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Contagious Progression and Distribution of Arsenic in India: A Key Towards Bioremediation

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ABSTRACT

Arsenic (As) is a renowned threat to the environment and human well-being. Its concentration is increasing year after year in several countries. The utmost pretentious are mining regions of India, as per government surveys and available research findings. Population residing near mining regions are bounded to consume arsenic tainted water in their routine life and evolve various hazardous health problems. Besides many physicochemical techniques at hand for its purification, none are promising. The microbial mediated arsenic detoxification involving oxidation/reduction and extrusion by a membrane-associated efflux pump may perhaps financially acuity and a promising method for bioremediation. The arsenic richness in mining regions triggered the evolution of bacterial cells to come up with a potential mechanism to survive in As rich environment. Microbial extrusion strategy of As in both As^{3+} and As^{5+} forms may also be involved in increasing As in abandoned mining regions in underground water. So, to understand the involvement of these bacterial cells in the increment of As in these regions the present study was performed by personally visiting these sites and conversation with local residents. We have witnessed many jaw-dropping truths about As exposure risk to humans and domesticated animals, which has been discussed in this article. This review comprehensively summarizes current studies associated with arsenic exposure, environmental dispersal and its bioremediation through arsenic metabolizing bacteria covering recent developments, pathways, action mechanism and understanding arsenic metabolizers with the depiction of future prospects on arsenic bioremediation from contaminated systems.

INTRODUCTION

Life standards have been increased in today's world with the realization of environmental threats and issues. Heavy metals besides PAHs (polycyclic aromatic hydrocarbons) are chronic contaminants broadly distributed in the surroundings, therefore simple remediation methods are privileged over high-priced physicochemical strategies (Singh et al. 2011).

Heavy metals persist in nature for a long time as they cannot be degraded by decomposers rather gets accumulated. Usually, arsenic remains along with minerals, like sulphur and iron. On average, its abundance in earth crust is 1-2 mg/Kg having two forms As^{3+} (arsenite) that is a pretty toxic form than As^{5+} (arsenate) (Meliker et al. 2008). Arsenic (As) tainting in groundwater is a prominent issue among mining regions and utilization of deep tube-wells for water supply, causing arsenic poisoning. Volcanic eruptions, weathering, and anthropogenic sources are accountable for arsenic release in groundwater including smelting, mining, etc. (Bhattacharya et al. 2007). Arsenic discharge from coal ventures likewise represents across the board pollution of soil and groundwater (Dontala et al. 2015).

According to the consolidated hazardous chemicals list of the Environmental Protection Agency (EPA) of 2001, the United States, exposure to arsenic causes acute and adverse health issues. In 2001, EPA declared arsenic limits for drinking water (10 µg/L) which is being employed till today. Arsenic pollution extent and its potential danger to human wellbeing have brought about extensive enthusiasm for concentrate microbial species in-charge of the diminishment of arsenic (Mirza et al. 2017). Drinking water mining from shallow tube-wells is among vital pathways for its entrance into human bodies (Chakraborti et al. 2017b). Human contact to arsenic predominantly occurs by utilizing groundwater (cooking or crop irrigation) which have increased inorganic arsenic levels (WHO) in humans as well as plant body. Various geological and other factors were acknowledged enhancing arsenic mobilization, affecting many regions in India (Philp 2015). Arsenic translocation and bio-magnification have also impacted numerous important crops (Chakraborty et al. 2014).

The As-toxicity relies on its biochemical nature and uses phosphate transporters for entering into a bacterial cell (Nordstrom 2002). Aquaporin mediated As entrance in the cell, blocks the function of many proteins by altering their functional groups. It also affects respiration by binding to PDh (pyruvate dehydrogenase), 2-oxoglutarate dehydrogenase and other enzymes leading to DNA damage by inhibiting its repairing mechanism (Bhattacharjee et al. 2005). Oxidative phosphorylation is caused by As due to hindrance created by it in enzymatic activity (Jomova et al. 2011). Like-wise As^{5+} structural similarity to phosphate is responsible for its access to active cells, interrupting oxidative phosphorylation (Kumari & Jagadevan 2016). The ingestion of large doses of arsenic causes fatal health problems (Fig. 1) and prolonged contact produces lesions in skin (Mazumder 2015). Arsenic carcinogenicity is already well known however, its lung cancer mechanism has not well been understood (Wei et al. 2019). Metals serve vital roles (in small quantities) in living beings, serving essential catalytic roles (Ryan et al. 2005). Microorganisms can metabolize metals through various methods (Silver & Phung 2005). Diverse detoxification strategies were developed by bacteria to encounter arsenic toxicity and among them, one is transforming it to a less lethal form (Turner 2001). Among reduced arsenic species, arsenite is more portable and dangerous while arsenate is less toxic in comparison (Edwards et al. 2000).

National rural drinking water (NRDW) programme has been adapted by State governments for providing clean water to affected habitations (Tomar 2017). An objective of making accessible safe and hygienic drinking water by 2021, is set by the Government of India (GOI) for 28,000 habitations extremely pretentious with arsenic contamination (Dey 2017). Due to increasing health issues, the Ministry of Drinking Water and Sanitation (MDWS) has launched 'National Water Quality Sub-Mission' for the habitants of affected regions (Chakraborti et al. 2017a). The MDWS has also commissioned around 35 developmental projects for water quality improvement in rural and urban areas (Omar et al. 2017).

According to a recent report demonstrated in Rajya-Sabha (Feb. 2017) from MDWS, various schemes have been executed equipping clean water in pretentious areas of the country. Expanding the reliability of numerous nations on groundwater has increased the focus on safe remediation strategies (Kadushkin et al. 2004). Arsenic expulsion technique in influenced regions could be the only option for a healthy water supply. The arsenic alleviation approach has to be implemented according to specific geographical and socio-economic characteristics of the area.

GLOBAL ARSENIC EPIDEMIOLOGY

Arsenic is affecting a major World population with several

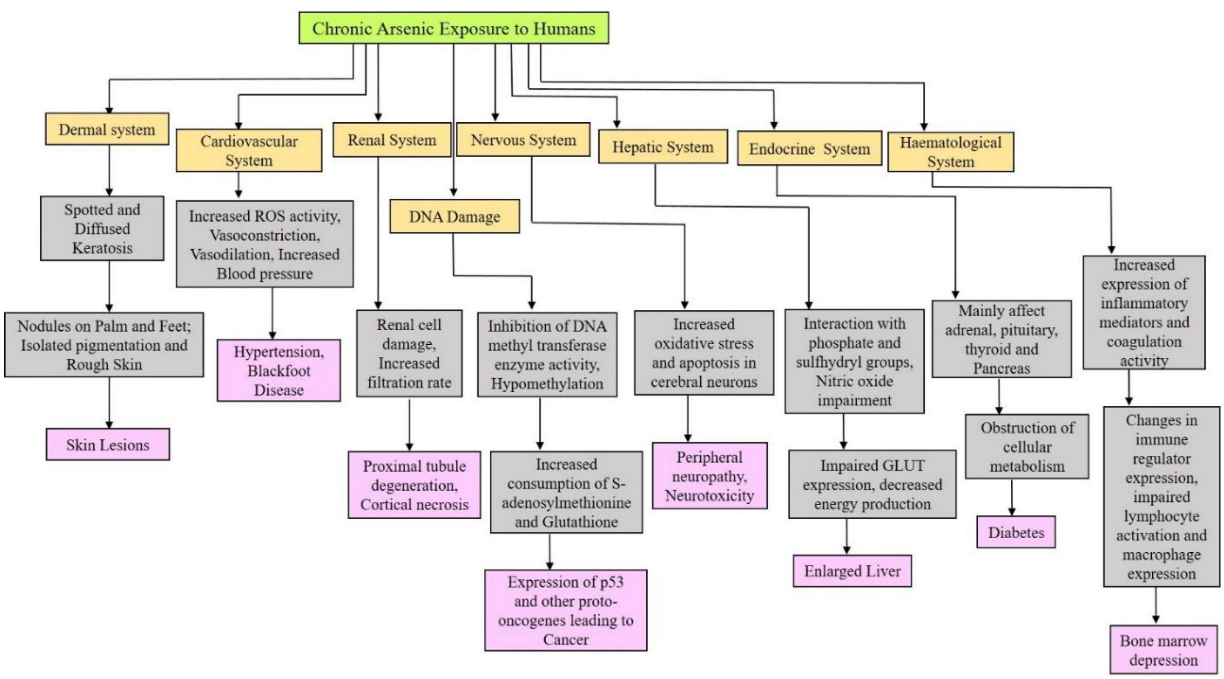


Fig. 1: Arsenic exposure and human health effects.

impacts and has now become a foremost environmental concern (Chikkanna et al. 2019). Detecting arsenic for a normal person is nearly impossible due to the absence of flavour, colour and aroma. People relying on groundwater having eminent arsenic level are vulnerable to its toxicity risk. Abandoned mines cause arsenic arrival in groundwater leading to an increase in its concentration (Hajalilou et al. 2011). Nowadays research on arsenic accumulation is going on to understand its speciation.

The application of arsenic-rich water for irrigation is a key factor responsible for high soil arsenic accumulation. Soil samples were investigated by Sandhi and co-workers during the ripening stage of rice from different paddy fields and arsenic aggregation was observed to be higher (90 to 210 μg/kg) in dehusked grains than in husked grains (Sandhi et al. 2017). Certain ecological advancements contain abnormal arsenic amounts that reach wells and various public water deliveries through leaching (McArthur 2019). Quantitative investigations of groundwater samples of Mongolian regions indicate that arsenic species are vastly associated with Fe species controlled by geographical and redox factors (Jiang et al. 2015). Arsenite was detected in rice samples from Korea with concentrations of 28.51 and 51.91 μg/kg (Kwon et al. 2017).

In Chile, the capital of Santiago, millions of tube wells were found containing arsenic (>50 μg/L) and 7600 people are affected (Quinodóz et al. 2019). Various nations specifically, India, Bangladesh and Vietnam are at great risk, where the one-third population is drinking arsenic-polluted water (WHO 2010). Around 57 million individuals are devouring arsenic-contaminated water above recommended limits in India and many other countries (Table 1) (Jha et al. 2017).

EPIDEMIC ARSENIC CONSEQUENCE IN INDIA

In India, groundwater contamination is severely problematic due to futile purification systems and intermittent floods and monsoon (Kadushkin et al. 2004). Mining, industrialization, ore processing has deleteriously impacted the environment through ecosystem alterations, biodiversity damage and accumulation of toxic pollutants (Singh & Singh 2016). These activities produce enormous hazardous wastes, left without treatment. Abandoned mines contaminate groundwater by redox events and anaerobic conditions,

Table 1: Worldwide distribution of arsenic (Santra et al. 2013, Herath et al. 2016).

S.No.	Country	Region	Groundwater Arsenic level (μg/L)	Permissible limit (μg/L)
1	Afghanistan	Ghazni	10-500	10 (WHO)
2	Australia	Victoria (around the gold-mining regions)	1-12 (Groundwater); 1-73 (Drinking-water); 1-220 (Surface water)	NIL
3	Bangladesh	Noakhali	<1-4730	50 (WHO)
4	Brazil	Minas Gerais (Southeastern Brazil)	0.4-350 (Surface water)	10 (WHO)
5	Cambodia	Prey Veng and Kandal-Mekong delta	Up to 900 1-1610	10 (WHO)
6	Canada	Nova Scotia (Halifax County)	1.5-738.8	10 (WHO)
7	China	-	50-4440	50 (WHO)
8	Finland	Southwest Finland	17-980	10 (WHO)
9	Greece	Fairbanks (mine tailings)	Up to 10,000	10 (WHO)
10	India	West Bengal Uttar Pradesh	10-3200	50 (WHO)
11	Japan	Fukuoka Prefecture (southern region)	1-293	10 (WHO)
12	Mexico	Lagunera	8-620	25
13	Nepal	Rupandehi	Up to 2620	50
14	Pakistan	Muzaffargarh (southwestern Punjab)	Up to 906	50
15	Taiwan	-	10-1820	10 (WHO)
16	Thailand	Ron Phibun	1->5000	10 (WHO)
17	USA	Tulare Lake Red River Delta	Up to 2600	10 (USEPA)
18	Vietnam	(Northern Vietnam) Mekong Delta (Southern Vietnam)	<1-3050	10 (WHO)

accumulating particulates in groundwater sources, hence, contaminated water treatment is considerably essential before consumption (Ayangbenro & Babalola 2017). It has been reported that even after abandoning the mining activity, the concentration of As increases, which may be due to the microbial transforming system (Zhang et al. 2019). It adversely affects the flora and fauna of mining regions.

With the aim of providing safe drinking water, a number of wells were developed earlier (advocated by UNICEF and World Bank) resulted in decreased newborn mortality and diarrheal disease by 50%. But as per investigation, approximately one among five wells are now arsenic tainted above EPA standard (Chakraborti et al. 2008). Nine hundred villages were found to have arsenic above the standard limit, where groundwater is mostly extracted by deep tube-wells having higher 'As' sediments (Shah 2010).

WHO has reported many regions of West-Bengal and other states, consuming high arsenic-contaminated drinking water (Ahamed et al. 2006). Government programs to provide 'safe' drinking-water, controlled arsenicosis, but in a few areas problem is still the same. The districts situated nearby Ganga and Gandaki river were scrutinized and found that the arsenic affected far above WHO defined limit (10 µg/L) (Shah 2010). In 2016, the Mahavir Cancer Institute, Bihar analysed 23,000 new malignancy patients, and these cases were due to arsenic poisonous quality (Chakraborti et al. 2017b). A number of other states like, Jharkhand, Madhya Pradesh, Chhattisgarh and Assam are facing arsenic tainted water issues. Sahibganj district of Jharkhand situated in the middle Ganges plain has arsenic >50 µg/L (Ramanathan et al. 2006). People residing in those areas have no alternative and are continuing with the same exposed risk (Chakraborti et al. 2003). Arsenic disasters are happening today and most outstandingly due to drinking water contamination. More than 40 million individuals in India, are exposed to 50 µg/L or above arsenic. The scenario is equally bad in some districts of Bihar, West Bengal and Uttar Pradesh (Milton et al. 2001). Chhattisgarh state was parted from Madhya Pradesh (year 2000). Border regions of MP and Chattisgarh district are mostly affected by arsenic contamination. In 1999 Rajnandangaon district of Madhya Pradesh was reported to have high groundwater arsenic concentration and above a million of the population of Rajnandangaon is consuming arsenic polluted water (Patel et al. 2017). Some dug-wells, along with hand tube-wells, are contaminated with higher (520 µg/L) arsenic concentration in West Bengal and Rajnandgaon (Saha & Ray 2019).

The As^{3+} concentration in groundwater of Barasat (Gangetic plain), West Bengal was found excessive than As^{5+} (10 to 538 µg/L), showing reducing conditions (Kar et al. 2010). The reduction activity of iron/sulphur oxides was a foremost mechanism for arsenic release into groundwater (Sichone 2019). Several states were also discovered as influenced with maximum arsenic level (3,700 µg/L) (Tchounwou et al. 2019).

Groundwater arsenic release mechanism was investigated in Balia district, U.P and 468 µg/L concentration was found at depths of 30-33 m (Chauhan et al. 2009). Groundwater arsenic concentration of Sahibganj district, Jharkhand was highest in post-monsoon (133 µg/L) compared to monsoon (98 µg/L) and pre-monsoon (115 µg/L) (Alam et al. 2016). The water standard of the Dhanbad area, Jharkhand was not up to the mark (Masto et al. 2011). Hydro-geochemical processes and isotropic rock tracing of aquifers were studied in the East-Singhbhum area of Jharkhand. Temporal and seasonal differences affect groundwater quality significantly (shallow aquifers) due to disparity in flow, recharge, geochemical processes. Groundwater arsenic concentration of Bishnupur locality, Manipur was found highest in post-monsoon and increase is anticipated in nearby future (Chakraborti et al. 2018).

Many people of West-Bengal are affected by arsenic exposure, as per the latest report presented in Lok-Sabha. Although State governments are determined to manage the arsenic peril, still more is left to finish (Gupta & Singh 2019). Since the skill for arsenic elimination from water is novel and expensive, there is a progress lag in setting water management plants (Shan et al. 2019). Comprehensive detail on groundwater, Kolkata Municipal Corporation (KMC) stated a higher arsenic level (>50 µg/L) (Chakraborti et al. 2017a). A proceeding of Lok Sabha, 2017 by MDWS, says that states like Himachal Pradesh, Punjab, Haryana, Assam, Arunachal Pradesh, Karnataka, Kerala and many additional regions are moderately or severely affected with arsenic (soil or water) contamination (Ali et al. 2019).

ARSENIC DETOXIFICATION GENES

The majority of microorganisms have evolved with arsenic detoxification systems (Yan et al. 2019). Various studies elucidating its molecular processes were conducted against many microorganisms. Microbes deliberate arsenic detoxification with the assistance of the ars operon framework (Thul et al. 2019). This operon possesses either three (arsR, B, C) or five (arsR, A, B, C, D) gene components (Table 2). This (ars) operon exists either on plasmids or integrated with genome (Firrincieli et al. 2019). The operon (ars) gene encrypts proteins for repression in absence of arsenic, arsenate reduction, its efflux supporting detoxification system. The ars homologs were studied in diverse biological structures like; fungi, plant and animals (Fernández et al. 2014).

MICROBIAL ARSENITE RESISTANCE

Microbes evolved mechanism for enzymatic oxidation (As^{3+} to As^{5+}) for arsenite or reduction (As^{5+} to As^{3+}) for arsenate. They do carry redox events and are imperative players in the arsenic geocycle (Turner 2001). Bacteria resist their toxic effects by preventing their intake or actively exporting the arsenicals or by modifying them enzymatically or chemically. Therefore paramount search for more oxidizing (arsenite) bacteria concerning bioremediation is greatly significant (Verma & Kuila 2019). Many microorganisms show resistance to arsenic especially arsenate, while there are only a few bacterial isolates known exhibiting arsenite resistance (Tian et al. 2019). Arsenite concentration was proved lethal on or above 200 µg/L for many bacteria. However, arsenite directly can be methylated by *P. alcaligenes* (arsenite S- adenosylmethionine methyltransferase) (Zhang et al. 2015). Arsenite oxidation attenuates toxicity, provided its re-conversion in the cell does not happen. Bacteria harbour transport protein for both arsenic valencies and their transformation catalysing enzymes (Oremland & Stolz 2003). Presently, it is typically assumed that arsenite oxidizing microbes can harbour both oxidase and reductase enzymes. Both enzyme system in arsenic resistant isolate was found, however, the simultaneous occurrence of two enzymatic systems jeopardize the bioremediation process (Dunivin et al. 2019).

SOLUBILIZATION, MOBILIZATION AND UPTAKE OF ARSENIC

Arsenic solubilization depends on its speciation and transformation (Smedley & Kinniburgh 2002, Cullen & Reimer 1989). Arsenic is additionally found in different methylated forms in the mine drainage system and geothermal inputs. Anaerobic condition in the underground region holds the highest arsenic concentration, where it favours the geochemical conditions for its solubilization (Cullen & Reimer 1989). Fe-Mn oxides at the water-sediment interface scavenge arsenic from mine tailings and from contaminated water that causes As concentrations to remain enriched in the upper sediments even after mine tailings have been ceased (Sprague & Vermaire 2018).

Structural similarity of As^{3+} and As^{5+} to phosphate

Table 2: Characterized arsenic resistance gene cluster among microorganisms.

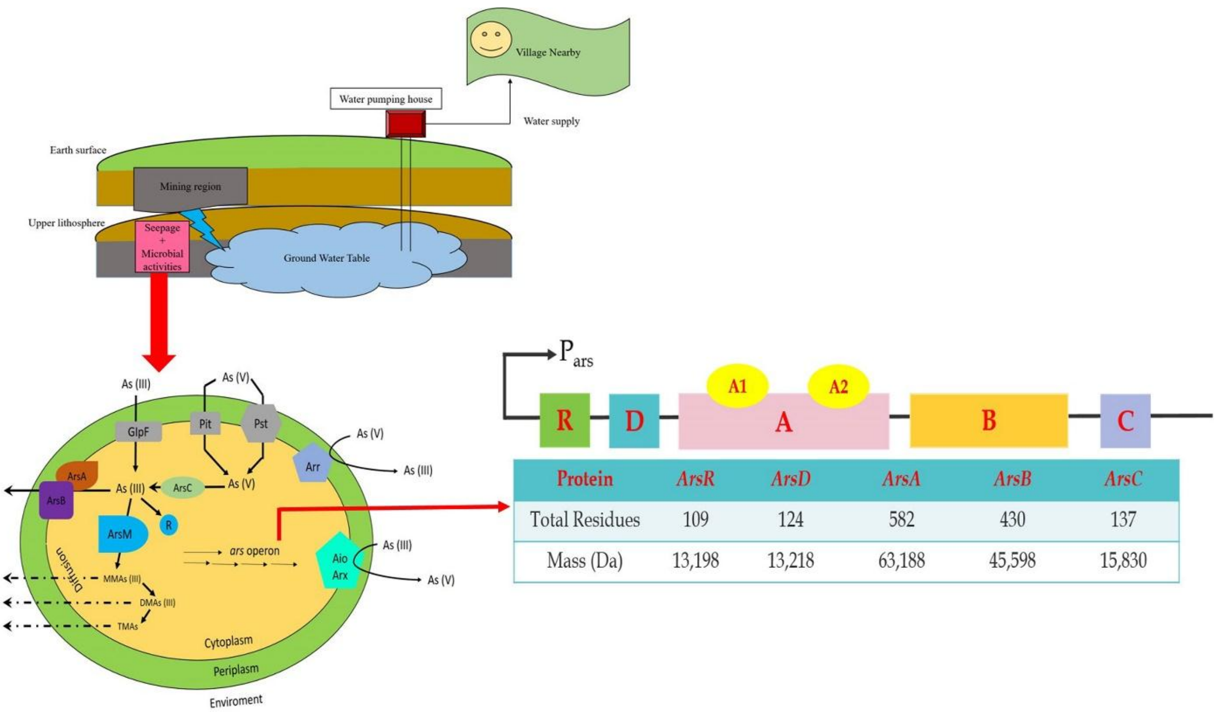
S.No	Name of organism	*ars* genes	References
1	*E. coli* plasmid R773.	arsR, A, B, D, C	Hedges & Baumberg 1973
2	*Staphylococcus xylosus* plasmid pSX267.	arsR, B, C	Rosenstein et al. 1992
3	*Staphylococcus aureus* plasmid pI258	arsR, B, C	Ji & Silver 1992
4	E coli W3110	arsR, B, C	Carlin et al. 1995
5	Yersiniae sp.	arsR, B, C, H	Neyt et al. 1997
6	*Acidiphilium multivorum* AIU 301 plasmid pKW301	arsR, D, A, B, C	Carlin et al. 1995
7	Bacillus subtilis	*arsR*, B, C *ORF2*	Sato & Kobayashi 1998
8	Pseudomonas aeruginosa	arsR, B, C	Cai et al. 1998
9	Acidithiobacillus ferrooxidans	*arsR, C* and *arsB, H*.	Butcher et al. 2000
10	*Pseudomonas fluorescens* strain MSP3	arsR, B, C	Prithivirajsingh et al. 2001
11	*Synechocystis* sp. Strain PCC 6803	arsB, H, C	López-Maury et al. 2003
16	*Shewanella* species ANA-3	arsR, B, C	Saltikov et al. 2003
12	*Halobacterium* sp. Strain NRC-1	arsA, D, R, C, arsR2, M	Wang et al. 2004
13	*Corynebacterium glutamicum* ATCC 13032	arsR, B, C	Ordóñez et al. 2005
14	Sinorhizobium meliloti.	arsR,apqs C	Yang et al. 2005
15	Acidithiobacillus caldus	arsD, A, B	Kotze et al. 2006
17	*Streptomyces* sp. Strain FR-008.	arsR, O, B, T, C	Wang et al. 2006
18	Leptospirillum ferriphilum	arsR, B, C, arsR, C, D, A, B	Fournier et al. 2006
19	Acinetobacter baumannii	arsR, B, H, C	Fournier et al. 2006
20	*Ochrobactrum tritici* SCII24T	arsR, A, B, D and arsR, C, H ,ACR3	Branco et al. 2008

Note: (i) arsR (arsenical resistance operon repressor), arsB (arsenical pump membrane protein), arsC (arsenate reductase), arsH (unknown), arsD (arsenic operon regulator), arsA (ATPase subunit). (ii) arsR, B, C makes the main detoxification system, arsD and arsA are supporting proteins.

typically aid prokaryotic cells for their uptake through various transporters (Fig. 2). More specifically, the Pit-phosphate transporter is in-charge of arsenate uptake in *E.coli* cells (Willsky & Malamy 1980). Pst-phosphate transporter is also found transporting arsenate in some bacterial cells but at a low level than "Pit" (Bertin et al. 2011). Microbial flora which is continually presented to high measures of arsenate express just "Pst" according to lessen arsenate take-up (Meng et al. 2004). In *E.coli*, GIpF (aquaglyceroporins) is stated for foremost As^{3+} uptake (Banerjee et al. 2018). Dissimilatory Arsenate Reducing Bacteria (DARB) phylogenetically belong to a diverse group and respire As^{5+} by reducing it anaerobically. DARB own a conserved arsenate reductase (arrA) gene, essential for reduction system (Burton et al. 2014). It is mostly utilized biomarker estimating variations in arsenic reducing microbes across different environments. Most bacteria utilize a similar process of As detoxification involving its uptake, reduction and extrusion using an efflux pump (Kumari & Jagadevan 2016).

ARSENITE OXIDATION (AS^{3+} TO AS^{5+})

Oxidation of As^{3+} represents a promising detoxification process that allows microorganisms to tolerate their toxic levels in tainted locations (Santini & Hoven 2004). So far, isolated arsenite oxidising prokaryotes can be grouped in two categories: (i) Chemolithoautotrophs (aerobes or anaerobes, using arsenite as electron donor and CO_2/ HCO_3^- as specific carbon source) (ii) Heterotrophs (grow in organic matter) (Oremland et al. 2002). Chemolithoautotrophic bacteria *NT-26* and *MLHE-1* oxidizes As^{3+} to As^{5+} using oxygen and NO_3^- as electron acceptor (Ellis et al. 2001). Several heterotrophic bacteria are persuaded to oxidise As^{3+} provided in growing media using arsenite oxidase. Arsenite oxidase activity was measured biochemically in presence of azurin or cytochrome-C (Pandey et al. 2009). Yet, at the alike period, it looks that most ecological isolates don't have this potential, in spite of the fact that many microorganisms were recognized having arsenite oxidase gene, which proves that arsenite oxidase is the most important factor for counteracting arsenic toxicity. Microorganisms can indirectly disturb arsenic mobility via sulphate reduction, iron-oxide reduction and mineral dissolution by oxidation (McArthur et al. 2004). The As^{3+} oxidation to As^{5+} (a less toxic form of arsenic) occurs due to bacterial arsenite oxidase in the peri-plasm of microorganism (Rosen 2002). A sensor kinase (AoxS) recognizes the presence of As^{3+} and activates a controller protein (AoxR). AoxR is a point control for aox operon clubbed with RpoN (an option of □54 of RNA polymerase) (Satyapal et al. 2016). The RpoN is the requisite factor for



Protein	ArsR	ArsD	ArsA	ArsB	ArsC
Total Residues	109	124	582	430	137
Mass (Da)	13,198	13,218	63,188	45,598	15,830

Fig. 2: Pandect of arsenic seepage in groundwater and bacterial interaction. The development of wells and human activities quickens this process by providing necessary oxidants.

the transcriptional onset of aox operon, the products of which are transferred to peri-plasm by 'Tat' protein in *A. tumefaciens*. In peri-plasm, 'Tat' is engaged in the oxidation of As^{3+} (Shankar & Shanker 2014).

ARSENATE REDUCTION (AS^{5+} TO AS^{3+})

Arsenate uptake by the bacterial cell is done by dual phosphate transporters Pst (specifically) and Pit (generally). Arsenite expulsion is finished by (i) carrier-mediated arsenite protein and (ii) through ATPase engaged in arsenite translocation (Shankar & Shanker 2014). Bacteria harbour two systems for arsenate reduction; cytoplasmic and periplasmic reduction systems. When As^{5+} is intruded by Pst/Pit transporters (membrane), the arsC (arsenate reductase) reduces it to As^{3+} followed by extrusion using arsAB pump (Biswas et al. 2019). In cytoplasmic reduction arsC uses glutaredoxins providing reducing potential. In the reaction cascade (Fig. 3), arsenate first unites with an anionic site of arsC forming an arsenate thioester transition with the active site and reduced by glutathione, extruding arsenite (Hare et al. 2019).

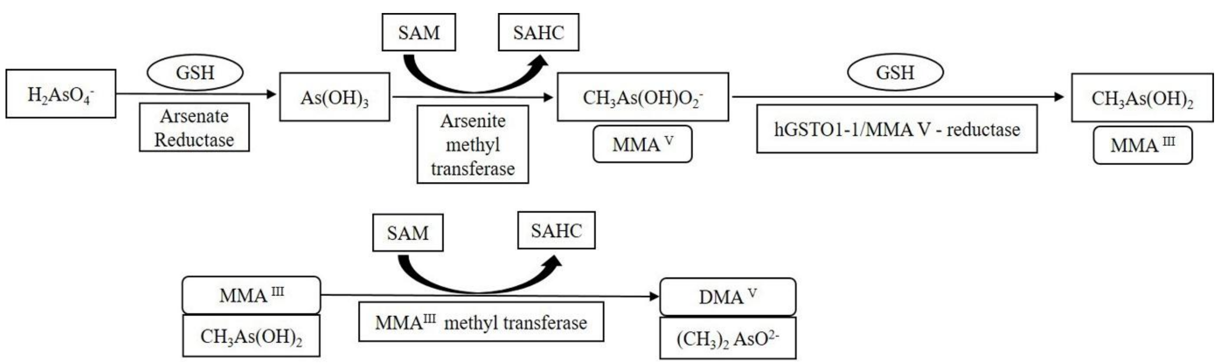
Various arsenic forms display different toxicity degrees. Among organic acids of arsenic MMAV (monomethylarsonic acid) and DMAV (dimethylarsinic acid) are barely harmful than inorganic arsenic, whereas MMAIII (monomethylarsonous acid) and DMAIII (dimethylarsonous acid) are highly toxic (Santra et al. 2013). Different arsenic metabolizing bacteria are classified into arsenite oxidizing and reducing (arsenate) bacteria which are skilled in coinciding in the environment, demonstrating a fact that they do play an essential part in metabolizing arsenic (Yan et al. 2019).

ARSENIC BIOREMEDIATION

Bioremediation technology for eliminating hazardous metals have gained considerable importance over the years. Microbial biosorbents are eco-friendly, cost-effective and a competent substitute for remediating arsenic (Sylvia et al. 2005). The flexibility of microorganisms to detoxify huge pollutants range makes bioremediation an innovative strategy in this regard (Singh 2014). Bioremediation skill relies on encouraging the growth of specific micro-flora that are indigenous to the specified contaminated sites and are capable to perform desired activities (Dixit et al. 2015).

The arsenic inescapability in nature has constrained the development of resistant mechanism in specific microorganisms utilising As in metabolism. *K. pneumonia*, a gram-negative, non-motile, rod-shaped bacteria possess the ability to oxidize As^{3+} and reduce As^{5+} (Daware & Gade 2015). It is also reported to have high resilience regarding both arsenate/arsenite. Through the transformation assay redox ability of *K. pneumonia* towards arsenic was determined to clarify the detoxification mechanism (Batool & Rehman 2017). Although arsenic bioremediation has received great consideration, still this technology hasn't been implemented at commercial or field-scale to rectify contaminated sites. For successful bioremediation, the design requires three main factors which are: bacteria should exist in arsenic-contaminated sites, should bear high metabolic capacities and could perform under distinct environmental conditions (Nookongbut et al. 2017). Recent investigation depicted, purple non-sulphur *Rhodopseudomonas* bacteria (*R. palustris* & *R. faecalis*) are attractive organisms for application in arsenic bioremediation as they possess the most versatile growth modes and can flourish under a variety of conditions (Zhao et al. 2015). Bacterial nucleotide sequence having ars operon encodes for various regulatory components like arsA, arsB, arsC, arsD and arsR respectively (Table 3).

Recent advances in various biotechnological tools have prompted the beginning of a few non-conventional bioremediation strategies advancing their components and



Note: GSH (Glutathione), SAM (S-adenosyl-L-methionine), SAHC (S-adenosyl-L-homocysteine), hGSTO1-1 (human glutathione-S-transferase omega 1-1), MMAIII (monomethylarsonous acid), DMAIII (dimethylarsinous acid).

Fig. 3: Enzymatic method for arsenate reduction.

Table 3: The *ars* operon genes and their putative function (Branco et al. 2008).

ORF	Putative function
arsR	Regulatory Protein
arsD	Regulatory Protein/Chaperone protein
arsA	Oxyanion-translocating ATPase
arsB	Arsenite membrane pump
arsC	Arsenate reductase

performance under diversified natural conditions (Gorny et al. 2018, Palit et al. 2019). Metallothioneins (MTs) are low molecular weight proteins, introduced recently and found in a variety of microorganisms. The cysteine-rich amino acid content of MTs have a greater binding affinity with heavy metals and are efficiently suitable for remediating them at low concentrations (Ma et al. 2011). Recombinant *E.coli* plasmid with a copy of the MT gene was constructed resulting in a three times increase in bioaccumulation of arsenic (Ma et al. 2011). Voluntary As^{3+} oxidation at the anode of microbial fuel cells (MFCs) with bioelectricity production was also studied and evaluated (Li et al. 2016).

CONCLUSION AND DISCUSSION

The bacterial arsenic encounter varies greatly among bacterial species. Various bacterial cells were recognized harbouring genes for metabolizing and transforming arsenic species. The Arsenic oxidizing ability of bacterial isolates are well understood and many microbes were being diagnosed and isolated. Arsenic reducing (As^{5+} to As^{3+} transformation) property of bacterial cells are not very well understood, so emphasis should be given in this area as it is too dangerous for the biotic community as well as humans. It has already been reported that in the abandoned mining regions the concentration of arsenite is increasing year after year minutely. This is because the native bacterial cells take up arsenate, reduce and extrude arsenite in the underground environment. This process increases the arsenite concentration in underground water severely, making it unfit for drinking and irrigation purpose. The arsenite when consumed by humans for a long period, leads to various abnormal deformities, as reported in this review. Crops if grown on As contaminated soil or irrigated with As contaminated water, either sequester it or accumulate it. This process further leads to the development of crops that are not suitable for human or animal consumption.

When we visited some contaminated regions of MP and Jharkhand, we came to know about a fact by talking to the native people, that none individual is survived above 52 years of age. The living population, especially adults have developed skin lesions (grey or black spots) on their palms, foot and front abdominal region. We also came to know that, if they use those crop plants which are being grown of contaminated soil, as fodder for milk yielding animals like cow and buffalo, they gradually become weak and decreases the milk yielding capacity and finally, dies within 10 to 12 months. Likewise, many regions are there in India, facing these types of moderate to severe problems. Government and non-government agencies are also playing their part to improve the vegetation and livelihood of arsenic affected regions, but it is not up to the mark. Every year the problem persists and increases in a dramatic mode. So, biological remediation methods employing bacterial community may prove a valuable and cost-effective method, to achieve arsenic decontamination from affected regions.

Bacterial activity and various factors (biotic or abiotic) including complexation, sorption, precipitation, detoxification, redox events impacts the fate of arsenic. More profound knowledge regarding As^{3+} oxidizers dispersal and metabolic pathways in the natural environment could be a plausible marker for arsenic remediation. However, there are many gaps in the area that needs attention and resolved before exploring in-situ bioremediation as a viable treatment option. Notwithstanding the antiquated origin and wide dispersion of arsenite oxidase in an anoxic environment, a comprehension on its part and effect on regular habitat is yet inadequate. The current improvements in metabolic pathways engaged in arsenic metabolization are yet incomplete and need exhaustive study. This review provides a deeper understanding of the distribution of its metabolizers in the natural environment and depicts future prospects for remediating arsenic. The use of an integrated approach of biomarkers of arsenic exposure and early genotoxic effects will provide a better understanding and mechanistic insight into the health risks of arsenic exposure. The information obtained here highlights the importance of the prevention of arsenic exposure and the need for effective strategies to reduce the risk for the development of diseases associated with such exposure.

FUTURE PROSPECTS

The profligate growing industry and advancement in technology have put heavy metals encumbrance on the environment contaminating water and soil. As the conventional approaches are slow, complex and expensive, microbial remediation strategies help to surmount such situation. Existing technologies still need modification on a pilot-scale and to be implemented effectively to remove these contaminants in a cost-effective and user-friendly mode. So, it is unambiguously suggested that sheltered and effective advances ought to indorse for arsenic expulsion from drinking water. Microbial assisted bioremediation strategy has some margins but still grants a secured and swift way for remedying contaminated sites. Microorganisms play a crucial role in remediating arsenic from the underground environment through the genes harboured by them. If the mechanisms and functions of these genes can be understood, they can be easily employed in the construction of genetically modified organisms (GMOs). The GMOs could be the best significant turning point that can concurrently endeavour on several heavy metals. Essentially, a more profound investigation is required to boast proficiency of GMOs. Manipulation in the exterior membrane can be discouraging for its uptake and even on expressing precise transforming gene, further boost the bioremediation capability by employing GMOs.

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