

1 **Recent advances in the bioremediation of arsenic-contaminated soils: a mini review**

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16 **Abstract:**

17 The carcinogenic metalloid arsenic (As), owing to its persistent behavior in elevated levels
18 in soils, aggravates environmental and human health concerns. The current strategies used in
19 the As decontamination involve several physical and chemical approaches. However, it
20 involves high cost and even leads to secondary pollution. Therefore, it is quite imperative to
21 explore methods that can eradicate As menace from the environment in an eco-friendly,
22 efficient, and cost-competitive way. Searching for such viable alternatives leads to the
23 option of bioremediation technology by utilizing various microorganisms, green plants,
24 enzymes or even their integrated methods. This review is intended to give scientific and
25 technical details about recent advances in the bioremediation strategies of As in soil. It takes

26 into purview the extent, toxicological manifestations, pathways of As exposure and
27 exemplifies the substantive need of bioremediation technologies such as phytoremediation
28 and biosorption in a descriptive manner. Additionally, the paper looks into the wide potential
29 of some plant growth promoting microorganisms (PGPMs) that improve plant growth on
30 one hand and alleviate As toxicity on the other. Furthermore, it also makes a modest attempt
31 to assimilate the use of nanoparticles, non-living biomass and transgenic crops which are the
32 emerging alternative bioremediation technologies.

33 **Introduction:**

34 Arsenic (As), a toxic metalloid, has sparked worldwide concern. Its rising prevalence in
35 biosphere (Sanyal, 2017) is alarming for environmental and human health (Mazumder et al.,
36 2013), specifically as a tenacious category 1 human carcinogen (Menon et al., 2020). As may
37 be found in the environment in both inorganic [arsine (As^{-3}), elemental As(0), As(III), and
38 As(V)] and organic [dimethylarsinic acid (DMA), monomethylarsonic acid (MMA),
39 trimethylarsine oxide (TMAO), arsenobetaine, etc] forms (Upadhyay et al., 2018). As
40 pollution of groundwater in South and Southeast Asia is mostly caused by natural
41 biogeochemical processes (Sengupta et al., 2021). In India and Bangladesh, the problem of
42 As poisoning is more acute, with groundwater As concentrations in about 50% aquifers
43 having > 2.50 mg/L, which is many magnitude greater than WHO recommended levels of
44 0.01 mg/L (Sanyal, 2017). Food crops, particularly paddy, grown with As-laden irrigation
45 water are a potent source of As exposure to humans through soil-crop-food transfer.

46 Rice and rice based products are considered to be the leading source of As pollution for
47 millions of people (Awasthi et al., 2017; Sengupta et al., 2022). In India and Bangladesh,
48 daily consumption of rice is high around 68.2 and 173.3 kg person⁻¹ year⁻¹ respectively.
49 Approximately 69.6% of the calorific intake is from rice in Bangladesh and for India it is
50 29.1% (Mandal et al. 2021; Sengupta et al. 2021).

51 Under anaerobic field conditions, the existence of As, mostly in the form of arsenite
52 [As(III)] is transferred via over-expressed silicic acid transporters in rice (Srivastava et al.,
53 2012). Other crops produced aerobically, such as wheat, maize, and Indian mustard, result in
54 an excess of arsenate [As(V)]. Furthermore, As(V) is taken up and transported by phosphate
55 transporters. Long-term exposure to As can have serious consequences for the functional
56 integrity of human tissues and organs such as the intestinal system, liver, skin, kidney,
57 nervous system, and so on. Animals also get impacted by As by ingestion of water and feed,
58 and those can function as a origin of As to following species in the food chain (Datta et al.,
59 2012). As poisoning affects plant growth and development, resulting in plant mortality or low
60 crop production and quality, even curtailing yield by more than 30% in some cases
61 (Bhattacharyya et al., 2021).

62 Arsenic pollution has sparked global interest, prompting the development of a number
63 of physical and chemical cleanup techniques. The current available remediation approaches
64 are mainly adsorption by using specific media, immobilization, modified coagulation along
65 with filtration, precipitations, immobilizations, and complexation reactions (Lim et al.,
66 2014). However, these mechanisms are costly and bear limited applicability among poorer
67 section of the society. Further, these methods can also cause secondary pollution in the soil
68 environment. This led to the search for alternative strategies. Microorganisms like
69 *Aspergillus*, *Candida*, *Scopulariopsis*, *Penicillium*, *Fusarium*, and *Trichoderma* are among
70 the fungi that may methylate inorganic As compounds to organic ones (Cullen and Reimer,
71 1989). Further, some microorganisms are resistant to arsine (AsH_3), monomethylarsine
72 (MeAsH_2), dimethylarsine (Me_2AsH), and trimethylarsine (TMA) and have the ability to
73 convert As into volatile arsine gases (Páez-Espino et al., 2009). Many microbes have the
74 ability to resist the As and they also contain a special type of As resistant system, ars operon

75 (Laha et al., 2021). So the biological methods may be an alternative as they are the low cost
76 and eco-friendly technique.

77 Simultaneously, the efficacy of plants for eliminating contaminants must be improved
78 in order to harness phytoremediation potential, as it is an affordable solar-driven technique.
79 As-resistant plant growth promoting microorganisms (PGPMs) might well be regarded as
80 benign, minimal cost incurring, efficient and long-term biological agents for reducing As
81 toxicity in plants and managing As accumulation in crops (Vejan et al., 2016). If appropriate
82 PGPM-based techniques succeed, they will give further benefits in terms of diminished
83 inorganic fertilizers use, economic savings, and environmental preservation.

84 **Global status of As contamination and toxicological manifestations**

85 Natural As pollution of groundwater has been recorded all over the world, with most of
86 events occurring in the South Asian and South American zones (Mandal et al., 2021).
87 Bangladesh, India, China, Nepal, Cambodia, Vietnam, Myanmar, Laos, Indonesia, the USA,
88 are among the worst-affected countries (Bhattacharyya and Sengupta, 2020). Several studies
89 show that high levels of As (0.5 to >4600 g/L) contamination in Bangladesh's shallow
90 aquifers have a negative impact on public health (Shaji et al., 2021).

91 In India, groundwater is critical for meeting the water demands of numerous sectors,
92 including household, industrial, and irrigational requirements. The alluvial tracts of Ganga
93 and Brahmaputra rivers are the country's richest groundwater provinces. The Ganga is
94 currently one of the earth's most contaminated rivers, with levels of Cr, As, Cd, Pb, Cu and
95 Hg, as well as pesticides and pathogenic bacteria, about >3000 times the World Health
96 Organization's acceptable limit (WHO, 2011). Groundwater with high As levels (>10 µg/L)
97 has been observed in ten Indian states; nonetheless, India's deeper aquifers (>100 m) are As
98 free (Sanyal, 2017). As pollution in West Bengal's groundwater was first discovered in 1978,

99 and roughly more than 50 million people are at danger in 12 districts and 111 blocks (Shaji et
100 al., 2021).

101 The elevated level of As concentration in ground water is associated with human
102 wellbeing. The formation of skin diseases, such as raindrop hypopigmentation, pigmentation,
103 keratosis (palmer and plantar), and even dermatological malignancies including basal cell
104 carcinoma (BCC), squamous cell carcinoma (SCC), and Bowen's disease, is a key symptom
105 of As toxicity. Surprisingly, only 15–20% of the population exhibits these symptoms.
106 Research studies with human cancer cells unearthed different toxicological mechanisms with
107 respect to skin-related outcomes and cancerous fates of bladder, lungs, liver etc (Sanyal,
108 2017). As research at the cellular level over the last decade has demonstrated that As changes
109 the cellular gene expression motif, as well as cell cycle, epigenomic profile, telomere length,
110 and other factors. DNA damage and repair mechanisms have been one of the most studied
111 area of As poisoning in the last decade. The identification of genetic damage as a valid
112 biomarker for As-triggered hazardous consequences has been demonstrated in both human
113 and cell line studies. When compared to unexposed human participants, both chromosomal
114 abnormalities and micronucleus were found to be highly related with As exposure and have
115 exhibited a strong relationship to As toxicity (Upadhyay et al., 2018).

116 **Remediation of As from the environment: the multi-faceted tools**

117 As elimination mechanisms should connect certain basic technological criteria, together with
118 hardiness, no additional environmental adverse effects, and ease of use. There are currently
119 several procedures for decontaminating As from As-contaminated area, the physical,
120 chemical, and biological techniques.

121 In the physical technique, the As content in soil can be lowered by combining
122 polluted and unpolluted soils to achieve an allowable quantity of As dilution. Soil washing is
123 one more treatment that belongs to physical methods, in which As-polluted soil is cleaned

124 with various concentrations of chemicals such as nitric acid, phosphoric acid, sulfuric acid,
125 and hydrogen bromide. The option of costly chemicals utilized for extractant is the limitation
126 of the use of soil cleaning into smaller-scale actions (Mahimairaja et al., 2005).

127 Meanwhile, cement can immobilise soluble As(III) and has been effectively utilised to
128 stabilise As-wealthy sludges, suggesting that it could be used to treat sludge from
129 precipitative shifting units. The dumping of As-containing water treatment lavishes has been
130 studied, with a specific focus on stabilisation/solidification (S/S) methods, for their suitability
131 in managing As-bearing lavishes. The brine produced by the recovery of operated alumina
132 filters is believed to speed up cement hydration in this process. Furthermore, because water
133 solubility is the managing techniques of pollutant dissolution, additives (surfactants,
134 cosolvents, etc.) have been employed to improve the abilities of soil flushing utilizing soapy
135 solutions. In laboratory trials, the use of surfactant alone yields roughly 80–85% efficiency.
136 Adsorption employing particular media, immobilisation, and altered coagulation with
137 filtration, precipitations, immobilizations, and complexation reactions are among the
138 currently available chemical remediation procedures. Coagulation with filtration is a cost-
139 effective approach for removing As from polluted sources (Lim et al., 2014).

140 The stabilisation technique benefits from the creation of stable phases, such as
141 insoluble FeAsO_4 and hydrous species of this compound, such as scorodite ($\text{FeAsO}_4 \cdot 2\text{H}_2\text{O}$).
142 Furthermore, because most of the contaminated area are polluted with numerous metal(loid)s,
143 using selected stabilising amendments is a difficult operation. Another promising boost for
144 the stabilising approach is nanosized oxides and Fe. Engineered oxide nanoparticles are
145 promising materials for the remediation of soils polluted with inorganic contaminants due to
146 their active and relatively high specified surface area (Waychunas et al., 2005).

147 Given the limits of traditional cleanup procedures, biological solutions involving
148 bacteria could be investigated as a potential alternative mitigation strategy (Laha et al., 2021).

149 Bioremediation of soils polluted with inorganic or organic As carried in pesticides and
150 hydrocarbons, has gained widespread acceptance in some areas. Despite the fact that
151 bioremediation has significant drawbacks, it is gaining popularity for the reclamation of
152 metal polluted soils because of its economic success. Two types of bioremediations are used:
153 intrinsic and designed bioremediation. Intrinsic bioremediation is the breakdown of As by
154 normally occurring microbes without human interference, and this procedure is better suited to
155 remediate soil with small levels of pollutants. Engineered bioremediation frequently relies on
156 human intervention to optimise environmental conditions so as to increase the proliferation
157 and activity of microbes living in the region. As a result, using a designed bioremediation
158 approach in a heavily contaminated area is more advantageous (Mahimairaja et al., 2005).
159 Absorption of As in the form of As(V) by phosphate transporters, uptake of As in the form of
160 arsenite by aquaglyceroporins, conversion of arsenate to arsenite by arsenate reductases, and
161 extrusion or sequestration of arsenite are the four mechanisms for As detoxification
162 (Garbinski et al., 2019).

163 A microbial community vigorously oxidised As(III), resulting in a considerable drop
164 in soluble As contents and a commensurate enhance in the As toxicity in the sediment
165 downstream of the hydrothermal origin. *In situ* oxidation studies verified the existence of
166 arsenite-oxidizing groups (aro A-like genes) in the structure. These findings suggest that
167 microbe-involved As oxidation aid in the depletion of As content and stabilisation of As in
168 the solid system, hence limiting the quantity of As carried downstream (Leiva et al., 2014).

169 Filtration, osmosis, adsorptions, and precipitations are among the physicochemical
170 methods used to reduce As levels in water, while biological procedures involve
171 phytoremediation or microbes assisted As decontamination (Singh et al., 2021). Biosorption
172 and biomethylation are two essential processes in microorganisms' elimination of As from
173 water. Biomethylation (by As(III) S-adenosylmethionine methyltransferase) has been

174 identified as the most reliable biological method for eliminating As from aquatic media. The
175 arsenite S-adenosylmethionine methyltransferase (ArsM) gene was recently introduced into
176 the chromosome of *Pseudomonas putida* KT2440 for possible environmental As
177 bioremediation as described by the first X-ray crystallographic structure (Lim et al., 2014;
178 Gupta et al., 2020).

179 **Microbes resistant to As**

180 Microbes contain a special type of As resistant system. They contain ars operon which enable
181 survival under As stress condition. The arsRDABC operon implicated in the As tolerance
182 phenotype was found by the nucleotide sequence of the determinants from the *E. coli* R773
183 plasmid, and *Staphylococcal* plasmids pI258 and pSX267. ArsR, a metalloregulatory protein
184 belonging to the SmtB/ArsR family, is encoded by the arsR gene. ArsR is a trans-acting
185 transcriptional repressor protein that attaches to the ars operon promoter and aids in
186 transcription (Ben et al., 2018). The arsC (reduction of arsenate to arsenite), arsR
187 (transcriptional repressor), and arsB (may also be a component of the ArsAB arsenite-
188 translocating ATPase, an ATP-driven efflux pump) genes make up the majority of
189 detoxification operons. Furthermore, two additional genes (arsD-metallochaperone and arsA-
190 ATPase) are found in several detoxifying operons. The metallochaperone ArsD binds
191 cytosolic As(III) and transfers it to the efflux pump's ArsA subunit. Arsenate that enters the
192 cell is converted to arsenite by the ArsC gene before being transported out by the ArsB gene
193 in a normal procedure. As a result, the environment will be exposed to a more hazardous
194 form of As . Microorganisms can also reduce arsenate to arsenite by a dissimilatory reduction
195 pathway, which can occur in either a facultative or stringent anaerobe state, with arsenate
196 functioning as the terminal electron acceptor. Those microbes having the capacity to eat other
197 microorganisms, oxidise inorganic (sulphide and hydrogen) and organic (e.g., nitrous oxide)

198 substances and use it as an electron donor (formate, aromatics, and lactase acetate) (Kabiraj et
199 al., 2022).

200 Bacteria with As-toleration ability include *Acinetobacter*, *Rhodococcus*,
201 *Agrobacterium*, *Staphylococcus*, *Thiobacillus*, *Escherichia*, *Achromobacter*, *Pseudomonas*,
202 *Alcaligenes*, *Microbacterium*, *Cupriavidus*, *Ochrobactrum*, *Desulfomicrobium*, *Fomitopsis*
203 *pinicola*, *Fusarium oxysporum*, *Penicillium gladioli*, *Fucus gardneri*, *Bosea sp.*,
204 *Psychrobacter sp.*, *Rhodobium*, *Bradyrhizobium*, *Sinorhizobium*, and *Clostridium* (Chandra
205 and Banik, 2021).

206 **Bioremediation as a potent weapon of alleviating As toxicity**

207 Bioremediation, a procedure that utilises microbes to remove contaminants from soils, and
208 phytoremediation, a method that involves plants to abolish heavy metals, are two biological
209 approaches that are regarded successful for heavy metal remediation (Adams et al., 2015).

210 Because of the extent and complexity of environmental issues by polluted soils, the fact that
211 plant-based reclamation methods causes little environmental disruption, favours
212 phytoremediation as the biggest profitable chances for As remediation (Susarla et al., 2002).

213 **Global status of research initiatives in As bioremediation techniques**

214 Various studies (Table 1) of biodegradation and bioremediation abilities involving specific
215 microbes or plant have been published, with various levels of success (Elekwachi et al.,
216 2014). However, little research into patterns and likely causes in the global use of these
217 procedures has been uncovered. In 1996, Kinya and Kimberly looked into how many
218 remediation companies and research institutes had adopted soil and groundwater cleanup
219 processes, compared cleanup prices, and expressed thinking on the use of non-indigenous
220 microbes for bioremediation (Kato et al., 1996). The previously recorded reports on the use
221 of bioremediation method for remediation of contaminated zones is scarcely inspiring. Only 4
222 of the 391 polluted land sites addressed between 2000 and 2007 were treated with *in situ*

223 bioremediation, according to the UK Environment Agency. Only two locations had *ex situ*
224 bioremediation proposed, but it was never implemented. According to the US-EPA, out of
225 997 source-control-treatment programmes completed between 1982 and 2005, 240 were
226 classed as "innovative technologies" with 60 *ex situ* and 53 *in situ* bioremediation operations
227 accounting for a minor share (12%). Monitored Natural Attenuation (MNA), Bio-
228 augmentation and Bio-stimulation are frequently used process (USEPA, 2007).

229 **Mechanism of bioremediation**

230 Bioremediation functions by reducing, detoxifying, degrading, mineralizing, or transforming
231 more toxic metals into less harmful ones. Toxic waste is removed from a polluted
232 environment using cleaning techniques. Bioremediation is extensively assumed in the
233 degradation, eradication, immobilisation, of numerous chemical lavishes and physically toxic
234 compounds from the neighbouring environment through microbes' all-encompassing and
235 activity (Sharma, 2020). Figure 1 shows the different bioremediation measures that have been
236 used till date.

237 **Phytoremediation strategies**

238 Phytoremediation of As requires the utilizations of green plants and associated rhizosphere
239 dwelling microorganisms for complete elimination or stabilization or breakdown of
240 contaminants from soil, sediment as well as surface and groundwater (Yadav et al. 2018; Wei
241 et al. 2021). The different approaches for phytoremediation of As are phytoextraction,
242 phytoexclusion, and phytostabilisation, wherein each of the techniques, has different set of
243 mechanisms for creating pollutant-free environment (de Souza et al. 2019).

244 **Phytoextraction**

245 Phytoextraction aims to eliminate the pollutants from soil through plant uptake followed by
246 translocation and accumulation into harvestable portion. Then, the harvestable biomass is
247 incinerated and metals are extracted from the resulting ash content. *Pteris vittata* L. (Chinese

248 brake fern) is the first reported As hyperaccumulator, having considerable ability to collect
249 As efficiently from disturbed soils. Its unique metabolic properties, which include effective
250 mobilization of As in the rhizosphere, efficient uptake by the roots, and translocation to the
251 shoots, are largely responsible for its successful decontamination of As-contaminated soil.
252 There are 11 Pteridaceae fern species have been discovered since the discovery of *P. vittata*
253 (de Souza et al. 2019).

254 **Mechanisms of As hyperaccumulation**

255 The unique processes of As hyperaccumulation have been gradually unravelled based on a
256 variety of investigations, and appear to require effective As mobilization in the rhizosphere,
257 quick root uptake, and accelerated As transfer by *P. vittata*. Various characteristics set
258 hyperaccumulator plants apart from regular or non-accumulator plants. The key mechanism
259 separating hyperaccumulator plants from non-accumulator plants is the very quick transfer of
260 As from the root to the aboveground portion, considerably stronger detoxifying ability, and
261 higher As sequestration capacity in the aboveground part (Saxena and Misra, 2010).

262 **Exudates from the roots are used to mobilize As**

263 Arsenic solubilization in the rhizosphere has been demonstrated to be aided by root exudes
264 and bacteria. *P. vittata* generate two times more diffused organic carbon (DOC) than the non-
265 hyperaccumulating fern *Nephrolepis exaltata*. In comparison to *N. exaltata*, organic acids
266 from root exudates caused three times or more greater mobilisation of As from unsolvable As
267 minerals ($AlAsO_4$ and $FeAsO_4$) in an As-contaminated soil (Wang & Ma, 2015). In addition
268 to root secretions, it has been demonstrated that As-tolerant bacteria from *P. vittata*'s
269 rhizospheric zones (*Pseudomonas sp.*, *Comamonas sp.*, and *Stenotrophomonas sp.*) may raise
270 the As content in the absorption solution from 5 g/L to 5.04–7.37 mg/L by resolving
271 insoluble $FeAsO_4$.

272 **Mycorrhizal symbiosis**

273 *P. vittata* colonized by a population of AMF accumulated 2–5 times more As than those
274 without colonization in a greenhouse trial with total soil As of 100 mg kg⁻¹, with the increase
275 becoming more pronounced with increasing soil P content (Wang and Ma, 2015).

276 **Phytostabilization**

277 Phytostabilization on the other hand, involves arresting of As through absorption and
278 accumulation in the rhizospheric region. This low-cost process is particularly important in
279 limiting the bioavailability as a whole and biomagnification in the food chain (Fernández et
280 al., 2016). This technology stabilizes it in a particular environment and does not lead to the
281 complete removal of pollutants which may trigger resurgence in future. Its potential can
282 further be enhanced using amendments like compost, phosphates, bone mill, furnace slag, fly
283 ash etc (Shackira and Puthur, 2019). Hammond et al. (2018) reported phytostabilization of As
284 by *Prosopis juliflora* in compost-amended pyritic mine tailings. Kowitwiwat and
285 Sampanpanish (2020) applied *Pennisetum purpureum* cv. Mott for phytostabilization of As in
286 contaminated metalliferous mine site amended with cow-manure and acacia wood-derived
287 biochar.

288 **Indigenous tolerant species with low Translocation Factor (TF)**

289 Native *Populus* and *Salix* could be used to phytostabilize As-contaminated areas, according
290 to the discovery that trace metals were deposited primarily in woody roots (84–89%) with
291 limited shoot translocation (Saxena and Misra, 2010). A 0.15-meter-thick layer of sand-filled
292 soil was spread over a 0.7-meter-thick layer of ashes containing heavy metals in a polluted
293 area (such as As, Cu, Zn etc.) to improve the uptake efficiency. Even with 16–92% lower
294 tissue biomass than the control, 100% survival of native *Populus* and *Salix* was obtained
295 following two years of soil improvement (such as blending with imported soils, ploughing,
296 and fertilisation).

297 **Legumes improve the substrate**

298 Legumes with a high N-Fixation capacity and a robust root system can be employed as
299 pioneering colonizer species to repair the substrate and revegetate the region in order to
300 restore the nutritional condition of degraded areas with As pollution. Additionally, the
301 majority of legume plants have a limited capacity to move their shoots (Vazquez et al., 2006).

302 **Biochar and iron oxides**

303 The metabolic behaviours of metals in soils are significantly impacted by biochar, a
304 promising soil amendment. At a normal environmental pH, biochar with relatively high
305 cation exchange capacity consistently exhibits adsorption capability toward metal cations but
306 low binding ability for As species, regardless of the kind of feedstock and pyrolysis. In some
307 circumstances, supplements like Fe oxides are required to reduce As pollution and therefore
308 help plants survive. For example, As levels as high as 6670 and 56,600 mg kg⁻¹ were found in
309 two extremely polluted mining tailings in South Korea, resulting in substantial As toxicity in
310 plants. Adding amorphous Fe with biochar precipitated most of the As bound to Fe, and thus
311 further resulted in reduction of 70–80% accessible As (Wang and Ma, 2015).

312 **Phosphorus**

313 Phosphorus (P) more specifically pentavalent P is a chemical analog of As(V) and effectively
314 competes with arsenate for binding sites in soils. Following P treatment, competitive anion
315 exchange led to increased bioavailability and plant uptake of As. In a pot experiment with
316 soil As at 0, 15, and 30 mg kg⁻¹, greater As buildup in both rice grain and straw was reported
317 with lower grain production after P treatment of 50 mg/kg P (Hossain et al., 2009).

318 **Organic matter**

319 Due to the type of compost employed, the level of humification, and pH fluctuations, organic
320 matter has inconsistent effects on As mobility as a complex mixture of varied components
321 (Juwarkar et al., 2008; Lagerkvist et al., 2008; Shiralipour, 2002). For instance, in a
322 greenhouse experiment, the treatment of municipal solid waste and biosolids compost led to a

323 significant increase in soil soluble As from 5.7 to 7.1 mg/L. Application of organics, on the
324 other hand, diminish As bioavailability in soils and therefore in plants, as hitherto showed for
325 sesame (Sinha et al., 2011), wheat, and maize (Mandal et al., 2019a; 2019b), and vegetables
326 (Bhattacharyya et al., 2021).

327 **Mycorrhiza**

328 Proper inoculates of As-tolerant mycorrhiza can serve as a practical way to give host-
329 enhanced tolerance and boost Phyto stabilization by preferentially accumulating P over
330 As(V). Mycorrhizal inoculation of *G. mosseae* enhanced plant P content by 50–200% using a
331 compartmented cultivation system. Shoot As was reduced by 9%–30% in the presence of 1
332 and 205 mg kg⁻¹ As (Wang & Ma, 2015).

333 **Phytoexclusion**

334 In As contaminated agricultural soils, it is impractical to use non-food crops for
335 phytoextraction. Due to an effective silicon transport channel, rice, which feeds half of the
336 world's population, accumulates As more quickly than other cereals, especially when it is
337 flooded. This has led to widespread observations of elevated As levels in paddy rice, with As
338 TF values frequently reaching unity. It is possible to remediate As-contaminated agricultural
339 soils and lessen rice's uptake of As by using a variety of agronomic approaches and
340 biotechnologies that have been developed to enhance food safety and agriculture
341 sustainability (Zhao et al., 2010).

342 **Water management**

343 Water management can aid in reducing the mobilization of As brought on by the reductive
344 dissolution of Fe hydroxides under anaerobic conditions since soil redox potential regulates
345 As mobility and toxicity in paddy soils. When compared to conventional flooding farming,
346 the availability and uptake of As by rice can be greatly reduced under aerobic conditions,
347 even to the tune of 80% (Sengupta et al., 2021).

348 **Silicon fertilization**

349 According to Ma et al. (2008), rice easily absorbs As(III) through the Si transport system,
350 which suggests that enhanced Si availability can decrease As transfer in the soil-rice system
351 while also boosting grain output. Under Si fertilisation (20 g SiO₂ kg⁻¹ soil) Despite a 1.5–2
352 fold higher As concentration in soil solution [with 78–100% As(III)], rice straw and grain had
353 As concentrations that were 78% and 16% lower, respectively.

354 **Arsenic sequestration by Fe plaque**

355 In paddy rice and other aquatic species, As inflow into rice roots may be successfully reduced
356 by the iron plaque that is produced on the root surface as a result of rhizosphere oxygenation
357 due to its high capacity to store As(V). As concentrations in the rhizosphere soil solutions
358 were noticeably reduced in a pot culture experiment with rice growing under flooded
359 conditions, being 2.5-fold and 16-fold lower upon the amendment of amorphous iron at 0.1
360 and 0.5 percent as a result of enhanced As sequestration by Fe plaque (Ultra et al., 2009). The
361 ability to control As inflow into rice roots by efficient As fixation by Fe plaque is higher in
362 rice cultivars with higher root porosity and rate of radial O₂ loss because they release more O₂
363 to the rhizosphere.

364 **Pre-treatment of As contaminated irrigating water/Phytofiltration**

365 The heavy irrigation with groundwater contaminated with As is the main cause of the
366 elevated levels of As in paddy soils in South and Southeast Asia. In addition to the above-
367 mentioned strategies for lowering As contamination in the soil-rice system, it is crucial to use
368 efficient methods for removing As from irrigation water. In both lab and pilot-scale studies,
369 the As phytofiltration technique has been tried on As hyperaccumulators like *P. vittata*. Both
370 *P. vittata* and *P. cretica*, another As hyperaccumulator, were able to reduce As to below the
371 drinking water limit of 10 g/L in less than 24 hours, with baseline As(V) concentrations
372 ranging from 20 to 200 g/L.

373 As concentration in the outflow was typically less than 2 g/L over the course of an 84-
374 day demonstration in a pilot scale phytofiltration system, with starting As between 6.6 and 14
375 g/L and flow rate between 255 to 1900 L/day. Elless et al. (2005) found that the effectiveness
376 of the As removal was unaffected by day length, light intensity, or humidity, demonstrating
377 the high dependability of the technique. Therefore, in regions where As contamination is an
378 issue, phytofiltration has the potential to lessen the buildup of As in agricultural soils by
379 eliminating As from irrigation water (Wang & Ma., 2015).

380 **Microbial remediation (biosorption, bioaccumulation, biotransformation etc.)**

381 A low-cost and environmentally benign method of lowering the expense of heavy metal
382 pollution removal is microbial bioremediation. In order for bacteria to withstand heavy
383 metals, there are five primary mechanisms. 1) Extracellular barriers: Metal ions cannot enter
384 the cell through the cell wall, plasma membrane, or capsule. 2) Active metal ion transport
385 (efflux): P-type ATPases, CDF (Cation Diffusion Facilitator), and RND (Resistance,
386 Nodulation, Cell Division) proteins work together to form a pathway to transport potentially
387 dangerous metalloids from the cytoplasm. 3. Extracellular sequestration: this process involves
388 the accumulation of metal ions in the periplasm, the outer membrane, or the complexation of
389 metal ions by cellular components into insoluble compounds. 4) Intracellular sequestration:
390 Metal resistance is based on the accumulation of metals in non-bioavailable forms inside the
391 cytoplasm, preventing exposure to critical cellular components. This kind of metal resistance
392 is demonstrated by the production of metallothionein by *Synechococcus* species and cysteine-
393 rich proteins by *Pseudomonas* species. 5) Metal oxidation, including Cu and As, is another
394 crucial detoxifying method (González Henao and Ghneim-Herrera, 2021).

395 **Bio adsorption**

396 Microbial Bio absorbent depends on the microbial species engaged in metal uptake, whether
397 it is an active or passive process, or both. The passive uptake mechanism is generic to metal

398 species, whereas the active process is a sluggish method that is dependent on cellular
399 metabolism. Specific proteins, such as metallothioneins, form compounds with heavy metals
400 throughout the active phase. Both active and passive processes can occur at the same time. In
401 addition to chitosan and glucans, microbial species with high cell wall chitin content operate
402 as an effective bio sorbent. Metal bio sorbents can also be found in the walls of fungus,
403 yeasts, and algae. Gram-positive bacteria have a greater ability to attach metals than Gram-
404 negative bacteria (Satyapal et al., 2016).

405 **Bioaccumulation**

406 Bioaccumulation is a heavy metal transport pathway that is energy-dependent. Ion pumps, ion
407 channels, carrier mediated transport, endocytosis, and lipid permeability are all potential
408 bioaccumulation pathways for heavy metal influx across bacterial membranes. Bioreporters
409 for inorganic species in the environment have been made using genetically engineered
410 microorganisms. These bioreporters are based on genetic constructs that combine an *arsR*
411 operator and promoter sequence with the reporter gene sequence, such as luciferase, -
412 galactose, an auto fluorescent protein, or cytochrome c peroxidase. The intracellular build-up
413 of As by bacteria is preferred among the various probable As bioremediation processes. A
414 mutant strain of *C. glutamicum* has been developed to accumulate As intracellularly
415 (Satyapal et al., 2016).

416 **Biotransformation**

417 Biotransformation is a process of transfer of a metal from its toxic form to less toxic for or
418 nontoxic form. Specific enzymes or respiratory chains in the bacteria are responsible for the
419 redox transformation of As. These microbes can use As either as an electron donor or
420 electronacceptor and thereby play a significant role in As detoxification mechanisms. A
421 group of bacteria (*Pseudomonas sp*, *Burkholderia sp*, *Bacillus sp*, *Rhodobacter sp*.) are able
422 to transfer arsenite (toxic form) to arsenate (less toxic form) (Laha et al., 2021). As(V) and

423 As(III) both can undergo chemical and or microbial oxidation-reduction and methylation
424 reactions in soils and sediments and can adsorb on hydrous oxides of Fe, Al, and Mn. The
425 most important natural attenuation process known for As(III) compounds is precipitation as
426 As sulfide (As_2S_3). As(III) is more toxic and mobile in soils than As(V), and methylated
427 species such as monomethylarsonic acid [MMAA, $\text{CH}_3\text{AsO}(\text{OH})_2$] and dimethylarsinic acid
428 [DMAA, $[(\text{CH}_3)_2\text{AsO}(\text{OH})]$] are also mobile (Figure 3) . However, these methylated forms are
429 volatile and unstable under oxidizing conditions and are cycled back into the soil
430 environment in inorganic forms.

431 **Bacterial metabolisms of As**

432 The microbes have evolved several metabolic processes to counteract hazardous effects of
433 As. According to certain theories, the enzyme arsenate reductase was not present during
434 origin of life. When the environment turned reducing, microorganisms quickly evolved this
435 enzyme. Bacterial As metabolism involves mainly four separate mechanisms, including
436 reduction, oxidation, methylation, and demethylation (Ospino et al., 2019).

437 Arsenic is taken up by prokaryotes because of its molecular resemblance to the
438 substrates of different membrane transporter proteins. $\text{As}(\text{OH})_3$, which is formatively similar
439 to glycerol, departs aqueous solution at physiological pH. AsIII and AsV, on the other hand,
440 functions as a phosphate structural analogue and is taken up by membrane phosphate
441 transporters. GlpF is an aquaglyceroprotein that helps AsIII pass the cell membrane (Kabiraj
442 et al., 2022). Several microorganisms have GlpF homologies. Pit and Pst are phosphate
443 transporter proteins that let AsV get into cells (Yang et al., 2012). Pst is a phosphate-specific
444 transport system, whereas Pit is a universal transport system. Because of its configurational
445 closeness to phosphate, arsenate is easily absorbed through the Pit transport system. Because
446 Pst transports AsV inefficiently, microbial communities revealed to elevated levels of
447 arsenate express Pst solely to limit arsenate uptake. Arsenic is extruded by bacteria by two

448 methods. The first is carrier-mediated efflux through an arsenite carrier protein, which is
449 powered by the cell's membrane potential, and the second is arsenite-translocating ATPase
450 (Yang et al., 2012).

451 In bacterial *ars* operon, ArsC enzyme plays key role in arsenate reduction and As
452 resistance which is previously discussed. Bacterial transformation of As includes respiratory
453 As(V) reduction and As(III) oxidation for autotrophic growth are called “arsenotrophy”
454 (Oremland et al., 2009). The interconversion of As(V) and As(III) in arsenotrophy is
455 catalyzed by one of the three enzymes of the dimethyl sulfoxide (DMSO) reductase family,
456 arsenate reductase (ARR) and two distantly related arsenite oxidases, AIO and ARX (Ospino
457 et al., 2019).

458 The *arsM* genes are directly involved in As methylation (Kabiraj et al.,2022). The
459 methylation pathway proposed for prokaryotes (Stolz et al. 2006) is same with *Scopulariopsis*
460 *brevicaulis* (Challenger,1945). It starts with As(V) and following a series of oxidative
461 methylations by S-adenosylmethionine methyltransferases and reductive steps with
462 glutathione and other thiol containing compounds to the end product TMAs, which is
463 currently thought to be rather safe (Cullen and Bentley 2005). An overview of bacterial As
464 metabolism are given in Figure 4.

465 **Unveiling the importance of plant growth promoting microorganisms in As** 466 **remediation**

467 **Arsenic resistance mechanisms in PGPB (Plant Growth Promoting Bacteria)**

468 Microorganisms that can tolerate high levels of As have a variety of As-resistance systems
469 that may be used in bioremediation procedures. Bacterial As-resistance pathways can be
470 found in a variety of bacteria. Sequestration can be divided into two categories: intracellular
471 and extracellular sequestration, as well as active sequestration. Biosorption and
472 bioaccumulation are two effective strategies for extracellular and intracellular As

473 sequestration, respectively. Due to their big surface-to-volume proportion and abundance of
474 active chemisorption capacities, microbial cells are frequently considered as effective bio-
475 sorbents (Sharma and Archana, 2016).

476 Biosorption is a physicochemical process which rummages heavy metals onto the
477 surface of bacterial cells without using any energy. The toxicants have no impact on bacterial
478 metabolism and have no toxic effects since they cannot enter the cell. Peptidoglycan and
479 phosphate groups found in both gram-positive and gram-negative bacteria can behave as
480 cationic and anionic binding sites. Extracellular polymeric substances (EPS), a large
481 molecular mass bacterial secretory derivative that can also operate as a hazardous metal(loid)
482 absorbent, are generally composed of mucopolysaccharides, polysaccharides, lipids, proteins,
483 uronic acids, and humic substances (Sengupta and Dey, 2019).

484 The presence of acetamido, amine, sulfhydryl, carboxyl, and phosphodiester groups in
485 proteins, as well as phosphate, hydroxyl, and polysaccharide groups, gives EPS a negative
486 charge that promotes metal sequestration (Mukherjee et al., 2019). The energy-intensive,
487 slow, and persistent process of bioaccumulation, in contrast, is how heavy metals build up
488 inside the microbial cell after passing through ion pumps, ion channels, endocytosis, and lipid
489 penetration. This, unlike biosorption, is a metabolism-dependent mechanism that has harmful
490 effects on bacteria. The metal is likely rummaged inside the cell by cysteine-rich
491 metallothionines (Mondal et al., 2021).

492 Biofilm development has also been observed among these PGPR. The biofilm
493 structure is normally made up of EPS, proteins, and extracellular nucleic acids. Armendariz et
494 al. (2015) and Vezza et al. (2020) found that it borders the cell populations across its surface,
495 which additionally impede metal diffusion. By far the most researched method is active
496 extrusion of the metal from the bacterial cell. Extrusion mediated by the ars operon
497 (ArsB/ArsAB) is broadly allocated and ably defined (Mondal et al., 2021). *Sinorhizobium*

498 *meliloti* has a novel detoxification route in which As(III) is extruded via an aquaglyceroporin
499 (AqpS)-encoded aquaglyceroporin. Surprisingly, AqpS replaces arsB inside the ars operon
500 (Yang et al., 2005). An overview of As resistant mechanisms and plant growth promotion
501 activities are given in Figure 5.

502 **Novel Technological advances in the As bioremediation field**

503 The application of nanoparticles, non-living biomass and genetically modified plants for
504 abolition of metal pollution from various origins attributed to having fast and big
505 bioremediation capacity (Gaur et al., 2014).

506 **Nanoparticle utilization**

507 Use of nanotechnology is being widely utilized for the progress of creative, methodical and
508 environment friendly nanomaterial structures in various domains of bioremediation. The
509 physiochemical characteristics of the nanoscale molecules vary notably from their bigger
510 counterparts. Due to high surface to volume ratio of nanomaterials it gives them big amount
511 of adsorption potentiality. They also have a low cost and increased bioavailability, making
512 them ideal candidates for bioremediation.

513 Because of this, nanoparticles have been discovered to be employed as an adsorbent;
514 in compared to macroparticles, this opens up a wider range of possibilities. Arsenic has
515 previously been removed using nanoparticle-based adsorbents generated from metals and
516 metal oxides such as TiO₂, Fe₂O₃, and NiO nanoparticles, as well as cupric oxide
517 nanoparticles. This method has been employed for nano iron (hydr) oxide impregnated
518 granulated activated carbon in this context (Hristovski et al., 2009). It is preferable to use
519 ecologically friendly materials in the production of metal nanoparticles. In this respect,
520 attempts have been made to produce silver nanoparticles using fungal extracellular enzymes,
521 such as the silver nanoparticles synthesised by using *Aspergillus foetidus* (Roy et al., 2013).

522 **Non-living biomass**

523 Heavy metals can be absorbed by nonliving marine algae from dilute water solutions. Such
524 sorbents have yielded promising results; many studies on the sorption ability of marine
525 organisms may be found in the literature (Jalali et al., 2002). Cationic metals, like as copper,
526 have been shown to be beneficial to macrophytes. Arsenic, lead, cadmium, zinc, and
527 chromium are just a few examples of such phenomenon (Mudhoo et al., 2012). *Cystoseira*
528 and *Dictyopteris* are the two brown algal candidates which have a high As adsorption
529 capacity. *Dictyopteris* performed admirably, with As-specific absorption values comparable
530 to the highest ever recorded (Pennesi et al., 2012).

531 **Transgenic crops**

532 Transgenic *Arabidopsis thaliana* plants that expressed the *C. reinhardtii* As (III)-S-
533 adenosylmethyltransferase (arsM) gene was also reported (Kabiraj et al., 2022). These plants
534 were able to convert the majority of inorganic As into DMA(V) and volatile As species in
535 their shoots. By heterologously indicating PvACR3 in the *athac1* background and beating
536 down the HAC1 gene, *Arabidopsis thaliana* was transformed into an As accumulator.
537 AtHAC1 is an As reductase (Wang et al. 2018). As efflux was decreased in the medium when
538 the As reductase was mutated (Zhang et al. 2018). The utterance of the vacuolar As
539 transporter ACR3 in the roots did not promote As(III) efflux into the medium or vacuolar
540 sequestration in these transgenic plants, but it did aid As loading into the vasculature and
541 encouraged translocation to the shoots. PvPht1;3 is expressed in stele cells in transgenic *A.*
542 *thaliana* and soybean, and it is thought to have contributed to P/As translocation. As move
543 and build-up in shoots are increased by PvPht1;3 expression, which may enhance As
544 phytoextraction in As-contaminated soils (Bertin et al., 2021).

545 **Conclusion and future scope**

546 The United Nations' Sustainable Development Goals (SDGs) place a major emphasis on
547 decreasing pollution. For example, SDG 3.9 seeks to “substantially reduce the number of

548 deaths and illnesses from hazardous chemicals and air, water and soil pollution and
549 contamination” by 2030. Achieving sustainable global development requires urgent action to
550 limit As pollution exposure through effective management of both historic pollution legacies
551 and contemporary releases of pollutant. To maximise the opportunity to manage or mitigate
552 As pollution effectively, bioremediation technique is effective and promising in terms of its
553 sustainability and eco friendliness. Use of PGPMs (bacteria, fungi and algae) for remediation
554 of As contaminated soils has been explored by researchers worldwide but it still warrants
555 refinement. More research is desired to screen the prospective microorganisms for
556 remediation of As polluted soils as well as to address the issues of co-contamination of other
557 heavy metals at a time and also to identify the potential combination of the PGPMs. Research
558 should be carried out on the feasibility and applicability of these potential bioremediation
559 techniques in field conditions. This will enable the researchers to identify and address the
560 issues faced by the stakeholders (farmers) in application of a technology at the real time field
561 condition.

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763 **Table 1:-List of the microorganisms which help in bioremediation**

Name of the microorganisms	As tolerance capacity	Mode of action	References
Bacteria			
<i>Bacillus flexus</i>	12%	Bioaccumulation	Upadhyay et al., 2018
<i>Bacillus sp. SF-1</i>	56% within 70 h	Mobilization by reduction	Wan et al.,2020
<i>Bacillus vietnamensis</i>	Tolerate 20mM of As(III)	Reduced bioavailability	Upadhyay et al., 2018
<i>Bacillus aryabhatai AS6</i>	20 mM 100 mM	Bioaccumulation	Mondal et al.,2021
<i>Bacillus tequilensis</i>	18 mM	Arsenite oxidation	Mondal et al.,2021
<i>Bacillus licheniformis</i>	30 mM	Biofilm formation	Mondal et al.,2021
<i>Burkholderia cepacia</i>	55% of arsenate	Biotransformation	Laha et al.,2021
<i>Burkholderia metallica</i>	73% of arsenate	Biotransformation	Laha et al.,2021
<i>Brevibacillus sp KUMAs1</i>	55% within 96 h	Oxidation	Wan et al.,2020
<i>Rhodopseudomonas palustris</i>	Remove 2.2%–4.5% of As	Bio volatilization	Wan et al.,2020
<i>Geobacter metallireducens GS-15</i>	-	Mobilization	Wan et al.,2020
<i>Acinetobacter junii</i>	Volatize 14% of As within 72 h	Biovolatilization	Wan et al.,2020
<i>Acinetobacter lwoffii (RJB-2)</i>	125mM As(V), 50mM As(III)	Biofilm formation	Mondal et al.,2021
<i>Sporosarcina ginsengisoli CR5</i>	99% within 10 d	-	Upadhyay et al., 2018
<i>Kocuria flava</i>	Can tolerate 35mM of As(III)	Reduced bioavailability	Mondal et al.,2021
<i>Methylobacterium oryzae</i>	580µM As(V)	-	Mondal et al.,2021
<i>Ralstonia eutropha</i>	Reduced As content (22–50%) of edible portion of crop	Mobilization	Mondal et al.,2021
<i>Rhizobium tropici</i>	Reduced As content (22–50%) of edible portion of crops	Mobilization	Mondal et al.,2021
<i>Exiguobacterium aurantiacum</i>	Reduced As content (22–50%) of edible portion of crops	Mobilization	Mondal et al.,2021
<i>Brevundimonas diminuta</i>	150 ppm As(V), 20 ppm As(III)	-	Mondal et al.,2021
<i>Brevundimonas diminuta NBRI012</i>	150 mM	Bioaccumulation	Mondal et al.,2021
<i>Exiguobacterium sp. As-9</i>	180 mM	-	Mondal et al.,2021
<i>Klebsiella pneumoniae</i>	50 mM	As(III) oxidation	Mondal et al.,2021
<i>Klebsiella oxytoca</i>	50 mM	As(III) oxidation/Arsenite	Mondal et al.,2021

		oxidase gene	
<i>Luteimonas aestuarii</i>	50 mM	As(III) oxidation/Arsenite oxidase gene	Mondal et al.,2021
<i>Escherichia fergusonii</i>	50 mM	As(III) oxidation/Arsenite oxidase gene	Mondal et al.,2021
<i>Psychrobacter faecalis</i>	50 mM	As(III) oxidation/Arsenite oxidase gene	Mondal et al.,2021
<i>Bacillus safensis</i>	50 mM	As(III) oxidation/Arsenite oxidase gene	Mondal et al., 2021
<i>Escherichia fergusonii</i>	50 mM	As(III) oxidation/Arsenite oxidase gene	Mondal et al., 2021
Rhizosphere Fungi			
<i>Trichoderma sp.</i>	650 ppm As(III)	-	Upadhyay et al., 2018
<i>Piriformospora indica</i>	100µM As	Bioadsorption	Upadhyay et al., 2018
Arbuscular mycorrhizal fungi			
<i>Rhizoglyphus intraradices</i>	100 ppm As(V)	Maintaining favorable P: As	Upadhyay et al., 2018
<i>Rhizophagus intraradices</i>	Decreased plant As accumulation from 7.8mg As kg ⁻¹ to 6.0mg As kg ⁻¹	Up regulation of phosphate transporter- <i>RiPT</i> , putative As efflux pump- <i>RiArsA</i>	Upadhyay et al., 2018
<i>Rhizophagus intraradices</i>	60 ppm As(V)	Biomethylation	Upadhyay et al., 2018
<i>Glomus geosporum</i>	50% reduction of arsenic	Through enhancing P/As ratios	Upadhyay et al., 2018
<i>Glomus versiforme</i>	50% reduction of arsenic	Through enhancing P/As ratios	Upadhyay et al., 2018
<i>Glomus mosseae</i>	50% reduction of arsenic	Through enhancing P/As ratios	Upadhyay et al., 2018
<i>Glomus etunicatum</i>	100 ppm As(V)	Maintaining favorable P: As	Upadhyay et al., 2018
Algae			
<i>Chlorella vulgaris</i> and <i>Nannochloropsis sp.</i>	-	Reduced oxidative stress, As toxicity 1000µM As(III)	Upadhyay et al., 2016
<i>Anabaena sp</i>	60µM As(V) and As(III). As transporter	Mobilization	Upadhyay et al., 2018
<i>Pseudomonas putida</i> and <i>Chlorella vulgaris</i> consortium	50µM As(V)	Biofilm formation	Upadhyay et al., 2018

764 **LIST OF FIGURES:**

765 **Figure 1.** Bioremediation approaches for environmental clean-up (Sharma, 2020).

766 **Figure 2.** Plant used in arsenic phytoremediation

767 **Figure 3.** Chemical reaction of arsenic (As) transformation/removal from soils (Shrivastava et al., 2015)

768 **Figure 4.** Bacterial arsenic metabolism mechanism. As enters in cell through glycerol or phosphate
769 transporters. Arsenate is transformed to arsenite which may then be extruded from the cell by ArsAB.
770 Further As is methylated by ArsM. During methylation, the formation of volatile intermediates
771 monomethyl arsenite (MMAs (III)), dimethyl arsenite (DMAs (III)), and trimethyl arsine (TMAs) is
772 found. Arsenite's presence regulates the ars operon's expression and has an impact on a number of
773 functions, including DNA repair, oxidative stress response, motility, and EPS synthesis. Arsenite
774 oxidation or arsenate reduction can be employed to get energy from arsenic that isn't inside the
775 cytoplasm.

776 **Figure 5.** Mechanisms of bioremediation and plant growth promotion by microbes









