Geomicrobiology & Microbial Biotechnology

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Microbial directors re-direct underground arsenic (As) horror story scaring millions of inhabitants worldwide

Arsenic horror story: Hundreds of millions are trapped

We are blessed because of our science discoveries and inventions and especially the chemists for letting us know about the periodic table of the elements. But, what if one of the member of the table is cruel to us. Yes!!!!! its Arsenic (As), a metalloid of the Nitrogen family and present in Earth's crust, sediment minerals, many pesticides/fertilizers, plasticizers and it is toxic and carcinogenic; means can cause cancer in humans and other animals. But over-burdening of As in groundwater and related health hazards are becoming a high-profile problems throughout the world and is called as "Worst mass poisoning in 21st Century". Horror stories of arsenic (As) poisoning in drinking water and groundwater have appeared in many news and press articles. The Economic Times in politics and nation section on Dec 24th, 2017 stated that 239 million people across 153 districts of 21 states in India drink water that contains unacceptably high levels of arsenic (> 10 µg/Litre) means being slowly poisoned, and 19 % Indians drink water with lethal As levels (https://economictimes.indiatimes.com/news/politics-and-nation/19-of-indians-drink-water-with-lethal-levels-of-arsenic/articleshow/62228448.cms) In Lok Sabha (the upper house), Ministry of Water Resource stated that 65 % of Assam's population, or about 21 million people drinking arsenic-contaminated water, while it's 60 % in Bihar, 44% in West Bengal, 7.4% I Punjab, M.P, 7 % in Gujarat and Haryana, and 4 % Andhra Pradesh, and Tamil Nadu. Uttar Pradesh has the largest number of people, over 70 million, exposed to arsenic-contaminated water, in absolute terms. The horror also applies to cross-border/neighbours in South-East Asia where 60 million inhabitants in Pakistan, 77 million in Bangladesh, another 150-200 million in China, Cambodia, Vietnam are under high risk of developing As health hazards, and it goes on. Other countries are not spared from its grudge, because many South-American and European countries are also affected with groundwater As problem and it has gone

global and will be bigger in future. Because As is in our every commodities, many new reports have come highlighting many cases of As in food and food products. UK's largest daily INDEPENDENT reported 80 % of infant formulas, 60 % of 530 different snacks, cereals, formulas and drinks shows levels of As. Another report in INDEPENDENT published three quarters of rice products sold as baby food found to contain illegal levels of arsenic marketed throughout European countries. Even, scientists are warning that common method of cooking rice (parboiling) leaves levels of arsenic in food and many feeds supplements for poultry are with dangerous levels of As and Bottled water of famous brands found to have high levels of arsenic and hence pulled from stores in many countries, thus As is everywhere now in the food chain and it will be there through root-to-gut.

Sources, causes and factors: Who to blame???

In India, until 1960 people were relying on surface water as primary water resources but due to intestinal disease incidents like cholera and diarrhoea, people started using groundwater by installing tube-wells/hand pumps of lower depth. In 1980s, cases of As in the urine and blood samples of inhabitants opened the eyes of scientist and policy makers to look deep into the problem and retraced its path to groundwater (1). The toxic As present in groundwater or drinking water comes from natural source that is geogenic (geological) means this As is naturally present in the sub-surface sediment of the aquifer (underground layer of water-bearing permeable rock). The sediment hosts or composed of various minerals rich in Fe, Al, Mn in the form of Fe-oxides, hydroxides, their pyrites, carbonates, sulphides, and other silicate minerals like phyllosilicates, onto the surface of which arsenic (As) adsorbs or precipitates or co-precipitates (2). It is long way back in geologic past of around million years back, where major rivers like The Ganges, Brahmaputra and several others were evolved from Himalayan glaciers and carried/transported all the sedimentary deposits with them (fluvial deposit) and deposited along their flow paths along the planes of India and Bangladesh and same with other countries. In the due course, the minerals weather, oxidize, precipitates with As and form sedimentary deposits with As in them. But, microbial members/community key players interact with sediment minerals to release the As and other sources like mines (coal, fossil fuels) activities, agricultural activities and run-off, severe groundwater abstraction, irrigation of As contaminated water, lesser groundwater recharge, contaminant percolation to sub-surface aggravates the situation further, which is depicted in Figure 1.

Microbial directors underground: Arsenic story makers

Several hydrogeologists have described certain sub-surface geological factors to be the main cause of As problem in the groundwater. But, in the recent decade geomicrobiologists have proved that microbial community members and their dynamic physiological role to be one of the key driver of As solubilisation or mobilization from sediment to the groundwater. As we all know that microbes are omnipresent and omnipotent.

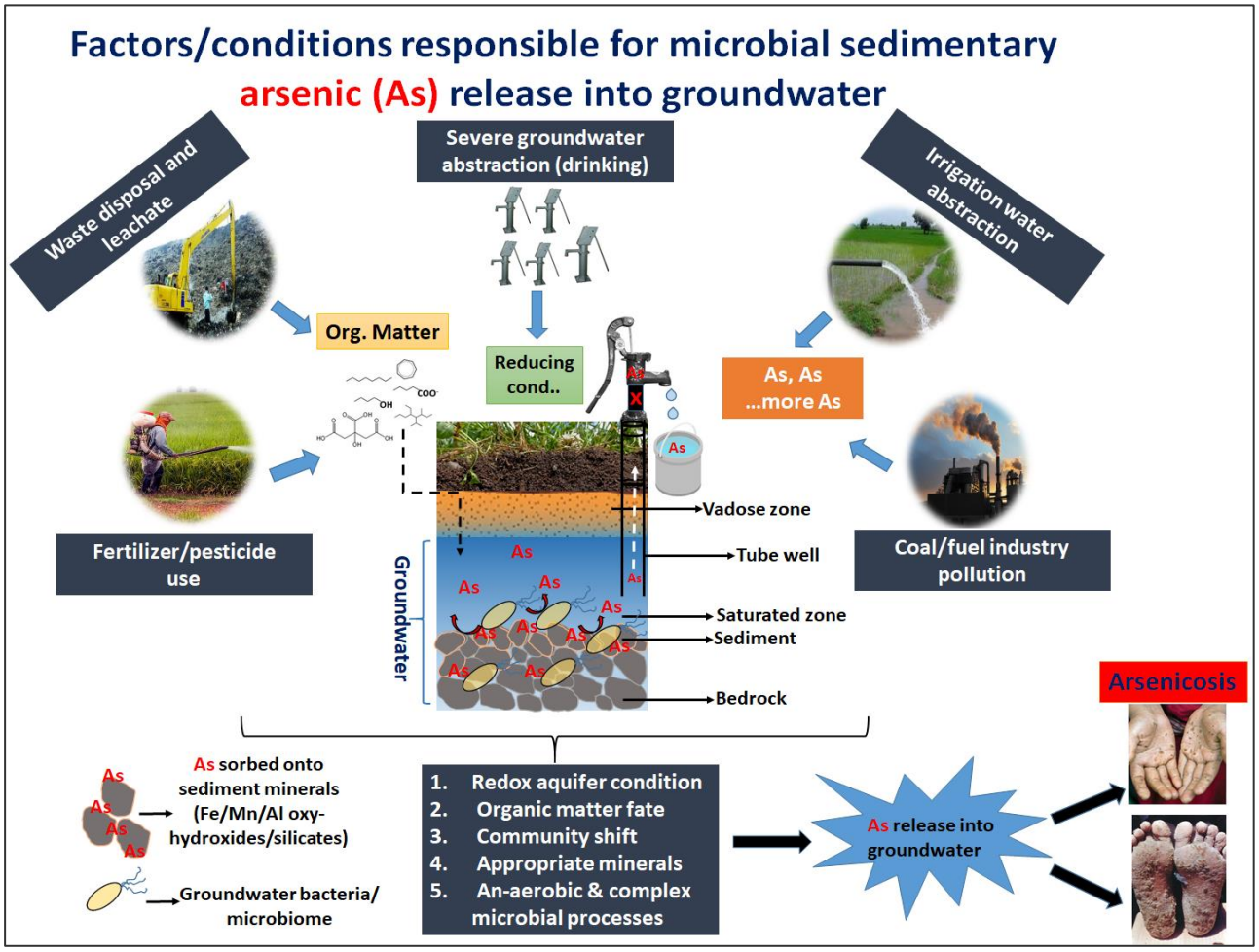

Figure 1: Schematic representation of the multipartite factors responsible for high arsenic condition in the groundwater of West Bengal in India as well as other areas and microbial members contributing in release of arsenic from sediment into the groundwater.

Bacteria-As-sediment interaction has been found to be a prominent mechanism for mobilization of sediment bound As into the groundwater of Bengal, India has been the point of greatest debate for sustainable groundwater management. Indian geo-microbiologists, especially researchers at IIT Kharagpur, India have deciphered the sediment-groundwater microbial communities and their diverse roles played underground (3-5) by isolating and characterizing more than 250 bacteria from groundwater and sediment samples of Bengal, India. Culture independent technologies involving next generation sequencing: metagenomics and Geo-chip etc. have emphasized the abundance of facultative anaerobic bacterial residents belonging to phylum *Proteobacteria* (*Rhizobium, Pseudomonas, Xanthomonas, Achromobacter), Actinobacteria (Rhodococcus, Arthrobacter*), Firmicutes (Bacillus) (images are presented) in theAs-contaminated groundwater of Bengal, India . These microbes are found to encode several genes like arsenite (As3+) oxidase (*aioAB*),

arsenate reductase (*arsC*), arsenite transporter (*arsB*), arsenite pump (acr3), As regulator (*arsR*), etc. to transform As from sediment to groundwater. For the first time, three bacteria were named and described as novel bacteria from As-contaminated groundwater of India as: *Rhizobium arsenicireducens* (published in Achieves of Microbiology), *Pseudoxanthomonas arseniciresistens* (published in Plos One), and *Achromobacter arsenitransformans* (published in Journal of Env. Science and Health Part-A), which showed versatile As transformative capacities (6-10). Next level studies like whole genome sequencing and X-ray based technologies (X-ray absorption of near edge structures, X-ray diffraction, and X-ray fluorescence) proved the multifaceted role of bacteria in metabolizing hydrocarbons, reducing Fe (dissimilatory Fe reduction), nitrate (NO3-), nitrite (NO2-) (denitrification), ammonia (NH4+) oxidation, methane production (methanogenesis), and subsequently mobilize As from sediment minerals to groundwater.

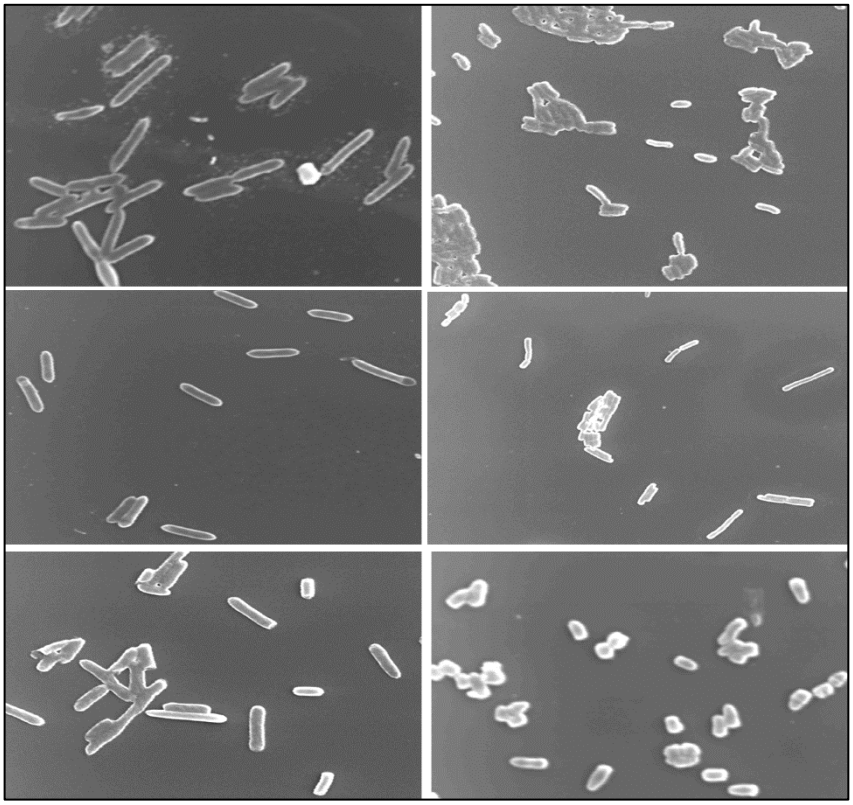

Figure 2: Electron micrographs show some of the isolated bacterial members from arsenic-contaminated groundwater of Bengal, India which show high arsenic transformation properties.

Arsenic removal technologies: The biological step and future prospects

Sub-surface ecology with changing nutrient flux, electron donors, acceptors, carbon, large surface, contrasting pH and temperature, hydrostatic pressure, and dissolved salts hosts dynamic microbial communities with vast array of metabolic pot-

ential and has been treated as “blackbox”. Since, aquifer or groundwater environment is ideal for sediment mineral-microbe interactions with metal and nutrient biotransformation abilities, more focus on groundwater-sediment microbiology and biogeochemistry is imperative to understand many geomicrobiological processes. With respect to role of microbes in As-geomicrobiology, key microbial members use the sedimentary organic matters (as hydrocarbons, sugar acids, etc.) and other energy rich inorganic nutrients for their survival or metabolism and acts upon sediment facies to remove or release As using oxidative-reductive reactions. Biological water treatment methods are considered to be a suitable approach to overcome these problems and they have attracted considerable research interest over recent years. Efforts on removal of arsenic has been performed by using bacterial consortia, pure cultures of arsenic resistant bacteria or iron- and manganese-oxidizing bacteria to transform and/or capture arsenic forms indirectly. One of the innovative technology for arsenic removal has been used biocolumn reactors consisting of immobilized bacterial cells capable of arsenic adsorption where a low cost biocomposite granules of cement coated with cysts of Azotobacter were used for arsenic removal from drinking water [93] that has claimed to remove 95 % of As. Besides bioremoval, bacterial oxidation of As (III) to As (V) is an alternative approach to treat contaminated water instead of using conventional oxidants (i.e., potassium permanganate, chlorine, ozone, hydrogen peroxide). Some recent studies have been conducted to assess the As (III) oxidation efficiency of different As(III)-oxidizing bacteria like Ensifer, Rhodococcus, Acinetobacter. Instead of pure cultures, use of consortia like CAsO1 has been attempted but pose several limitations in viability, efficiency, and effects on the treated water. Hence it is imperative to explore the indigenous groundwater microbiome or microbial strains to decipher their complete metabolic processes in terms of As biotransformation. With advent of genome sequencing many bacterial genome sequencing is undertaken to study the evolution of arsenic metabolism. In addition, next-generation sequencing based microbial community metagenomics of As-contaminated environments and gene expression patterns is providing immense insight into the microbial potential to predict their behaviour under sub-surface environmental conditions to design, develop and succeed in strategizing As removal or management options in a sustainable way.

Author’s Biography

Dr. Balaram Mohapatra is a passionate environmentalist and an enthusiastic plant lover. Dr Mohapatra is a post-doctoral fellow in the Department of Biosciences and Bioengineering at the Indian Institute of Technology (IIT) at Bombay in India. His research activities are focused on gaining insight into the genomic and metabolic ways of microbial pollutant biodegradation. He received PhD in Biotechnology from the Department of Biotechnology, IIT Kharagpur in 2019 in the area of groundwater arsenic geo-microbiology and genomics. He has published over 15 international research publications based on innovative concepts of

arsenic detection, and filtration. His research paved the way in the discovery and naming of three novel bacterial strains from arsenic-impacted groundwater in the eastern states in India. With considerable research experience in cutting edge areas including genomics, community genomics, proteomics, and X-ray based advanced analytical technologies, Dr Mohapatra has expertise and unique skills to study and understand microbial communities and metabolic pathways for hydrocarbons degradation and metal-microbe interaction. Based on his outstanding reputation and his excellent research, he received several prestigious awards such as DST-INSPIRE (2013-2018), Young scientist award at IISF, DST, and National entrepreneurship competitions for pitching idea for start-ups on developing arsenic detection kit and filtration units. Dr. Mohapatra has been associated with international association on hydrological sciences (IAHS), global microbial identifier (GMI), society for human ecology (SHE), and student member of Deep Carbon Observatory (DCO). In near future, he aims to work on microbial metal bio-geo-cycling and pollutant biodegradation at soil-root-plant interface. Dr. Mohapatra can be reached at balarammohapatra09@gmail.com.

Prof. Pinaki Sar is currently a mentor and professor nurturing a group of young microbiologists and ecologists at Environmental Microbiology and Genomics Laboratory in the Department of Biotechnology, IIT Kharagpur. He has great interests in Microbial genomics, Geomicrobiology, and Environmental Biotechnology to explore and understand the microbiomes from ecological hot-spots like deep terrestrial subsurface, arsenic contaminated groundwater, impacted sites (Acid mine Drainage, Petroleum refinery, etc.) using next generation sequencing and culturomics technologies, their biogeochemical role relevant for environmental processes and solution strategies. His current focus aims to understand landfill microbiome and its application for Bioremediation of Environmental Pollutants with Tata Consultancy Services Limited. Other projects are on Arsenic from Root to Gut and the effect Arsenic contaminated groundwater on Rice Paddy Soil and its microbiome, biogeochemical cycles, and impact on Arsenic (As) Accumulation by the Rice plants. In one of the interesting ongoing project, Prof. Sar aims to understand the microbiology of deep granitic subsurface of Koyna-Warna region, where the team found digs up life in India from 2.5 billion years ago corresponding to great oxidation event and the news that has been highlighted and covered by Times of India (https://timesofindia.indiatimes.com/city/kolkata/iit-kharagpur-digs-up-life-in-india-from-2-5-billion-yearsago/articleshow/67617949.cms). He has been recipient of several awards and honors like Competitive Seed Challenge Grant, Young Investigator award from DBT, BOYSCAST fellowship, Atomic Energy Young Scientist award, DST Fast Track award and with several research publications in journal of national and international repute and has guided 11 PhD till date and several others are still continuing. Prof. Sar can be reached at sarpinaki@yahoo.com.

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