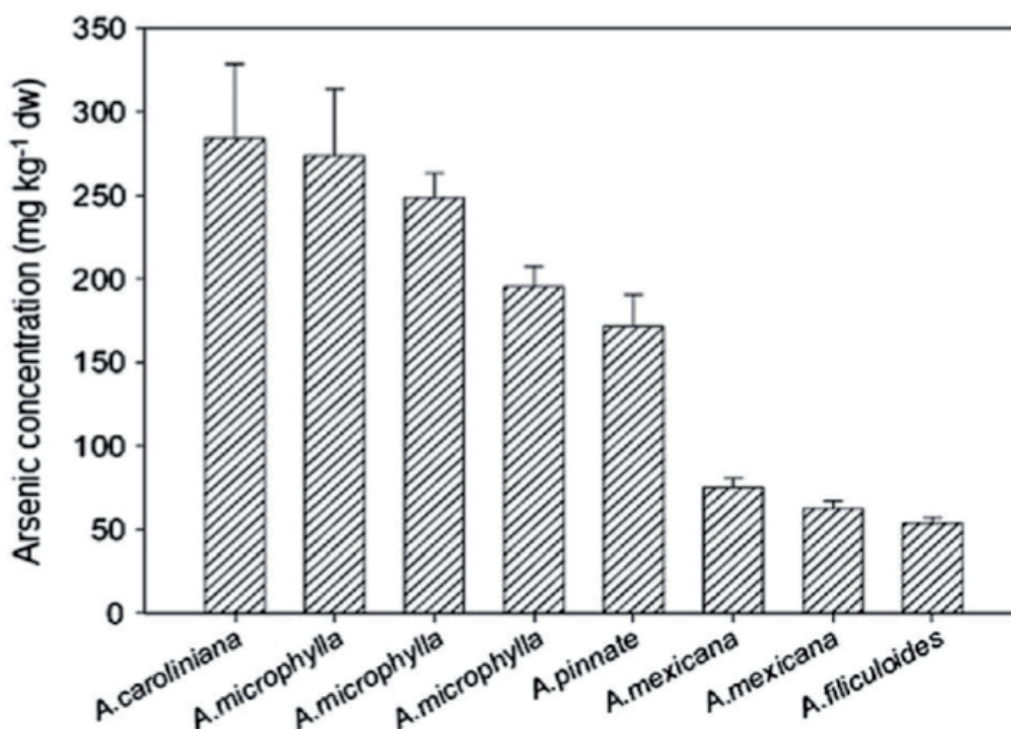
**Lemna, Spirodella and Wolfia:** Weeds belongs to Lemna, Spirodella and Wolfia are generally known as Duckweeds. Duckweeds are small free-floating aquatic weed plants which generally found in water bodies, mainly comprises of four genera, *Lemna*, *Spirodela*, *Wolfia*, and *Wolffiella*, and of 34 species. Among these Lemna, Spirodela, and Wolfia have been widely reported to accumulate arsenic from contaminated water [13, 32–34]. Research studies indicated that, total As accumulation in *Lemna gibba* was more in field condition compared to laboratory conditions due to higher exposure time in field condition [32]. However higher accumulation of As in plant parts is not always correlated with bio-concentration factor (BCF). It was found that total As accumulation plant parts may be higher in field condition, but higher BCF was obtained at laboratory conditions [32] due to better availability of external nutrients.

However nutrients like phosphate addition may suppressed As uptake by duckweeds as both phosphorus and arsenic belongs same group-V(b) element family in periodic table [33]. In most of the phytoremediation study carried out in laboratory condition, As is provided either in the form of arsenite ( $\text{As}^{3+}$ ) or arsenate ( $\text{As}^{5+}$ ). But some studies included dimethyl arsenic acid (DMAA), an organic form of arsenic for evaluation of As phytoremediation potential of duckweed species. In a lab study, *Spirodela polyrhiza* was exposed to two forms of As species, arsenate and DMAA with concentrations ranged from 1, 2, and 4  $\mu\text{M}$  and their interaction with phosphate (100 to 500  $\mu\text{M}$ ) was studied [33]. Results obtained showed that arsenate uptake was affected by higher phosphate concentrations whereas DMAA uptake was not influenced by phosphate concentration indicating that *Spirodela polyrhiza* had separate mechanisms for DMAA uptake. Duckweeds showed contrasting As uptake behavior when provided in two separate inorganic forms ( $\text{As}^{5+}$  vs.  $\text{As}^{3+}$ ) and maximum As uptake was reported with arsenite form ( $\text{As}^{3+}$ ) [34]. *Spirodela polyrhiza* extracted 17408 and 8674  $\mu\text{g g}^{-1}$  As (dry weight basis) respectively from solutions containing As in the form of  $\text{As}^{3+}$  and  $\text{As}^{5+}$  (64  $\mu\text{M}$  As each) respectively within 6 days [34]. Maximum amount of As extracted by duckweeds is still questionable and it is varied with As exposure time, concentrations of As in contaminated solution, and research type (laboratory vs. field study). *Spirodela polyrhiza* reported to uptake 400  $\text{mg kg}^{-1}$  As (dw basis) without showing any toxicity symptoms, but can accumulate up to 900  $\text{mg kg}^{-1}$  As (dw basis) when subjected to 320  $\mu\text{M ml}^{-1}$  As containing solutions [35]. Under natural condition, *Lemna minor* was found to accumulate 430  $\text{mg kg}^{-1}$  As (dry weight basis) under As contaminated environment [36]. There are few studies on As uptake by *Wolfia globosa* (rootless duckweed). *Wolfia globosa* had been reported

to extract more than  $1000 \text{ mg kg}^{-1}$  (frond dry weight basis) from contaminated water [37]. Like other duckweeds, *Wolffia globosa* also uptake more arsenite form compared to arsenate form [37]. Later studies confirmed that *Wolffia globosa* produced phytochelatins which played an important role minimizing toxic effects of As in their body parts [38]. These above cited studies showed that *Lemna minor*, *Spirodela polyrhiza* and *Wolffia globosa* are suitable for phytoremediation of As from contaminated water.

**Salvinia:** *Salvinia* is a floating fern belongs to genus salvinaceae, commonly called as butterfly fern. The genus salvinaceae contains 12 different species, out of them only 3 had been investigated for As phytoremediation were namely *Salvinia molesta*, *Salvinia minima* and *Salvinia natans* [39–41]. *Salvinia minima* have been reported as an efficient scavenger of Pb ( $34 \text{ mg g}^{-1} \text{ dw}$ ) and less efficient remover of As ( $0.05 \text{ mg g}^{-1}$ ) from contaminated medium and uptake of both Pb and As increased with exposure time duration and concentration of the element in the medium concerned [40]. The plant showed toxicity symptoms when  $\text{As}^{3+}$  concentration was more than  $100 \mu\text{M}$  and tolerates up to  $300 \mu\text{M}$ . Addition of phosphate in solution, reduced As uptake of as occurred in other aquatic weed plant also been recorded in their study. Similarly negative impact of phosphate and iron on As uptake by *Salvinia natans* was observed [41]. Phosphate addition reduced As uptake when provided in the form of arsenate ( $\text{As}^{5+}$ ), in contrast no impact when As was provided in the form of DMAA. Like other aquatic weeds (*Eichhornia*, *Pistia* and *Spirodela*), *Salvinia molesta* also showed As toxicity upon exposure to higher concentration. To counter As stress, antioxidant enzyme activities and reactive oxygen species (ROS) were increased in floating leaves [39]. These studies indicated that *Salvinia* can play an important role for As phytoremediation as it had own defense mechanism.

**Azolla:** *Azolla* is a small, free floating aquatic fern commonly found in paddy fields, ponds, river and lakes. There are numerous studies carried out globally showed that *Azolla* can remediate heavy metal toxicity from contaminated water [42–44]. But studies on As phytoremediation capability of *Azolla* were scarce. In As contaminated area of Bangladesh, Mahmud et al. [45] evaluated 49 different plant species for As uptake and BCF; found that *Azolla pinnata* along with



**Figure 1.** Arsenic uptake pattern in different *Azolla* sp. (adapted from Zhang et al., 2009).



*Eichhornia crassipes* and *Spirodella polyrhiza* showed higher BCF and TF in paddy field. Among 49 plant species, *Azolla pinnata* showed highest BCF 10.92 indicated its suitability to reduce As uptake by paddy plants in field condition. A study using *Azolla* conducted in China using 50 different strains of *Azolla* spp. based on their uptake and speciation [46]. As uptake was ranged from 29 to 397 mg kg<sup>-1</sup>; *A. caroliniana* accumulated maximum As followed by *A. macrophylla* and minimum accumulation was associated with *A. filiculoides* when all strains were grown in 50 µM As<sup>5+</sup> solution for 10 days (**Figure 1**). Arsenic speciation followed in the order of arsenate (As<sup>5+</sup>) > arsenite (As<sup>3+</sup>) > DMAA and MMAA accounting 50–60, 25–40 and 1–5% of total arsenic in *A. caroliniana* respectively. In contrast, arsenite (As<sup>3+</sup>) was dominant As species in *A. filiculoides* governs 55–69% of total As [46]. Another study was conducted on phytoremediation of As by *A. caroliniana* wild using various As concentrations (0, 0.25, 0.5, 1.0 and 1.5 mg L<sup>-1</sup>) and impact of As exposure on plant enzymatic properties were investigated [47]. Maximum As uptake (386 mg kg<sup>-1</sup>) was reported at highest As concentration (1.5 mg kg<sup>-1</sup>). It was observed that peroxidases, glutathione reductase, catalase and superoxide dismutase activities were enhanced at lower As doses and reduced at higher doses. In exposure to higher As concentration, thiol content and anthocyanin production were increased and correlated with higher As uptake.

### 3.2 Phytoremediation of arsenic by semi aquatic weeds

Some semi aquatic weed such as *Alternanthera philoxeroides*, *Arundo donax*, *Vetivaria Zizinioids*, *Typha latifolia*, *Phragmites* spp. and *Canna* spp. had been widely reported to accumulate As in their body parts from contaminated soils and water [16, 17, 48–51]. *Alternanthera philoxeroides* had potential to extract As from contaminated water and stored in root system [52, 53]. Reports from previous studies indicate that As accumulation in *A. philoxeroides* followed in the order of root > stem > leaf and average BCF for root ranged from 106 to 191, when exposed to various doses of As containing solutions (1, 2 and 5 mg kg<sup>-1</sup>) under laboratory condition [52]. Under natural condition, *Alternanthera philoxeroides* observed to uptake 12.94 mg kg<sup>-1</sup> total As dw from pulp paper industry water with average BCF- 3.58 and TF-0.51 [53]. Higher BCF under laboratory condition observed due to used of higher As containing solution and availability of external nutrients for weed plants which may trigger As uptake through phosphate uptake pathway.

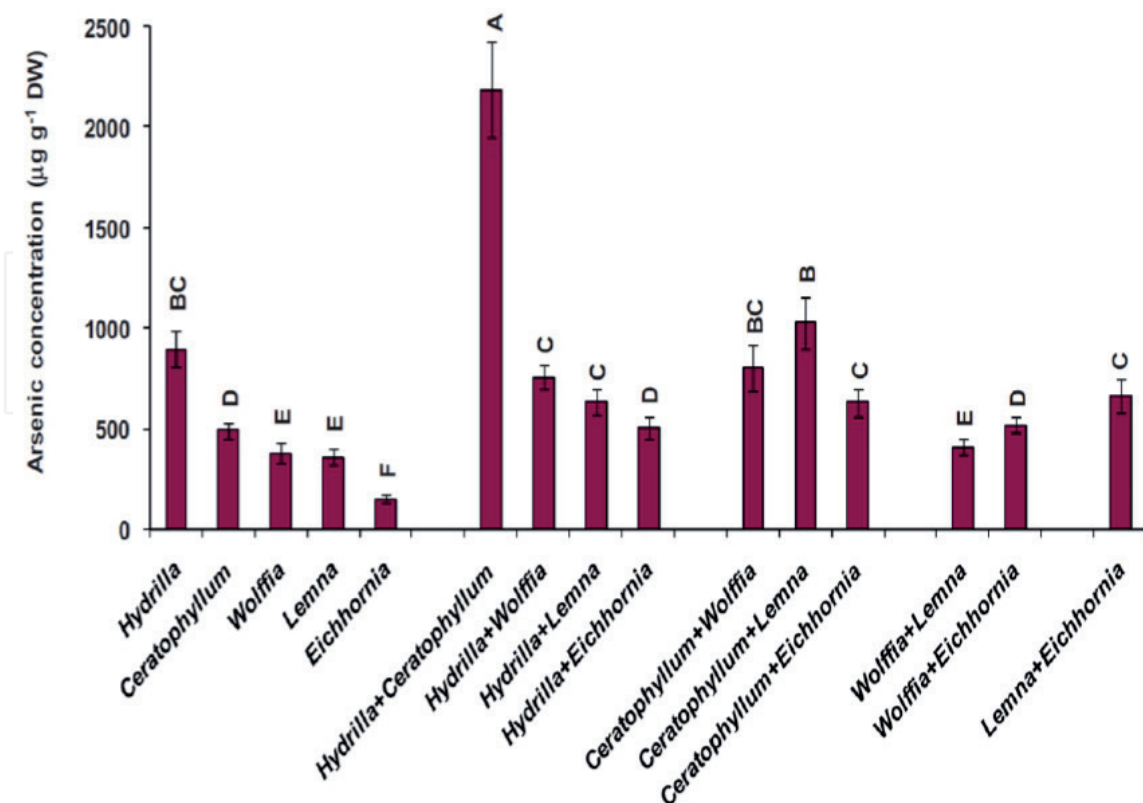
*Arundo donax* is a perennial semi aquatic weed mostly found in submerged condition offer a tremendous potential to uptake As from contaminated water. Earlier research work showed that *Arundo donax* can grow efficiently up to 50–600 µg L<sup>-1</sup> As concentration without showing any toxicity symptom and maximum As uptake, BCF (15), TF (4.93) were recorded at 600 µg L<sup>-1</sup> [16]. Toxicity symptoms appeared when plants were exposed to solutions containing 1000 µg L<sup>-1</sup> As [16]. Further, combined use of plant growth promoting rhizobacteria (PGPR) such as *Stenotrophomonas maltophilia* and *Agrobacterium* sp. increased bioaccumulation of As in roots of *Arundo donax* plant upon exposure to higher concentration As (20 mg kg<sup>-1</sup>) and enhanced overall phytoremediation efficiency of *Arundo donax* in presence of PGPR bacteria [51]. The As accumulation in *Phragmites australis* followed in the order of roots > rhizomes > leaves and maximum total As uptake was registered 32.5 mg kg<sup>-1</sup> [54]. *V. zizinioids*, another semi aquatic weed reported to be capable of extracting As from contaminated water [17, 55]. In a hydroponic study (21 days), root to shoot As uptake it was increased with increase in As concentrations by *V. zizinioids* can uptake [17]. The BCF and TF for As were 10 and 0.86 indicates that *V. zizinioids* was an As hyper accumulator and stored higher proportion of As in their root system. Combined use of arbuscular mycorrhizal fungi (*Glomus*



spp.) enhanced As uptake capability and growth of vetivar grass (*Chrysopogon zizanioides*) [55]. *Typha latifolia* also had the potential to uptake higher proportion of As from contaminated environment (soil), but most of the studies conducted using *Typha latifolia* were focused in soil. Most of the studies showed that semi aquatic weeds store more As in their root system and lower in upper vegetative parts. Higher plant vigor, higher As extraction capacity and perennial nature make them suitable phytoremediation agent for constructed wetland system. Combined use of submerged weeds like Hydrilla, Ceratophyllum, Potamogeton along with semi aquatic weeds (*Arundo donax*, *Vetivaria zizanioides*, *Phragmites* spp. and *Typha* sp.) and PGPR like VAM, As oxidizing bacteria may be highly useful to treat and remediate As contaminated water in constructed wetland system. Semi aquatic weeds are highly efficient when As present in higher concentrations and when As concentration in the system become lower submerged weeds come to play their role, as they are highly efficient As remover at lower concentrations. Again use PGPR will increase overall phytoremediation efficiency. Future research may be undertaken in these aspects for better information and output.

### 3.3 Phytoremediation by submerged aquatic weeds

Among the submerged aquatic weeds *Hydrilla verticillata*, *Ceratophyllum demersum*, *Potamogeton crispus*, *Valisnaria natans*, *Eleocharis acicularis* and *Elodea Canadensis* widely reported by many researchers to extract As from contaminated water. Studies conducted in laboratory and field conditions indicated that *Hydrilla verticillata*, and *Ceratophyllum demersum* can uptake higher proportion of As from contaminated water depending on exposure time and concentration of metalloid [22, 56, 57]. Unlike *Spirodela polyrhiza*, *Hydrilla verticillata* also uptake



**Figure 2.** Arsenic uptake comparison between various weed plants (*Hydrilla*, *Ceratophyllum*, *Wolffia*, *Lemna* and *Eichhornia*) grown in singly or in various combinations upon arsenic exposure for 30 days (adapted from Srivastava et al., 2014).

more arsenite ( $\text{As}^{3+}$ ) form rather arsenate ( $\text{As}^{5+}$ ) form [50]. Under simulated field condition (aquatic pond) *Hydrilla verticillata* alone removed sum total 8546  $\mu\text{g}$  ( $348 \mu\text{g g}^{-1}$ ) of As from contaminated water (As concentration  $1500 \mu\text{g L}^{-1}$ ) which is 72% of the total arsenic supplied [56]. *Ceratophyllum demersum* reported to accumulate  $76 \mu\text{g g}^{-1}$  in 4 days and As accumulation further increased to  $201 \mu\text{g g}^{-1}$  in 7 days when exposed to  $50 \mu\text{M}$  As solutions [22]. Maximum As accumulation by *Ceratophyllum* was recorded  $525 \mu\text{g g}^{-1}$  dw when subjected to with  $250 \mu\text{M}$   $\text{As}^{5+}$  solution for 7 days [22]. Uptake of As by *Ceratophyllum demersum* depends on species of As present ( $\text{As}^{3+}$  vs.  $\text{As}^{5+}$ ) and pH of the medium. Maximum uptake of  $\text{As}^{3+}$  by *Ceratophyllum* was reported at pH 6.5 [58]. This variation in selective uptake of As species largely depends on uptake pathways and plant metabolism.

In natural conditions, submerged weeds grow in water bodies in association with floating macrophytes. Use of Combinations of submerged and floating weeds found more effective for phytoremediation purpose than submerged and floating weeds alone. Research work carried out using *Hydrilla*, *Ceratophyllum*, *lemna* and *Wolfia* at various combinations showed that *Ceratophyllum* + *lemna* combination ( $3326 \mu\text{g}$ ) combination removed maximum total As followed by *Hydrilla* + *Wolfia* ( $1896 \mu\text{g}$ ) (Figure 2). When the contribution of single plant considered, contribution of *Hydrilla* is more than 50% [56]. Arsenic phytoextraction potential of five different submerged weeds namely *Ceratophyllum demersum*, *Potamogeton crispus*, *Myriophyllum spicatum*, *Hydrilla verticillata* and *Vallisneria natans* were compared

Name of the plants	Key findings	Reference
<i>Eichhornia crassipes</i>	Removed $600 \text{ mg As ha}^{-1} \text{ day}^{-1}$ within 21 days with 18% removal efficiency when As was applied @ $0.15 \text{ mg L}^{-1}$	[13]
<i>Lemna minor</i>	Removal rate $140 \text{ mg As ha}^{-1} \text{ day}^{-1}$ within 21 days with 5% removal efficiency when As was applied @ $0.15 \text{ mg L}^{-1}$	[13]
	Removed relatively higher $\text{As}^{3+}$ ( $17408 \mu\text{g g}^{-1}$ ) and lower $\text{As}^{5+}$ ( $8674 \mu\text{g g}^{-1}\text{As}$ ) from As containing solutions ( $64 \mu\text{M}$ As each)	[35]
<i>Pistia stratiotes</i>	Accumulates $1120 \mu\text{g g}^{-1}$ As in roots and $31.60 \mu\text{g g}^{-1}$ As in leaves (dry weight basis) from $10 \mu\text{M}$ As containing solution	[31]
<i>Salvinia natans</i>	Accumulates $50 \mu\text{g g}^{-1}$ As in roots	[41]
<i>Hydrilla verticillata</i>	Removed sum total $8546 \mu\text{g}$ ( $348 \mu\text{g g}^{-1}$ ) of As from contaminated water (As concentration $1500 \mu\text{g L}^{-1}$ )	[49]
<i>Ceratophyllum demersum</i>	Accumulates $525 \mu\text{g g}^{-1}$ (dry weight basis) from $250 \mu\text{M}$ $\text{As}^{5+}$ solution for 7 days	[22]
<i>Potamogeton crispus</i>	Accumulates $1000 \text{ mg kg}^{-1}$ (dry weight basis) from As contaminated environment	[52]
<i>Myriophyllum spicatum</i>	Accumulates $1000 \text{ mg kg}^{-1}$ (dry weight basis) from As contaminated environment	[52]
<i>Vallisneria natans</i>	Accumulates $1000 \text{ mg kg}^{-1}$ (dry weight basis) from As contaminated environment	[52]
<i>Alternanthera philoxeroides</i>	Extract $12.94 \text{ mg kg}^{-1}$ total As (dry weight basis) from pulp paper industry effluents	[61]
<i>Arundo donax</i>	Accumulates As at the rate $9 \text{ mg kg}^{-1}$ with TF = 4.93 and BF = 15.00 for the arsenic containing solution $600 \mu\text{g L}^{-1}$ .	[16]
<i>Phragmites australis</i>	Accumulates $32.5 \text{ mg kg}^{-1}$ As in root	[62]

**Table 1.**  
Phytoremediation ability of various aquatic and semi aquatic weeds.

under natural As contaminated environment [59]. Results showed that all plants accumulated more  $1000 \text{ mg kg}^{-1} \text{ dw As}$ ; highest and lowest As accumulation and BCF were associated with *Vallisneria natans* (BCF- 361) and *Ceratophyllum demersum* (BCF- 221) [59]. Similarly ability of potamogeton spp., Myriophyllum spp. and Valisnaria spp to uptake As from contaminated water were also been reported by many authors [60–62]. Arsenic uptake by various types of aquatic, semi-aquatic and submerged weeds has been outlined in **Table 1**.

## 4. Mechanisms of arsenic uptake and detoxification in aquatic weeds

### 4.1 Mechanisms of arsenic uptake in aquatic macrophytes

Three pathways for arsenic uptake in marine macrophytes have been described – (i) active uptake through phosphate uptake transporters, (ii) passive uptake through aquaglyceroporins, and (iii) physicochemical adsorption on root surfaces. Plants mainly uptake As(V) through phosphate uptake transporters [63, 64]. As(III), DMAA and MMAA gets into the plants by passive mechanism through the aquaglyceroporin channels [64].

#### 4.1.1 Active uptake through phosphate uptake transporters

As(V) and phosphate are chemical analogs, and compete for uptake carriers in the plasmalemma [65]. As a result, as the phosphate content rises, more As (V) is required to be desorbed in the solution. Mkandawire and Dudel. [32] and Rahman et al. [33] showed that As (V) is taken up by aquatic plants through the phosphate uptake pathway, it competes with phosphate for uptake in tissues of *L. gibba* L. and *S. polyrhiza* L.

#### 4.1.2 Passive uptake through aquaporins/aquaglyceroporins

Physiological studies indicate that these arsenic species are transported in rice through aquaporins /aquaglyceroporins via passive uptake mechanisms [66, 67]. Molecular studies revealed that Nodulin26-like intrinsic membrane proteins (NIPs), one of the major subfamilies of aquaporins transporters that promote the transport of neutral molecules like water, glycerol, and urea, are responsible for transporting As(III) into rice roots [68]. Aquaporins and aquaglyceroporins are two of three subfamilies of water channel proteins (WCPs), the transmembrane proteins that have a specific three-dimensional structure with a pore that permeates water molecules [69], which are permeable to water, glycerol, and/or other small, neutral molecules. Glycerol and As(III) compete for uptake in rice (*Oryza sativa* L.), indicating that this arsenic species is carried via the plasma membrane by aquaporins/ aquaglyceroporins [67].

#### 4.1.3 Physicochemical adsorption on root surfaces

Arsenic is adsorbing and accumulating on the surfaces of aquatic plants due to suspended iron oxides (Fe-plaque). Robinson et al. [70] discovered a strong association between arsenic and iron concentrations in aquatic plants, which is believed to be due to arsenic adsorption on plant surfaces' iron oxides. Rahman et al. [14] investigated arsenic species adsorption on precipitated iron oxides on *S. polyrhiza* L. roots/fronds and revealed a strong association between arsenic and iron concentrations in tissues when the plant was exposed to As (V). There was no association

between arsenic and iron in plant tissue when *S. polyrhiza* L. was exposed to As (III), DMAA, and MMAA. As (V) is primarily adsorbed on precipitated iron oxides on the roots of aquatic plants and deposited by a physicochemical adsorption process, according to the findings.

#### 4.2 Arsenic metabolism and detoxification in aquatic macrophytes

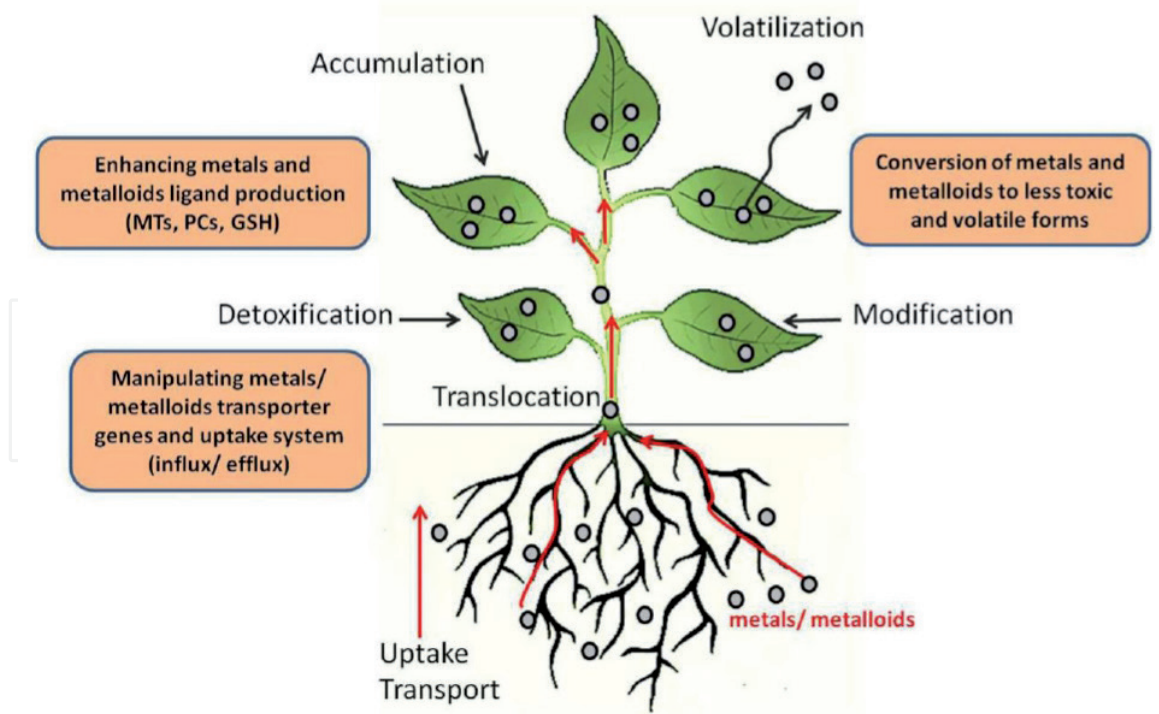
Arsenic occurs primarily as As (V) in an oxic environment and as As (III) in a reduced environment [64]. In plants, As (V) and phosphate share the same transporter, while As(III) enters plant cells through NIPs/aquaporins [57, 64]. Because of their distinct molecular properties, these two types of arsenic elicit different biochemical responses in aquatic plants [71]. As (V) has no affinity for thiol ligands, while As(III) has a strong affinity for peptides with sulfhydryl (-SH) groups, such as glutathione (GSH) and phytochelatins (PCs) [64, 72]. Even though plants had been exposed to As, arsenic speciation in plant tissues indicates that arsenic is primarily present in the As(III) oxidation state (V). This suggests that As(V) is effectively reduced to As(III) in plant cells after uptake, and that most plants have high As(V) reduction competence [64]. The reduction of As(V) to As(III) is mediated by GSH [73] and by enzyme [74], which is thought to be a detoxification mechanism of the plants. As(V) and As(III) have been shown to generate reactive oxygen species (ROS) within cells when they are taken up [75], and plants counteract the generation of ROS by various enzymes and cellular compounds [76]. The GSH can act as an antioxidant and is required for the synthesis of Phytochelatins which are required for metalloid chelation [71].

The mechanism of arsenic accumulation and detoxification was studied by many others in aquatic plant *H. verticillata* [57, 71]. In the presence of As (III) or As(V), *H. verticillata* enhanced the biosynthesis of thiols such as PCs, and increased antioxidant enzyme activity. Although the levels of thiolic compounds such as NP-SH, cysteine, GSH, and oxidized glutathione (GSSG) were significantly enhanced in *H. verticillata* upon exposure to both As(III) and As(V), As(III) was found to enhance the activities of cysteine synthase and c-glutamylcysteine synthetase and the amount of cysteine and GSH to higher levels than As(V). The analysis of PCs indicates that the accumulation of PC1 and PC2 in *H. verticillata* was enhanced with the increase of both As(III) and As(V) concentrations [71]. Thus, during As (III) and As(V) stress, phytochelatins and antioxidant systems in *H. verticillata* react differently, which is considered to be the plant's detoxification mechanism.

### 5. Biotechnological interventions for phytoremediation

Plants have been utilized for phytoremediation of toxic metals and metalloids, however due to heavy metal phytotoxicity to plants; this process has been slow and largely rendered ineffective [77]. Natural heavy metal hyperaccumulators are also available, however, they are limited to specific geo-climatic conditions and also lack the crucial biomass required for efficient phytoremediation. Phytoremediation has a lot of potential using genetic engineering technologies to improve plant tolerance and heavy metal accumulation. Furthermore, various new studies using omics technologies such as genomics, transcriptomics, proteomics, and metabolomics to elucidate the genetic determinants and pathways involved in heavy metal and metalloid tolerance in plants have been identified. Presently there are three main biotechnological approaches for the phytoremediation of heavy metals and metalloids are currently being used to engineer plants for phytoremediation of heavy metals and metalloids: (1) manipulating metal/metalloid





**Figure 3.**

Potential biotechnological strategies for phytoremediation. Heavy/toxic metals can be mobilized and transported (influx) into roots through plasma membrane transporters. They can then be transported (efflux) out of the roots into the xylem and translocated into the shoots. At this stage, plant tolerance to toxic elements may be enhanced through manipulation of influx/efflux transporters or by increasing the levels of ligands/chelators. Volatilization of the toxic elements can be achieved through enzymes that modify these toxic elements. Chelators or efflux transporters can also be used to export the toxic elements out of the cytosol and into vacuoles or the cell wall. Adapted from Dhankher et al. (2011).

transporter genes and uptake systems; (2) enhancing metal and metalloid ligand production; (3) conversion of metals and metalloids to less toxic and volatile forms [78] (**Figure 3**).

### 5.1 Manipulating metal/metalloid transporter genes and uptake system

Enhanced heavy metal tolerance and bioaccumulation has been attained in different plant species by genetic manipulation of metal transporter genes. For example, the overexpression of full length *NtCBP4* (plasma membrane channel protein) in *Nicotiana tabacum* showed  $Pb^{2+}$  hypersensitivity and enhanced accumulation of  $Pb^{2+}$  in the genetically manipulated plants. However, the overexpression of a truncated version of *NtCBP4* generated by deletion of its C-terminal, calmodulin-binding domain and part of the putative cyclic nucleotide-binding domain showed improved tolerance to  $Pb^{2+}$  and less accumulation of  $Pb^{2+}$  [79]. *Nicotiana tabacum* plants expressing *CAX2* (calcium exchanger 2) gene accumulated more  $Ca^{2+}$ ,  $Mn^{2+}$  and  $Cd^{2+}$  and also showed enhanced tolerance to elevated  $Mn^{2+}$ . It was also observed that overexpression of *CAX2* gene in *Nicotiana tabacum* increased  $Mn^{2+}$  and  $Cd^{2+}$  transport in the root tonoplast vesicles in the transgenic plants [80]. Moreover, T-DNA mutants of the *Arabidopsis CNGC1* (cyclic nucleotide-gated ion channel 1) gene, that encodes a homologous protein to *NtCBP4*, also showed  $Pb^{2+}$  hypersensitivity and enhanced accumulation of  $Pb^{2+}$  in the genetically manipulated plants. These findings suggest that *NtCBP4* and *AtCNGC1* play an important role in the transport pathway of  $Pb^{2+}$  [79, 81]. The overexpression of yeast *YCF1* (Yeast Cadmium Factor 1) gene in *Arabidopsis thaliana* resulted in enhanced accumulated higher amounts and tolerance to  $Pb^{2+}$  and  $Cd^{2+}$  metals in plants [82].

Recent research findings have revealed arsenite is transported in plants by proteins belonging to the aquaporins [83, 84]. It is observed that in efficient arsenic hyperaccumulators such as *Pteris vittata* has highly well-organized system of arsenic translocation from root to shoot tissues [85, 86], However, most non-hyperaccumulators show low mobility rate compared to *P. vittata*, also variable Arsenic mobility rate is observed among different plant species, suggesting that it is controlled by genes. Arsenic loading to the xylem is a critical stage in arsenic translocation from root to shoot, however it is a poorly known mechanism. Ma et al. [87, 88] has identified and characterized *Lsi2* gene encoding an efflux protein, plays an important role in loading arsenite into the xylem. Mutation in *Lsi2* gene caused about 50% reduction in arsenic accumulation in the shoot. The *Lsi2* gene is a homolog of the *E. coli* *ArsB* gene, an As (III)/H<sup>+</sup> exchanger that confers bacterial arsenite tolerance [89].

Genome-wide gene expression analysis in *Oryza sativa* roots treated with different heavy metals and metalloids; As(V), Cr(VI), Pb, and Cd, showed numerous differentially expressed genes as well as unique genes. Various genes belonging to different transporter families were identified [90]. Recently Wang et al. [91], has identified genes for Cu tolerance in the *Paeonia ostii* with the help of *de novo* transcriptome sequencing approach. Such genes may further be transferred to crop plants for enhancing heavy metal tolerance. Therefore, strategies of developing transgenic plants for arsenic (As) phytoremediation include enhancing plant uptake for phytoextraction, decreasing plant uptake, improving the plants' tolerance to As contamination, and increased methylation for enhanced food safety.

## 5.2 Enhancing metals and metalloids ligand production

Complexation of Arsenic with phytochelatin (PCs), or metallothionein (MTs) or glutathione (GSH) is an proficient way to detoxify As(III), since these complexes are sequestered in the vacuoles, this process is catalyzed by the homologs of multidrug resistance proteins (MRPs) [92, 93]. Enhancing the accumulation or synthesis of PCs and/or GSH and/or MTs may be one way to increase phytoremediation of arsenic. The overexpression of *PCS* in *Brassia juncea* enhanced its tolerance to arsenic but no significant increase arsenic accumulation was observed, this may be due to the fact that PC synthesis is also limited by the production of GSH [94]. The overexpression of *AtPCS1* and *GSH1* genes, that encode g-glutamylcysteine synthetase (g-ECS), the rate-limiting step in GSH biosynthesis, individually in *Arabidopsis thaliana* increased both arsenic tolerance and as well as accumulation [95].

Arsenic (As) tolerance in plants can also be increased by modifying GSH and PCs. Dhankher et al. [96] transferred and co-expressed two bacterial genes, *E. coli* arsenate reductase (*arsC*) and  $\gamma$ -glutamylcysteine synthetase ( $\gamma$ -ECS), in *Arabidopsis thaliana*, the transgenic plants grown in the presence of 125  $\mu$ M sodium arsenate accumulated threefold more arsenic in the aboveground biomass and showed almost 17-fold higher biomass than wild type WT plants. The overexpression of *AtPCS1* under constitutive promoter in *A. thaliana* enhanced tolerance to arsenate but failed to enhance arsenic accumulation [97]. These studies showed that manipulation of genes for increasing the production of metal chelation agents hold great potential for improving heavy metal and metalloid tolerance and accumulation in plants.

The *de novo* transcriptome sequencing analysis in *Raphanus sativus L.* roots under cadmium stress was carried out to discover differentially expressed genes and microRNAs (miRNAs) involved in Cd-responsive regulatory pathways. Various candidate genes encoding PCs, GSHs, and MTs; and other genes belonging to zinc iron permease (ZIPs) and ABC transporters were identified [98]. Likewise, in *de novo* transcriptome analysis in radish roots under chromium stress, showed that

1561 unigenes down-regulated and 1424 unigenes were up-regulated, various transcription factors such as Chromium stress-responsive genes involved in chelate compounds, signal transduction and antioxidant biosynthesis were discovered [99]. Such candidate genes can further be transferred into the crop plants to enhance heavy metal tolerance as well as accumulation.

### 5.3 Conversion of metals and metalloids to less toxic and volatile forms

There are several reports for developing phytoremediation strategies for heavy metals with the help of biotechnological interventions by conversion of these metals to less toxic and volatile forms. It is observed that many organisms, including bacteria, fungi, and animals, methylate arsenic. Methylated arsenic have been discovered in several plant species, including rice grain [100, 101], and suggest that this is the process is a result of endogenous methylation by the plants themselves. The final product of this pathway is the gas trimethylarsine (TMAs(III)), that can be volatilized from the plant. Qin et al. [102] have cloned a gene encoding an As(III)-S-adenosylmethionine methyltransferase (*arsM*) from the soil bacterium *Rhodospseudomonas palustris*. Expression of the *arsM* gene in an arsenic-sensitive strain of *E. coli* that resulted in the biosynthesis of several methylated forms of arsenic, including volatile TMAs(III) and conferred arsenic tolerance in the plants. These findings show that the expression of the single methyltransferase (*arsM*) gene is sufficient to produce both volatilization and tolerance to arsenic (As). A gene for an ArsM homolog in a primitive plant, the eukaryotic alga *Cyanidioschyzon merolae* has been identified [103]. Cells expressing *CmArsM* methylates As(III), as like the purified enzyme. In a rice microarray study, a putative gene annotated as a methyltransferase was found to be upregulated upon exposure to arsenate in the growth solution [104]. These findings indicate the possibility of engineering arsenic volatilization for the phytoremediation of arsenic-contaminated water and soil and also to improve the safety of the food supply.

## 6. Conclusions

Contamination of soils and water by arsenic is one the serious threat for food security and human health in throughout the world. Some severe skin and other diseases occur due to continuous consumption of As contaminated foods and water. This necessitates a suitable technology to handle arsenic contaminated water carefully, so that above mentions points can be satisfied. Phytoremediation of arsenic contaminated water by aquatic and semi aquatic weeds offers low cost, economically feasible and eco-friendly technology to remove arsenic from contaminated water for long term. Some weeds have tremendous potential to accumulate higher amount of arsenic in their plant parts such as *Eichhornia crassipes*, *Hydrilla verticillata*, *Spirodella polyrhiza*, *Arundo donax* and *Vetivaria* spp. More specifically semi aquatic weeds like *Arundo donax* and *Vetivaria* sp. (perennial) can be used with in combination with *Eicchornia*, *Spirodella* and *Hydrilla* to remove arsenic more efficiently from treatment tanks or constructed wetland system. Although management of plant biomass will be another concern for disposal, but these plant materials can be used for making fiber (water hyacinth), handcraft items (*Arundo* and *Typha* stems) and biofuel purpose. Moreover, with advancement of molecular genetics in future As tolerance genes can be transferred to food crops (specially rice) which can store huge amount of As in their roots or very low transfer co-efficient from root to grain so that transgenic rice crops will able to grow using As contaminated water and contribute in food security in upcoming days.

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