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 arsenic 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 (Cu₃AsS₄) and realgar (As₄S₄). In some bacteria, the As-operon machinery thiol group bind with As, it detoxifies 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 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 arsenic acid [DMA], tri-methyl arsineoxide [TMAO], arsenobetaine [AsB], arsino-choline (AsC), arsenosugars (AsS), arsenolipids etc. (Tangahu et al., 2011). Among them, dimethyl arsonic 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 thiois (-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 exposor 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 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, hyperkeratosis, 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, biominneralization, 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. Zuzzolo 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 resesemblances, As-contaminated regions are subdivided (Mukherjee et al., 2019). For instance, in Asia, most of the affected areas are the Ganges–Brahmaputra in Meghna-plane 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 (Chandarajith 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 Sibera (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 lgl (Acharaya 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, Swe-
den, 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 (As₂S₃) 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 (H₃AsO₃) 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 methanearsionate, disodium methanearsionate, and caco-dylic acid (National Research Council (US), Environmental Protection Agency, (1980). According to this Council, 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 arseni 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, phytosequestration, phytovolatilization process. *Pteris vittata* has been reported as one of the best hyperaccumulator 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-

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

BYQ-12

The maximum As leaching ratio obtained from realgar in the presence of mixed adopted cultures was 28.6 %. Leng et al., 2016

Acidithiobacillus ferrooxidans

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

Rhizogonium 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

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

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organisms 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 methaneproducing 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 bacterium *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*, and *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 organometheic 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 flav 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 [Ca₅(H₂)(AsO₄)₃·H₂O] (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 SSA1.

**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](image-url)
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 arsenate and monomethyl arsenate by the application of Penicillium brevicaulis, 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 disulphide 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, Trichodermaasperellum, Penicillium janthinellum and Fusarium oxysporum intracellular methylation. During the process biomethylation, metal-resistant fungi generated volatile As-species (biolvatilization). Some fungi were reported to generate arsine such as Penicillium sp., Uplcladium sp., Neosartorya fisch, eri, Aspergillus clavatus, etc. (Visoottiviseth and Panviraj, 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 RiarS, 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
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 peti-oles of plants treated with AM fungi as compared to non-treated plants. The effect of AM fungi in the reme-
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.e RiArsB 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 with 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. 4. Mechanism of As-detoxification by the As-tolerant/hyper accumulator plants**
Medicago sativa and culture substances also noticed the remarkable up-regulation of RiMT-11 gene 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, phytoextraction, 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–adenosylmethionine 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 (*Pseudomonas gessardii* and *Brevundimonas intermedia*) and two fungi (*Fimetiariella rabenhortti* 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 *Piniformospora 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 PC₅₁ and PC₅₂ 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 (Garcia-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 (dimethylarsinyladensinase) undergoes glycosidation to produce Oxaloarsenosugar-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*, *Synechocystis* sp. known for arsenosugar production. Xue et al. (2017) treated *Nostoc* sp. with arsenite As (III) for fourteen days and separated monomethylarsionate, dimethylarsionate, a glycerol arsenosugar (Oxo-Gly) and a phosphate arsenosugar (Oxo-PO₄). 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 thermophila* 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). Casciò 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 is also 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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