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# Implementation of Biotechnological Techniques in Treatment of Groundwater Contaminated with Arsenic

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**Abstract:** *Over the course of time, there seemed to be an increase in rate of Arsenic (As) contamination in groundwater found around the world. Several organic or/and anthropogenic resources have emerged to be the primary cause in contributing to a negative impact on public health as well as on the environment. A large number of people throughout the world rely on groundwater containing toxic levels of As for their drinking needs. When As is exposed to drinking water in a significant amount for a great period of time, it may lead to several disabling and weakening diseases. the most common one being a dermatological condition known as Arsenicosis. The sources, distribution, migration of As, as well as a worldwide summary of its contamination in drinking water are discussed in this paper. The research also examines the human health hazards associated with As, as well as its absorption process and contribution in the poisonous pathways alongside providing an introduction of modern evidence on treating groundwater with several Biological/Biotechnological techniques as a substitute or potential replacement of the existing and well known physical-chemical methods used in the purification process.*

**Keywords:** *Arsenic, Biological Iron Oxidation, Arsenite Oxidizing Bacterium;*

**ABBREVIATION:** Arsenous acid ( $H_3AsO_3$ ), Dihydrogen arsenate ( $H_2AsO_4$ ), Hydrogen arsenate ( $HAsO_4$ ), Disodium arsenate ( $Na_2HAsO_4$ ), Fourier-transform infrared spectroscopy (FTIR), X-ray fluorescence (XRF), X-ray diffraction (XRD), Scanning electron microscope (SEM), Energy Dispersive X-Ray Analysis (EDX), Arsenite Oxidizing Bacteria (AOB), Arsenite-resistant bacteria (ARB), Hydraulic retention time (HRT), Nanofiltration (NF), Reverse Osmosis (RO).

## I. INTRODUCTION

Nearly most of the water flows calmly and remain unnoticeable on the surface, however sometimes it makes a glorious display mainly in a large spring, geyser or cave and these prominent features of groundwater were the only knowledge of the prehistoric man. Thus, with course of time people start settling down near the springs and started digging wells to find more water where it was not clearly visible on the surface.[1]. Groundwater can be defined as water existing beneath the Earth's surface in between the soil pores and rock and rock formation fractures. An aquifer defined as a small segment of rock or an unconsolidated sediment. Water table is formed at an abyss where no spaces are present in between the soil pores, rocks or fractures. The surface of actually responsible for groundwater recharge mainly at seeps and springs forming wetlands or oasis. Extraction wells are constructed and utilized for collecting groundwater for industrial, agricultural and municipal use. A survey also stated that almost 50% or more people of United States, mainly people dwelling in rural areas survive by using groundwater for their drinking purposes.

## II. ARSENIC CONTAMINATION IN GROUNDWATER:

In today's world Arsenic contamination is imposing a serious threat to the health of people consuming Arsenic in groundwater for drinking purposes. A majority of people from the rural areas of India, Bangladesh, Canada, China and several parts of world uses groundwater for drinking and domestic purposes like irrigation are affected by Arsenic poisoning. The chief natural sources of Arsenic are:

The erosion and weathering of rocks and soil having presence of Arsenic.

- 1) Gold mine operations
- 2) Wood preservative services
- 3) Several anthropogenic sources [2,4]

A number of geochemical and hydrologic factors affect the Arsenic solubility in groundwater. [3]

The World Health Organization (WHO) fixed  $10 \mu\text{g L}^{-1}$  to be the safe limit of Arsenic in drinking water. A recent survey showed that almost 9 and 42 districts of West Bengal (in India) and Bangladesh respectively have Arsenic level over  $50 \mu\text{g/L}$  in groundwater used for drinking and domestic purposes. In these 9 districts of West Bengal, almost 69 police stations/blocks and 985 villages have been found to be already affected by Arsenic. Since Arsenic contaminated groundwater has been used for agricultural purposes thus increasing the level and longevity of Arsenic in soil finally making the soil unsuitable for irrigation. This situation leads to increase the risk of livestock and human health. [2]

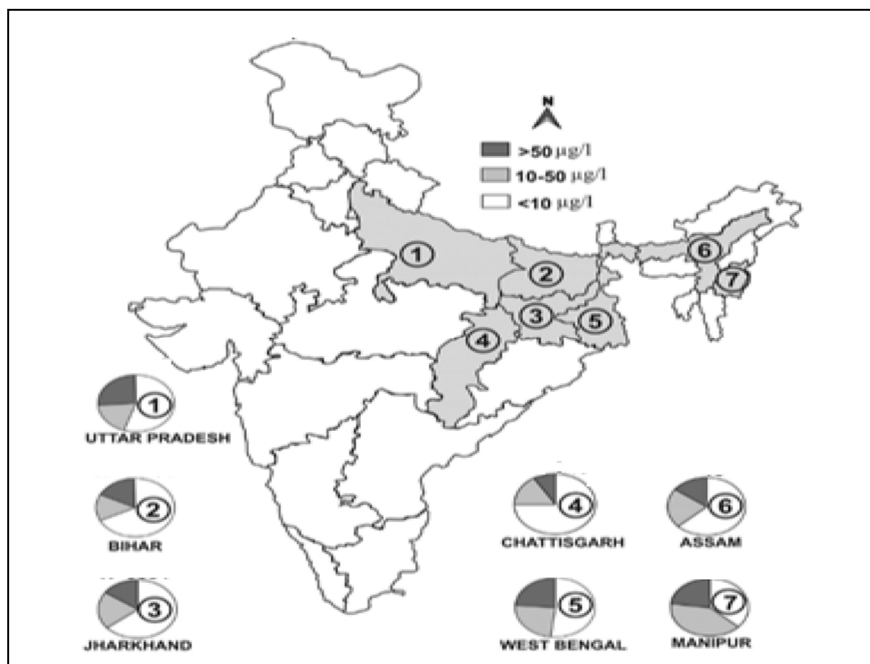


Figure 1: Mapping of Arsenic affected states of India. [5]

As Arsenic has no specific taste, colour, odour of its own so it is impossible to avoid and detect the presence of Arsenic by a normal individual.[6]

A report estimated that almost 150 million people in today's world is already affected by As poisoning.[7]

Worldwide large deltas and river valleys or basins are majorly affected by Arsenicosis [8] like Bengal delta [9-11], Paraiba do Sul delta, Brazil [12], Danube River basin, Hungary [13], Mekong delta, Cambodia [14], Hetao river basin, Mongolia [15], Zenne river basin, Belgium [16], Duero Cenozoic Basin, Spain [17] and Tulare Lake, USA [18]. Along term risk is imposed to the ecosystem and human when As gets transferred to the food chain. [19]

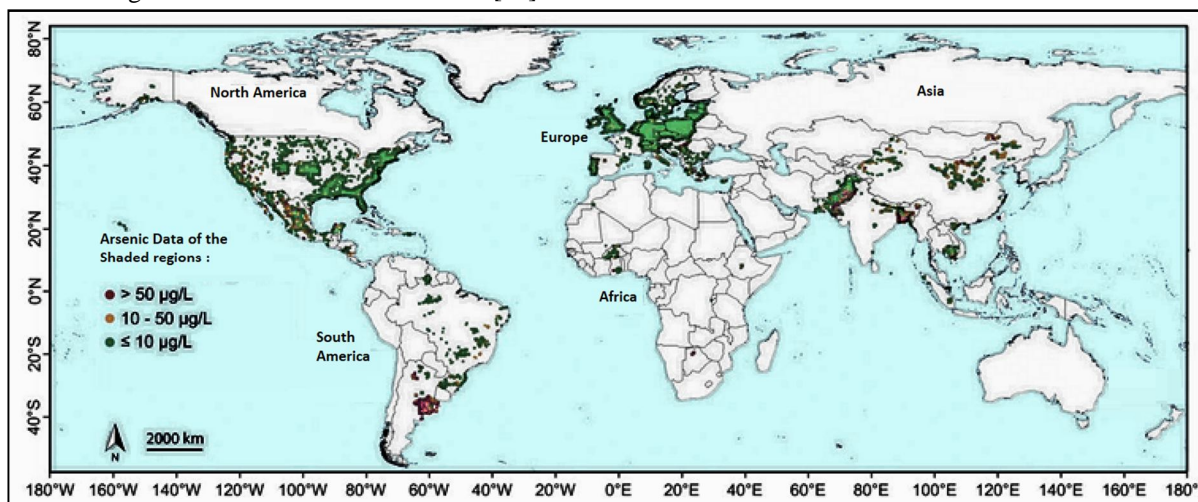


Figure 2: Worldwide Arsenic threat in groundwater.[20]

### III. IMPACT OF ARSENIC CONTAMINATION ON HUMAN HEALTH:

Arsenicosis, a common health problem caused by long term intake of Arsenic, a common carcinogen through food or water.

Arsenicosis involves several disorders like:

- 1) Skin disorders
- 2) Internal cancers like bladder, lung and kidney
- 3) Skin cancers
- 4) Malfunctioning of blood vessels in legs and feet
- 5) Diabetes
- 6) Hypertension
- 7) Reproductive disorders [21-23]

Pentavalent arsenate and trivalent arsenite are more common and toxic than any other organic forms in the terrestrial environment. They react with sulphhydryl groups present in cysteine residues harmfully effect the protein metabolism as their toxicity increases.[24] Arsenicosis victims faces severe consequences in their family life, livelihood and earning capability. Women eventually isolates themselves from the society as her physical appearances deteriorates succeeding Arsenicosis.

The pH conditions and redox potentials are the two factors chiefly determining the distribution of the two primary species As(III), As(V) of Arsenic.[25] Since the surface waters get dominated mostly by oxidizing conditions making pentavalent Arsenic as the most predominant species, primarily existing in its oxyanionic forms  $H_2AsO_4^-$ ,  $HAsO_4^{2-}$  with  $pK_a \frac{1}{4} 2.19$ ;  $pK_b \frac{1}{4} 6.94$  respectively. While the prevailing reducing conditions in groundwaters make the trivalent Arsenic As(III) as the most predominant and thermodynamically stable species which exist as non-ionic form of  $H_3AsO_3$ , arsenious acid having  $pK_a \frac{1}{4} 9.22$  It is present in almost all-natural water having vast pH range. It is thus very difficult to get rid of trivalent Arsenic by conventional treatment methods like precipitation, adsorption, etc. as it may show minor reaction with most solid surfaces.[26] The other treatment technologies are coagulation/ filtration, lime softening, adsorption on activated alumina or iron oxides, ion exchange, reverse osmosis which are employed for removing Arsenic from water. [27-29] A preoxidation step is usually employed convert the trivalent form of Arsenic to the pentavalent form

by adding certain chemical reagents like potassium permanganate, hydrogen peroxide, chlorine, ozone or manganese oxides. [27,30,31] On one hand these chemical reagents successfully oxidize the trivalent Arsenic but on the other hand their residuals or by-products may give rise to several secondary problems. These chemical reagents can also abruptly increase the operational cost of the entire process. Now a days, these chemical reagents are often replaced with biological iron oxidation, employed for oxidizing trivalent to pentavalent Arsenic. [32,33] Several microorganisms like *Leptothrixochracea* and *Gallionella ferruginea* which are originally found in groundwater are employed as catalyst for this iron oxidation reaction. [34] Low level iron oxides containing organic matter in significant amounts are produced as a result from the iron biological oxidation. The intermixing of these resultant iron oxides, existing bacteria and organic matter give rise to solids which have the capability to attract and hold substances by the process of absorption and adsorption.

These solids are called sorbent showing unique retention properties of metals.[35] Even though Arsenic can be easily eliminated by co-precipitation or direct adsorption on bioactive iron oxides that have already been synthesized, however the iron oxidizing bacteria increases the overall removal efficiency of the trivalent Arsenic oxidation.[36]

### IV. RESULTS AND DISCUSSIONS

Our objective is to review the biological/biotechnological processes (notfocusing on the well-known chemical processes) that can be used for effective purification of groundwater contaminated with Arsenic:

#### A. Purification Processes in General

The purification process for removing Arsenic from groundwater may involve several typical and well known physical-chemical methods which includes adsorption, ion exchange, membrane technology and precipitation. The precipitation agents used for precipitation methods are mostly ferric sulphate, Portland cement, hydrogen peroxide and calcium oxide. With time several methods like membrane separation, adsorption and chemical coagulation-precipitation which removes Arsenic removal from drinking watersuccessfully. But, here, we are only going to focus on the biological/biotechnological methods that can be introduced for the desired purification process.

The Biological/Biotechnological methodologies deemed to be effective in the purification process:

1) *Using Biological Iron Oxidation*

The conditions maintained while removing trivalent Arsenic from the groundwater containing ferrous oxide:

- a) pH around 7.2
- b) Redox potential: 270–280 Mv
- c) Dissolved oxygen concentration of about 2.7 mg/L. [37]

A sample of groundwater was collected and checked for its Arsenic level. If the Arsenic level was found to be too low then more amount of Arsenic is externally added to have a considerable amount which successfully helps in investing the removal process. The stock solution of As (III) was prepared by adding Arsenic oxide ( $As_2O_3$ ) as analytical reagent (AnalaR) and 10 ml/ L of HCl acid and then dissolving them in de-ionized water. The final solution is then heated. As(V) was prepared as a stock solution by adding  $Na_2HAsO_4 \cdot 7H_2O$  in distilled water. A unit of fixed-bed up flow filtration is set up for Arsenic elimination using biological iron oxidation. The apparatus involves a Plexiglas column stuffed with polystyrene beads which represents the filtration media. After 3 months the microorganisms employed in the process are deposited and collected in the filtration column.

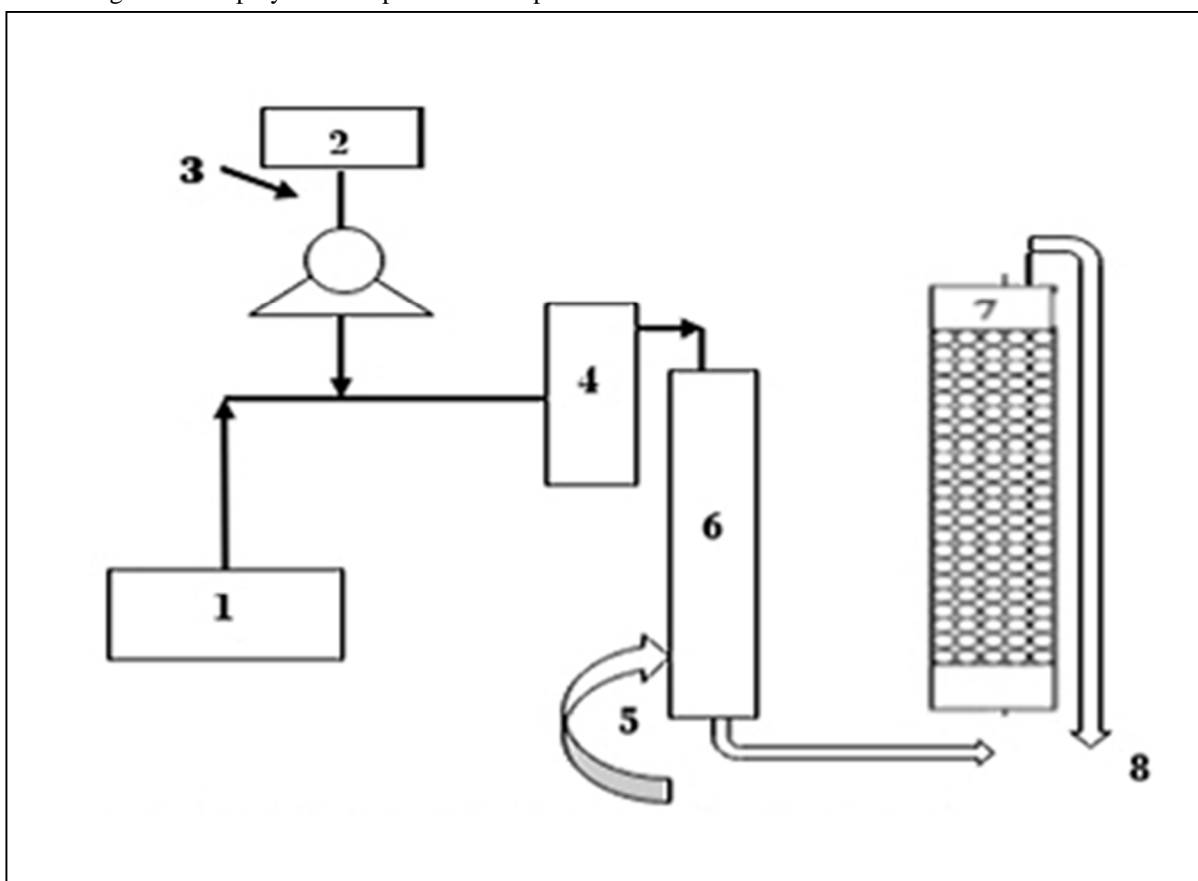


Figure 3: Schematic diagram of Biological Iron Oxidation.

The schematic diagram above represents the several parts of the biological oxidation and filtration system.

- Continuous flow of Arsenic contaminated groundwater
- Arsenic stock solution
- Peristaltic (feeding) pump
- Influent sampling vessel
- Air injection
- Aeration column
- Filtration column
- Effluent

Traits of Plexiglas column:

- 1 m of active height
- Inner diameter of 68 mm
- Surface area of 0.0036 m<sup>2</sup>
- Bed volume of 3.6 L
- Total bed porosity of 0.37
- Bead diameter of 3–4 mm.

In a ferrous iron rich oxidizing environment, *Leptothrixochracea* and *Gallionella ferruginea* respectively form sheaths and stalks. The formation of sheaths and stalks provide protective mechanism to these microorganisms as they become unstable in an oxidizing environment which is formed when the reducing capacity of ferrous iron level start increasing. The exhausted bacteria finally reach the filtration media where they start gaining their energy from oxidizing ferrous iron: Gallionella or by ingesting organic matter: Leptothrix [38,39]

The plastic vessel serves as the first sampling point where the two streams of Arsenic stock solution is dissolved in contaminated groundwater stream followed by aeration in a distinct column and then lead to the filtration unit. The concentration of iron in the effluent is increased to prevent the bubbles colliding with the deposited sludge. Several samples are collected from the effluent and then evaluated for iron, As(III) and total As(III and V). The treated water is then released from the topmost point of filtration column.

$$\text{Re\% As} = \left[ \frac{\text{As}_{\text{in}} - \text{As}_{\text{out}}}{\text{As}_{\text{in}}} \right] 100$$

The above equation is employed to calculate the percentage of Arsenic removal

$t = \text{bed height/linear velocity}$

The above equation is used to calculate the residence time of water present in the filter.

The filter column is cleaned by backwashing after every 3 days to prevent filter clogging as the amount of deposited iron increase eventually.

In accordance with Driehaus and Jekel improved method, two approaches of atomic absorption spectrometry (Perkin Elmer 2380) and hydride generation (Perkin Elmer-MHS 10) are employed to calculate the total amount of Arsenic present.[40] The sample is pre-treated with acetic acid followed by hydride generation to specifically calculate the As(III) form as this method involves quick analysis of inorganic Arsenic species when the concentration is lower than 1 mg/L. Spectrophotometric is used for iron speciation (between FeII and FeIII) and determination[41]. Redox potential is measured by using redox) meter (E396 B, Metrohm, with two electrodes (Ag/AgCl and Pt). Dissolved Oxygen Meter (Consort, Z 521) was used for measuring the dissolved oxygen level. The different products of biological precipitation are classified by:

- Z-potential (Rank Brothers Mark II)
- FTIR (Perkin Elmer 1600)
- XRF (Spectro-Xepos)
- XRD (Philips PW 1830)

SEM analysis (SEM, Philips 555M) together with energy dispersive X-ray microanalysis (EDX) is employed for detecting the microorganisms and guaranteeing the formation of stalk and sheath during the process.[34]

#### B. Using Immobilized Arsenite Oxidizing Bacterium

In former times, Arsenic oxidation was usually carried out by several chemicals like ozone, potassium permanganate, chlorine and hydrogen peroxide. [42-44] However, these chemical processes evolved to be quite expensive and are quite difficult to handle. Eventually these chemical processes were replaced with biological oxidation using Arsenite Oxidizing Bacteria (AOB) to win over these drawbacks. These bacteria are present in numerous ecological specimens like Arsenic-contaminated water, mines, raw sewage geothermal waters, soils and residues. [45-52] It was also discovered that chemoautotrophic and aerobic AOB like *Thiomonas arsenivorans*, collected from goldmine site, utilize As(III) as the primary source of energy. It is used for As(III) oxidation which further purify the contaminated groundwater making it acceptable for drinking purposes.[53] It was also reported that activated sludge can contain Arsenic-resistant bacterium capable of oxidizing As(III). This bacterium can develop in an aerobic environment without needing any organic carbon source.

A study was conducted to know how AOB collected from activated sludge get effected by pH, concentration of initial nitrogen source, water temperature. Several batch experiments were also conducted to investigate the characteristics of collected AOB using the prior stated conditions. AOB were made immobilized using a polyvinyl alcohol gel carrier before the experiments were conducted. Activated sludge was collected from aeration tank and from there arsenite-resistant bacteria (ARB) were separated.[54] In order to separate out the chemoautotrophic ARB from mixed culture, an artificial medium devoid of sodium lactate was used as a carbon source. Finally, based on the different morphology of pure and mixed culture of ARB, the pure form of ARB was collected. The phylogenic analysis of the obtained pure culture AOB was conducted. A bioreactor was used to detect how the continuous biological oxidation of As(III) was influenced by the hydraulic retention time (HRT).[49,55,56]

Biological or Biotechnological Process	Merits	Demerits
I. Biological Iron oxidation  II. Immobilized Arsenite Oxidizing Bacterium	<ul style="list-style-type: none"> <li>• Microorganisms can be used to biodegrade organic pollutants in a simple, cost-effective, and community-accepted approach.</li> <li>• A large variety of species are employed using either mixed or pure cultures.</li> <li>• In terms of simplicity, economics, and community acceptance, the use of microorganisms for biodegrading organic pollutants is an excellent idea.</li> <li>• Removes biodegradable organic compounds quite effectively. Large quantities of both suspended solids and biochemical oxygen demand are removed quite effectively. Microbiological processes will play a significant part in upcoming technological breakthroughs for eliminating emerging pollutants from water.</li> </ul>	<ul style="list-style-type: none"> <li>• Specific compounds have a poor biodegradability.</li> <li>• There is a high possibility of solid sludge to form foam and bulk up. This may also be accompanied by high amount of microbial sludge generation along with unrestrained degradation products.</li> </ul>

[57-64]

Table: Critical Analysis of the various biological/biotechnological processes.

### V. CONCLUSION

The increased challenge in the Arsenic contamination have enlarged the number of Arsenic victims. Few studies have been conducted which provide aid to these Arsenic victims. These studies mainly focus on the groundwater or drinking water treatment to get rid of any Arsenic impurities if present before they are consumed. Massive physico-chemical treatment plants were established for this treatment process. However, all the physico-chemical treatment process excluding the membrane-based technology showed several weaknesses. The membrane-based technology only evolved to be an effective one resulting in Arsenic-free water. Though both Nanofiltration (NF) and Reverse Osmosis (RO) evolved to successfully remove Arsenic from groundwater but the increased cases of membrane fouling emerged to be their drawback. Soon, the physico-chemical treatment methods were replaced with biological processes. This has made us turn our attention to the biological/biotechnological methods discussed in the paper that can be put into use to obtain the desired results.

From our study, we can propose that the process of biological oxidation using the microorganism *Leptothrixochracea* and *Gallionella ferruginea* can be an effective method for removing Arsenic from groundwaters. It can be speculated that during the entire process, iron oxides got continuously accumulated in the filter medium accompanying the exhausted microorganisms which further provide an advantageous environment for Arsenic to get adsorbed and eliminated from the aqueous stream. The As(III) oxidation by these specified microorganisms was observed under suitable experimental conditions which further enhances the process of Arsenic removal.

These experimental conditions also helped the successful removal of As(V). Since it does not employ the use of chemical reagents for the process of Arsenic oxidation, the method evolved to be more environment friendly and economical. It even does not involve continuous auditing of the breakthrough point. Since it is a united treatment process of biological oxidation–filtration–sorption, it can be employed for removing a wide range of inorganic contaminants like manganese, iron, Arsenic from groundwater.

The phylogenetic analysis helped to compare the rDNA sequence of the AOB isolated with its correlated sequences and also helped to detect their phylogenetic relationship. The optimum ratio of the concentration of nitrogen source to As(III) concentration was detected to be around 0.5. The optimum pH and water temperature for As(III) oxidation was found to be in the range of 6 to 8 and beneath 20°C respectively. Thus, the usage of the proposed biological/biotechnological techniques in the purification of groundwater contaminated with Arsenic may actually turn out to be very effective and may open up avenues for further research.

- *Conflict of Interest:* The authors declare no Conflict of Interest.

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