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Review Article

Bioremediation of arsenic contamination from the environment: New approach to sustainable resource management

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Abstract
Present acceleration of Arsenic [As] exposure leads to severe health problems. Modern scientific approaches look towards potent bio-agents for the removal of such types of contaminations in sustainable ways. Microbes can potentially change the redox potential, solubility, pH by different complex reactions during bioremediation. There are many enzymes present in the microbial system which are involved in methylation such as As (V) reductase, monomethyl arsonic acid reductase, As (III) methyltransferase, and MMA (III) methyltransferase. On the other hand, microbes have As transformation ability and changed into different extractable forms with sulfide minerals such as arsenopyrite (FeAsS), enargite (Cu_3AsS_4) and realgar (As_4S_4). In some bacteria, the As-operon machinery thiol group bind with As, itdetoxifies its toxicity. *Ars R* gene and arsenic reductase enzyme (Ars C) play the key role in the reduction of As (V) to As (III) and detoxify by being transported outside of the cell by Ars AB As chemiosmotic efflux system. In fungi, As (V) is reduced to As (III) by the arsenate reductase and GSH glutathione converted into GSSH glutathione disulfide. In plants, As (III) conjugates with phytochelatin (PC) or GSH glutathione and accumulates in the vacuole or is converted into less toxic forms in the presence of arsenic reductase enzyme. This review focused on the potentiality and mechanisms of different microbes for As-detoxification in a sustainable manner.

Keywords: Algae, Arsenic [As], AM fungi, Bacteria, Fungi, Hyperaccumulator, Protozoa

INTRODUCTION

Arsenic [As] is a semi-metallic compound with atomic number 33 in the periodic table. The discovery of As might be in the early Bronze Age (2500 BC). Albertus Mannus (1250) was the first to isolate in the form of arsenic sulfide. As is found on earth in solid grey, yellow and black colours with brittle and relatively low Mohs-hardness. There are four oxidation states *viz.*, -3, 0, +3, and +5 of As in which As (0) is elemental form and As (+3) and As (+5) mainly existing forms in the environment. Out of four oxidation states, As (V) is the most stable form (Sharma and Sohn, 2009; Zhao *et al*., 2010; Gupta *et al*., 2011). As exists in many different organic forms such as mono-methyl arsenic acid [MMA], dimethyl arsinic acid [DMA], tri-methyl arsineoxide [TMAO], arsenobetaine [AsB], arseno-choline (AsC), arsenosugars (AsS), arsenolipids etc. (Tangahu *et al*., 2011). Among them, dimethyl arsinous acid and monomethyl arsonous acid are more toxic, whereas As

(III) is usually more toxic than As (V) in inorganic forms (Mass *et al*., 2001; Abedin *et al*., 2002a and b). As (III) can oxidize cysteine residues thiols (-SH) group plays an important role in the citric acid cycle. It can inhibit succinate dehydrogenase activity, ATP production in mitochondria and reduce NAD^+ during oxidative phosphorylation in plants (Mazumder, 2005). As-toxicity may lead to cancer and its exposer to man kind cause different health problems. IARC (International Agency for Research on Cancer) accepted it as a group first carcinogenic compound. WHO set the standard of As quantity in drinking water that is 0.01 mg/l whereas Food and Agriculture Organization (FAO) declared permissible limit for As limit 0.10 mg/l in irrigated water. WHO advisory conference of As scheduled to consider 200-300 ppb for rice. Under normal conditions, As-concentration in land plants are usually less than 10000 ppb (Matschullat, 2000).

As-contamination comes in the environment by natural as well as man-made sources. Shallow or main aquifers are natural sources and come into the water by rock weathering. However, the present deep water irrigation and other industrial effluents, mining, pesticides, wood preservatives, etc., are other anthropogenic sources. As contamination occurs globally *viz*., Argentina, Bangladesh, Chile, China, France, Germany, India, USSR, Peru, Namibia, Mexico, Sweden, and USA (Nelson 1977; Mandal and Suzuki, 2002; Shaji *et al*., 2021) are affected. In South-East Asia, Ganga-Meghna-Brahmaputra (GMB) plains are highly contaminated. The presence of arsenic in groundwater is associated with health issues (Zhang *et al*., 2020; Medunić *et al*., 2020). Weak health and poor nutritional condition of the body may accelerate its severity. Exposure to high arsenic water causes ulceration, hyperkeratoses, pigmentation, skin cancer and also affects the kidney, liver, lungs and heart (Sun *et al*., 2019). In recent years, As contamination has become a serious concern in view of its toxicity to humans being (Shaji *et al*., 2021). There are various techniques to reduce arsenic contamination in water (Mendoza-Chávez *et al*., 2020; Nguyen *et al*., 2020). However, microbes and hyperaccumulator plants play a key role. They transform the As by different activities such as biosorption, bioaccumulation, microbes mediated oxidation-reduction, methylation-demethylation, biostimulation, biovolatilization, bioleaching, biomineralization, biofilm formation. The process of phytoremediation of As can be done through the process of phytostabilization, phytoextraction, and phytovolatilization. This review discusses the various microbes mechanism and ability to remediate As toxicity in a sustainable manner.

Sources of arsenic contamination in the environment

As-contamination has also been reported from Pre-Cenozoic provinces (Peters *et al*., 1999). As concentrations in soil, rivers, lakes, estuaries groundwater and marine in different part of the world. Zuzolo *et al*. (2020) reported groundwater contamination of As from central parts of Italy. According to Medunić *et al*., (2020) Botswana, Burkina Faso, Ethiopia, Ghana, Morocco, Nigeria, South Africa, Tanzania, Togo, and Zimbabwe are worst affected African countries.

Further, based on some geomorphic and geologic resemblances, As-contaminated regions are subdivided (Mukherjee *et al*., 2019). For instance, in Asia, most of the affected areas are the Ganges–Brahmaputra in Meghna-plain and delta in India, Bangladesh (Bhattacharya *et al*., 2007; Das, 2019), Pakistan Indus plain (Rabbani *et al*. 2017; Zubair *et al*., 2018; Ali *et al*. 2019), Myanmar Irrawaddy delta (WRUD, 2001), Sri Lanka (Chandrajith *et al*., 2020), Vietnam (Stopelli *et al*., 2020), Cambodia and Laos Mekong river delta (Feldman *et al*., 2007). Likewise, some arid and semi-arid climatic regions are also reported to be contaminated with a critical level of As. For instance, Argentina (Bhattacharya *et al*., 2006), the Atacama Desert area of northern Chile (Borgono and Greiber, 1971), and Nevada of the USA (Fontaine, 1994). From the marine sediments, As-contaminated aquifers are reported from Australia,*i.e.,* southeastern parts (Smith *et al*., 2008) and Taiwan (Tseng *et al*., 2000). However, As-enriched sulfide mineralization is also a major source of As-contamination and some of the examples of As-contaminated area are Ghana (Smedley, 1996), northern Chile (Oyarzun *et al*., 2004). Nigeria (Gbadebo, 2005), Central Balkan Peninsula in Siberia (Dangic' and Dangic', 2007); Albania (Lazo *et al*., 2007) and Appalachian belts of northeast USA (Peters, 2008). As contamination in different systems is widespread, it is severe enough to exceed guideline values in many areas. Stroud *et al*. (2011) reported that As has affected more than 150 million people worldwide with its increasingly elevated concentrations in drinking water. The major As affected regions are presently found in large deltas and along major rivers emerging from the Himalayas, with the Bengal delta being the worst affected area where >88% of the 45 million inhabitants are at high risk of exposure to As concentrations >50 lg/l (Acharyya and Shah, 2007; Ravenscroft *et al*., 2011; Uddin and Huda, 2011).

The sources of As-contamination in the environment include both natural and anthropogenic. Natural sources of As-contamination are by desorption and dissolution of naturally occurring As-bearing compounds adsorbed onto pyrite ores into the water by geochemical factors and over-exploitation of shallow (or main) aquifers. About 90% of the world production of As was contributed by the countries like China, France, Germany, USSR, Peru, Namibia, Mexico, Sweden, and the USA (Nelson 1977; Mandal and Suzuki, 2002). Anthropogenic sources include, i.e., by use of insecticides, pesticides, herbicides and the use of phosphatic fertilizers, mining, smelting, burning of fossil fuel, timber and feed additives, etc. (Bundschuh et al., 2011; Singh et al., 2015). However, anthropogenic sources exceed by the ratio of 3:1 (Anthropogenic: Natural) in the environment (Woolson, 1983). Some of the examples of important sources of As-contamination are discussed in detail below.

Groundwater

Over-exploitation of groundwater has been a major source of As problems all over the world. Mexico, USA, China, Bangladesh, Vietnam, and Pakistan were found to be contaminated with As. Very high concentrations of As in groundwater was reported globally (Yoshizuka *et al*., 2010; Ahn, 2012; Liu *et al*., 2019; Le Luu, 2019; Shaji *et al*., 2021).

Minerals

As occurs as a major constituent in more than 200 minerals and common As- minerals are arsenides, sulfides, oxides, arsenates, and arsenites.. These As-minerals are moderately rare in the natural environment and are usually found in close association with transition metals such as Au, Cu, Pb, Zn, Sn, Ni, and Co in ore zones. Some abundant As-minerals found in the environment are arsenopyrite (FeAsS), realgar (AsS) and orpiment (As_2S_3) and it is believed that under the temperature of earth's crust, the formation of arsenopyrite, along with the other abundant mineral such as As-sulphide minerals realgar and orpiment take place (Smedley and Kinniburgh, 1996). It has been reported that China has a large number of As-reserves and it is to be 3977 kt, and 2796 kt preserved reserves, of which 87.1% existed in paragenetic or associated ores up to the end of 2003 (Xiao *et al*., 2008).

Wood preservatives and desiccants

In many parts of the world, As such as arsenic acid is widely used as a cotton desiccant. In 1981, Fluor–Chrome–Arsenic–Phenol (FCAP), the first wood preservative, was used in the USA. Later on, in 1964, 2500 tons of arsenic acid (H_3AsO_4) was used as desiccants on 495,000 ha of US cotton (Fordyce *et al*., 1995). However, in earlier times, 99% of the wood preservatives were prepared from Chromated Copper Arsenate (CCA) and Ammoniacal Copper Arsenate (ACA) (Rahman *et al*., 2004).

Pesticides and herbicides

As is being extensively used in insect and pest control programs,*i.e.,* for the preparation of an insecticide and pesticides. Many As-compounds are used for the production of cotton as pesticides, such as lead arsenate, copper acetoarsenite, Paris-Green (copper acetoarsenite), calcium arsenate, arsenic acid, monosodium methanearsonate, disodium methanearsonate, and cacodylic acid (National Research Council (US), Environmental Protection Agency, (1980). According to this Counicl, inorganic arsenicals, first and foremost, sodium arsenite, were extensively used in 1890 as weedicide, chiefly for sterilization of non-selective soil. Ure and Berrow (1982) reported very high As concentrations (366–732 mg/kg) in orchard soils due to the historical application of arsenical pesticides to fruit crops.

Feed additives

According to the European Commission's Feed additives are products used in animal nutrition to improve the quality of feed and the quality of food from animal origin or improve the animals' performance and health. Several compounds which are rich in As are used for feed additives and some of the examples are arsenic acid, 3-nitro-4-hydroxy phenylarsonic acid, 4-nitrophenylarsonic acid, etc. (Mandal and Suzuki, 2002).

Drugs

The medicinal value of As has been acclaimed for nearly 2500 years. The period between the seventeenth and nineteenth centuries resulted in the development of new As-based drugs, which were applied for the treatment of skin diseases and acute promyelocytic leukemia. arsenic trioxide (ATO, Trisenox) is an important antileukemic drug and malignancy (Chen *et al*., 1996; Shen *et al*., 1997;). There are some medicine, which include As in their medicinal preparations, for instance, potassium arsenite used in Fowler's solution, As and mercuric iodides in Donovan's solution, arsenic trioxide and black pepper in the preparation of Asiatic pills, liquor arsenii chloridi in de Valagin's solution, neoarsphenamine, oxophenarsine hydrochloride (Mapharsen), arsthinol (Balarsen), acetarsone, tryparsamide and carbarsone (Vallee, *et al*., 1960; Bates *et al*., 1992; Mandal and Suzuki, 2002).

Mitigation strategies for arsenic contamination

As is extremely harmful, keeping in view of its toxicity, [As] is categorized as a group first carcinogenic element. The extant exposure of a large population towards As-toxicity is related to the drinking of As contaminated water and agriculture on contaminated soils. So, the reclamation programs are intended to first reduce its toxicity from contaminated water and soils. Modern technologies are being utilized for this purpose (Srivastava *et al*., 2012; Irshad *et al*., 2021). Various physical and chemical techniques are available and applied according to their need; however, their effectiveness is in doubt.

Some physical technologies utilize sulfuric acid, nitric acid, phosphoric acid, and hydrogen bromide As-detoxification. While other physical technologies utilize pre-landfilling with cow dung mixture for stabilization and reduction of As (Sullivan *et al*., 2010). Some selective pore-based separation of molecule membrane technologies *viz.,* microfiltration (MF), ultrafiltration (UF) and nano-filtration (NF), etc., are efficient in the removal of As from water contamination (Figoli *et al*., 2010). On a very small scale, osmosis membrane (reverse osmosis (RO) or forward osmosis (FO) is being utilized in treatment plants for treating As containing industrial wastes (Cath *et al*., 2006). Many chemical compounds that are reactive or interact with As are utilized for the As-precipitation as a byproduct. Many oxidant compounds such as chlorine, chlorine dioxide, hydrogen peroxide, chloramine, permanganate, ferrate, and ozone, etc., can precipitate arsenic as in less toxic forms (Lee *et al*., 2003; Vasudevan *et al*., 2006; Sharma, 2007; Mondal *et al*., 2013). Some coagulant agents like alum, ferric oxide, sulfate, etc. help in As removal through the process of coagulation-flocculation (Singh *et al*., 2015).

The physical and chemical methods described above are practiced on small level remediation because it is not feasible to remediate As from large water bodies or large agricultural fields. Moreover, these techniques are very costly and hard to apply in remote areas. So emerged the concept of bioremediation, where efficient flora and fauna for in situ remediations is used. Soil microbes and plants are utilized for remediation of As-contaminated soils. Many biochemical processes are reported for As-remediation by these microbes, e.g. bioaccumulation, biosorption, oxidation-reduction, methylation-demethylation, bioleaching, biomineralization, biovolatilization, and biofilm formation, etc. As-hyperaccumulator plants also utilize some mechanisms for the removal of As like phytostabilization, phytoaccumulation, phytovolatilization process. *Pteris vittata* has been reported as one of the best hyperaccumulor plant (22,630 mg/kg) (Ma *et al*., 2001).

Arsenic *vs.* microbial remediation

With greater public awareness of As-poisoning in animal and human nutrition, there has been growing interest in developing guidelines and remediation technologies for mitigating As contamination in ecosystems. A range of technologies, including chemical immobilization and bioremediation, has been applied with varying levels of success, either to completely remove As from the system or to reduce its bio-toxicity. Bioremediation with special reference to the microbial approach is an emerging technology that uses microbes to remove or stabilize contaminants. It may offer a low-cost and ecologically viable means for the mitigation of heavy metals toxicity in the environment.

Soil microbes thus play a crucial role in environmental As detoxification by biotransformation of inorganic arsenicals into organic forms. Some microbes showed the potentiality of As remediation (Table 1). Soil microbes generally belong to the monera and fungi group, in which many indigenous microbes perform well in the safe removal of As into less hazardous forms. Microor-

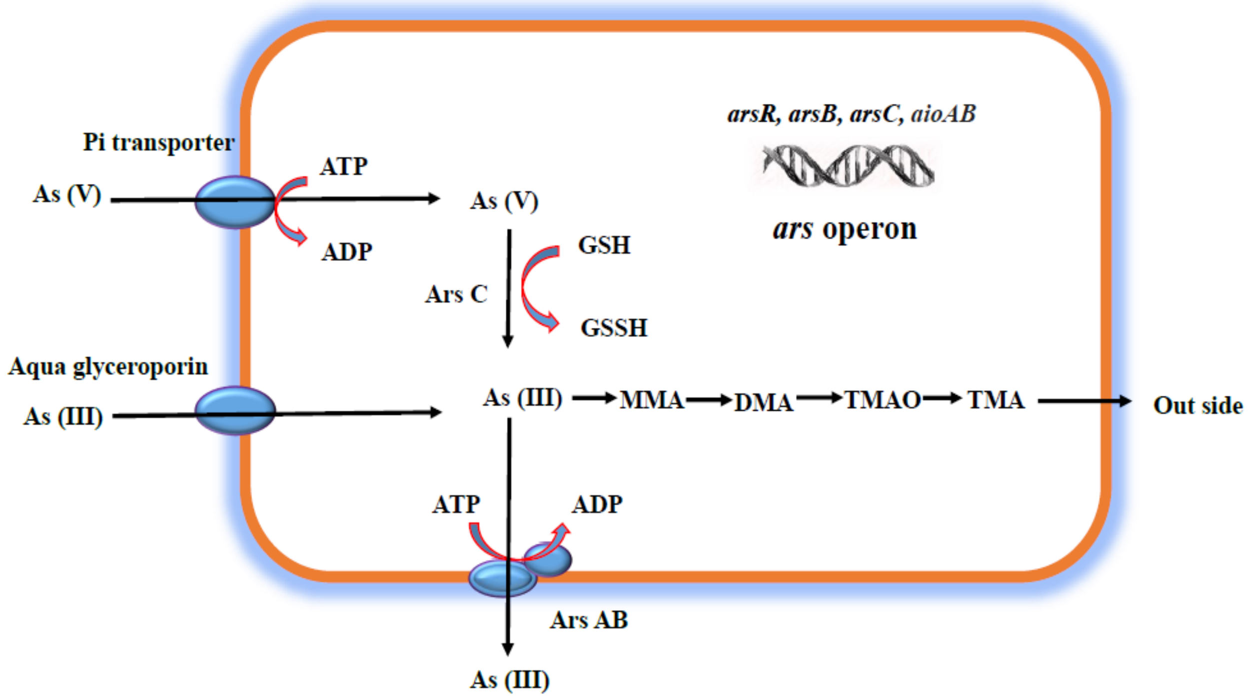

Fig. 1. *Mechanism of As-tolerant bacterium for As-detoxification*

Table 1. List of some important microbes showing the potentiality of As remediation

Microbes/Bio-agents	Remarks/Conclusions	References
Acidithiobacillus thiooxidans	Leaching efficiency tended to decrease with increasing temperature due to the decrease in the bacterial growth rate at higher temperature	Lee *et al*., 2015
Acidithiobacillus thiooxidans and *A. ferrooxidans*	The results showed that mixed cultures were more efficient than each bacterium for Cu and As, while *A. ferrooxidans* demonstrated faster extraction efficiency for Mn and Zn.	Diao *et al*., 2015
Fusarium oxysporum CZ-8F1, *Penicillium janthinellum* SM-12F4, and *Trichoderma asperellum* SM-12F1	All three fungus found capable in As- volatilization, and demethylation.	Feng *et al*., 2015
Acidithiobacillus ferrooxidans and *A. thiooxidans*	The maximum As leaching ratio obtained from realgar in the presence of mixed adopted cultures was 28.6 %.	Leng *et al*., 2016
Acidithiobacillus ferrooxidans BYQ-12	The maximum As bioleaching rate was 73.97% under optimum conditions, and the most effective factor for As leaching was initial ferrous ion concentration.	Yan *et al*., 2017
Bacillus flexus strain As-12	As-tolerant and efficient remediate As contaminations *in vitro* conditions.	Jebeli *et al*., 2017
Nostoc sp. PCC 7120	Workers proposed the biosynthesis pathway of arsenosugar phospholipids production in *Nostoc* sp. PCC 7120.	Xue *et al*., 2017.
Dunaliella salina	The absorption of As (III) or As (V) significantly reduced by an increased phosphate supply.	Wang *et al*., 2017
Rhizoglomus intraradices and *Glomus etunicatum*	AM fungi inoculated plants reduced oxidative stress significantly and maintained favorable P: As ratio.	Sharma *et al*., 2017
Microvirga sp.S-MI1b	oxidize arsenite at broad pH ranges from 4.0 to 9.0	Tapase, and Kodam, 2018
Kocuria flava AB402 and *Bacillus vietnamensis* AB403	As-resistant halophilic bacterial strains	Mallick *et al*., 2018
Ancylobacter sp. TS-1	As-oxidizing bacterium	Anguita *et al*., 2018
Trichoderma sp. MG	This fungus can tolerate high concentrations of As (500 mg/L) and Pb (650 mg/L) and can help in the bioremediation of metal contaminated soils.	Govarthanan *et al*., 2019
Aspergillus flavus	*Aspergillus flavus* capacity to reduce the mobilization of As in rice and converted As into less toxic forms.	Mohd *et al*., 2019
Bacillus sp. XZM	*Bacillus* sp. XZM found able to reduce As toxicity of *Vallisneria denseserrulata*.	Irshad *et al*., 2020
Bacillus firmus L-148	*Bacillus firmus* L-148 exhibited a hyper-tolerant and fast As (III) oxidizing nature and this study also confirm the presence of ars and *aio* operon in cells.	Bagade *et al*., 2020
Bacillus subtilis S4	*Bacillus subtilis* S4 with iron oxide nanoparticles (IONPs) reduced arsenic (As) stress and improve the growth in seedlings of *Cucurbita moschata*.	Mushtaq *et al*., 2020

Contd……..

Table. 1. Contd…..

Fomitopsis betulina	Different As species such as As (III), As (V), DMA, TMAO, etc. detected in dried samples of fungus growing on contaminated sites.	Button *et al*., 2020
Aspergillus niger strain CBS 140837	This fungus affects the manganese speciation along with bioaccumulation of potentially toxic metals such as As that associated with manganese oxides.	Farkas *et al*., 2020
AM fungi	Inoculation of AMF in *Pteris vittata* improved the As remediation with up to the depth 0–0.2 m significantly.	Cantamessa *et al*., 2020
AM fungi	As concentration reduced significantly in grains by the inoculation of AM fungi in *Pisum sativum.*	Alam *et al*., 2020
Leptolyngbya boryana	*Leptolyngbya boryana* showed an ability of accumulation, biotransformation of As.	Zhu *et al*., 2020
Bacillus flexus strain SSAI1	*Bacillus flexus* strain SSAI1 confirms to have *glpF, aioS, aioE*, and *aioE* genes seem to be a potential tool for arsenite bioremediation.	Mujawar *et al*., 2021
Aspergillus niger strain	*Aspergillus niger* strain exhibit a significant heavy metal bioextraction capacity from the natural ochres.	Urík *et al*., 2021
Rhizophagus intraradices	*Rhizophagus intraradices* reduced the toxic effect of As in *Triticum aestivum* by the promotion of plant growth and As-mediated impairments in plant physiology.	Gupta *et al*., 2021
Chlorella sp.	Rice seedlings co-culturing with the *Chlorella* sp. reduced the As-toxicity through the enhanced expression of GRX, GST, SAMT genes in plants.	Ranjan *et al*., 2021

ganisms have developed various strategies to counteract As toxicity: like active extrusion of As; intracellular chelation (in eukaryotes) by various metal-binding peptides including glutathione (GSH), phytochelatins (PCs), and metallothioneins (MTs), and As transformation to various organic forms which could be potentially less toxic.

Bacteria as As-remediator

The prokaryotic cell membrane has hydroxyl, amino, and amide groups, which help in the As sorption (Mukhopadhyay *et al*., 2002). The mechanism of As-tolerant bacterium for As-detoxification has been represented in Fig. 1. Inside the cell, there is As-operon machinery associated with genes and arsenic reductase enzyme (Ars C) and chemiosmotic efflux system that play the key role in the reduction of As (Macur *et al*., 2001). Many enzymes were discovered in methane-producing bacteria, including As (V) reductase, monomethylarsonic acid reductase, As (III) methyltransferase, and monomethylarsonous acid methyltransferase, were revealed to be involved in As methylation, according to Wu*et al*. (2005). Green (1918) was the first to report As-oxidation by using the bacteria *Alcaligenes faecalis*, *Corynebacterium glutamicum* term as model As-resistance bacteria in industrial wastewater (Villadangos *et al*., 2011). Sher *et al*. (2021) utilized *Bacillus licheniformis* for mitigating the As toxic effect from contaminated industrial wastewater. Model As-resistant bacteria, *Corynebacterium glutamicum* tolerant up to 400 µM arsenite and another bacteria *Pseudomonas aeruginosa* PAR 3 show the highest level growth at 200 mg/l of polluted wastewater (Anyanwu and Ugwu, 2010). *Microbacterium lacticum* identified as tolerant can tolerate up to 3000 mg/l arsenites (Pal and Paknikar, 2012). Methanogen bacteria, *Methanobacterium bryantii* MOH strain utilized for As biovolatilization for industrial effluent (Stolz *et al*., 2006).

Enzymatic machinery inside the bacterial cell evolved for energy utilization as well as for the protection of cell. Many enzymes and transporter proteins such as phosphates (Pi) transporter and aquaglyceroporins provide passage to enter inside the cell for As (V) and As (III) respectively whereas, Ars A combined with B (*Ars AB*) make efflux pump which throws As (III) to the environment with ATP driven system (Tripathi *et al*., 2007). These types of machinery are operated by *ars* operon that consists of three genes *ars R, ars B, ars C* which encode trans-acting repressor, arsenite permease pump, arsenate reductase in cell respectively (Tripathi *et al*., 2007). The arsenate reductase (*Ars C*) converts As (V) to As (III) by using glutathione (GSH) as a reducing agent. Lin *et al*., (2007) identified two additional genes *ars A* and *ars D,* in some gram-negative bacteria. Inside the bacterial cell, As (III) converted into different organomethenic forms *viz*., MMA, DMA, TMAO, and TMA and after they finally export to the outer environment. The addition of few other genes were also identified *viz., acr 3, ars R, ars B* and *ars C* in *Lysinibacillus sphaericus* B1-CDA strain (Rahman *et al*., 2015). Yang and Rosen (2016) recognized another distinguish parallel pathway of As metabolism in the bacterial cell, including *ars M, ars I* and *ars H* genes for encodes As (III) S-adenosylmethionine methyltransferase, C-As bond lyase, methyl arsenate oxidase, respectively. ArsAB chemiosmotic As-efflux mechanism flung As outside the cell and As (V) reductase (Ars C) of small molecular mass (13 to 16 kD), one of the principal reductive enzymes present in microbial cytoplasm (Silver and Phung, 2005; Macur *et al*., 2001). Some chemolithoautotrophic microbes utilized [As] as an energy source and an electron donor in process of As (III) oxidation for the reduction and cell growth maintenance (Wang and Zhao, 2009). Some methanogen bacteria demethylated As under anaerobic conditions but it is not stable and rapidly oxidized when coming in contact with oxygen (Kuehnelt and Goessler, 2003).

Under natural conditions, microbial As-demethylation occurs both aerobically as well as anaerobically (Huang *et al*., 2007). Generally, As-demethylation is not good for remediation (Sierra-Alvarez *et al*., 2006). Transformation ability of microbes to convert As in different extractable forms in the process of bioleaching (*et al*., 2006). Some inorganic elements act as a stimulator to increase microbial As remediation. Márquez *et al*. (2012) reported role of oxidation of ferrous to ferric ions of sulfide that helps in As extraction. *Thiobacillus caldus Thiobacillus ferrooxidans* and *Leptospirillum ferrooxidans* etc. were investigated and have the potency to extract As under optimal laboratory conditions. Elemental sulfur and carbon source accelerated the rate of mitigation of As and helped in their bioleaching from soils or other substrates (Bayard *et al*., 2006; Mc Lean *et al*., 2006 Chen *et al*., (2017) reported bioaugmentation of rice straw (5%) with *Pseudomonas putida* KT2440 increased As volatilization up to 483.2 µg/kg/year. Mallick *et al*. (2018) investigated As-resistant halophile bacterial strains *Kocuria flava* AB402, *Bacillus vietnamensis* AB403 from Sunderland mangrove and reported strain AB402 and AB403 tolerated 35 mM and 20 mM of arsenite, respectively *in vitro* conditions. In nature, the mineralization process is involved in the hardening or stiffening of As. Iron, manganese, and sulfide, etc., help in As precipitation in the calcium-rich environment in the form of calcium arsenate [Ca_5H_2 $(AsO_4)_4 \cdot cH_2O$] (Martínez-Villegas *et al*., 2013). Soil pH and indigenous microbial population affect the rate and mineralogical composition of the arsenic-sulfide minerals in groundwater (Rodriquez-Freire *et al*., 2016). *Pseudomonas putida* KT2440 exhibited a high capacity of As-volatilization when applied with rice straw raised arsine fluxes in the reclamation of As-contaminated soils (Chen *et al*., 2013, 2014, 2017). Mujawar *et al*. (2021) identified the role of *aioAB* gene expression association with arsenite oxidase enzyme in *Bacillus flexus* strain SSAI1.

Fungi as As-remediator

There are various mechanisms by which fungi tolerate and detoxify metals such as extracellular/intracellular precipitation, complexation, biomethylation, bio-

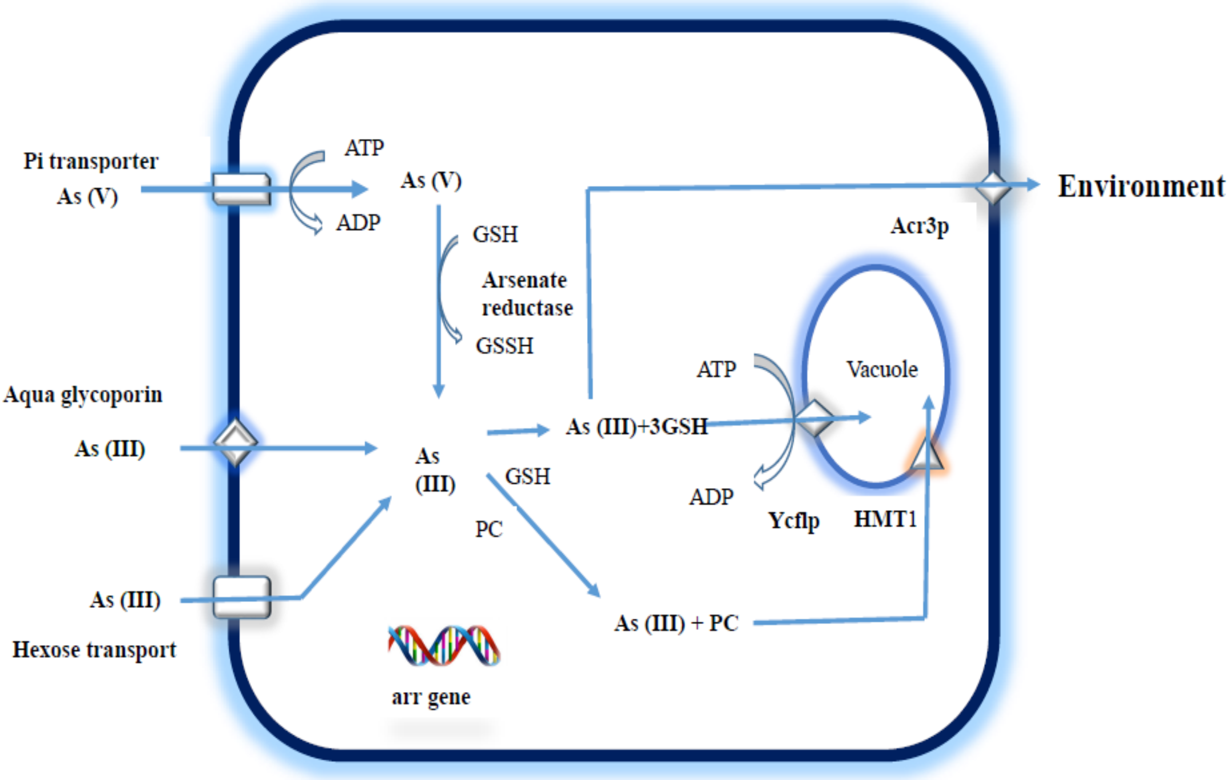

Fig. 2. *Mechanism of arsenic tolerant filamentous fungi for As-detoxification*

accumulation, biovolatilization, biosorption, impermeability, active uptake and sequestration. Some of the mechanism arsenic tolerant filamentous fungi for As-detoxification is represented in Fig. 2. Aqueous arsenite and arsenate from wastewater converted into dimethyl arsine and monomethyl arsine by the application of *Penicillium brevicaule*, *Scopulariopsis* sp., *Aspergillus glaucus*, *Candida humicola*, and *Gliocladium* sp. Under aerobic conditions, As (V) enter the fungal cell via the phosphate transporter, whereas aquaporins and hexose provide a passage for As (III) species to enter the cell (Persson *et al*., 1999; Liu *et al*., 2004). In the presence of arsenic reductase, glutathione (GSH) was oxidised into glutathione disulfide along with As (V) was reduced to As (III). With the help of transporter proteins HMT1 and YcFIP, conjugated As (III) (with phytochelatin (PC) or GSH glutathione) accumulate in vacuoles (Tsai *et al*., 2009). The efflux machinery can also expel the conjugated form As (III)-3GSH via the Acr3p protein.

Many fungi, such as *Penicillium chrysogenum*, *Penicillium purpurogenum*, and *Aspergillus niger*, can absorb As from polluted substrate (Loukidou *et al*., 2003; Pokhrel and Viraraghavan, 2006). Tani *et al*. (2004) reported that the fungus KR21-2 might accumulate a lot of arsenate during manganese oxide production in the lab. Different fungus species have been found to have both external and intracellular methylation. This As biomethylation converted inorganic to different organic forms, MMA, DMA or TMAO, MMA (III), DMA (III) or gaseous arsines, etc. (Oremland and Stolz, 2003; Jia *et al*., 2013). Su *et al*. (2012) reported some fungi reported for their extracellular methylation, such as *Apiotrichum humicola*, *Scopulariopsis brevicaulis, Trichoderma asperellum, Penicillium janthinellum* and *Fusarium oxysporum* intracellular methylation. During the process biomethylation, metal-resistant fungi generated volatile As-species (biovolatilization). Some fungi were reported to generate arsine such as *Penicillium* sp., *Ulpcladium* sp., *Neosartorya fischeri*, *Aspergillus clavatus,* etc. (Visoottiviseth and Panviroj, 2001; Edvantoro *et al*., 2004; Čerňanský *et al*., 2009).

Waghunde *et al*. (2016), observed that *Trichoderma* sp. belonging to Ascomycota found more important plant growth-promoting microbes helping in the enhancement of soil fertility and stress tolerance activity. *Trichoderma* was also capable of increasing plant growth hormone production and root system for uptake of nutrients from the soil. These microbes have a variety of functional groups on the cell surface that allow them to bond with metals. According to Tripathi *et al*. (2017). *Trichoderma* sp. potentially improved the chickpea plant under As (V) contamination. Tripathi *et al*. (2013) added that these fungal microbes were capable of restoring growth deformities as well as induced the nodule formation, chlorophyll content, and proline content in chickpea plants under the high level of As contamination. Caporale *et al*. (2014), described that *T. harzianum* and *T. atroviride* were reduced the As toxicity level by improved growth and ‘P’ uptake in lettuce under As stress conditions. Srivastava *et al*. (2011) isolated 15 fungal strains from As contaminated soil, belongs to *Westerdykella, Aspergillus, Trichoderma, Neocosmospora, Rhizopus, Lasiodiplodia*, *Sordaria,* and *Penicillium*. They further reported that *Westerdykella* and *Trichoderma* were the better performing isolates than *Rhizopus* and *Lasiodiplodia* under As stress.

AM fungi as As-remediator

Arbuscular mycorrhizal (AM) fungi are best known for plant growth-promoting activity and for having remedial activity of several heavy metals (Smith and Read, 2008; Brundrett and Tedersoo, 2018). These AM fungi are obligatory in nature (Bogo *et al*., 2000) and require lipid during their life span and to complete their life cycle (Bravo *et al*., 2017; Jiang *et al*., 2017). In contrast, on a return basis, AM fungi improve the host plant by improving their nutrient status, water uptake and protecting the host plant from other biotic and abiotic stresses (Lenoir *et al*., 2016). AM fungi were reported from highly metal toxic soils and found associated with metal tolerant plants (Tonin *et al*., 2001). After struggling with the stress conditions, these AM fungi developed the resistive mechanism against the heavy metals. When these AM fungi were further provided unstressed conditions, they lose their tolerating properties against the heavy metals (Shalaby, 2003). This tolerating property is mainly due to phenotypic plasticity compared to genotypic. Garg and Singla (2012) experimentally observed that the AM colonized pea plant exposed to As (V) showed enhancement in relative water content (higher turgor) and chlorophyll content. They also reported that the AM fungi increase the uptake of sucrose and glycine betaine in the cell and Latef (2011) added that AM fungi also increase the proline content under metal stress conditions.

AM fungi were found to help the plants fight against As-induced phosphorus deficiency and maintain the P: As ratio in plants by decreasing As-translocation (Spagnoletti *et al*., 2016). Molecular studies revealed that up-regulation of *RiPT* showed a high affinity with AM fungi and *RiArsA*, a putative efflux pump in rice plants under high As treatments (Wu *et al*., 2015). Pathare *et al*., (2016) indicated that the specific genes are involved in AM fungi-mediated As toxicity amelioration in rice plants. The AM fungi inoculation was found to tackle As stress with a decrease in As (V) uptake, increase in plant growth, and N, P, K levels (Garg and

Singla, 2012).

Smith and Read (2008) reported that AM fungi often protect plants against high concentrations of non-essential metals, in addition to the improvement of 'P' status and uptake of some essential elements resulting in greater plant biomass. This is due to arsenate As (V), which has great similarity in chemical behavior to phosphate. It is thought that AM fungi may also influence As dynamics in soils. *Triticum aestivum* plants associated with AM fungi exhibited much better growth and were healthier than non-associated plants under contaminated soil (Sharma *et al*., 2017; Gupta *et al*., 2021). AM fungi also enhance the expression of phosphate transporter genes (*PHT1*) in many plants (Chen *et al*., 2007). These *PHT1* genes were isolated from As (V) tolerant *Arabidopsis thaliana* (Catarecha *et al*., 2007). These AM fungi *i.e.* species of *Glomus* suppressed high-affinity arsenate and phosphate transport into the roots. Conversely, mycorrhizal association with the fern *Pteris vittata* has been reported to stimulate more As accumulations (Liu *et al*., 2005). Jankong and Visoottiviseth (2008) experimentally reported that As concentration was lowered in petioles of plants treated with AM fungi as compared to non-treated plants. The effect of AM fungi in the remediation of heavy metal stress is well known. Mechanism of the potential of AM fungi adopts amelioration of heavy metal including As has been presented in Fig. 3.

In rhizospheric soil, AM fungi release glomalin that binds heavy metal in soil, and also binds in their cell wall components. This process plasma membrane act as a barrier (Khan, 2005). Different chelators present in cytosol, e.g., metallothioneins, organic acids, amino acids, and metal-specific chaperons, act on entering heavy metal and sequestration into the vacuole and also export to plant (hyperaccumulator) help in phytoremediation. (Göhre and Paszkowski, 2006). Plant-AM fungi symbiosis is mutual and contributes resource exchange nutrient transfer, water transfer, and sometimes carbon transfer to plant, but AM fungi' effectiveness varies from plant to plant (Johnson *et al*., 2002; Nottingham *et al*., 2010). AM hyphal network makes a broad network and reaches beyond the deficit zone from the root and absorbs nutrients, water, and other trace nutrients in limiting soil and also regulates carbon dynamics ecosystem (Clemmensen *et al*., 2013). For the proper growth of plants especially reproductive phase development phosphorus (P) is very important, generally in immobilized form (orthophosphate) in soils. AM fungi are well known as P-solubilizer and store 'P' as poly-

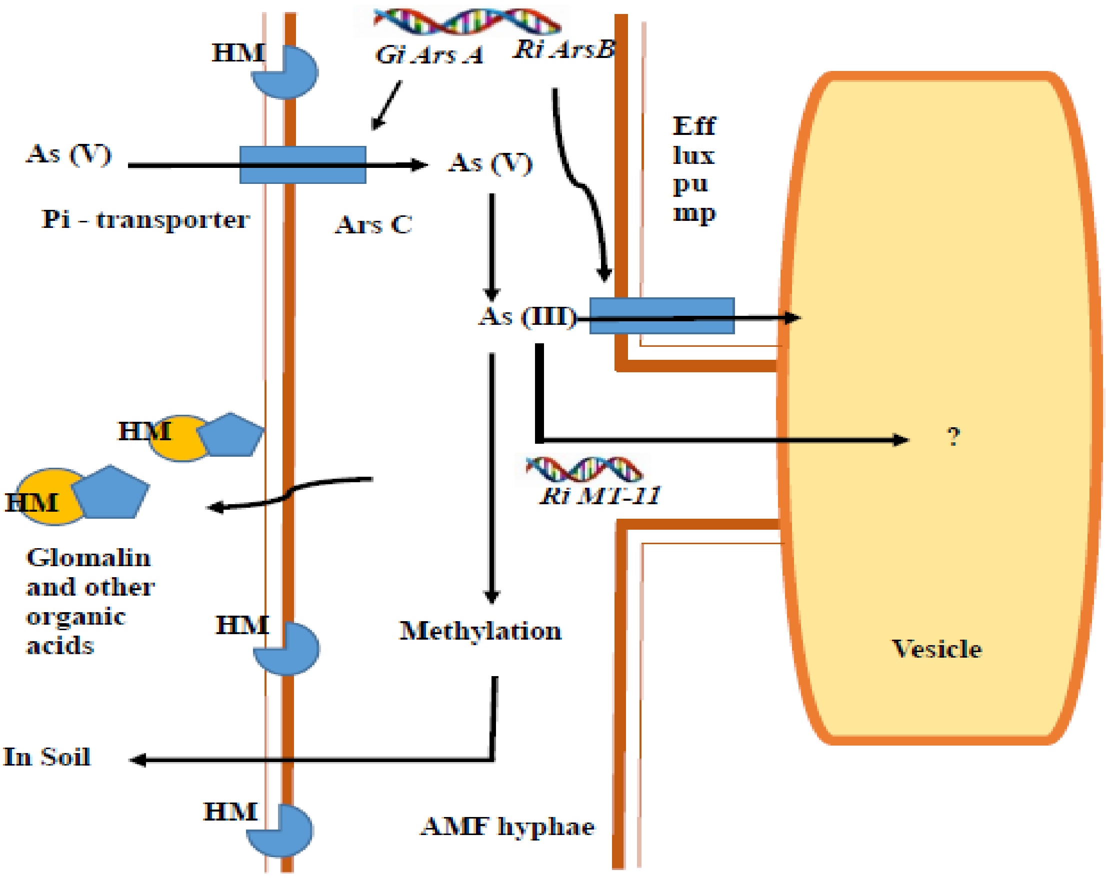

Fig. 3. *Mechanism of potential AM fungi adopt amelioration of heavy metal including As (HM = heavy metals)*

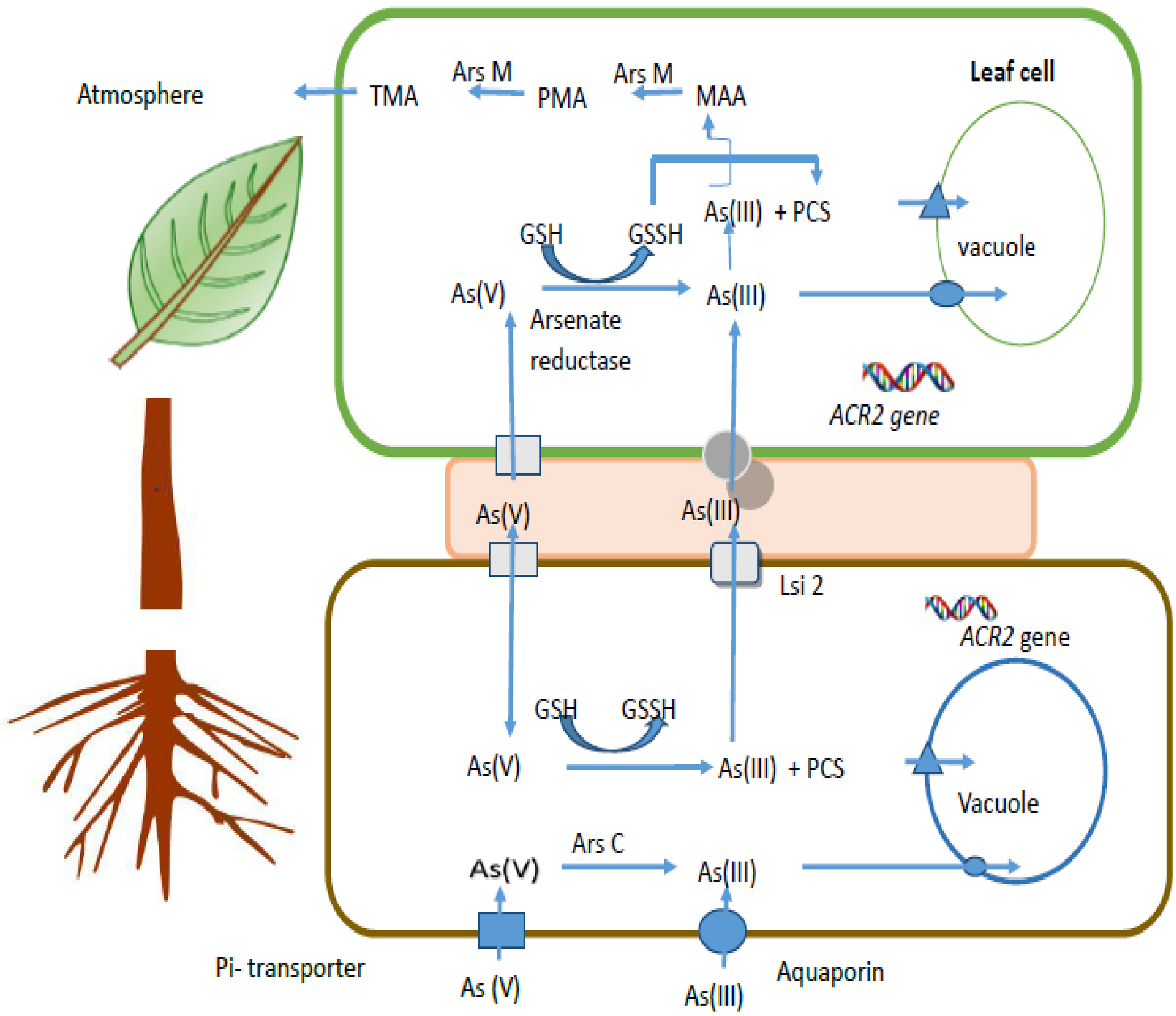

Fig. 4. *Mechanism of As-detoxification by the As-tolerant/hyper accumulator plants*

phosphate, which helps keep relatively low Pi concentration in AM hyphae, so the proper 'P' transfer to plant root cell maintained (Hijikata *et al*., 2010).

As and 'P' belong to the same group of elements in the periodic table and 'P' is a chemical analog of As and utilized the same transporter for their entry into root cell (Wu *et al*., 2011). Cozzolino *et al*., (2010) reported the inoculation of native AM fungi in consortium with 'P' increased plant biomass and noticed a decrease in As accumulation in plants. Pigna *et al*. (2010) revealed that 'P' can elevate As toxicity in wheat-growing in As contaminated soils.

Researches specify the As a metal import, transportation, detoxification, etc., within hypha and discoveries of specific genes which are present to regulate the transportation, reduction, efflux pumping, etc. Two novel genes have been discovered *i.eRiArsB* and *RiMT-11* involved in As detoxification in AM fungi (Zhang *et al*., 2015; Maldonado-Mendoza and Harrison, 2018). *RiMT-11* gene was discovered in *Rhizophagus irregularis* and the other one *GiArs A* was also described previously in the same species which involved in As intake through phosphate transport channel (González-Chávez *et al*., 2011). *RiArsB* gene is associated with As (III) efflux pump towards outside of mycelia. It would be part of Ars AB, whereas RiMT-11 gene plays a role in methylation of As (Maldonado-Mendoza and Harrison, 2018). Christophersen *et al*. (2012) investigated the effect of AM fungi symbiosis in *Medicago truncatula* amended with As (V) and 'P' and concluded that the expression of *MtPT4* gene was maximum in plant inoculated *Glomus mosseae* while *MtPHT1; 1* expressed maximum in plant inoculated with *Glomus intraradices*. Further, they reported that the inoculation of the *G. mosseae* enhanced the uptake of 'P' as compared to As by the application AM fungi. These genes were coded arsenate/arsenite permease component and As methyltransferase, respectively. Li *et al*. (2021) experimented that *Rhizophagus irregularis* symbiosis successfully remediate the As from

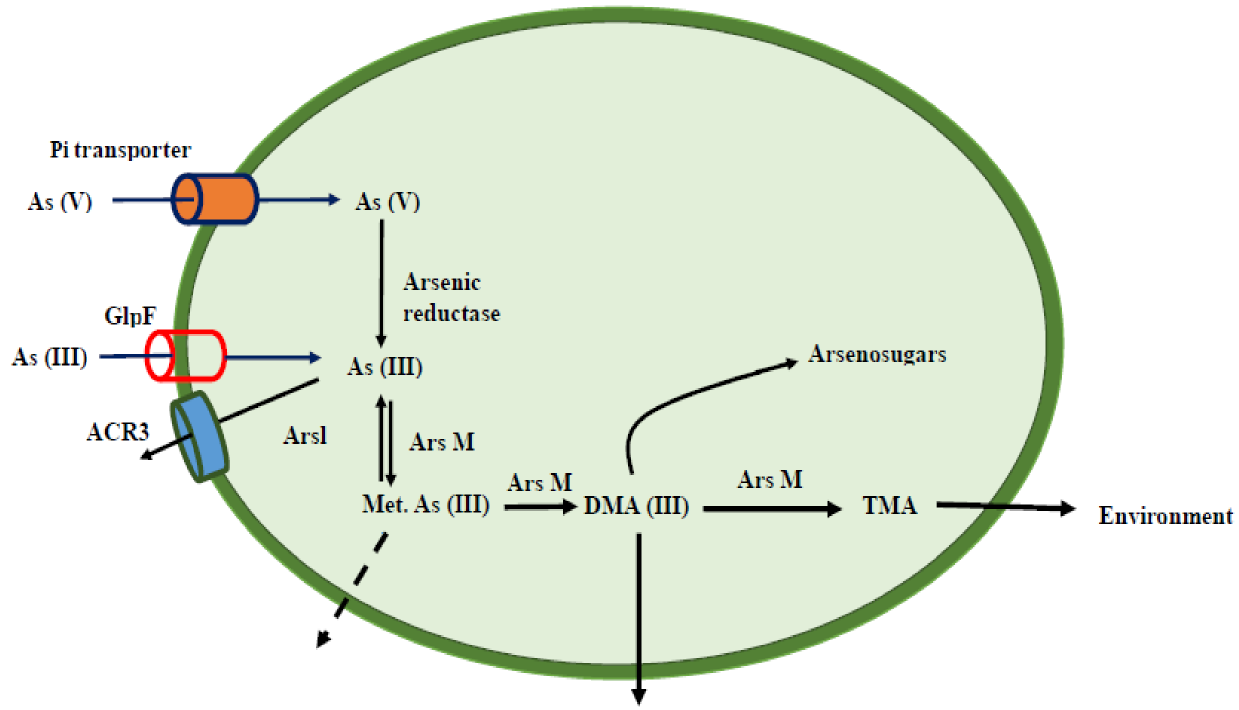

Fig. 5. *Mechanism of As-detoxification in Nostoc*

Medicago sativa and culture substances also noticed the remarkable up-regulation of *RiMT-11gene* in *Rhizophagus irregularis.*

All types of plant stresses generated reactive oxygen species (ROS) that cause oxidative stress, which disturbed intracellular activities such as oxidation of proteins, lipids peroxidation, and enzyme activity inhibition that cellular damage in the plant (Sharma *et al*., 2012). AM fungi upgraded and enhanced the antioxidant defense system like isoprenoid production, higher hydraulic conductivity, increased antioxidant production by increased 'P' uptake, glomalin protein production, improving osmotic adjustment and modification in growth and physiological of host plants (Rapparini *et al*., 2008; Wu and Zau, 2009; Evelin *et al*., 2009). AM fungi also help in the decontamination of metal-polluted soils and their broad application practicing in phytoremediation (Vallino *et al*., 2006; Bhargava *et al*., 2012; Meier *et al*., 2012). AM fungi check or slow the translocation of heavy metals from soil to root and reduce to reach in above-ground parts (Wu *et al*., 2015), thereby improving the plant health so food quality and safety are constant (Xie *et al*., 2015). The phosphate-solubilizing microbes affect the As-uptake and mitigate the stress through the process of precipitation, complexation, redox reactions, and nutrient availability (Rahman *et al*., 2015). Biosorption, exclusion, binding to cysteine-rich peptides/proteins, methylation, and volatilization, etc., were different mechanisms involved in the As-transformation (Upadhyay *et al*., 2018).

Metallophytes as As-remediator

[As] phytoremediation comprises different mechanisms which make plant tolerant against As stress such as accumulation, phytostabilization, phytoextraction, and phytovolatilization (Barbafieri *et al*., 2013; Favas *et al*., 2014). Metallophytes release root exudates which stimulate microbial activity and convert As into complex immobilizing forms through several chemical reactions such as acidification, chelation, complexation, precipitation, and redox reactions (Wuana and Okieimen, 2011; Bolan *et al*., 2011). As- hyperaccumulator plants accumulate As in different plant parts and also convenient means of As mitigation from soil (Fig. 4). The highest amount of As accumulated in *Pteris vittata* (22, 630mg/kg) followed by *Pityrogramma calomelanos* (8350 µg/g) (Ma *et al*., 2001; Francesconi *et al*., 2002; Fitz *et al*., 2003). In the process of As-phytoremediation, plants generate gaseous arsine species also and released into the environment.

In-plant, intake of As via phosphate and aquaporin transport channel enzyme arsenic reductase which helps in conversion of As (V) and As (III) and converted As (III) stored in the vacuole(Ma *et al*., 2008; Wu and Okieimen, 2011). Yamaji and Ma (2011) reported *Lsi 2* transporter responsible for inner translocation from the root cell to the xylem. The As reduction is governed by some functional genes in metallophytes. Nahar *et al.* (2017) investigated the role of cloned *At ACR2* gene (arsenic reductase 2) As reduction in *Arabidopsis thaliana.* As reaches in different plant parts *via* vascular translocation through xylem cells and stabilized or volatilized in presence of As (III)-S–adenocylmethionine methyltransferase (arsM) (Qin *et al*., 2006). Pigna *et al*. (2010) investigated the role of phosphorus in detoxification and As uptake and their distribution in the root, shoot, and grain when wheat grown in As-polluted soil and concluded that enhanced phosphorus reduced As toxicity in the plant.

Sharma *et al*. (2017) experimented with the *Triticum aestivum* plant noticed the role of AM fungi amendment in artificially As contaminated medium with different doses. Their study revealed that AM fungi reduced oxidative stresses significantly and reduced As translocation from root to grains. AM fungi also increase different physiological, biochemical, and antioxidant enzymes in wheat. Soto *et al*. (2019) investigated the role of isolated two As-resistant bacteria (*Psedomonas gessardii* and *Brevundimonas intermedia*) and two fungi (*Fimetariella rabenhorttii* and *Hormonema viticola*) in the alleviation of As toxicity in *T. aestivum* and noticed that *P. gessardii* and *Brevundimonas intermedia* inoculation increased plant growth and also recorded overexpression of antioxidant synthase gene in plants. Ghorban *et al*. (2021) evaluated the inoculation effect of *Piriformospora indica* in As-stressed *Oryza sativa*, and found their positive role in restoring the photosynthetic pigments as well as tolerance by the down-regulating Lsi2 expression and up- regulation of PC_S1and PC_S2 protein expressions.

Algae as As-remediator

Algae developed multiple detoxifying mechanisms that involved redox potential, methylation, and demethylation etc. In algae, both methylation and demethylation processes coexist, but the reason for this coexistence and their metabolisms are unknown (Yan *et al*., 2017). Some marine alga contains As in their hydrocarbon chains in cellular bodies (García-Salgado *et al*., 2012). *Nostoc* showed the ability to methylation and demethylation of As with help of enzyme the S- adenosylmethionine methyltransferase (Ars M) (Yin *et al*., 2011) whereas, Ars I worked reverse to process (demethylation) (Yan *et al*., 2015). Methylated As (III) bound to Ars M and further converted to DMA (III) and TMA (III) (Yin *et al*., 2011). Xue *et al*. (2017) postulated and described arsenosugar phospholipid biosynthesis in *Nostoc*. In this process, S-adenosyl- L-methionine (SAM) provides methyl group to As (III) to generate TMA (III) with help of S- adenosylmethionine methyltransferase enzyme and further provides adenosyl group to DMA (III) for generating the basis of arsenosugar. The DMA synthesis (dimethyl arsinyladenasine) undergoes glycosidation to produce Oxo-arsenosugar-glycerol (Oxo-Gly), which acts as the precursor of arsenosugar phosphides. Details of enzymes involved in arsenosugar synthesis are not yet identified as well. Murray *et al*. (2003) identified arsenosugars in *Chlorella vulgaris* there are some other algal species *viz., C. vulgaris, C. reinhardtii, Synechacystis* sp. known for arsenosugar production. Xue *et al*. (2017) treated *Nostoc* sp. with arsenite As (III) for fourteen days and separated monomethylarsonate, dimethylarsonate, a glycerol arsenosugar (Oxo-Gly) and a phosphate arsenosugar (Oxo-PO_4). These microalgae are applicable in As contaminated paddy crops. Paddy fields are suitable for algal growth and microbial activity helps As detoxification.The detailed mechanism of As detoxification by *Nostoc* is given in Fig. 5.

Protozoa as As-remediator

Protozoans are sensitive to toxic material and environmental pollutants. These characteristics make them ideal for use as experimental test organisms in ecological investigations (Yin *et al*., 2011). Yin *et al*. (2011) reported that *Tetrahymena thermophile* SB-210 accumulates 187 mg/kg dry weight when it is exposed to 40μM with As (V) methylated species (MMA, TMA), accounting for 66% of the total amended As. In natural conditions, *Euglena mutabilis* tolerated up to 260 mg /l of As in medium (Squibb and Fowler, 1983). Casiot*et al*. (2004) studied *E. mutabilis* in severely loaded sulphate (1.9-4.9 g/l), iron (0.7–1.7 g/l), As (0.08–0.26 g/l) environments and found able to oxidized As (III) to As (V) but no substantial methylated As species were noticed. Yin *et al*. (2017) reported *Tetrahymena pyriformis* As biotransformation abilities in response to various ‘P' and As concentrations. When exposed to As (V) for twenty hours and at low ‘P' concentrations (3.6 and 15 mg/l), As demonstrated dominance, whereas methylated forms such as MMA, DMA, and volatile As species showed a positive connection with initial ‘P' concentrations.

Conclusion

As contamination in terrestrial and aquatic ecosystems is a very sensitive environmental issue due to its adverse impact on human health. It enters into the terrestrial and aquatic ecosystems through several natural processes such as weathering reactions, biological activity, volcanic emissions, and anthropogenic activities. The natural soil is a heaven of microbial diversity. In Agriculture, soil microbes are intimately associated with plant health and their production. The soil microbes have the potential for remediation of such soils. Biological remediation may play a great role to cope up with such pollutants. Soil microorganisms are responsible for the dynamics of the transformation and development of soil structure. Metal tolerant bacteria, saprophytic and mycorrhizal fungi have recently received great attention for establishing vegetation in heavy metal contaminated soils. These microbes have developed several mechanisms for the detoxification of heavy metals. The exploration of microbes for sustainable agriculture and the improvement of degrading habitats is a new approach to modern steps. The work on utilization of the microbes in their best way isalso needed to understand better their mechanisms, physiology, molecular and genomic details for the potential application at a large scale. In natural degrading habitats, microbes develop multiple mechanisms to cope up with such stresses. These mechanisms made of microbes can serve as bioremediation tools that transform toxins into less toxic substances. Some microbes volatilize As added in the environment (dilution effect) and the association of such potent microbes may help in bioremediation. On the other hand, some microbes are utilized as bio-barrier of As when it is amended with phosphorus. Thus, these studies suggest that microbes could be safely used to manage As contaminated areas.

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Conflict of interest

The authors declare that they have no conflict of interest.

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