

BIO-REMEDIATION OF HEAVY METALS FROM DRINKING WATER BY THE HELP OF MICRO-ORGANISMS WITH THE USE OF BIOREACTOR

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Abstract— On Earth water has too many forms and variety which are necessary specifically for particular geographical as well as environmental surroundings. Below 1% of the world's fresh water (0.007% of all water on earth) is reachable for direct human uses. Water pollutions now become a part of concern and disquiet in country like India. Large parts of water which are life supportive get contaminated because of illegal activities of human beings. Water effluence is a major problem globally. It is the leading worldwide cause of deaths and diseases, and that it accounts for the deaths of more than 14,000 people daily. In addition to the acute problems of different problems in developing countries, industrialized countries continue to struggle with water pollution problems as well. There are many inorganic metals which are contaminating water bodies which serve life to large part of India, Arsenic (As) is one of the biggest threats for water bodies. High toxicity of Arsenic poses a serious risk not only to ecological systems but also for human health. There is availability of sophisticated techniques for arsenic removal from contaminated water, development of new laboratory based techniques along with cost reduction and enhancement of conventional techniques are essential for the benefit of common people. This paper is based on the future aspects, for removal of Arsenic from drinking water or the water of different rivers like Ganga, Gomti and Yamuna etc which humans are consuming for domestic purpose. Demograph estimate that around 52 millions peoples are drinking ground water with arsenic concentrations above the guidelines of World Health Organization. WHO proposed a parameter or MIC for Arsenic i.e. of 10 parts per billion (ppb) or 0.010 Mg/L, it is found that level of Arsenic has been increased vigorously in many rivers. Objective is to apply Bioremediation technique with the help of batch culture that needs Bioremediators to detoxify contaminated water and helps in maintaining the original quality of water.

Key words— Arsenic, MIC, Bioremediation, Batch culture, Bioremediators

I. INTRODUCTION

A. Water

Our Earth is called a “blue planet” as it contains a large and enormous percentage of water than land. On earth water has too many forms and variety which are necessary specifically for particular geographical as well as environmental conditions. It was found that only < 1% of water is available for drinking or domestic use and rest of the part is saline. Human being, plants & animals i.e. living being needs water for their survival. < 1% of the world's fresh water (~0.007% of all water on earth) is accessible for direct human uses. Rivers, lakes, ponds, estuaries, waterfalls, sea, oceans, deltas these are the different types of varieties of water bodies and have different percentage of water in such forms as it

covers 71% of the Earth's surface. It is vital for all known forms of existence. On Earth, 96.5% of the total water is found in seas and oceans, 1.7% in groundwater, 1.7% in glaciers and the ice caps of Antarctica also small fraction in other large water bodies and 0.001% in form of haze, (water cycle), and rainfall. Domestic water (fresh water) constitutes only 2.5% of the Earth's water and from that 98.8% of water is in the form of ice and ground water. [1] Less than 0.3% of all freshwater is in rivers, lakes, and the atmosphere, and smaller amount of the Earth's freshwater (0.003%) is contained within biological bodies and manufactured products. It's a fact that origin of life takes place in water on earth “Oparin- Haldane theory”. Long voyage from Unicellular to Multicellular organisms' water is necessary either for their survival or for their basic needs. As unicellular get developed into multicellular organisms, this development took place in water itself, different chemical as well as biological reactions took place this accident had occur millions of years ago this also defines origin of life due to water and just because of that all living being needs water for their survival[2]. For the subsistence of life on Earth the existence of liquid form of water and some extent its gaseous and solid forms are vital. Our Earth has geographical relationship with water so it is necessary to know the features of water on earth. The Earth is located in the “zonal habitat” of the solar system, if it were slightly closer to or farther from the Sun (about 5%, or about 8 million km.), the conditions which allow the forms of water to be present simultaneously would be far less likely to exist. Gravity exists on earth that allows it to hold an atmosphere. Carbon dioxide and Water vapour in the atmosphere provide a temperature buffer (greenhouse effects) which helps to maintain a relatively steady surface temperature. If Earth were smaller, temperature extremes would allow by a thinner atmosphere, thus the accumulation of water except in ice caps of polar region get prevented [3]. Water is necessary in many geological processes. In most of the rocks Groundwater is present, and the pressure of this groundwater affects Faulting patterns. Water present in the mantle play a very major role as it is responsible for the melt that produces volcanoes at subduction zones. On the surface of the Earth, water is obligatory in both physical and chemical weathering processes. For a large amount of sediment transport that occurs on the surface of the earth's water, and to a lesser but still significant extent, ice, are also responsible. Many types of sedimentary rocks are formed by deposition of transported sediment which makes up the geological record of earth's history [4]. Water scarcity is the major concern in most part of

the world, and its availability is a major social and economic issue. Currently, about a billion people around the world consume contaminated as well as unhealthy water. Up to 2015 the number of people worldwide who do not have access to safe water and sanitation, many countries accepted the goal of halving the rate during the 2003 G8 Evian summit [5]. [6] Water, however, cannot be a finite resource, but rather it will be re-circulated as potable water in precipitation in quantities, many degrees of magnitude higher than consumption by human beings. If this difficult goal is met then also it will still leave more than an estimated half a billion people without access to adequate sanitation and over a billion without access to safe drinking water. Bad or poor water quality and bad sanitation are deadly; some five- six million deaths a year are caused by polluted or contaminated drinking water. Developing world scenario said that, 90% of all waste water still goes into local rivers and streams without proper treatment. The strain not only affects surface freshwater bodies like rivers and lakes, but it also degrades groundwater resources. Demograph of Some 50 countries show that roughly a third of the world's population also suffer from medium or high water scarcity stress, and 17 of these extract more water annually than is recharged through their natural water cycles this is the statically proved data. Due to water pollution there are different types of diseases occurring and are also getting increase day by day like Diarrhea, Cholera, Jaundice and other Gastro problems are the major issue now these days. Not only this, there are also harmful chemicals which are polluting water; these pollutants are release as non-biodegradable waste product which is easily thrown to the river. There are many heavy metals who show their presence in our rivers which are boon to life and prove that these are also contributing in contamination of water. Improper sewage, industrial waste, illegal human activities all are responsible for water pollution [8].

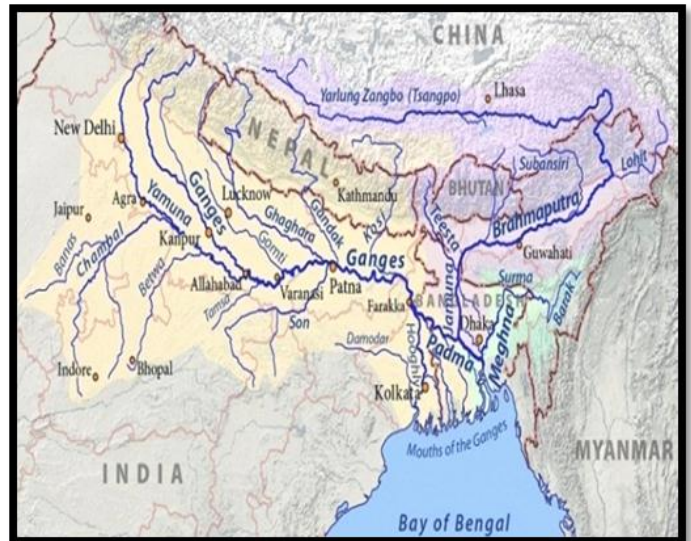
B. India and Water

Indian scenario is also not good as we all had got highest rank amongst the top polluted water countries because we did not know how to make water safe, because we don't know how to conserve water, because we are not concern about sustainable development and for our mistake the upcoming generation has to pay for it. Condition of Major River like Ganga Yamuna and their substitutes are not good, taking the example of river Yamuna, in New Delhi the river is actually dead because of 0.0 B.O.D the water which is now serving the capital of India is just a flow of sewage which is a mix up of sewage and industrial waste and that water is consumed by the citizens of New Delhi that's why the water scarcity problem in New Delhi is the biggest issue, think that when the condition of capital of India is worst what is the condition of rest of the country[9].

II. THE GANGES

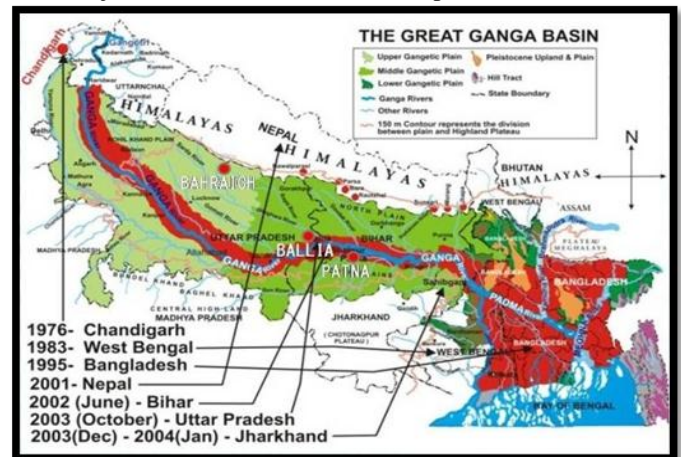
River **Ganga** is a national river of India serving maximum Northern Plains of India in many ways like for domestic purpose, for ethical purpose, for industries, for irrigation, for hydroelectricity that is in the form of energy resource but can anybody ask that Ganga is clean river or not, river Ganga exists on earth since from that era when there was no life on earth in form of human beings. River Ganga originates from Gangotri glaciers over Himalayas and ends her passage to Bay of Bengal it covers about 2,525 km distance overall, but before that this river has taken so many turns as well as burden in form of waste, toxic products and buried parts of human body.

The Ganges River contains the utmost freight of silt than any other river in the world and the deposition of this material in the delta region resulting in the largest river delta in the world (400 km from north to south and 320 km from east to west). The affluent mangrove forests of the Gangetic delta called as Sunderban delta contain very incredible and valuable species of plants and animals which are consummate among many forest ecosystems existing on the earth [10]. The expansion of groundwater in different parts of the country has not been uniform. Highly exhaustive development of ground water in certain areas in the country had resulted in over exploitation leading to decline in the levels of ground water and sea water intrusion in coastal areas. There is unremitting growth in dark and overexploited areas in the country [11].

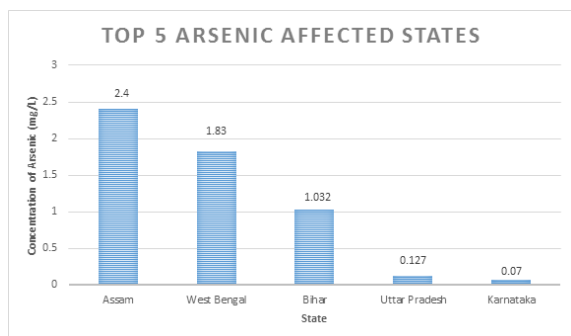


The Ganges has to suffer from extreme pollution conditions, a demographic study said that this affects near about 400 million people who exists close to the river. Suspended Particulate matter is also responsible for contamination of water quality of the rivers. This major problem is exacerbated due the fact that many people who live their life to poverty line and their life dependently rely on the river on a daily basis for different domestic purpose like bathing, cooking and washing [12]. The world economy representative "WORLD BANK" estimates that three percent of India's GDP is equal to that the health costs of pollution of water in India It has been proposed statically that 80% of all illnesses in India and one-third of deaths can be contributed to water-borne diseases. Rapid deforestation in the last few decades, increment in silt deposits occur due to topsoil erosion in the lose area which lead to the raise the river bed and also lead to devastating floods in the rainy season and stagnant flow in the dry season [13]. Along the main river course there are 25 towns with a population of more than 100,000 and about another 23 towns with populations above 50,000. In addition there are 50 smaller towns with populations above 20,000. There are also about 100 identified major industries located directly on the river, of which 68 are considered as grossly polluting. Fifty-five of these industrial units have complied with the regulations and installed effluent treatment plants (ETPs) and legal proceedings are in progress for the remaining units. The natural assimilative capacity of the river is severely stressed [14]. Cultural, Historical, Educational capital of India "Varanasi", a city of more than one million people that many pilgrims explore this holy place to take a "holy dip" in Ganga, statics shows around 200 million litres of untreated human

sewage flows into the river each day, which leads to large concentrations of “coliform” bacteria. According to official principles, it is estimated that water is only safe for bathing should not contain more than 500 “faecal coliforms” per 100ml, and upstream of ghats of Varanasi, the water of Ganga river already contains 120 times as much, sixty thousands “faecal coliform” bacteria per 100 ml which is now comes in major concern by many non- governmental organizations. The basin of Ganges is significantly influential to the agricultural economies of India and Bangladesh just because of this India is called for Agriculture Based Country. River Ganga and its tributaries provide for irrigation, a perennial source by covering a large area. Chief crops cultivated in the area include rice, wheat and potatoes [15]. There are also many fishing opportunities to many small scale industries along the river, though it remains highly polluted. The major industrial towns like Varanasi, Kanpur etc situated on the banks of the river with the preponderance of tanning industries add to the pollution [16]. A rich growing area for crops such as legumes, chillies, mustard, sesame, sugarcane, and jute along the banks of the river because of the presence of swamp and lakes provided naturally. A plan is made seeing the worst condition of Ganga i.e. “The Ganga Action Plan”, which was taken on priority and with much enthusiasm but unfortunately it was delayed for two years. The result was not very appreciable after the expenditure was almost doubled. The concerning governments and the agencies which got the responsibility for the plan were not very prompt to make it a success. Social Audit was not taken into consideration which can help to reduce the time in surveying. The releasing of industrial and urban wastes in the river was not controlled fully. The flowing of dirty water through drains and sewers were not adequately diverted as they are diverted to directly into the river. The continuing customs of throwing carcasses, burning dead bodies, washing of dirty clothes by washer men, immersion of idols cattle, wallowing etc were not checked properly. Ignorance and avoidance to all these points all these made the Action Plan a failure, has also been variously attributed to "environmental issue without proper understanding of the interaction or relationship between human and environment the failure of the Ganga Action Plan occur. Customs, tradition, culture ethical issues, beliefs, corruption and a lack of technical acquaintance and lack of support from religious authorities. These are some highlighted points which are now leading to destroy the quality of water of river Ganga. In 2009 December the World Bank agreed to pass loan for India of US\$1 billion over the next five years, for achieving the objective of cleaning The Ganges. According to Budget 2010 Planning Commission estimates, an investment of almost Rs. 70 billion (approximately US\$1.5 billion) is needed to clean up the river[17].



In November 2008, the Ganges, alone among India's rivers, was declared a "National River", facilitating the formation of a “National Ganga River Basin Authority (NGBA)” that would have greater powers to measures aimed at protecting the river as well as set plan, implementation is also necessary and finally a monitoring cell has to form which look after all the central issues [18]. The incidence of water borne and enteric diseases such as intestinal diseases, different types of infections like typhoid, cholera etc significantly among people who are using the water of river Ganga for bathing, washing dishes and brushing teeth is high and other domestic purpose, demographically 66% per year. Recent studies by Indian Council of Medical Research (ICMR) proved that the river is now full of pollutants which are highly toxic that those are living along its banks in Uttar Pradesh, Bengal and Bihar [19]. Bacteriophages are also found in river Ganga designated as “super bugs”, helpful in phage therapy against patients of Diarrhea, phages play a medicinal role in formation of phage therapy which will be very helpful for the treatment of patients of Diarrhea [20]. It has now be proven that as The Ganges came to northern plains region of India it start accumulating itself with lots of unpredictable stats and results in form of toxic contamination but as the fact says that Ganga flow with very high speed so all the contaminated also flow to the deeper regions and West Bengal as Indian state suffers a lot by this. Bangladesh a sub continental country a very close to West Bengal also suffers the devastating features of river Ganga in Bangladesh called as “JAMUNA”. Ministry of Health and ruling authorities for Ganga proved that accumulation of heavy metals in the water of river Ganga made it toxic in lower regions of India [21]. The figure shown above proved that not only the river but nearby places are also contaminated due to Arsenic poisoning. If we scrutinize the figure it proves that the eastern part is highly contaminated than Northern part but both the regions are toxic but eastern part is showing maximum toxicity rate because at northern plains of India Ganga flow with a continuous speed that means water of river Ganga is not in rest form but in Maximum part of India as well as in Bangladesh water of river Ganga is in state form basically in Sunderban delta region that’s why the accumulation of contaminated water is higher in eastern region rather than Northern Plains of India, Biochemical Oxygen Demand start decreasing as we start moving from higher to eastern part eventually proves that Ganga is contaminated in Eastern India [22].

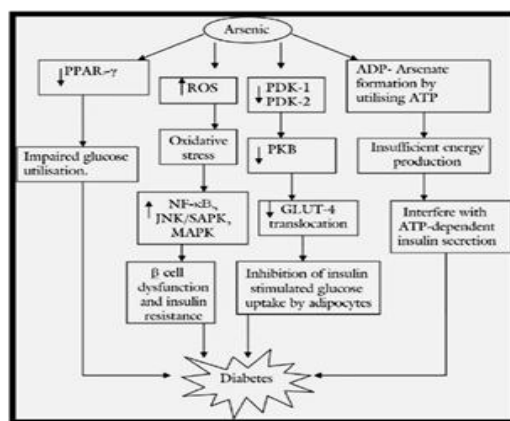


One more report by the government of India shows that Royal Bengal Tigers of Sunderban delta are also anguish from different diseases or genetic disorder because their body is consuming that Arsenic as heavy metal and they are also getting dead in that toxic environment [23]. Loss of Dissolved Oxygen, Loss of Biochemical Oxygen Demand etc also leads to a threatening for aquatic life, for example Gangatic Dolphins are very famous all over world but not as single authority is considering that a great loss to the Dolphins going to occur which are living in river Ganga. The dolphins of river Ganga, which used to exist in near to urban centres in the river Ganga, is now seriously in danger of extinction by pollution and dam constructions. Their population have now dwindled to a quarter of their numbers of fifteen years before, and now they have become extinct from the main streams of river Ganga [24]. A recent survey by the WWF reported only 3,000 dolphins left in the water catchment of Ganga river system. Indirectly or directly these contaminated water is responsible for many losses, it is now found that the rice or wheat products which is growing by the farmers contains parts of heavy metals included and when it serves to us then our body consumes those poisonous food which is really toxic and can destroy us [25].

III. ARSENIC

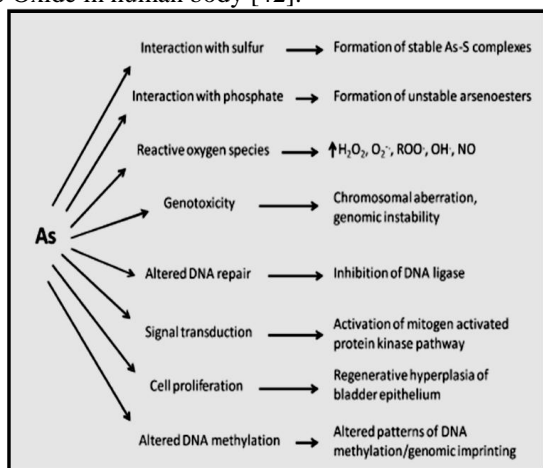
Arsenic (As) is a chemical element with atomic number 33. It occurs in many minerals and in a pure form of existing elemental crystal. Albertus Magnus was the first person to document Arsenic in the year 1250 [26]. Actually Arsenic is a metalloid and can be found in the form of different allotropes. There are many fundamental uses of Arsenic like it is used in underpinning alloys of copper [27]. In the field of semi conductors Arsenic is an N- type dopant. In doping techniques Arsenic is used like "Gallium arsenide" is a common semiconductors used after doped silicon. In formation of herbicides, pesticides Arsenic are used but now a day's its use are banned in many fields. Arsenic is a poison for multicellular life existing on the earth, it's a fact that some species of bacteria can able to use arsenic compounds as primary metabolites or authoritarian metabolites. The trioxide form of Arsenic is used in the pesticides industry, industry of insecticides as well as herbicides [27]. Arsenic being a heavy metal it is a metalloid and poisonous to living being this came to know by understanding the analysis of physio-chemical properties because sometime objective is hiding in these properties only and without understanding the physio-chemical properties it is not possible to cure it [28]. It is stable in nature; [29] Yellow arsenic is tetraphosphorus (P_4) is very soft as well as waxy as both have 4 atoms precise in a tetrahedral type in which each atom is bound to each of the other 3 atoms by a single bond. An unstable allotrope, being molecular, is the most volatile, low density as well as high toxicity. One more form Black Arsenic very similar to red phosphorus [30]. By cooling vapour state at 100-220^oC Black

Arsenic is formed which is a poor conductor of electricity. One stable isotope of natural occurring Arsenic is ^{75}As , this is said to be monoisotopic element but many radio isotopes have also been synthesized and their atomic mass ranges from 60 to 92. Out of this the most stable form is known as ^{73}As has half life of 65.30hrs. , ^{72}As has half life of 26.00hrs. , ^{77}As has a half life of 38.83hrs. Isotopes which are lighter than the stable [31]. ^{75}As tend to decay by β^+ decay, and those that are heavier and tend to decay by β^- decay with some expectations. Least ten nuclear isomers have been described, ranging in atomic mass from 66 to 84. The most stable form of Arsenic isomer is ^{68m}As Arsenic with a half life of 111 seconds. These values define atomic properties as many times these metals or metalloid are exist in different forms not in their native state so atomic properties helps to determine that these forms are new elements or they are the isotopes of any known element [32] [33]. Arsenic in form of sulphur compounds is also found. Realgar (As_4S_4) and Orpiment (As_2S_3) are abundantly found in nature and formerly used as in paint industry. In As_4S_{10} , arsenic has exclusive oxidation state of +2 in As_4S_4 which describe bond between the As atoms so that the valancy of As is fixed as 3 [34]. Industries waste management program has to be made so to control and check the level of waste which is toxic in nature and provide a specific area so that these toxic resources are thrown into that place [35]. These toxic compounds which are used to make highly toxic materials like in pesticides poison etc. The proton acceptance steps between the Arsenate and Arsenic acid are similar to the Phosphate and Phosphoric acid as somewhat structures are also same. Unlike phosphorous acid, Arsenous acid is genuinely basic in nature, with the formula $\text{As}(\text{OH})_3$. The total volume of Arsenic makes up about 1.5 ppm (0.00015%) of the total Earth's crust and just because of 53rd most abundant element in Earth. Soil contains 1–10 ppm of arsenic which is an inactivated or normal range for Arsenic also recognized by WHO. Statistics said that water in form of oceans and sea has only 1.6 ppb Arsenic (As). Many lower Arsenic containing minerals are known. An organic form of Arsenic also occurs in the environment [36]. Arsenic is obtained mainly as a peripheral product from the Copper purification. Furthermore purification from chalcogens and sulfur which is achieved by Sublimation process in vacuum or in a hydrogenic atmosphere or simply by distillation from Lead-Arsenic mixture present in molten form. Dust from gold, copper, and lead smelters Arsenic is just a part of that smelter only [37].



Arsenic in inorganic form is considered the most potential form of human carcinogen, and humans are exposed to it from water, soil, air and food. In the system of arsenic metabolism,

inorganic arsenic is get methylated to monomethylarsonic acid (MMA) and finally to dimethylarsinic acid (DMA), which get excreted through urine. Thus, DNA hypomethylation may cause due to arsenic exposure resulting continuous methyl depletion, initiating aberrant gene expression that results in carcinogenesis. Further, though arsenic is non mutagenic, it interacts significantly with gene based toxic agents in the assembly of different types of mutation, and also different chromosomal disorders which get induces and proliferation of cell also occur. In the arsenic endemic regions of West Bengal (India) due to arsenic contamination this region is declared as epidemic and epidemiological research have established that inorganic arsenicals have the capability to cause lung and skin cancers in human being. Research on the genetic polymorphism like SNP in the arsenic methyltransferases enzyme with the population exposed to arsenic, and quantification in the arsenic-induced mutational spectrum may be necessary for the development of molecular markers and therapeutics and for simplifying the knowledge and features of arsenic-induced carcinogenesis [38][39]. Arsenic contamination is understood to be of geo-genic origin that means released from soil under conditions inductive to dissolution of Arsenic from solid phase on grains of soil to liquid phase in water and fertilizer percolation of residues must played a modifying and significant role in imminent exaggeration. There are large number of hypotheses and predictions about the source of Arsenic and sufficient reasons of occurrence in groundwater [40]. Symptoms of arsenic poisoning begin with headaches, confusion and drowsiness. As the poisoning develops, convulsions and changes in fingernail pigmentation may occur. When the poisoning becomes acute, symptoms may include diarrhea, vomiting, blood in the urine, cramping muscles, hair loss, stomach pain, and more convulsions. Inorganic arsenic show the mechanism of action of leading to cancer remains a mystery. There is no proved report which can justify that inorganic arsenic species can react covalently with DNA just like organic carcinogens. But, to act as carcinogens (which cause cancer) it has to be act in some way to alter or restrict the regulation of cell replication. In the study with human keratinocyte cultures, increase in cell proliferation arsenite was shown the formation of keratinocyte-derived growth factors [41]. This significant activation process of Arsenic and its substitute salt appeared to generate Oxidative Stress which initiate cardiac problem as the free radical scavenger N-acetylcysteine inhibit the activation of the kinases. Directly or indirectly Arsenic also plays an important role in increasing oxidative stress by lowering the Nitric Oxide in human body [42].



A research study on rats in the field of immunology has done in which rats are daily dosed with arsenic in their water every month the level of arsenic get increases by the researches, it is found that arsenic is not effecting the rats and rats are curved towards the garlic, this experiment is done in levels equivalent to those found in groundwater in West Bengal in India and Bangladesh and the consequences were found to respond towards garlic extracts. It was found that rats which are tested for arsenic are recover with 40 percent less arsenic in their blood and liver, and 47 percent more arsenic get passed from their urine. The conclusion is conforming that sulphur-containing substances in garlic, eradicate arsenic from blood and tissues. The whole observation concludes that people who are living in areas at risk of arsenic contamination in any kind of water supply should eat one to three peels of garlic per day which will really help out human to be safe, garlic is also helpful for the patients of blood pressure.

Removal Methods

Natural as well as feasible methods are necessary to remediate it on a large scale keeping in view that those removal methods may not be disadvantageous for humans as well as different living beings. Water pollution and its remediation are now a part of major concern and cost effective techniques could help it out [43].

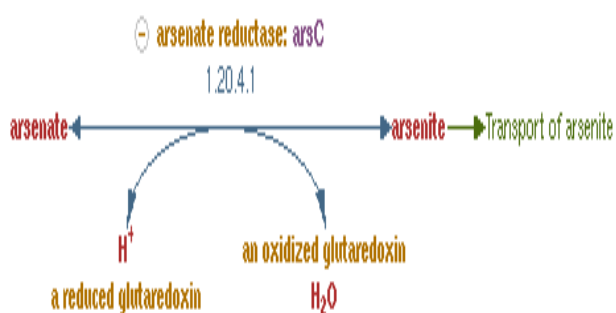
Chelation Processing

Chemical and synthetic- Artificial methods are used to treat arsenic poisoning or Arsenicosis. Chelating agents such as Dimercaprol and Dimercaptosuccinic acid are help in removing the arsenic away from blood proteins in the human body and are also used in treatment of acute arsenic poisoning. But it has too many side-effects also; most important side effect is Hypertension. Hypertension may lead to death of human being also so its use is not in medical field. Dimercaprol is considerably more toxic than succinic acid but DMSA monoesters e.g. MiADMSA are helpful antidotes for arsenic poisoning. It is found that over dose of such drugs may lead to serious infection in human body. Synthetic materials are not always suitable for human body.

IV. Bioremediation

It is a technique of waste management which involves the significant use of different types of organisms to remove neutralizes and eradicates pollutants from the site of contamination and toxification. According to official as well as governmental authorities bioremediation is a “biological treatment that utilize naturally occurring organisms or micro-organisms to break down hazardous as well as catastrophic substances into less toxic or non-toxic as well as reaches to inactive state; substances”. Bioremediation technologies can be generally classified into two categories as 1st *In situ* bioremediation and 2nd *ex situ* bioremediation. *In situ* bioremediation means involving treatment of the contaminated material at the site itself, whereas *ex situ* means “involves the removal of the contaminated material to be treated somewhere else. There are many examples of bioremediation related techniques are phytoremediation, biostimulation, rizofertilizers, biopesticides etc [44]. Current advancements and development have also proved successful by the addition of similar microbial strains having such genes which can deliberately help in removing toxicity from environment to the medium and to enhance the population of microbe which has ability to break down contaminants and toxic properties. Microorganisms which are used for the

intensive function of bioremediation are called as **Bioremediators** [44]. However, all contaminants are not able to treat through bioremediation by using microorganisms. For example, different heavy metals such as lead and cadmium are not readily absorbed or disrupt by microorganisms. A dynamic question arises as the primary detoxification process regarding the determination of methylation, since some of the mammals are deficient in the primary enzyme of this metabolic process. In case of human beings who have been exposed to a high levels of arsenic in drinking water due to many unsolved reasons, during treatment with sodium 2,3- dimercapto-1-propane sulfonate DMPS urinary MMA levels were found to increase several turns, a chelating agent is also used to treat metal intoxication from natural resources [45]. It is uncommon that over accumulation of arsenic exposure had predisposed abruptly the methylation of MMA to DMA which is very important to know, though it is also a fact that the actual physiological and metabiological cause of reduced rate of methylation due to arsenic exposure is yet unclear. Arsenite also have a high affinity as well as compatibility for "Thiol" groups in many proteins which can form various complexes with vicinal implicative thiols and directly inhibit more than 200 enzymes at a time. So, inhibition or restriction of methyltransferase by arsenite could affect arsenic metabolism positively [46]. Second aspect, genetic polymorphism in the arsenite MMA (monomethylarsonic acid) methyltransferases might contribute to the observed disruption in arsenic metabolism and sharp variations in degree of susceptibility and accountability of exposure to arsenic within a population or of different geographic regions. So, research in the genetic polymorphism, using different amino acid sequences or the coding gene of arsenite methyltransferases can be taken as a probe in the arsenic-endemic large population of West Bengal should provide just round the corner knowledge into the genetic study of arsenic metabolism [47].



As shown in figure that arsenic reductase is working in conversion of Arsenate V to Arsenite III similarly Arsenic oxidase is responsible for the conversion from arsenite III to arsenate V. Arsenic is also defined as pro-oxidant and thus may cause lipid peroxidation, protein and enzyme oxidation and GSH depletion, DNA oxidation and DNA adducts. Further, arsenic generates reactive oxygen species like nitric oxide; reactive oxygen species are known to induce poly ADP-ribosylation which is implicated in DNA repair, signal transduction and apoptosis. As a result, arsenite may induce DNA strand-breaks and NAD depletion.

Hence the genotoxic effects of arsenic compounds may be connected with an inhibition of DNA repair or the induction of oxidative stress. In fact, metabolic methylation of inorganic arsenic to DMA is involved in induction of DNA damage and DNA single-strand breaks resulting from the

inhibition of repair polymerization and hence is a genotoxic-enhancing process. It is thus likely that arsenic-mediated DNA-protein interactions may play a major role in arsenic carcinogenesis and the induced protein associated DNA-strand breaks could provide an explanation for chromosome aberration [47]. Studies on the basis of Molecular biology, Genetics, Biochemical studies, microbiological, biotechnological, bio-informatical and structure biological are going on but yet the consequences and conclusions are not verified up to that clinical extent [48]. There are so many techniques already implemented for remediation of arsenic but all cannot as useful for removal of such toxic metal in mass. It is very necessary to find such micro-organisms which are helpful in removing arsenic but their estimation in different river is not yet proved though it is found that bacteria which have the property of methylation can methyl arsenic in detoxify form. It is mandatory to found such bacteria that will not able to spread disease [49][50]. It is a contradiction or may be exceptional case that such detoxifying bacteria are found only those places which are arsenic affected. But their populations are very low at that place and are not able to detoxify on a mass level. National Institute of Technology Rourkela has isolated some unknown strains of bacteria which are helpful in removing Arsenic from water especially in this report we have to focus on those remediators which provide a least cost effective approach [52][53].

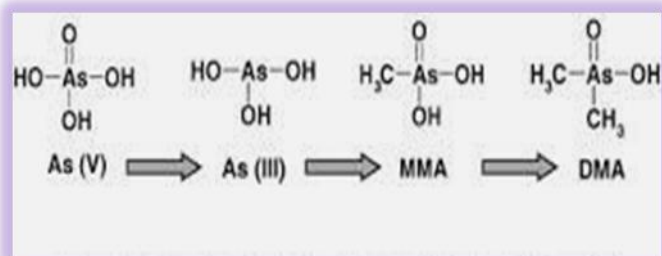
There are many microorganisms found on earth since from very long time. The fact provided against arsenic is that not a single vision is applied to bioremediate it from water. Metabolic pathway of Arsenic is based on the two enzymes arsenic reductase and methyltransferase. It is found via KEGG genome that Arsenic inhibits most of the enzyme regulation but being nontoxic form it can be excrete through Urine. It is found that the pentavalent metabolites MMA V and DMA V are less toxic than arsenite or arsenate Excretion of Arsenic in the urine primarily through the kidneys occurs. Humans excrete a mixture of inorganic, monomethylated, and dimethylated but not the trimethylated forms of arsenic as it is very toxic and this form cause disease [54]. Identification of such microorganisms is necessary so to compare the metabolic pathway of such organism as well as metabolic pathway of Arsenic in Human body as we are searching towards the basic aspects which can prove that this bacterium will help in bioremediating heavy and toxic metals from water. It also to find that this bacteria is found in water or not and if it is not found in water then what are the specialization to associate both of them at a very low cost. It is also necessary to find different techniques which can prove that in human body the detoxification would occur in similar manner.

V. EXPERIMENT

Bio-remediation of heavy metals from drinking water by the help of micro-organisms with the use of bioreactor

Brief Study-- World Health Organization. WHO proposed a parameter or MIC for Arsenic i.e. of 10 parts per billion (ppb) or 0.010 Mg/L, it is found that level of Arsenic has been increased vigorously in many rivers. Objective is to apply Bioremediation technique with the help of batch culture that needs Bioremediators to detoxify contaminated water and helps in maintaining the original quality of water. Aim is to identify that will this strain of E.coli K12 sub strain MG-1655 can be act suitable for detoxification process of water which is

contaminated due to arsenic. This strain theoretically proved for detoxification process against arsenic but yet regarding practical analysis has not been proven so for justification it is necessary to follow some new procedure to analyze that it is confirmed or not. Bioreactor can be further used for mass production of such micro-organisms which would surely help in improving treatment plant so that our rivers get also purified from such harmful effects.



VI. MATERIAL REQUIRED

Petri plates, L.B. media, Agar, conical flask, DO probe detector, sterile condition, Incubator, Inoculation loop, Test tubes, cotton, paper, spirit lamp.

VII. PROCEDURE

- A. Collection of water sample of river Ganga from different cities like Varanasi, Gazipur, Allahabad and Kanpur.
- B. Sample received from MTCC- IMTECH was provided in a glass tube sealed from all side. It is packed in a very sophisticated manner in such way that it cannot be broke by chance.
- C. Sealed sample is crushed safely under sterile condition; this sterile condition will help to protect culture safe free from contamination.
- D. Now it was the time to analyze the culture,
- E. Formation of L.B. media of Broth nature and inoculate this bacterial strain into the media and incubate it for 24hrs at 37°C.
- F. After incubation up to 24hrs it is found that this strain of E.coli gives a translucent formation in conical flask containing L.B. media; Preparation of different aliquots of enriched culture. Stored at 15-20°C so that its properties remain sustained.
- G. Now preparation of water sample with Arsenic trioxide that means 0.001gm of Arsenic trioxide has been taken in 100ml of water.
- H. Measurement of Dissolved Oxygen in different water sample collected from different ghats of Varanasi and from other cities like Gazipur, Allahabad and Kanpur.
- I. Preparation of solidified L.B. media and this solidified media pour on different Petri plates this method is applied for screening test of strain producing or following which pathway.
- J. With the help of micropipette at same concentration of water sample has been taken and enriched culture has been spread over four solidified LB media plate and two controls are also formed out of that one contains the untreated water sample and other contains enriched culture.
- K. To find the original concentration it is necessary to find the CFU, which means how many colonies are necessary to convert toxic into detoxification.
- L. for that ready all apparatus for Serial dilution technique, take 9ml water which is distilled and take 1ml enriched sample serially dilute it for 10-12 times and finally spread 1ml of that to new solidified plate.

M. On a colony counter it is found that in 1 section 6 colonies are formed.

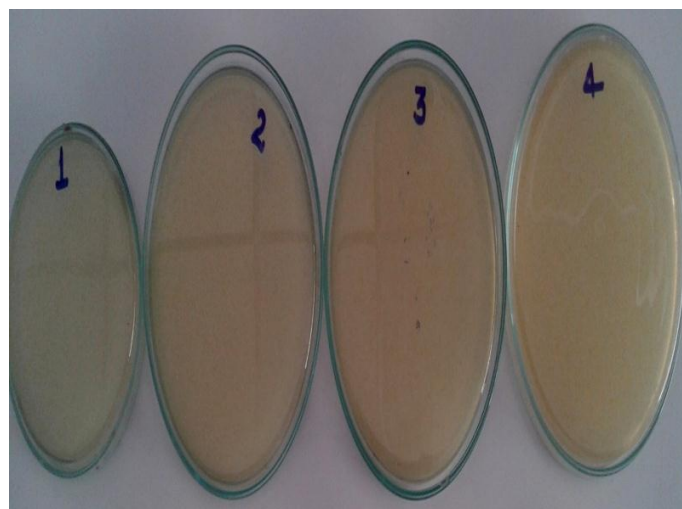
N. One more method can be applied as it is a gram negative bacterium therefore “Gram- staining technique” has been applied to identify the bacteria which have been done after centrifugation process. Centrifuge the culture with 3ml water discard the supernatant and dry the pellet then stain the pellet with Gram’s stain and identify how many colonies are there present the pink colored one will tell no. of colonies present in the serial dilution.

O. Arsenic quantification test has been done also to get the exact value of level of Arsenic in water with the help of Arsenic quantification system in ppb.

P. Further this bacterial strain is now taken into consideration as work is going on to continuation; different reactors can be used to grow this strain on a large scale.

VIII. OBSERVATION & RESULT

1. It is observed that this strain of E.coli is giving Arsenic oxidase as an enzyme.
2. Arsenic oxidase gives yellowish color in the process of detoxification.
3. Arsenic oxidase or arsenite oxidase convert Arsenite into Arsenate which is further methylated into less toxic forms with the help of enzyme Arsenite methyltransferase.
4. It is also confirm that there are approx. 6 colonies are responsible for this particular conversion.
5. There are 4 samples and 2 controls have been observed result shown that no color is observed in both the controls; controls are of water containing arsenic trioxide and other contains enriched sample.
6. It has been proven that appearance of color is like $1^{st} < 2^{nd} < 3^{rd} < 4^{th}$ that is 4th sample on plate of concentration 100µl untreated water and 40µl of enriched sample showing biggest change on the plate which is finally screened out.



7. Consequences said that arsenic in river Ganga is very low in Stretch of Varanasi and Allahabad but then also there are some places where B.O.D is very low as shown in table 2, this is because of other reasons but Assam, West Bengal and Bangladesh as shown in figure 3 is highly affected from such heavy metals.

8. Other medicinal properties of river Ganga nearby West Bengal has been lost and West Bengal royalty by Royal Bengal Tigers are also getting died just because they are consuming this toxic water.

9. As now it is confirmed that *E.coli* K12 sub strain MG 1655 is responsible for Arsenic detoxification therefore it is necessary to increase the production of such microorganism is so that they can act as bioremediator in large purification or water treatment plants.

10. Different In silico study can also help in getting that how drugs can be design or can be improved in a better way if we find the molecular data of such enzymes.

11. The results which proved that keeping the concentration of untreated water same and varying the volume of enriched culture of bacterial strain proves that as we are moving in increasing order from lower to higher concentration the plates are observed to be give a yellow color appearance as shown in figure 11 .

12. Finally it has been practically proved that *E.coli* K12 sub strain MG 1655 is responsible for detoxification of water from arsenic and can be awarded as good bioremediation agent or Bioremediator. Hence it has been proved that two important enzymes namely—Arsenite Oxidase and Arsenite Methyltransferase are going to provide a significant marker in Arsenic detoxification from water and from human body from different metabolic pathways.

IX. CONCLUSION

Large parts of water which are life supportive get contaminated because of illegal activities of human beings. Water pollution is a major problem globally. It is the leading worldwide cause of deaths and diseases, and that it accounts for the deaths of more than 14,000 people daily. On earth water has too many forms and variety which are necessary specifically for particular geographical as well as environmental conditions. Less than 1% of the world's fresh water is accessible for direct human uses. Water pollutions now become a part of concern in country like India. In addition to the acute problems of different problems in developing countries, industrialized countries continue to struggle with water pollution problems as well. There are many inorganic metals which are contaminating water bodies which serve life to large part of India, Arsenic (As) is one of the biggest intimidations for water bodies. High toxicity of Arsenic poses a serious risk not only to ecological systems but also for human vigor. There is availability of sophisticated techniques for arsenic removal from contaminated water, enlargement of new laboratory based techniques along with cost reduction and augmentation of conventional techniques are indispensable for the benefit of common people. Demograph estimate that around 52 millions peoples are drinking ground water with arsenic concentrations above the guidelines of World Health Organization. WHO proposed a parameter or MIC for Arsenic i.e. of 10 parts per billion (ppb) or 0.010 Mg/L, it is found that level of Arsenic has been increased vigorously in many rivers. This paper is based on the future aspects, for removal of Arsenic from drinking water or the water of different rivers like Ganga, Gomti and Yamuna etc which humans are consuming for domestic purpose. Objective is to apply Bioremediation technique with the assist of batch culture that needs Bioremediators to detoxify contaminated water and helps in maintaining the original quality of water. Hence it has been proved that two important enzymes namely—Arsenite Oxidase and Arsenite Methyltransferase are going to be a significant marker in

X. FUTURE ASPECT & ANALYSIS

- The detail characterization of the material synthesized before & after the experiment.
- Studies of various Mass production models.
- Effect of competitive anion present in the removal efficiency of arsenic
- Practical applicability of the material
- Calculation for the cost of the implementation of this technique.

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