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Research Article

BIOMAGNIFICATION AND BIOREMEDIATION OF ARSENIC IN AFFECTED REGIONS OF MURSHIDABAD DISTRICT

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A B S T R A C T

Arsenic contamination of soil and drinking water is one of the major problems of the modern world. In India, the state of West Bengal is severely affected by Arsenic toxicity. Nine districts of West Bengal are arsenic affected. Amongst them, Murshidabad shows very high levels of Arsenic in ground water and soil. U U.S. Environmental Protection Agency and WHO has set the recommended limit of arsenic to 10 ug/l in drinking water. In India, permissible limit of arsenic is 50 ug/l, in the absence of any alternative source of drinking water. In Murshidabad district, Blocks situated in the western side of Bhagirathi river are less affected by Arsenic toxicity (30.1% above 10 ug/l and 11.7% above 50 ug/l) than the blocks located on the eastern side (64.7% above 10 ug/l and 32.5% above 50 ug/l). The most common and toxic forms of arsenic are arsenate and arsenite. Arsenite is more toxic than arsenate. Both arsenite and arsenate are soluble, but arsenite has a higher solubility, bioavailabilty and mobility than arsenate. Skin lesions are signs of advanced stages of arsenic poisoning. Long term intake of arsenic causes cancer of skin, lung, liver, bladder and kidney. Other adverse health effects include pulmonary diseases, neurotoxicity, diabetes, cardiovascular diseases and developmental defects. Bioremediation is a natural process in which living organisms like bacteria, fungi, algae and plants are used to degrade or transform hazardous organic contaminants, or to reduce the toxicity of inorganic contaminants. Certain microorganisms are resistant to arsenic toxicity. Some of them use arsenite oxidase enzyme to convert arsenite (more toxic form) to arsenate (less toxic). The genes encoding proteins important for arsenite oxidation reside in an operon, named as *aox* operon. Biomagnification is a process where the concentration of certain substances moves up in a food chain. Rice accumulates arsenic into the grains, much more efficiently than any other staple cereal crops. To combat this, transgenic rice plants could be developed, where the genes involved in the pathway of uptake of arsenic could be mutated, so that bioaccumulation of arsenic does not take place in rice.

INTRODUCTION

Many regions of developing and developed countries are being contaminated by human activities over centuries. Heavy metal contamination of soil and water is one of the severe problems of modern world. Arsenic is an important environmental pollutant which shows health risk to human and other living organisms in long term. Currently, contamination of drinking water and soil by arsenic is a matter of concern, both nationally and globally. Although, arsenic occurs naturally in earth crust, various anthropogenic activities like excessive use of arsenic in soil as disinfectant, pesticide, herbicide, wood preservatives and medicinal products, act as a major cause of arsenic contamination in soil (Pais IJ and Benton Jons JR, 1997). Being a major constituent of more than 200 minerals, desorption and dissolution of naturally occurring arsenic bearing minerals and sediments results in high arsenic contamination in groundwater. Currently, U.S. Environmental Protection Agency has set the recommended limit of arsenic to10 ug/l in drinking water (EPA, 2006). But the permissible limit of arsenic in India is

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50 ug/l, in the absence of any alternative source of drinking water (WHO, 2011). Although, methods like coagulation/ adsorption, precipitation, oxidation and ion exchange can be employed, till date there is no so efficient, cost effective method for removal of arsenic from drinking water and soil, suitable for rural areas in particular. Removal of arsenic from water at low concentration is one of the most difficult tasks that require addition of various chemicals to treat the water. Even after the treatment of water, careless disposal of sludge will return the contaminant back to ground water and soil (Mandal P *et al*., 2016). Therefore, innovative removal and disposal methods are needed urgently for this purpose.

These innovative methods could be used to solve the menace of arsenic contamination in the affected areas of Murshidabad district. To address the problem of arsenic contamination, biotechnological tools can be used. The methods can be based on two pillars i.e. Bioremediation and to inhibit Biomagnification. Arsenic contaminated areas of Murshidabad district could be identified. Strategies should be designed to reduce the toxicity of arsenic in contaminated water and soil. Plants and bacteria could be used for this purpose. Along with this, arsenic resistant *Oryza sativa L. ssp.* could be generated, which is resistant to arsenite and arsenate (Chen Y *et al*., 2017). An attempt could be made for recombinant expression of arsenite oxidase and other proteins required for arsenite oxidation (Branco R *et al*., 2008). After the successful expression of these proteins and analysis of their functionality, contaminated soil and water could be treated with these proteins. Thus, there could be application of biotechnological tools for remediation of arsenic biomagnification in rice and for promotion of bioremediation and phytoremediation of arsenite and arsenate.

Environmental chemistry

Arsenic is a heavy metal with atomic number 33. It is considered to be a human carcinogen. White arsenic powder is odourless, tasteless and readily available. Therefore, it is widely known for its criminal use in homicide. Arsenic exists in four valence states: +5, +3, 0 and -3. The most common and toxic forms of arsenic are +5, As (V) or arsenate, and +3, As (III) or arsenite. Arsenite is much more toxic than arsenate. The oxidation state of arsenic is -3 in arsenides, which are alloy-like inter-metallic compounds. Arsenic occurs in many minerals. It combines easily with sulphur and metals. Arsenic is highly reactive in nature. Arsenic forms arsenic oxides upon exposure to oxygen. It forms acidic solutions with water. It reacts with metals to form arsenides. Arsenic acids and salts are the most common arsenic contaminants of ground water. With the variation of arsenic's valence state, variation can be observed in its solubility, toxicity, bioavailability and mobility. Both arsenite and arsenate are soluble, but arsenite has a higher solubility, bioavailabilty and mobility than arsenate (Yang HC and Rosen BP, 2016, Dey U *et al*., 2016).

Health effects

Long term intake of arsenic through contaminated food and water causes many types of cancer such as cancer of skin, lung, liver, bladder and kidney (Smith AH *et al*., 1992). Other adverse health effects associated with long term ingestion of arsenic include pulmonary diseases, neurotoxicity, diabetes, cardiovascular diseases and developmental defects (Hendryx M, 2009, Ratnaike RN, 2003). It also causes dermatological and respiratory disorders. Skin lesions are signs of advanced stages of arsenic poisoning. Arsenic inhibits ATP production through various mechanisms. It acts as a phosphate analog and is easily taken up by the cell. Mitochondrial respiration, ATP synthesis and reduction of NAD+ are inhibited by arsenic (Gresser MJ, 1981). It uncouples oxidative phosphorylation and inhibits lipoic acid, the cofactor of puruvate dehydrogenase. It also induces oxidative DNA damage and disrupts repair mechanisms (Li D *et al*., 2001).

Arsenic toxicity in Murshidabad district

West Bengal is one of the most arsenic affected states of India. Arsenic poisoning in West Bengal has been first reported in 1984 (Garai R *et al*., 1984). Since then various studies have been done in this direction. Nine districts of West Bengal are arsenic affected. Four (North 24- Pargana, Nadia, Malda and Murshidabad) of them are severely affected. South 24- Pargana is moderately affected, and the remaining four (Barddhaman, Howrah, Hooghly and Kolkata) are less affected (Rahman MM *et al*., 2005).

Murshidabad district is divided into 26 blocks. There are 2414 villages and wards in this district. The river Ganga separates it from Bangladesh. The Bhagirathi river divides it into two parts. Blocks situated in the western side of Bhagirathi river are less affected (30.1% above 10 ug/l and 11.7% above 50 ug/l) than the blocks located on the eastern side (64.7% above 10 ug/l and 32.5% above 50 ug/l) (Rahman MM *et al*., 2005). Only groundwater of Bharatpur-II block is within the WHO guideline value of arsenic at 10 ug/l. Suti-I and II, Raghunathganj-I are some of the blocks of western bank showing high level of arsenic contamination in groundwater. Arsenic contamination in the hand tubewells of West Bengal usually decreases with depth. In Murshidabad, all the tube-wells including unsafe ones, shows increase in arsenic contamination with depth of up to 30.7 m. In case of unsafe ones, this increasing trend is observed up to 45.9 m below the ground level. Thus, the study of groundwater arsenic contamination indicates the severity of the problem and an urgent need of methods for arsenic removal.

Bioremediation

Bioremediation is a natural process in which living organisms like bacteria, fungi, algae and plants are used to degrade or transform hazardous organic contaminants, or to reduce the toxicity of inorganic contaminants. Although, arsenic is toxic to most organisms, certain microorganisms are resistant to arsenic toxicity. Some of them use arsenite oxidase enzyme to convert arsenite (more toxic form) to arsenate (less toxic). For instance, bacteria like *Agrobacterium tumifaciens, Alcaligenes faecalis, Pseudomonas arsenitoxidans* and *Centribacterium arsenoxidans* are chemolithotrophic arsenic oxidising bacteria (Silver S and Phung L Te, 2005, Majumdar A *et al*., 2013, Chang JS, 2015). The genes encoding proteins important for arsenite oxidation reside in an operon, named as *aox* operon. These genes are *aoxS, aoxR, aoxA, aoxB*, $cytC_2$ and *chlE* in *Agrobacterium tumifaciens* (Kashyap DR *et al*., 2006). *aoxR* codes for a response regulator, which is a part of two- component signal transduction system. *aoxS* is a sensor histidine kinase. *aoxA* and *aoxB* code for small and large subunit of arsenite oxidase enzyme, respectively. $CytC_2$ is a cytochrome isoform and the protein product of *chlE* is important for molybdopterin biosynthesis (Branco R *et al*., 2009). Molybdopterin is a cofactor of arsenite oxidase enzyme (Kashyap DR *et al*., 2006). Therefore, recombinant expression of these proteins and their application in arsenic contaminated soil can reduce arsenic toxicity. Similarly, some plants like *Azolla caroliniana, Populus nigra, Pteris vittata* and *Pteris multifida* are also resistant to arsenic. They are arsenic accumulators and thus, can be used for phytoremediation of arsenic from soil (Tangahu BV *et al*., 2011). Therefore, bioremediation and phytoremediation provide an alternative tool for the treatment of mining drainage, sewer sludge and contaminated soil and water.

Biomagnification

Biomagnification is a process where the concentration of certain substances, such as toxic chemicals, heavy metals and pesticides, increase as they move up in a food chain. Arsenic contamination in surface soil and in plants is elevated by the use of arsenic contaminated ground water for irrigation in crop fields. Rice is a staple cereal crop in India and Bangladesh. Rice is known to accumulate arsenic into the grains, much more efficiently than any other staple cereal crops. Rice is generally grown in waterlogged flooded condition, where arsenic bioavailability is high in soil. Irrigation of a rice field with arsenic contaminated groundwater (0.55 mg/l) of 1000 mm results in an estimated addition of 5.5 kg of arsenic per hectare per annum (Huq Imamul SM *et al*., 2006). Therefore, knowledge of mechanism of arsenic uptake by rice will provide ways to mitigate this problem.

Arsenate and phosphate share the same transport pathway in higher plants, with the transporters having higher affinity for phosphate than for arsenate. The uptake mechanism involves co-transport of phosphate or arsenate and protons (Yang HC and Rosen BP, 2016). In *Oryza sativa*, two phosphate transporters Pht1;1 and Pht1;4 play a significant role in phosphate and arsenate acquisition from environment having both low and high concentration of arsenic. These phosphate transporters are strongly expressed in roots. In rice, arsenite is mainly taken up by members of nodulin 26- like intrinsic protein family (NIP) (Chen Y *et al*., 2017). In rice, OsNIP1; 1, OsNIP2; 1(Lsi1), OsNIP2; 2(Lsi6) and OsNIP3; 1 are major arsenite importers. Lsi1 and Lsi2 proteins are localized at exodermis and endodermis of rice roots, Lsi1 at the distal side and Lsi2 at the proximal side. Therefore, after uptake of arsenite by Lsi1 and others in root, Lsi2 is important for efflux of arsenite towards stele. These will lead to accumulation of arsenite in shoots. Therefore, mutation in *pht1*, *pht2* and *lsi2* gene can result in decreased acquisition and accumulation of arsenite and arsenate in rice.

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