

**“Studies on the arsenic affected paddy grown area of  
Uttar Pradesh, India and its remediation approach”**

**THESIS**

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**Babasaheb Bhimrao Ambedkar University  
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*Submitted by*  
**VISHVAS HARE**  
M.Sc.

*Under the supervision of*  
**Dr. V.S. Baghel**  
Assistant Professor



**Department of Environmental Microbiology  
School of Environmental Science  
Babasaheb Bhimrao Ambedkar University  
(A Central University)  
Vidya Vihar, Raebareli Road,  
Lucknow-226025, India**

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## 5. SUMMARY AND CONCLUSION

Arsenic (As) is an environmental contaminant gaining attention these days due to its carcinogenicity (chronic and acute) to humans. Rice (*Oryza sativa* L.) is a staple diet for more than half of the world's population. Rice is particularly efficient in accumulating As and paddy fields in South and South-East Asia, which are highly contaminated with As. Particularly, in Uttar Pradesh, India, where almost every people depend on rice as subsistence diet are getting affected. After that secondly contamination of arsenic in the soil and water of Uttar Pradesh was determined Survey because Geographically Uttar Pradesh situated in the northern region of India and border of Nepal. The river Ganga and Ghaghara are major river flows northeast to southeast. It is also a threat to sustainable agriculture in affected areas. The soil arsenic level ( $13.12 \text{ mg kg}^{-1}$ ) crossed the global average ( $10.0 \text{ mg kg}^{-1}$ ), but within the maximum acceptable limit for agricultural soil ( $20.0 \text{ mg kg}^{-1}$ ) recommended by the European Union. The total arsenic concentration on food crops varied from 0.000 to  $1.464 \text{ mg kg}^{-1}$  of dry weight. The highest mean arsenic concentration was found in potato ( $0.456 \text{ mg kg}^{-1}$ ), followed by rice grain ( $0.429 \text{ mg kg}^{-1}$ ). Arsenic above the 50 ppb limit for drinking but arsenic according to WHO limit found in 31 district. The acceptable level of arsenic by WHO for maximum concentrations of arsenic in safe drinking water is  $0.01 \text{ mg L}^{-1}$ . In case of soil, maximum arsenic was detected in soil of Sohaon (15.43 ppm). Arsenic levels were higher in soils collected from 15–30 cm depth than 0–15 cm from the soil surface. The total amount of arsenic in raw rice is not taken in human body due to its distribution in follows the order of root>straw>husk>grain. An appreciable high efficiency in translocation of arsenic

from shoot to grain was observed compared to the translocation of from root to shoot. The work entailed studying the effects of As on plant metabolism, uptake and toxicity along with the understanding the role of thiol metabolism and various antioxidative mechanisms in alleviation of deleterious effects of As in rice plants of Uttar Pradesh.

Based on this hypothesis, a field study was conducted involving 100 day rice from contaminated field of Baharaich, Uttar Pradesh and five different sites (*viz.*, Mari Matamandir (Block-Mihinpurwa), Govindpur (Block-Payagpur), Rashulpur (Block-Phakharpur), Lalpur (Block-Payagpur) and Kochwa (Block-Rupaideeh) of Uttar Pradesh are contaminated with arsenic.) for evaluating accumulation of As in fields varying in As contamination. A hydroponic study, based on previous field analysis concluded that Uttar Pradesh rice genotypes have great diversity for As accumulation.

The arsenic concentration in rice and other food plants was found to be high in Asia, especially in Bangladesh after that Uttar Pradesh, India, indicating that rice from this region would be a significant source of dietary. Arsenic-contaminated irrigation water could increase the As level in soil and its subsequently accumulation in food; however, the arsenic risk assessment of food based on the total content of As in the soil and irrigated groundwater can be misleading because arsenic accumulation in food plants is largely influenced by a variety of factors, including soil physicochemical parameters; other elements such as iron, phosphorus, sulfur, and silicon concentrations; and environmental conditions that control As availability and uptake in the soil-rhizosphere-plant system.

Arsenic is a ubiquitous metalloid found in the earth's crust. Arsenic is present in III and V oxidation states. Arsenic concentration in soil, water, and air varies from place to place. Arsenic comes into the environment by both, natural and anthropogenic

ways. Natural sources of arsenic contamination in soil and water are mainly geogenic and the anthropogenic sources are mining, smelting of arsenic-rich minerals, use of arsenic pesticides, wood preservative, etc. Soil and water pH, prevailing redox condition, the presence of organic matter, ions in water greatly influence the forms and stability of arsenic species present in soil and water.

Arsenic content in edible crop, its relationships with soil and irrigated groundwater and the factors controlling As mobilization and uptake in edible crops. Arsenic-contaminated irrigation water could increase the As level in soil and its subsequently accumulation in rice grains; however, the arsenic risk assessment of rice based on the total content of As in the soil and irrigated groundwater can be misleading because arsenic accumulation in plants is largely influenced by a variety of factors, including soil physicochemical parameters; other elements such as iron, phosphorus, sulfur, and silicon concentrations; and environmental conditions that control As availability and uptake in the soil-rhizosphere-plant system. Environmental conditions can be managed by changing irrigation practices. For example, the flooding of the paddy soil mobilizes As in the soil solution and can increase As accumulation in rice. Therefore, changing agricultural practices to aerobic rice cultivation throughout the entire season may be a viable strategy to mitigate this problem. However, there are arguments in certain cases because under flooding conditions.

In addition, the selection of rice cultivars based on high root aeration and formation of Fe-plaques on the roots can be an effective way to inhibit As uptake in areas having high As contamination in soil. The uptake of arsenite in rice usually occurs through the silicon transport pathway. Therefore, the application of silica fertilizer in soil is suggested to decrease the transfer of arsenic from the soil and

irrigation water to rice. Furthermore, phosphate fertilization is suggested to lower arsenate uptake in plants because both compounds enter rice via the same transporters. However, there are arguments in certain cases because under flooding conditions, As is present as arsenite, which cannot compete with phosphate; furthermore, phosphate increases As mobility because it competes with arsenate for the adsorption site on Fe oxides/hydroxides. In addition, the accumulation of As in the presence of Fe-plaques and other soil parameters is still confusing. For a better understanding of As toxicity in the water-soil-plant system, more work should be performed.

Arsenic toxicity has global concern in two ways first its strong carcinogenic nature secondly its presence in the food chain. Arsenic exposure through food consumption is major concern for arsenic affected area of Uttar Pradesh. Arsenic is a toxic material and is able to cause acute and chronic toxicity in humans. A major part of the global population is under threat of arsenic poisoning. Inorganic arsenic compounds have been more toxic than its organic forms. Especially the As(III) is very toxic to humans. Presence of arsenic in food plant is badly affecting a huge number of population causing skin lesions, cancer and other health problems. Acute arsenic poisoning causes vomiting, nausea, diarrhea, abdominal pain. Skin pigmentation, keratosis, Blackfoot disease, DNA impairment, inhibition of enzymatic activities and cancer results from chronic arsenic toxicity. MMA, DMA are found to have geno and neurotoxic effects among the organic forms of arsenic.

Arsenic removal techniques are mainly based on oxidation, coagulation, adsorption, precipitation and filtration. As-III is difficult to remove compared to the removal of As-V; almost every process of removal tumbles to remove it efficiently. Efficient removal of arsenic requires a pre-oxidation process where As-III oxidizes to

As-V. Conventional arsenic remediation technologies are good for treating higher concentration of arsenic but often fail to remove arsenic from water having lesser arsenic concentration. Application of the current remediation technologies in rural areas has hardly been successful and the challenge for the researchers is to develop such technologies which are able to work in rural conditions and are affordable to the rural poor people.

As is present as arsenite, which cannot compete with phosphate; furthermore, phosphate increases As mobility because it competes with arsenate for the adsorption site on Fe-oxides/hydroxides. The use of vegetation directly or indirectly to remove contaminants from water or soil is an important innovative remediation technology potentially applicable to a variety of contaminated sites. Selection of the appropriate plant species is a critical process for the success of this technology. Approaches to reduce arsenic uptake in crops, especially in the edible parts, would provide a viable alternative. Many natural substances are expected to exhibit substantial effects on the microbial processes and subsequently change the environmental behaviour of As, either directly or indirectly. Researching microorganism-As interactions also provides the opportunity of studying As remediation taking advantage of microbial activities.

The species of As significantly reduced the root length and Shoot length of rice as increased concentration of AsIII and AsV followed by a decline at higher doses. Photosynthetic pigments showed significant decline in chlorophyll *a*, *b* and total chlorophyll at As exposures with BBAU/MMM<sub>1</sub>. Carotenoid content showed increase at low concentrations but decrease in higher concentration.

It is well documented that various abiotic stresses lead to the overproduction of ROS in plants which are highly reactive and toxic and ultimately results in oxidative

stress. Overall, the involvement of ROS in various metabolic processes in plant cells might have general implications. Oxidative stress is a condition in which ROS or free radicals, are generated extra- or intra-cellularly, which can exert their toxic effects to the cells. These species may affect cell membrane properties and cause oxidative damage to nucleic acids, lipids and proteins that may make them nonfunctional.

However, the cells are equipped with excellent antioxidant defense mechanisms to detoxify the harmful effects of ROS, The antioxidant defenses could be either non-enzymatic (e.g. glutathione, praline, a-tocopherols, carotenoids and flavonoids) or enzymatic (e.g. superoxide dismutase, catalase glutathione peroxidase and glutathione reductase). ROS are now also considered as key regulatory molecules vital for cells, but they cause cellular damage when produced in excess or when the antioxidant defense system is not properly functioning. The free radicals also can interact with each other and with antioxidant systems. It is the balance of all constituents that determines their good or bad effects of ROS. ROS play dual role and it has been first described in pathogenesis but now also shown under various abiotic stress conditions. For such kind of roles, the concentration of ROS in cell must be controlled. Furthermore, the mechanism of ROS production and its scavenging, its targets and molecular functions must be explored.

It is well known that plant cells and its organelles like chloroplast, mitochondria and peroxisomes employ antioxidant defense systems to protect themselves against ROS induced oxidative stress. A great deal of research has also established that the induction of the cellular antioxidant machinery is important for protection against ROS. Overexpression of ROS scavenging enzymes like isoforms of SOD (Mn-SOD, Cu/Zn-SOD, Fe-SOD), CAT, APX, GR, DHAR, GST and GPX

resulted in abiotic stress tolerance in various crop plants due to efficient ROS scavenging capacity. Pyramiding of ROS scavenging enzymes may also be used to obtain abiotic stress tolerance plants. Therefore, plants with the ability to scavenge and/or control the level of cellular ROS may be useful in future to withstand harsh environmental conditions.

Increase in NPT content was observed with increasing exposures of As more in Rice roots and shoots. GSH and GSSG content increased in concentration dependent manner during both AsV and AsIII stress. GSH/GSSG ratio varied with concentrations and durations in both AsV and AsIII.

The positive correlation was observed between As accumulation and level of MDA, EC, H<sub>2</sub>O<sub>2</sub> and activity of LOX in rice roots and shoots. Conversely, Arsenic treatment significantly reduced the level of MDA, EC, H<sub>2</sub>O<sub>2</sub> and activity of LOX in roots in comparison to As treated plants. Similar response were observed for shoots during As treatment and the level of these parameters decreased when compared to As alone exposure. The decrease in the oxidative stress during Se supplementation might be attributed to directly or indirectly via regulation of antioxidant system.

The SOD activity was found to increase in both As species treatments with the increase concentration. The activity of CAT, APX and GPx was found to significantly decline upon As exposure with the level of decline being variable for roots and shoots. Upon Se supplementation, activity of these enzymes was found to significantly increase to even higher levels as compared to control.

The thiolic compounds were measured in terms of Cys, NPT, GSH, GSSG, GSH/GSSG ratio and PCs level. Results indicated that As exposure significantly elevated the level of Cys in roots and shoots as compared to control. The NPTs and

GSH content were found decreased in both roots and shoots under single As treatment, however, As+ BBAU/MMM<sub>1</sub> treatments enhanced the NPT and GSH contents when compared to control but the GSH content was negatively correlated with As accumulation in root and shoot. Further, GSH/GSSG ratio was negatively correlated with As accumulation in root and shoot. The PCs level was found to be increased in roots and shoots during As exposure. GST,  $\gamma$ -ECS and PCS activities were positively correlated with As accumulation in roots and shoots. Further, The PCS activity was positively correlated with PCs content in roots and shoots.

Arsenic contamination in soil and ground water is a major global problem and local geochemical cycles have been intensified by either geogenic or irresponsible industrial and mining activities. If these problems are not addressed, these could create disastrous effects on human and animal health as arsenic is carcinogenic in nature. Arsenic contamination is a major global problem and local geochemical cycles have been intensified by irresponsible industrial and mining activities. Fortunately, many microorganisms have already evolved mechanisms to cope with this environmental challenge. The fundamental understanding of the biochemistry and metabolic pathways involved in arsenic resistance are now being gradually translated into strategies for engineering microbes for effective arsenic remediation. Although the initial reports are promising, substantial improvements are necessary to move these approaches from the bench to practice. In this respect, new tools in synthetic biology will certainly enable us to increase our efforts toward this end.

Many microorganisms have already evolved mechanisms to cope with this environmental challenge and these noble organisms could be exploited properly to remediate arsenic contaminated soil and water. The major advantage of microbial

remediation is that it is a natural process with huge economic superiority over other methods. The fundamental understanding of the biochemistry and metabolic pathways involved in arsenic resistance are now being gradually translated into strategies for engineering microbes for effective arsenic remediation.

In this regards, bioremediation process provides and effective innovative measures for treatment of a wide variety of contaminants. Amongst the various known bioremediation process phytoremediation, rhizoremediation and bioremediation by microbes could be efficient methods to reduce the arsenic and lead contaminants of soil. Microbially mediated oxidation and reduction reactions may produce less mobile As species and mixed solid phases capable of sorbing As, thus enhancing the immobilization processes. The government should monitor industrial and agricultural activities leading to As pollution. The awareness of the population is deemed equally important in maintaining and choosing mitigation. However, even for well-aware population, the dilemma is often the ability to meet prohibitive costs versus the wish to improve their situation. Supervision departments should increase the frequency of sampling and analysis of the discharge from industrial plants. We sincerely hope that this study will be of considerable interest to the readers and reflects the latest state of the art on understanding of various interdisciplinary facets of the problem of arsenic in environmental realm, mechanisms of mobilization in groundwater, biogeochemical interactions, and the measure for remediation. However, the immobilization processes by sorption is reversible and the remobilization of sorbed As may occur when the site biogeochemical conditions change with time. Microbially enhanced phytoextractions hold greater promise to clean up the arsenic contaminated soil because this green cure technology being inexpensive exploits the plant and rhizosphere microorganisms with

special reference to arbuscular mycorrhizae. Moreover, the selection of appropriate plant genotypes could improve phytoremediation technologies. Detailed investigation and long-term continued monitoring are absolutely necessary to ensure that soil ecological factors are optimum for proper functioning of the microbes to alleviate arsenic toxicity.

The chemistry of soil and water (i.e., pH and Eh) and predominantly microbial assemblages play a major role in As dynamics. Although bioaccumulation of As in plants and organisms has been reported, its biochemical transformations within the plant and other biota are still largely unknown. Engineering strategies using environmentally benign products may be considered to enhance the remediation rates and efficiency. However, most of these technologies have been tested only at the laboratory and pilot scale levels. Large-scale application of such technologies requires trained personnel for the operation of equipment to treat soils and waters. Although the initial reports are promising, substantial improvements are necessary to move these approaches from the bench to practice. In this respect, new tools in synthetic biology will certainly enable us to increase our efforts toward this end. The increase global population, industrialization and urbanization are the some major reasons to contaminate the environment.

From the results it may concluded that microbes could influence toxicity produced by abiotic stress. Increased growth of plants under As(III) reflects role of bacterial strain (BBAU/MMM<sub>1</sub>) assisting in nutrient acquisition through phosphate solubilization and secretion of different exudates. Reduced TBARS content concomitant increased in enzymatic and non enzymatic antioxidant protects plants against stress and provides tolerance. In addition, approx. 43% reduction in As

accumulation in root and reduced translocation in shoot, proved a strong candidature for As bioremediation as well as safeguard of rice crop in agriculture field. In this way bacterial strain (BBAU/MMM<sub>1</sub>) could be used in agricultural fields for enhanced production of rice and minimization of As translocation in shoot.