Critical review on arsenic: Its occurrence, contamination and remediation from water and soil

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Abstract
With the increasing pollution in today’s world, importance is being given to solve a problem and do it in a sustainable, eco-friendly manner. Arsenic is a class-1 carcinogen and also causes many other side effects to humans, plants and animals. The utilization of arsenic as wood preservatives, pesticides, or its historical overuse by some military units for rice killing operations has led to the increase in the toxic effects of arsenic like its carcinogenicity, decreased immune response etc. Although conventional methods like coagulation, lime softening, adsorption, membrane technology are effective, they have their disadvantages like additional waste generation, causing increased pollution and are expensive. The better alternative is phytoremediation. Appropriate plants like Brassica juncea, Hydrilla verticilata, Pteris vittata L., Vallisneria natans, can be chosen based on the method of the remediation like phytoextraction, phytostabilization and phytofiltration or phytovolatilization. This review provides the list of a few plants which can be likely chosen for the purpose of both water and soil remediation. Advancements are occurring in bioremediation studies with the development of transgenic plants like transgenic tobacco, transgenic Arabidopsis thaliana for better phytoremediation. Understanding the mechanism employed by the plant for its uptake/detoxification can aid in the enhancement of the process of remediation with the external supply of phosphorus. Along with this, the proper and safe disposal of plants is crucial for the remediation process. In addition, awareness of this solution to the general public is to be made for its effectiveness.

Keywords: Arsenate [As(V)], Arsenite [As (III)], Contamination, Phytoremediation, Toxicity

INTRODUCTION

Arsenic poisoning has been common in various countries like India, Argentina, Bangladesh, China, European countries, Nepal, the United States, Vietnam (Krämer, 2005; Shaji et al., 2021). In addition to its natural sources of occurrences, the excessive usage of arsenic as wood preservatives, pesticides, food additives led to the increased arsenic concentration in soil and underground water (Peryea and Creger, 1994; Hingston et al., 2001). Consumption/drinking of this arsenic polluted water is causing serious health effects to humans, plants and animals. The carcinogenicity of arsenic is also quite high and can cause cancer of the skin, bladder, kidney or lung even with minute exposure. In plants, necrosis, stunted growth, low yield, withered leaves and even death are observed with arsenic exposure (Mandal and Suzuki, 2002; Abbas et al., 2018; Palma-Lara et al., 2020). Therefore, this pressing problem has to be addressed efficiently. Conventional physical and chemical methods like coagulation, ion exchange, lime softening, adsorption, filtration etc., though are effective to an extent, can be expensive, involve high labour in handling, and create additional waste products during the process (Srivastava and dwivedi, 2015). An environmentally friendly process with little maintenance and cost-effectiveness is the use of plants (phytoremediation) or bacteria (bioremediation) for the removal/stabilization/conversion of arsenic into a less toxic form. With the advancements in genetic engineering, the usage of transgenic plants or bacteria for better and easy removal of arsenic is also in practice (Irshad et al., 2021). This review gives a detailed idea of the conventional methods in practice and their better alternatives, emphasising on the types of plants that aid in the phytoremediation of arsenic. The safe disposal of arsenic adsorbed plants has also been discussed here.
SOURCES AND OCCURRENCE OF ARSENIC

Arsenic (Z=33) is the 20th most abundant element in the geosphere and it has an average abundance of about 5 mg/kg in the earth’s crust (Garellick et al., 2008). It is a colourless, odourless and tasteless poisonous element (Katsoyiannis and Zouboulis, 2006). It occurs naturally as ores, usually in combinations with sulphur like Realgar (AsS), Orpiment (As₂S₃), Arsenopyrite (FeAsS) (Magalhães, 2002). Weathering of these minerals or rocks would form the particulate arsenic (as Arsenic trioxide), which then dissolves with rainwater and enters the soil /groundwater. Elevated concentration of arsenic is seen in the groundwater in countries like Argentina, Bangladesh, Chile, China, India, Mexico and the United States of America (Melkonian et al., 2011). In the soil system, the pH governs the availability of arsenic (Signes-Pastor et al., 2007).

Volcanic eruptions, dust storms, forest fires are some of the natural causes of the availability of arsenic. The process of arsenic occurrence in the environment is enhanced due to anthropological usage like mining, metallurgy, processing of ores, burning of fossil fuels, industrialization, its use as a wood preservative etc. (Taylor et al., 2003; Raj, 2019). Apart from these, the use of pesticides and herbicides containing arsenic, inclusion as an additive in the feed of livestock has led to the drastic elevation of the arsenic concentration in the soil and water, causing several toxic effects to plants and animals (Smedley and Kinniburgh, 2002; Irshad et al., 2021).

Arsenic is found mostly in four oxidation states: arsenate (+5), arsenite (+3), arsenic (0), and arsenide (-3). Usually, arsenic in -3 and 0 oxidation states are found to be unstable in soil (Xie and Huang, 1998). The inorganic forms of arsenic are common in mineral forms and are highly toxic. The inorganic form when enters the food chain gets methylated and less toxic organic forms like MMA (Monomethyl arsenic), DMA (Dimethyl arsenic), TMA (Trimethyl arsenic) are formed. Other organic forms of arsenic like arsenobetaine (which is possibly produced by zooplanktons or phytoplanktons) (Edmonds and Francesconi, 1988; Lee and Wen, 2019), arsenocholine (immediate precursor of arsenobetaine) (Landner, 1998; Chen et al., 2020) can be taken up by some fish, shellfish and can be buildup in its tissues. This is usually referred to as ‘fish arsenic’ and is less harmful (Chou and De Rosa, 2003). Arsenate is the chemical analogue of phosphate and is the thermodynamically stable form in aerobic conditions, while arsenite is dominant in anaerobic conditions like submerged soil conditions (Abedin et al., 2002; Signes-Pastor et al., 2007) and also in flooded water conditions. This is the reason for increased arsenite concentration in plants like rice which need flooded water conditions (Yamaguchi et al., 2011; Awasthi et al., 2017).

HISTORICAL USE OF ARSENIC

Arsenic was first isolated in 1250 CE. And ever since it has been used historically as a drug in the treatment of skin infections and for beautification (Shrivastava et al., 2015). It has also been utilized as a pesticide for grape plants, cotton and orchards in arsenate (AsO₃³⁻) form (Taylor et al., 2003). About 50% of arsenic is used in the production of pesticides, and 30% in the formulating of wood preservatives (e.g. chromated copper arsenate- CCA) to make the wood resistant to decay (Garellick et al., 2008; Rahman and Hasegawa, 2011). CCA has been applied onto the timber that is specially used for marine conditions as it preserved it from being damaged by wood-boring crustaceans and molluscs and decay by soft rot fungi and lignolytic bacteria (Brown et al., 2001).

Arsenite has been used as rodenticide, herbicide, and insecticidal bait because of its high solubility and rapid toxicity. Johnson grass (Sorghum halepense), grown primarily in cotton fields, can be controlled by the use of dimethylarsinic acid and disodium methyl arsenate. Cocadylic acid, marketed with the name ‘Agent Blue’ used by military forces in the 1960s for ‘rice-kill operations’ of their enemies, is an organic arsenic compound with high solubility (Bencko and Foong, 2017).

Arsenic was used as an additive to livestock feed until its ban at the end of the 20th century (Jones, 2007). Roxarsone (3-nitro4-hydroxyphenyl arsenic acid) was used in chicken farming feed, as it aided in weight gain, control of infectious agents, enhanced feed utilization and improved meat pigmentation (Fisher et al., 2015). During 1900 - 1955, arsenic was observed to be used to control ticks in cattle (Rahman et al., 2019). Other applications of arsenic usage are lead-acid batteries for automobiles, light-emitting diodes, and semiconductors (Chou and De Rosa, 2003).

Incidents related to the consumption of arsenic-contaminated food were also recorded historically. The Manchester epidemic of 1900 due to consumption of arsenic-contaminated beer (Phillips and French, 1998) and the acute arsenic poisoning outbreak due to contaminated soya sauce in Japan during 1956 (Mizuta et al., 1956) are noteworthy episodes in history (Naidu et al., 2006).

TOXIC EFFECTS OF ARSENIC ON BIOSPHERE

World Health Organization (WHO) has set a limit with a concentration of arsenic below 10 μg/L as safe for drinking water (https://www.who.int/news-room/fact-sheets/detail/arsenic) while the national standard for drinking water in Bangladesh is 50 mg/L. (Islam et al., 2015). However, some studies show that even that leads to the mortality risk (D’ippoleti et al., 2015). Arsenic is a highly toxic metal and a class- 1 carcinogen affecting...
the liver, lung, skin, kidney and bladder (Raj and Singh, 2015). Substances that are categorized under class-1 carcinogens can definitely cause cancer with enough supporting evidence (McGregor et al., 2010). In addition to causing cancer, inorganic arsenic is also seen to act as a potential endocrine disruptor, specifically influencing glucocorticoid receptor (GR)-associated gene expression, although detailed mechanism is yet to be under stood (Meakin et al., 2019).

In the plant system, arsenic competes with phosphate and enters through phosphate channels, leading to phosphate imbalance, formation of unstable adducts and thereby leading to lesser production of ATP in the cell (Sayantant and Shardendu, 2017). As(V) also hinders the phosphate of nucleic acid, thereby the DNA synthesis, while As(III) binds to sulfhydryl groups of peptides and proteins and interferes with their activities (Mishra et al., 2017). Additional toxic effects of arsenic include oxidative stress, alterations in cell signalling and DNA repair (Kozul et al., 2009). Toxicity of arsenic is in the order: arsenite > arsenate > MMA > DMA (Carbonell-Barrachina et al., 2000).

### Plants
Arsenic is a non-essential element and is toxic to plants as well (Garg and Singla, 2011). When arsenic enters the plants, they interfere with the various metabolic processes, induces oxidative stress due to the formation of reactive oxygen species (ROS) like superoxide radical (O²⁻), hydroxyl radical (OH), and hydrogen peroxide (H₂O₂) during the conversion of As(V) to As (III) (Sharma, 2012). Diminishing in the tissue respiration and energy supply can also be seen in the plant due to impaired glycolysis and the TCA cycle. This is due to the replacement/interaction of phosphate and sulfhydryl groups (-SH) of enzymes/biomolecules with As (V) and As (III) respectively (Thakur et al., 2020). Visible toxic effects observed in plants include inhibition of seed germination, discoloured-stunted roots, necrosis, chlorosis, decreased photosynthetic activity, withered leaves, reduced fruit and grain yield, and in extreme cases, even death. In most plants, the arsenic toxicity threshold limit in sandy and clay soils is 40 and 200 mg kg⁻¹, respectively (Vithanage et al., 2012).

Literature supports the higher accumulation of arsenic in plants like paddy because of higher bioavailability in soil, which is due to the fact that they are to be cultivated in water bed conditions. Therefore, they (Oryza sativa) are seen to accumulate 10 times more arsenic than other cereal plants. Reduced amylolytic activity is seen in wheat in arsenic toxic conditions. Arsenic is seen to obstruct the pigment biosynthesis through inhibition of tetrapyrrole synthesis when it reaches the chloroplast (Mishra et al., 2017).

### Animals
Animals also show certain toxic effects when exposed to arsenic like abdominal pain, weakness, nausea, diarrhoea and death. Mucosal epithelial necrosis and renal tube and gastrointestinal capillaries degeneration are seen. Exposure to a long time can induce depression, dehydration, frequent urination, imbalance in body temperature. Cutaneous arsenic exposure can cause drying up and deadening of the skin (Shrivastava et al., 2015). It has also been reported that arsenic inhalation in rodents can lead to nasal flow discharge and irritation in the eye. Autopsy reports showed reddened edematous gastric and intestinal mucosa, yellowing of liver, and lung edema in arsenic exposed animals (Shrivastava et al., 2015). Partial fibrosis is noticed in cattle if arsenic in the feed concentration in higher than 250ppm leading to stiffness and unsymmetrical growth of hocks and limb joints. In goats, an increase in the heart rate and respiratory rate, congested mucosa with drooling of saliva, convulsion, polyuria and reduced weight is observed (Mandal, 2017).

### Humans
Humans show many effects due to exposure to arsenic through water and food. Almost every food like rice, vegetables and even meat is seen to be contaminated with arsenic and with imports and exports of food, even people living in non-arsenic contaminated areas are getting exposed to arsenic (Upadhyay et al., 2019). Oral exposure of arsenic can show certain long term effects like melanosis (hyperpigmentation), the appearance of small corns or warts on the palms, soles, and torso; leukomelanosis (hypopigmentation), carotid athrosclerosis, and cardiovascular diseases (Chou and De Rosa, 2003). Inhalation of arsenic is seen to cause respiratory diseases, impaired lung functions (Slavkovich et al., 2013), peripheral nervous disorders and impaired cognitive abilities and motor functions. Exposure to arsenic is also observed to be associated with diabetes, hypertension (Lalita et al., 2012), development of arsenicosis, reproductive and developmental defects (Visoottiviseth et al., 2002). As defined by WHO, arsenicosis is a “chronic health condition arising from prolonged ingestion (not less than 6 months) of arsenic above a safe dose, usually manifested by characteristic skin lesions, with or without the involvement of internal organs” (Das and Sengupta, 2008).

Arsenic is observed to impede the metabolic pathways, alter the metabolites specially involved in the metabolism of aminoacid, pyruvate and Krebs cycle (Martin et al., 2015). They also act as endocrine and mitochondrial function disruptors (Howard, 2018; Sodhi et al., 2019). It also shows effects on hormonal regulation via retinoic acid, thyroid hormone and estrogen receptors.
Arsenic exposure may even compromise the body’s immune response (Kozul et al., 2009). The methylation of inorganic arsenic, which is an important process can be affected by factors like smoking tobacco. It is shown to decrease the methylation process and causes the deposition of inorganic arsenic in hair, bone and skin, and altering the DNA repair process (Melkonian et al., 2011).

**METHODS TO REMEDIATE ARSENIC CONTAMINATION**

The contaminated sites are hazardous and serve as a potential threat to all life forms. Therefore, it is necessary to remediate the contaminated sites. For this conventionally, the methods implemented may be to dig up the contaminated soil and transfer it to a landfill (Joseph et al., 2018), usually away from the human activity, or contain the area of contamination or leaching (use of strong chemicals to desorb/leach metals from the soil) (David, 1995). Any of these conventional methods do not solve the issue but simply would carry the problem from one place to another. It would also create a risk of toxicity during handling, transportation and excavation in addition to the high-cost factor (Mary, 2011).

Therefore, proper methods are to be employed which can destroy or convert the contaminant to a less-toxic form. Many physico-chemical methods are employed for this process like precipitation, filtration, sedimentation, ion exchange, etc. Alternatively, biological methods like phytoextraction, phytostabilization, phytodegradation, rhizodegradation, phyto filtration etc. are also used.

**Physico-chemical methods**

Physico-chemical methods involve the separation or removal of arsenic from the contaminated soil or water samples with the help of certain physical methods like sieves, electrodes or through chemicals like alumina, alum or the combination of both. Most electro/chemical methods help take off or separate As(V) than As (III). Therefore, treatment with strong oxidizing agents like chlorine, ferric chloride, permanganate, ozone or hydrogen peroxide is recommended to convert As(III) into As (V) (Ortega , 2017). The most commonly used methods are discussed below:

**Coagulation**

Coagulation is the process through which the dissolved arsenic can be converted into a solid or a semi-solid form with the addition of certain coagulants. The rough aggregation with the destabilized metal particles along with the coagulants is known as floc (Hashmi and Pearce, 2011). The most commonly used coagulants for arsenic remediation are alum (aluminium sulphate) and ferric chloride. Both of these chemicals are equally effective for the removal of arsenic on a molar basis. When the low doses of these coagulants are added, it leads to the formation of flocs with the rough aggregation of the colloidal particles, while an amorphous metal hydroxide floc is formed with the high doses of coagulants into which the colloidal particles get entrapped (Hering et al., 1996). The pH range for coagulation with iron is seen to be 5-8, while with alum is 5–7 (Grill et al., 1987). Adsorption, occlusion, filtration or sedimentation methods can be used to remove the coagulated material. The disadvantage of this method is the use of chemicals that can add colour and/or odour to the sample. Also, large volumes of contaminated sludge are generated with this method which causes disposal related problems (Kochian, 2004).

To prevent this problem, electrocoagulation can be used where coagulation can be achieved with the help of electrodes. The amount of sludge generated gets reduced as no coagulants are added. Hence, electrocoagulation is observed to be effective than the chemical coagulation method in treating water bodies (Nidheesh and Singh, 2017). The steps involved in this method are: Formation of coagulants by electrolytic oxidation are i) Contaminants destabilization, suspension of particulate and breaking of emulsion and ii) Floc formation by aggregation of the destabilized particles (Ali et al., 2011). Iron and aluminium electrodes are most commonly used for this process. Titanium, copper and zinc electrodes can also be used. The major drawback of this method is passivation and severe corrosion of electrodes in field conditions (Nidheesh and Singh, 2017).

**Lime softening**

Lime softening, a method commonly used for removing the hardness of water can also be used to remove arsenic in dissolved form. The addition of Ca(OH)₂ and Na₂CO₃ to water will increase its pH due to hydroxide ion release and precipitates substances other than magnesium and calcium. Arsenic also precipitates along with this and comes out in the calcifier with the lime sludge produced by the process (Kochian, 2004). The process is highly pH-dependent with an optimum 10.5 – 11. About 95% of As can be removed from the contaminated water at this pH, whereas only 30% can be removed at pH 8.5 (Litynska et al., 2017). The use of Cl⁻ can increase the removal efficiency of arsenic. At the end of the process, treatment with acid is probably necessary to lower the pH to the optimum drinking levels (Choong et al., 2007). Litynska and Babakov (2019) assessed this method as a non-environmental friendly one with high need for chemicals, large quantities of
organic nanoparticles, silicon-based nanomaterials, carbonaceous nanomaterials are in use. Sadeghi *et al.* (2020) synthesised graphene oxide ribbon (GONR), by unzipping of multwall carbon nanotubes. GONR are seen to be efficient in this ultrasonic assisted adsorption of arsenic.

**Membrane technology**

This technology typically includes methods that use membranes as filters to remove the pollutant (arsenic) from the sample. Reverse osmosis and dialysis can be included in this process. The success of filtration will depend on choosing the proper membrane with the appropriate pore size. The particulate arsenate larger than the given pore size will be rejected/ retained because of the size exclusion. Improvements with this method can be made by choosing membranes with certain physicochemical properties like hydrophobicity or charge, leading to adsorption or repulsion (Katsiyanni and Zouboulis, 2006).

Nano filtration and hyperfiltration are the two processes of reverse osmosis. Nanofiltration operates at relatively low-pressure reverse osmosis. It is primarily used to exclude larger dissolved solids. Because of its ability to remove the divalent ions like calcium and magnesium that causes hardness in water, this method can also be called membrane softening. Hyperfiltration, however, operates at higher pressures with a greater rejection of dissolved solids (Kartinen and Martin, 1995). The optimum pH for reverse osmosis is between 7.0 and 9.0 (Katsiyanni and Zouboulis, 2006). Of the different membranes used, chitosan, zeolite membranes like Fe-exchanged natural zeolite, Alumina-modified zeolite and synthetic zeolite etc (Khatamian *et al.*, 2017; Li *et al.*, 2018c). A cross-flow filtration experiment performed with a combination of zeolite modified chitosan membrane is seen to show a higher rejection rate for As(III) (Mukhopadhyay *et al.*, 2018). Cellulose acetate-zinc oxide combined matrix membrane prepared by Durthi *et al.* (2018) is shown to exhibit the capability to remove arsenic with high efficiency and flux rates when compared with that of the cellulose acetate membranes without the nanoparticles.

Electrodialysis is the process by which the separation of particles occurs through the membrane with the application of electric current (Ali *et al.*, 2011). The potential difference applied will aid in the transfer of ions through the alternating anionic and cationic membranes, which are aligned in between positive and negative electrodes. This results in two flow systems- one with concentrated ions and the other diluted stream. The efficiency of the arsenic removal is seen to be dependent on the applied voltage and the initial concentration (Pham *et al.*, 2021). No addition of chemicals is required and this method can also tolerate feed waters with high chlorides and sulfates. Highly cleanable, easily recoverable and durable membranes are being utili-
lized for this process, which is an added advantage (Taylor et al., 2014). The major disadvantage of the filtration process is creating larger volumes of sludge, causing the problem with disposal.

Other physicochemical processes of separation of arsenic include distillation- where the water is made to evaporate with thermal energy and then made to condense onto the roof of the cooler surface. Impurities like arsenic will be left behind and thus can be separated (Hashmi and Pearce, 2011). Therefore a fraction of the contaminant can be removed from the soil. (Nedjimi, 2021). Thus, a fraction of the contaminant would be taken up by the roots and translocated to the leaves, which can then be harvested (Abdulsalam et al., 2011). Biological remediation is an option in which the degradation or conversion of harmful contaminants occurs by employing plants or microorganisms. Thus, the remediation process occurs through a natural biological phenomenon, causing no damage to the soil texture/properties (Joseph et al., 2018). In fact, employing plants/soil microorganisms would enhance the quality of the soil in addition to remediation of arsenic and usually do not involve high expenditure.

Phytoremediation
As discussed earlier, biological remediation is a means to remediate the soil using plant and microbes. It is divided into phytoremediation and microbial remediation. Remediation of soil or water sample with the help of plants is called phytoremediation and remediation with microbes is microbial remediation (Tripti et al., 2017). A combination of these can also be used for the efficient removal of contaminants. Phytoremediation includes phytoextraction, phytostabilization, phytodegradation and phytofiltration.

Phytoextraction
Phytoextraction, also known as phytoaccumulation, or phytosequestration is the easiest and desirable way of removing a contaminant from the soil or water medium. It involves the extraction of contaminants from the soil or water with the help of terrestrial/aquatic plants. The contaminant would be taken up by the roots and translocated to the leaves, which can then be harvested (Nedjimi, 2021). Thus, a fraction of the contaminant can be removed from the soil. The plants that are chosen for this method are known as ‘Hyperaccumulator’ plants. Plants that can accumulate more than 0.1% DW of a contaminant are called hyperaccumulators (Nedjimi, 2021). The ideal characteristics of these plants involve greater above-ground biomass, higher growth rate, efficient translocation, and easy to cultivate and harvest. Hyperaccumulators, which can accumulate metals naturally in their tissues without developing any toxic conditions and plants with highly branched root system would be ideal for the better uptake of contaminants (Bhargava et al., 2012).

Hyperaccumulator plants that have the shoot to root metal concentration ratio greater than one are chosen for this method. Arsenic is usually localised in epidermal cells, mesophyll cells, and xylem tissues (Vithanage et al., 2012). Plants generally tend to store metals in the roots and prevent their transfer to the shoots as they can affect the photosynthesis, flowering capacity, etc., which is not observed in hyperaccumulators and thus as ideal for phytoextraction. Examples of hyper accumulator plants include *Hydrilla verticillata*, *Vallisneria neotropicals* (Chen et al., 2015; Li et al., 2018a), *Pteris vittata L.*, (Poynton et al., 2004; Xie et al., 2009) *Pityrogramma calomelons* ( Francesconi et al., 2002; Luongo and Ma, 2005). Hyperaccumulators might not always have high biomass, yet they would have a very high accumulation rate of target metal. Non-hyper accumulating plants with high biomass can also be employed, even though they do not have high specificity to the target metal as they can give a promising result overall, like *Brassica juncea* (Niazi et al., 2017). Once the contaminants get depleted from the soil to a certain extent, the plants are harvested. The harvested plants can either be smelted for potential metal recovery/ recycling or are to be disposed of safely as hazardous waste.

The translocation efficiency of the plant can be assessed with the calculation of certain factors like translocation factor (TF), enrichment coefficient of the shoot (ECS), bioaccumulation factor (BF). The phytoremediation efficiency of the plant is calculated based on the amount of arsenic translocated from the roots to shoots (Rahman and Hasegawa, 2011).

Translocation factor (TF)
An important aspect of characterising plant capacity in phytoremediation techniques is calculating TF. TF determines the ability of the plant to translocate a heavy metal from root to shoot parts. It is the ratio of the concentration of an element in the shoot (mg g$^{-1}$) to the concentration of the same element in the root (mg g$^{-1}$). Hyperaccumulators show high TF value, while the non-hyper accumulators usually have a value of less than one (Francesconi et al., 2002).

\[ TF = \frac{\text{conc. of element in shoot}}{\text{conc. of element in root}} \]  

\[ \text{.........Eq.1} \]

Enrichment coefficient of shoot (ECS)
The ECS is another factor that can be used to assess the heavy metal uptake capacity of the plant. It is the ratio of the concentration of metal in the shoot to that of...
its concentration in the soil. When the ECS of a plant is greater than 1.0, it represents the typical capacity of that plant to transfer the metal to the shoot, mostly to the vacuoles (Elshamy et al., 2019).

\[
ECS = \frac{\text{conc of metal in shoot}}{\text{conc. of metal in soil}} \quad \text{.........Eq.2}
\]

Bioaccumulation factor (BF)
The bioaccumulation factor (BF) is used to evaluate the capability of the roots to take up the metals from the soil. BF is the concentration of an element collected in root tissues (mg g\(^{-1}\))/concentration of the same element in soil (mg g\(^{-1}\)).

\[
BF = \frac{\text{conc of metal in root tissue}}{\text{conc. of metal in soil}} \quad \text{.........Eq.3}
\]

Plants like *Trifolium spp.* with multiple harvest in a single growth period can be used. Grasses like barnyard grass (Sultana and Kobayashi, 2011), rice cutgrass (Klaber and Barker, 2014) are preferred to shrubs/trees as they have high above ground biomass, growth rate and are more adaptable to stress (Ali et al., 2013). Care should be taken to prevent herbivores from consuming these plants leading to the contaminant entry into the food chain.

Phytostabilization
Phytostabilization or phytoimmobilization or in-place activation is a very efficient managing strategy in mining areas. This method stabilizes the contaminants, and thereby reduces their bioavailability and mobility. Thus, phytostabilization aids in reducing off-site contamination (Shrivastava et al., 2015). Plants secrete certain redox enzymes such as arsenate reductase and stabilize the contaminant (arsenic) through sorption, complexation/metal valence reduction or precipitation it to less toxic forms (Thakur et al., 2020) and stabilize the contaminant (arsenic) through sorption, complexation/metal valence reduction or precipitation and therefore convert it to less toxic forms. Thus, this method does not lead to the formation of any secondary waste, but it enhances soil fertility. Plants suitable for phytostabilization should develop an extensive root system, provide good soil cover, possess tolerance to the contaminant metals, and ideally immobilize the contaminant in the rhizosphere, reducing leaching or bioavailability of arsenic and wind erosion (Silva Gonzaga et al., 2006). Ideally, plants chosen for Phytostabilization should have low pollutant accumulation in the shoots. If the plants chosen to accumulate the contaminant into the leaves/tissues, contaminants can enter food chain; hence mostly the non-edible plants like *Eucalyptus, Arundo donax* L. can be chosen (Bolan et al., 2011; Mirza et al., 2011). When introduced with similar arsenic load, Woody plants like Eucalyptus species gather arsenic at much lower levels than grasses, ferns, or other plants. Although this makes them less than ideal for phytoextraction, they are still a good choice for phytostabilization. Also, the leaves of these plants are rarely consumed by mammals because of certain defense compounds like terpenes, phenolics and cyanogenic glycosides, preventing their entry into the food chain (King et al., 2008). However, it is to be noted that in the process of phytostabilization, only the movement of the metal is restricted and is not a permanent solution to the problem (Ali et al., 2013). The site is to be monitored at regular intervals to ensure that the optimal conditions are maintained. However, it is to be noted that in the process of phytostabilization, only the movement of the metal is restricted and is not a permanent solution to the problem (Ali et al., 2013). The site is to be monitored at regular intervals to ensure that the optimal conditions are maintained.

Phytofiltration
In general, Phytofiltration can be used to filter underground water, stormwater, subsurface water, wastewater etc. with a low concentration of contaminants and other effluents using aquatic macrophages or macroalgae (Garg and Singla, 2011; Shrivastava et al., 2015). In this method, the metal contaminants are adsorbed/absorbed onto the plant’s surface, leading to lessening the concentration of pollutants in the water, i.e., filtering metals from water into the root system (Mykolenko et al., 2013). Therefore, the plants with high absorption surface area and those able to tolerate hypoxia are to be chosen. For example, *Micranthemum umbrosum* is seen as a strong accumulator of arsenic with the accumulation of about 1000 mg As g\(^{-1}\) in its stem and leaf biomasses, potentially reducing the arsenic concentration in the solution of about 10-fold (Islam et al., 2015). In phytofiltration, the expulsion of arsenic from streaming water must be quick and so relies upon the water stream rate (Sandhi et al., 2018). Phytofiltration can be of three types based on the part of the plant used for this purpose- rhizofiltration (roots), blastofiltration (seedling) and caulofiltration (excised shoots) (Ali et al., 2013). For Rhizofiltration to be effective, non-efficient metal transporters (as metal transport to the shoot makes rhizofiltration less efficient) and plants with extensive root system (e.g. *Eucalyptus globulus*, *Acacia tortilis*, *Faidherbia albida*) (Anawar et al., 2008) are to be chosen. Although to treat surface contaminated water, plants with shallow roots like grasses, Indian mustard or ferns can be used. At the end of the process, the roots can be harvested and dried. Depending on the purpose, the metals can be extracted by acid treatment or can be burned at hazardous waste sites (Dushenkov et al., 1995). Thus, phytofiltration
becomes a very effective eco-friendly method to reduce contamination in the natural wetlands and estuary areas. Even arsenic-contaminated run off water from mines can be remediated by using *Lemma gibba*, which has high arsenic accumulation capacity (Anawar et al., 2008).

### Phytovolatilization

The process involves the taking up of the contaminant from the soil and its release into the environment in the gaseous state in modified/ unmodified form at low concentrations through transpiration (Ranjan et al., 2020). The toxic pollutant is observed to be diluted in the atmosphere or is likely converted into a less toxic form. (Guarnio et al., 2020). It can be divided into two types: direct and indirect. Direct involves the volatilization from the stem or roots or the leaves, while indirect involves the volatilization from the subsurface due to root activities (Pandey et al., 2018). It is the most controversial form of phytoremediation techniques. It involves the transfer of contaminant from one medium to the other, which could be redeposited back to the original medium. Thus, there seems to be little or no control over the movement of the contaminants (Bolan et al., 2011). In contrast, certain studies show that the contaminants might not cause any harm to the environment. An additional advantage of this method is that no labor or effort is needed to transfer or dispose of the contaminated plant materials physically, thus requiring less management force (Heaton et al., 1998). It is seen that the presence of sulphate and salinity in the soil can hinder the process of volatilization (Vithanage et al., 2012).

Usually, arsenic is phytovolatilized in the form of trimethylarsine [TMA(III)], the final product of methylation pathway in which As(III) is methylated to dimethylarsinic acid [DMA(V)] and then to trimethylarsine oxide (TMAO) which is finally reduced to the volatile trimethylarsine [TMA(III)] (Mirza et al., 2011). *P. vittata*, the well-known hyperaccumulator of arsenic, can also be used in phytovolatilization. It is seen to release arsenic compounds into the environment through its secretory glands at the edges of the fronds (Sakakibara et al., 2007).

### PHYTOREMEDIATION OF ARSENIC CONTAMINATED WATER BODIES

Contamination of water bodies is a serious threat to the living systems and it is to be addressed properly. Fig.1 and 2 show the arsenic contaminated water in different districts of India (Reddy, 2019). Among other chemical and physical methods, the natural bioremediation/ phytoremediation is a cost-effective and efficient method. The utilization of rapidly growing plants, like *Eichhornia crassipes* (water hyacinth), whose overpopulation is usually not desired, can be used to our advantage in decontaminating / remediating the polluted bodies (Ajayi and Ogunbayio, 2012). The metal removal rate of water hyacinth was reported to be 600 mg As/ha by (Alvarado et al., 2008). In addition to water hyacinth, those showing hyper tolerance to As and capable of hyperaccumulating arsenic are also the key players in the game of phytoremediation. Over the years, these plants are identified, like *Rorippa nasturtium-aquaticum* L., *Mentha* spp. (Robinson et al., 2006), the *Pteris* ferns, *Hydrilla verticillata* (Xue Yan, 2011), *Lepidium sativum* (Robinson et al., 2003), *Spirodela polyrhiza* L., (M. A. Rahman et al., 2007) *Eleocharis acicularis* (Ha et al., 2011), *Arundo donax* L. with a high growth rate, the Macrophyte *Lemma valdiviana* is observed to reduce the arsenic concentration in water to about 82% of initial concentration under controlled factors like pH, nitrate, phosphate concentrations (de Souza et al., 2019). In water bodies where the arsenic concentration is less than 30ppm, the naturally grown *Neptunia oleracea* (Water mimosa) that has Rhizofiltration can be employed (Atabaki et al., 2020). Identifying plants with higher biomass having greater metal uptake capacity and employing them for the process of remediation is a sustainable and eco-friendly method to address this serious problem. Destruction of these plants from the water surface is to be avoided as they help in the control of heavy metal pollution and prevent their entry into the food chain (Sasmaz and Obek, 2009).

Another process employed for the phytoremediation of polluted water bodies is through constructed wetlands. Interactions between plants, soils, sediments and microbial communities always exist in a wetland and hence they are considered to be complex bioreactors (Corroto et al., 2019). The concept of constructed wetlands has been put into practice ever since the mid 1980’s. Precipitation, coprecipitation and sorption are the major arsenic expulsion mechanisms incorporated in this system (Lizama et al., 2011). It is important to select metal tolerant plants, immobilize the contaminant in roots, and prevent the movement of these metals to the aerial parts of the plant, environmentally sustainable and easily implemented (Corroto et al., 2019). Table 1 represents various aquatic plants that are involved in the remediation of water bodies.

### PHYTOREMEDIATION OF ARSENIC CONTAMINATED SOIL

Soil health, defined by the Natural Resources Conservation Service (NRCS, USA) as “the continued capacity of soil to function as a vital living ecosystem that sustains plants, animals and humans” (Sanchez-Hernandez et al., 2019). Of the other factors affecting soil health like soil erosion, organic matter decline, biodiversity loss, contamination, loss of salinity etc.,
Growth and establishment of hyperaccumulating fern species like *Pteris vittata* can take up arsenic up to 4100 mg Kg$^{-1}$ (Fayiga et al., 2004), *Pityrogramma calomelanos* up to 8350 μg As g$^{-1}$ dry mass (Frances coni et al., 2002) in arsenic-contaminated area has shown to give promising results. *Pteris vittata* can accumulate about 10 times the concentration of arsenic in the soil when grown in arsenic-contaminated sites (Raj Singh, 2015). Harvesting these mature fronds on a regular basis will aid in the maximum removal of arsenic from the site of contamination. *Brassica* species like *Brassica juncea*, *B. carinata* and *Isatis capadocica* with the ability to produce high aboveground biomass and accumulate high arsenic concentrations in their shoots also aid in extracting high quantities of the heavy metals from soil (Karimi et al., 2003). Apart from these, even *Mimosa pudica*, a herb and *Melastoma malabathricum*, a shrub, are also shown to phytoremediate the arsenic-contaminated soils (Rahman et al., 2007). Hyperaccumulator plants are generally used for phytoextraction. However, non-hyperaccumulating plants can also be manipulated to enhance their arsenic uptake efficiency by providing certain factors or conditions, e.g. the use of non-As-hyperaccumulating *Brassica* sp. like *Brassica juncea*, *Brassica napus* with the assistance of phosphate, the chemical analogue of As(V) (Niazi et al., 2017). Table 2 represents various terrestrial plants that are involved in the remediation of soil.

<table>
<thead>
<tr>
<th><strong>PLANTS</strong></th>
<th><strong>CHARACTERISTICS</strong></th>
<th><strong>SOURCE</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrilla verticilata</td>
<td>It is a submerged macrophyte which acts as a bio-indicator of arsenic pollution. It is also a phytotransformer, hyperaccumulator with fast growth and high biomass.</td>
<td>(Chen et al., 2015)</td>
</tr>
<tr>
<td></td>
<td>It is a submerged rooted macrophyte, hyperaccumulator, phytofiltrator with greater survival and growth potential.</td>
<td>(Li et al., 2018a)</td>
</tr>
<tr>
<td>Vallisneria natans</td>
<td>It is a free-floating perennial extensively used to phytoremediate with rapid multiplication and greater biomass production. It can survive in wide range of temperatures between 1-40°C</td>
<td>(Rahman and Hasegawa, 2011)</td>
</tr>
<tr>
<td>Eichhornia crassipes</td>
<td>It is a free-floating macrophyte and is an efficient phytofiltrator in contaminated water bodies and paddy soils. It has high multiplication rates, short life spans and easy to grow in various habitats.</td>
<td>(Islam et al., 2015)</td>
</tr>
<tr>
<td>Spirodea polyrhiza</td>
<td>It is a rootless submerged aquatic plant shown to accumulate arsenic with a 20000-fold concentration factor.</td>
<td>(Zhang et al., 2011)</td>
</tr>
<tr>
<td>Micranthemum umbrosum</td>
<td>It is a rooted vascular plant, a strong accumulator and an efficient phytofiltrator of arsenic without showing any phytotoxic effect.</td>
<td>(Islam et al., 2015)</td>
</tr>
<tr>
<td>Azolla caroliniana</td>
<td>It is an annual floating fern and is a very good arsenic indicator.</td>
<td>(Islam et al., 2015)</td>
</tr>
<tr>
<td>Ulothrix cylindricum</td>
<td>It is a green algae and has been used as a cost effective method of biosorption of As(III) from solutions.</td>
<td>(Favas et al., 2012)</td>
</tr>
<tr>
<td>Cladophora sp.</td>
<td>It is a filamentous algae. It can be used to remediate arsenic-bearing waste water and make it suitable for irrigation</td>
<td>(Jasrotia et al., 2014)</td>
</tr>
<tr>
<td>Lemna gibba L.</td>
<td>It is a free-floating aquatic angiosperm and an efficient accumulator of arsenic.</td>
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</tr>
<tr>
<td>Ceratophyllum demersum</td>
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</tr>
<tr>
<td>Myriophyllum propinquum</td>
<td>It is a submerged macrophyte with high arsenic accumulation potential. It is fast-growing shrub. It has high arsenic uptake ability even from water containing relatively low concentration of this element.</td>
<td>(Favas et al., 2012)</td>
</tr>
<tr>
<td>Lepidium sativum</td>
<td>It is a root less duckweed with higher uptake efficiency and tolerance competence.</td>
<td>(Rahman and Hasegawa, 2011)</td>
</tr>
<tr>
<td>Wolffia globosa</td>
<td></td>
<td>(Islam et al., 2015)</td>
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**Table 1**: List of aquatic plants involved in phytoremediation of arsenic.

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<tr>
<td>Hydrocharis sp.</td>
<td>It is a submerged macrophyte which acts as a bio-indicator of arsenic pollution. It is also a phytotransformer, hyperaccumulator with fast growth and high biomass.</td>
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Heavy metal pollution is also a very serious threat and is to be addressed in an eco-friendly manner.
GENETIC ENGINEERING FOR PHYTOREMEDIATION

Genetic engineering can also aid in phytoremediation by creating transgenic plants that have the ability to phytoremediate the metal. Higher tolerance is seen in the plants with higher AR activity. AR possesses the CDC25 – tyrosine phosphatase activity with a conserved HCX5R motif. The genes encoding AR are found in *Arabidopsis* (AtAsr / At ACR2), *Holcus* (HlAsr) and *Pteris* (PuACR2) (Tripathi et al., 2007). Transgenic tobacco is a genetically modified plant that had the AtACR2 gene of *Arabidopsis thaliana* expressed in them, and this plant was seen to survive in concentrations up to 200µM As, where the wild type cannot survive. Also, the arsenic accumulation in the above-ground biomass (edible part) was seen to be much lower than wild, preventing arsenic entry into the food chain even when grown in contaminated lands (Nahar et al., 2017).

Another strategy for enhancing metal remediation was by elevating the synthesis of chelators like GSH and PCs, as the overexpression of phytochelatin synthase (PCS) showed encouraging outcomes in plants and bacteria. ATP-binding cassette class-C (ABCC) transporters, involved in ATP- powered translocation of many substrates across the membranes are also involved in the active transport of PC-conjugated As(III) into vacuoles. The over-expression of these transporters of *Saccharomyces cerevisiae* origin enhanced arsenic tolerance and accumulation in *Arabidopsis thaliana* (Song et al., 2010). The PCS enzyme is seen to be active only during the stressed conditions; otherwise, they remain inactive (Grill et al., 1987; Vatamaniuk et al., 2000).

In certain edible plant cases, the alternate mechanism of preventing the accumulation of arsenic in the plant is also in practice. The potential of biochar-DOM (Dissolved organic matter) interactions is found to be crucial for the suppression of movement and bioaccumulation of arsenic which is brought about by amending the agricultural paddy soils with biochar (Li et al., 2018b).

FATE OF ARSENIC IN PLANTS

Uptake of arsenic from soil to plants

The process of transpiration serves as a driving force to absorb nutrients and other soil substances into the plant root and further to the shoots (Taylor et al., 2003). As (V) and P are chemical analogues and therefore As (V) enters the plant through the inorganic phosphate...
ARSENIC DETOXIFICATION MECHANISMS IN PLANTS

One of the protection mechanisms from the generation of ROS due to arsenic presence in plants is the production of antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), and ascorbate peroxidase (APX) to balance the free radicals. The production/accumulation of certain osmolytes like proline (Sayantan and Shardendu, 2017), glycinebetaine and mannitol are also seen in plants under oxidative stress as a means of protection and survival (Abbas et al., 2018). The major pathway of antioxidant defense to detoxify $H_2O_2$ is the Ascorbate-Glutathione pathway (AsA- GSH). Along with AsA and GSH, four enzymes viz. ascorbate peroxidase, monodehydroascorbate reductase, dehydroascorbate reductase, and glutathione reductase, play a vital role in detoxifying ROS in this pathway and plays a vital role in protecting the plant from various abiotic stresses as well (Hasanuzzaman et al., 2019).

Another mechanism includes the complexation of arsenic with ligands followed by the vascular compartmentation. Once arsenic enters the plant, As(V) would be reduced to As(III) form, with the help of enzyme arsenate reductase (AR) (Zhao et al., 2003). Arsenite is highly disruptive to the metabolomic process in the cytoplasm and hence detoxification occurs. This is observed in many plants like *H. verticillata* (with >94% in As(III) form in shoots), *Brassica juncea* (with 96 -100% in roots and shoots), tomato and rice (about 92-99%) (Chen et al., 2015). Methyl arsenite [MA(V)] also reduces to MA(III) in rice shoots (Mishra et al., 2017). Inorganic arsenic and MA form complexes with metal-binding proteins like glutathione (GSH) or phytochelatins (PCs), which are then sequestrated into the vacuoles (Mishra et al., 2017; Pickering et al., 2000). GSH is a precursor of phytochelatins (PCs) (Thakur et al., 2003).

Table 2. List of hyperaccumulator plants involved in phytoremediation of arsenic.

<table>
<thead>
<tr>
<th>PLANTS</th>
<th>CHARACTERISTICS</th>
<th>SOURCE</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pteris vittata L.</em></td>
<td>It is a fern with high translocation factor that can grow well on highly arsenic contaminated sites. It also has the potential to phytoremEDIATE multiple toxic chemicals.</td>
<td>(Taylor et al., 2003)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(Chen et al., 2015)</td>
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<tr>
<td></td>
<td></td>
<td>(Raj and Singh, 2015)</td>
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<td></td>
<td></td>
<td>(Visootiviseth et al., 2002)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(Kochian, 2004)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(Xie et al., 2009)</td>
</tr>
<tr>
<td><em>Pteris cretica</em></td>
<td>It is a fern with greater arsenate influx in roots.</td>
<td>(Raj and Singh, 2015)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(Poynton et al., 2004)</td>
</tr>
<tr>
<td><em>Pityrogramma calomelanos</em></td>
<td>It is a fern that accumulates arsenic mostly in the fronds with ability to grow in highly contaminated areas. It can take up arsenic even from less contaminated areas. However, it is seen to be used as food in countries like Thailand.</td>
<td>(Luongo and Ma, 2005)</td>
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<tr>
<td></td>
<td></td>
<td>(Luongo and Ma, 2005)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(Francesconi et al., 2002)</td>
</tr>
<tr>
<td><em>Pteris umbrosa</em></td>
<td>It is a fern and are efficient root uptakers. They are fast growing plant and are also aesthetically pleasing.</td>
<td>(Luongo and Ma, 2005)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(Koller et al., 2007)</td>
</tr>
<tr>
<td>*Adiantum capillus ven-</td>
<td>It is a fern with a strong arsenic resistance.</td>
<td>(Raj and Singh, 2015)</td>
</tr>
<tr>
<td>eris*</td>
<td></td>
<td>(Singh et al., 2010)</td>
</tr>
<tr>
<td><em>Brassica juncea</em></td>
<td>It is a herbaceous plant. It is a highly resistant plant with ability to grow on soils of different nature.</td>
<td>(Pickering et al., 2000)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(Vocciante et al., 2019)</td>
</tr>
</tbody>
</table>
Phytochelatins are cysteine-rich, low molecular weight peptides. The exposure of arsenate induces the synthesis of PCs in several plant species (Mirza et al., 2014). This is the general strategy of detoxification employed by all plants irrespective of being hyperaccumulators or hypertolerants or non-hyperaccumulators (Chen et al., 2015). In hyperaccumulators, thiols have limited role and most a rsenic is stored as As (III) (Mishra et al., 2017). In leguminous plants, homo-PCs (hPCs) are synthesized along with PCs. Increased synthesis of PCs is observed in tolerant plants like H. latus (Tripathi et al., 2007).

DISPOSAL OF PLANTS AFTER REMEDIATION

The objective of phytoremediation will not be met if the plants used are not properly disposed of or handled after removing metals from the environment due to their storage in the biomass of the plants (Ghosh and Singh, 2005). The reutilization of end products of phyto remediation makes the process even more eco-friendly.

One option for the safe disposal of heavy metal-laden remediator plants would be composting. Composting will aid in the reduction of the volume of the biomass and also help in easy transport (Mohanty, 2016; Newete Byrne, 2016). The major drawback would be to transfer the hazardous substance from one place to another (Ghosh and Singh, 2005). Although techniques like stabilization/inertization by the application of lime can reduce the leachability of the metals (Voccia et al., 2019), the plants have to dispose of not just anywhere but in specialized areas like tail mining. The disposal on the slopes of the tailing dam can act as mulch to put down the dust and would also give an opportunity for heavy metals to go back to where they belong after the process of decomposition and can reinstate the soil fertility for revegetation(Ghosh and Singh, 2005). Another method commonly used is incineration and the produced charcoal can be used as an energy source for cooking fires(Ghosh Singh, 2005). This method would obviously make sure that the biomass cannot be used for any other purposes like fertilizers or animal feed. But this could be a source of air pollution. Hence, its recommended that incineration is not performed in the open.

The next best alternative is pyrolysis – heating the biomass at temperatures typically between 350 and 650 °C in anaerobic conditions (Voccia et al., 2019). The end products of pyrolysis are pyrolytic fluid oil and coke (Newete Byrne, 2016). The coke contains heavy metals which can be used in the smelter. The arsenate is also seen to reside in the pyrolytic residue when CCA treated wood is pyrolyzed at low temperatures, hence a better alternative (Helsen et al., 1997). Also, the gases like methanol or other liquids can be produced through the process of bio gasification, which can be utilized as a source of fuel (Mohanty, 2016). It is also seen that the biochar produced can be used to adsorb dye like methylene blue (Gong et al., 2018).

Conclusion

Arsenic, a potent carcinogen is seen to show its toxicity in both plants and animals. It is seen to impede the body’s metabolism and hormone regulation. The increased anthropological activities leading to arsenic contamination in both soil and water systems is an issue in many countries and is to be properly addressed. Employing conventional methods like coagulation, lime softening, and adsorption may be effective to a certain level but might cause additional problems like large volumes of sludge generation, soil texture destruction, and being non-environmentally friendly. The greener alternative is phytoremediation. Proper identification and use of the arsenic hyperaccumulators and metal-tolerant plants like Pteris vittata, Hydrilla verticilata, Micranthemum umbrosum will aid in the management of the arsenic problem naturally. In addition, genetically modified plants like transgenic tobacco, transgenic Ar¬bidopsis thaliana can be utilised. However, the whole process would be to no purpose if the proper disposal methods are not followed. Apart from the scientific advancement, it is also crucial to increase awareness in the society regarding the measures that can be taken up, the type of plants that can be grown to control the arsenic pollution and the necessary precautions to be taken to prevent the pollution.

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