2.1 Preamble

In view of the environmental and human health adversities due to heavy metal pollution, there have been a large number of investigations on the role of microbes to solve/reduce the problem. This is particularly to recognize the fraction of native microflora capable of not only tolerating heavy metal toxicity but also to use their physiological pathways to investigate problems due to heavy metal contamination. Many metals are modulated by microbes using physiological processes microbial communities, or a versatile population within them, transform soluble and insoluble states. As Filali et al. (2000) point out some bacteria for instance many strains of *Pseudomonas* spp even use some heavy metal ions for energy and growth.

Under natural conditions, arsenic is cycled at the earth surface where the breakdown of rocks has converted arsenic sulfides into arsenic trioxide (Mandal and Suzuki, 2002; Oremland and Stolz, 2005). Furthermore, as Sanders (1980) and Tareq et al. (2003) report, arsenic is known to have multiple oxidation states where they are present in either organic or inorganic compounds in an aquatic environment. Both Zobrist et al. (1998) and Root et al. (2009) indicated that the mobility of arsenic inorganic compounds in contaminated aquatic and sediment environment is controlled by redox processes, precipitation, sorption, and dissolution processes.

2.1.1 Arsenic and its Uses

Although arsenic has been used as a poison, it has many chemical uses and is quite an important element. The first usage of arsenic in medicine could be dated around 2500 years ago where it was mainly consumed for the improvement of breathing problems as well as to give freshness, beauty, and plumpness figures in women (Mandal and Suzuki, 2002). Arsenic in the form of arsenical salvarsan (an As containing drug) was the initial antimicrobial agent used in the treatment of infectious diseases such as syphilis and sleeping sickness in 1908 (Rosen, 1999). Arsenic in the form of arsenic trioxide (As$_2$O$_3$) is one of the most common forms of arsenic, which is often used in manufacturing and agriculture industry and for medical purposes (Ratnaike, 2003).

Arsenic trioxide is also proven to be useful in criminal homicides due to its characteristic, which is tasteless, colorless, highly toxic, and soluble in water (Mandal and Suzuki, 2002). The high usage of arsenic trioxide in suicide cases had made it to
be often referred as the “inheritance powder” in the 18th century (Oremland and Stolz, 2005). During the 1970s, arsenic was mainly used in agriculture industry as a component insecticide in order to get rid of the insects (Cervantes et al., 1994; Mandal and Suzuki, 2002; Spiegelstein et al., 2005). As was also used as cotton desiccant and wood preservative in the United States (Mandal and Suzuki, 2002). Besides that, it is also being used in ceramic and glass industry, pharmaceutical industry, as food additive and, pigmenting agent in paints (Cervantes et al., 1994; Ratnaike, 2003). Arsenic in the form of 4-aminobenzenearsenic acid (p-arsenic acid, p-ASA) has been used as animal food additive for feeding of boiler chickens (Jackson and Bertsch, 2001).

2.1.2 Arsenic in the Environment

Affecting countless people worldwide and a widely distributed toxic metalloid, the As, is high in waste dump-yards (Pontius et al., 1994). It causes cancer and other chronic and acute problems, among an array of toxic effects. Among flora and fauna it complexes with C and H. Arsenic usually exists in four oxidation states: As\(^{-3}\) (arsine), As\(^{0}\) (arsenic), As\(^{+3}\) (arsenite), and As\(^{+5}\) (arsenate) (Oremland and Stolz, 2005; Afkar, 2012). In water, two primary forms of arsenic are arsenite (As\(^{+3}\)) and arsenate (As\(^{+5}\)). Arsenite is ~100 times more toxic than arsenate (Neff, 1997; Mukhopadhyay et al., 2002; Muller et al., 2003) and is shown to inhibit various dehydrogenases (Ehrlich, 1996). With its ability to bind to sulfur bearing peptides/proteins and dithiols (e.g. glutaredoxin) the AsO\(_2\)\(^{-}\) causes severe damages. As an identical analogue of PO\(_4\), the AsO\(_4\)\(^{3-}\) can alter/cease phosphorylation. Unlike arsenate and arsenite, arsine is often available as highly toxic gases such as (CH\(_3\))\(_3\)As (Oremland and Stolz, 2005) and often present in low concentrations in the environment.

Smelting of non-ferrous ores and river drainages are substantial local input sources for arsenic into marine environment. Mining leachates, increased land erosion and domestic sources (Cullen and Reimer, 1989) are other sources. Volcanoes and bed-rock leaching are the natural sources (Huysmans and Frankenberger, 1990; Rosen, 1999; Nair et al., 2003). Use of pesticides, burning of coal, gasoline, oil and wood (Hingston et al., 2002; Weis and Weis, 2002) also add As to aquatic systems.
Drainages of As containing pesticides, weedicides and other agro-chemicals (Diorio et al., 1995) heavily pollute the aquatic bodies. The As concentrations in freshwaters greatly differ but mostly under 10 µg L\(^{-1}\) (Quentin and Winkler 1974). The anthropogenic releases far exceed releases happenning naturally (Ferguson and Gavis, 1972).

### 2.1.3 Arsenic Biogeochemical Cycle

After the introduction of arsenic into the environment through natural or anthropogenic sources, it enters the biosphere (Figure 2.1). Its biogeochemical cycle impacts “mobility and the distribution of arsenic species” (Tamaki and Frankenberger, 1992; Quinn and McMullan, 1995). Vital role microorganisms play in As cycle is summarized by Oremland and Stolz (2003) and Akai et al. (2004). They suggest accumulation of arsenic in the subsurface waters due to microbial transformations. Masscheleyen et al. (1991), McLean et al. (2000) and Duquesne et al. (2008) reported arsenate to be the major species in well oxidized waters, and arsenite in reduced systems due to latter’s slower redox alterations.

![Arsenic Cycle](image)

**Figure 2.1 The global arsenic cycle (from Mukhopadhyay et al., 2002)**

Among bacteria the ARB are reported to control cycling and speciation of As (Mandal et al., 1996; Smith et al., 1998; Fukushi et al., 2003; Oremland et al., 2004).
The versatility of *ars* mechanisms are reported to “affect the transformation between soluble and insoluble arsenic forms and toxic and non-toxic arsenic forms” (Turpeinen et al., 2002; Malasarn et al., 2004; Jackson et al., 2005; Zouboulis and Katsoyiannis, 2005). Some bacterial species resistant to a variety of antibiotics are known to possess toxic metal resistance (eg. *Staphylococcus* sp; Ug and Ceylan, 2003, *Escherichia coli*; Alam and Imran, 2014, *Salmonella typhimurium*; Garhwal et al., 2014 and, *Pseudomonas aeruginosa*; Matyar et al., 2010).

### 2.1.4 Toxicity of Arsenic

In Bangladesh, over 40 million consume As-laced groundwater (Smedley and Kinniburgh, 2002). Its presence and higher concentrations are a great concern elsewhere in the globe as well. Many studies on a variety of life-forms noted far higher tolerance than humans to As (Goering et al., 1999; Patel et al., 2007). Therefore, the USEPA in 2001 reduced the safe level of arsenic from 50 ppb to 10 ppb in drinking water.

The organic forms of arsenosugars, arsenobetaine, etc are less toxic, small and natural accumulations in food. Its presence in soil and water has become an increasing problem in many countries around the world, including Bangladesh, India, Chile and Taiwan (Lodh et al., 1996; Tseng, 2005; Hadi and Parveen, 2004), and natural geological source is one of the main causes of contamination (Ratnaike, 2003). Consumption of drinking water that has been contaminated by hazardous level of As could cause diseases such as arsenic dermatosis, lung cancer, liver cancer, uterus cancer, skin cancer and occurrence of skin and bladder and, hepatocellular carcinoma that will result in slow and painful death (Hadi and Parveen, 2004; Lu et al., 2004; Duarte et al., 2009).

As an element, As possesses some chemical similarities to phosphorous and has received attention because of its possible role in metabolic processes as a phosphate analogue (Tawfik and Viola, 2001; Knode et al., 2012). Although known to be noxious to many higher life-forms, studies have shown that microorganisms use its ions as donars/acceptors of electrons (Ji and Silver, 1992a; Ahman et al., 1994; Cervantes et al., 1994; Macy et al., 1996; Newman et al., 1997). Some bacteria, as Oremland and Stolz (2003) note, modify its states during their physiological
processes. These processes bring about ionic modifications of As and aid in its transformation.

2.1.5 As Toxicity and Adaptive Responses of Native Microflora

Recently, there have been increasing concerns on arsenic toxicity issues. Arsenic contamination is reported from worldwide (Smedley and Kinniburgh, 2002; Mukherjee et al., 2006; Halem et al., 2009; Singh and Kumar, 2012). Consequent to human additions of over tens to thousands of times IMAC permitted limits, new amendments are available from some areas for water, soil, air, some life forms etc (Mukherjee et al., 2006; Armienta and Segovia, 2008; Sambu and Wilson, 2008). Since As is toxic in its inorganic forms its presence itself is an indication of impending harm. Arsenic poisoning of aquifers and possible health threats are reported from the Americas (Smedley and Kinniburgh, 2002), Asia (Chakraborti et al., 2002 and 2010) and also in central Europe (Lindberg et al., 2006) among human populations.

Arsenite is a toxic metalloid and its detoxification mechanism by most microbes frequently involves its oxidation to less toxic forms. The first arsenite oxidizing bacterium Bacillus arsenoxydans was isolated from South Africa by Green (1918). Since then numerous bacteria capable of arsenite oxidation are isolated and studied (Ilyaletdinov and Abdrashitova, 1981; Santini et al., 2000; Mokashi and Paknikar, 2002; Kashyap et al., 2006; Chang et al., 2010; Rehman et al., 2010). These encompass Pseudomonas arsenitoxidans, Alcaligenes faecalis, Microbacterium lacticum, Agrobacterium tumefaciens 5A, Microbacterium oxydans (Aksornchu et al., 2008), Pseudomonas strutzeri, Pantoea sp, Pseudomonas sp, Agrobacterium sp, Aeromonas sp, Enterobacter sp and Comamonas sp and Pseudomonas lubricans.

Once bioavailable, the arsenic stress to environmental microbiota is inevitable. In order to survive the As stress, microorganisms have been shown to develop resistance mechanisms. The arsenic resistant bacteria can survive in presence of arsenic but usually their growth declines with the increase in arsenic concentration and, at a point, the growth ceases. This dose is considered as minimum inhibitory concentration (MIC). Various workers have computed (e.g., Joshi et al., 2009; Dave et
al., 2010) As MIC for the isolates they studied and the MIC is reported to range from 2-120 mM.

The ARB occurrence and presence is a consequence of As contamination. Many ARB strains in some bacterial genera are known. Some of these are: *Acidithiobacillus* (Dopson et al., 2001), *Bacillus*, *Deinococcus* (Suresh et al., 2004a and b), *Desulfitobacterium* (Niggemyer et al., 2001) and *Pseudomonas* (Prithivirajsingh et al., 2001; De Vicente et al., 1990).

The As redox reactions are affected by bacteria in numerous ways. In the reducing situations as in ground water, As(V) is converted to As(III) for detoxifying the former. Bacteria can also convert arsenite to less toxic arsenate. Phillips and Taylor (1976) isolated several strains of *Alcaligenes faecalis* from raw sewage enriched with arsenite, which oxidized the arsenite to arsenate. Oxidation of arsenite to less toxic arsenate is brought about by *Pseudomonas* sp. (Turner, 1949; 1954; Turner and Legge, 1954). Arsenite oxidation is vital too in As cycling (Oremland et al., 2004). Several isolates were reported by Oremland and Stolz (2003) as capable of accepting electrons from arsenite and some bacterial strains detoxified arsenite by oxidizing it.

Arsenic oxidation is mediated by both heterotrophic and chemoautotrophic microorganisms. Some microbes gain energy from oxidizing arsenite (Inskeep et al., 2007), although this activity could be an exception limited to chemolithotrophic bacteria. Under standard conditions, arsenite oxidation is a thermodynamically exergonic reaction and can provide sufficient energy to support microbial chemoautotrophic cell growth (Ehrlich, 1996). Heterotrophic bacteria have not been shown to derive major energy from arsenite in growth experiments. In heterotrophic bacteria, this is generally considered to be a detoxification mechanism instead of supporting the growth.

Supposedly, the oxidation of arsenite is a detoxification process in some cases. Salmassi et al. (2002) isolated a bacterium namely, *Agrobacterium albertmagni* strain AOL15 is shown to oxidize arsenite as a detoxification step. The gene, *aox* is reported to perform arsenite oxidation to arsenate (Chang et al., 2009). Oremland and Stolz
(2003) suggested that arsenite [As(III)] impairs the function of many proteins and affects respiration.

2.1.6 Genetic Components Involved in As Transformation

A number of strains of Bacteria and Archeal capable of oxidase activities are reported (Muller et al., 2003). Various arsenite oxidizing bacteria that possess aox genes responsible for arsenite oxidation have been reported (Anderson et al., 1992; Vanden and Santini, 2004; Branco et al., 2009; Cai et al., 2009). The aoxB gene acts as a functional marker for aerobic arsenite oxidizers and is responsible for the oxidation reaction which results in the formation of an enzyme, arsenite oxidase, facilitating arsenite oxidation to arsenate (Quemeneur et al., 2008). The bacteria possessing aoxB are reported by Inskeep et al. (2007) and, Garrido and Joulian (2008).

2.1.7 As Oxidation and Efflux Mechanism

Selection of diverse ARB is likely in water and soil exposed to As for a long time. Under elevated arsenite concentrations such strains armed with efflux or oxidation and/or both processes are likely to perform efficiently. They can therefore be used as potential candidates for bioremediation. Further, by characterization of arsenite detoxifying bacteria and by determining the mechanism of arsenite detoxification, suitable in situ methodologies may be developed for the isolated strains and there is this potential of improvement in strains by genetic engineering. Most studies of arsenic resistant bacteria have been based on culturing them from environmental locations, which contain high concentrations of arsenic. For example Deinococcus indicus and Bacillus indicus were obtained from aquifers of West Bengal, India, Staphylococcus and Citricoccus from gold mine reactor (Sato and Kobayashi, 1998; Suresh et al., 2004a and b). However, research shows that common microorganisms such as Escherichia coli, Pseudomonas aerugenosa and Staphylococcus sp. also exhibit arsenic resistance.

2.1.8 Bacterial Resistance to Arsenic

Rosen (2002) and, Silver and Phung (2005) have provided overviews of As toxicity resistance possibilities. The following are the reported pathways to deal with As toxicity: (i) “minimizing the uptake of arsenate through the system for phosphate
uptake” (Cervantes et al., 1994) (ii) “by peroxidation reactions with membrane lipids: (Abdrashitova et al., 1986), and (iii) “using microbial arsenic detoxification pathways involving the \textit{ars} operon” (Silver and Phung, 1996). Among these the last is the best investigated (Stolz et al., 2006). In the main components are (ArsC), (ArsB) and (ArsA). When arsenate is taken in it is reduced and expunged using ArsB and ArsA-mediated ATP hydrolysis (Figure 2.2). Also ArsB can expunge As(III) if and when it enters the cell.

The primary role detoxification process is to cope and grow in the presence of a toxic substance. Methylation is another means of As detoxification. Several methylation steps producing penta-, tri- and di- and mono-methyl plus gaseous arsene are involved (Stolz et al., 2006). Many strains capable of methylation processes are available (Cullen and Reimer, 1989; Dowdle et al., 1996; Newman et al., 1998; Stolz and Oremland, 1999; Salmassi et al., 2002; Silver and Phung, 2005). It is however important to note that microbial arsenite oxidation is quicker than chemical processing (Tamaki and Frankenberger, 1992). In this regard, microbes with right set of genes either chromosomal or plasmid are relevant. The mechanism of resistance made possible by efflux pumping and enzymes, as Silver (1996) proposed, are useful in detoxifying many ions of heavy metals.

Figure 2.2 Bacterial As resistance mechanisms (after Hudson-Edwards and Santini, 2013)
Conversion of arsenite to less mobile less toxic arsenate that can be effluxed with ease is the primary survival mechanism. Many studies have recognized this fact (Ehrlich, 1996; Inskeep et al., 2002; Macy and Santini, 2002; Vanden and Santini, 2004). Genes that bring about detoxification are also studied from a variety of microbes (Silver and Phung, 1996; Weeger, 1999). Occurrence of bacteria from polluted locations capable of As tolerating are reported in several previous studies in particular by McNellis and Anderson (1998) and Kulp et al. (2004).

Arsenate resistance/tolerance in *Escherichia coli* is known to be achieved either by a chromosomal or plasmid encoded system. Silver and Nakahara (1983) suggest that the resistance mediated by chromosome activates a $\text{PO}_4^{3-}$ uptake pump. This enables reduced uptake for arsenate by enhanced phosphate intake. Resistance enabled by plasmid-encoding lead to rapid expunging of arsenenate (Mobley and Rosen, 1982). According to Bentley and Chasteen (2002), methylation of As could be other strategy for its detoxification. In methanogenic bacteria under anaerobic systems methane generating detoxification. In many fungi aerobic As detoxification process is modulated by unique methylation steps.

Encoded chromosomally or by plasmid, the *ars* operons are the mediators of As resistance. The two most common types of these operons contain either the five (*arsRDABC*) or three (*arsRBC*) gene operons (Silver and Phung, 1996) are the most common type in Bacteria. In Gram-negative bacteria such as *E. coli* R773, the former type is reported to be on the plasmids. The three gene ones can be on chromosome or plasmids. Examples are “*Staphylococcus aureus* pI258 and *Pseudomonas putida* KT2440” (Owolabi and Rosen, 1990; Diorio et al., 1995; Bruhn et al., 1996; Jimenez et al., 2002). The strain NRC-1 of *Halobacterium* sp. possesses a replicon pNRC100 which is a large extra-chromosomal unit. It contains *arsADRC-R2M*-type. In this is a gene cluster for arsenic resistance. It is known to possess a putative arsenite methyltransferase (Wang et al., 2004). A unique circular plasmid pWCFS103 is credited to carry *arsRDDB* in *Lactobacillus plantarum* conferring As resistance (Wang et al., 2006). This has two regulatory gene copies but does not possess arsenate reductase gene *arsC* and *arsD*.

Now known for long, the plasmid-associated As efflux systems are reviewed extensively (Cervantes et al., 1994). While the composition efflux processes is known
to vary, up to five \((\text{arsRDABC})\) genes are reported from the plasmid pKW301\(f\) Acidiphilium multivorum and plasmids R773 and R46 of Escherichia coli (Suzuki et al., 1998), the transcription of genes in R773 are from a single operon. In the strain FR-008 of Streptomyces sp., a linear plasmid pHZ227 as reported by Lianrong et al. (2006) possesses \(\text{arsRBOCT}\) conferring As resistance. The expression of \(\text{ars}\) operon at basal and secondary stages in \(\text{arsR}\) and \(\text{arsD}\) genes is controlled by repressors. Where as structural systems of As resistance is encoded by \(\text{arsABC}\) genes. Trans-membrane efflux pump is empowered by ArsA which is an ATPase. This and ArsB form a complex to facilitate As effluxing. Located in cytoplasm the ArsC is a small arsenate reductase. Further, Dey and Rosen (1995) had reported that in the absence of ArsA too ArsB can expunge arsenite.

Only three genes, \(\text{arsRBC}\) are known to confer As resistance by plasmids in Staphylococcus (with pl258 and pSX267) and by chromosome in E. coli (Carlin et al., 1995) as well as Pseudomonas aeruginosa (Cai et al., 1998). The \(\text{arsRBC}\) and a fourth ORF of yet to be known function was reported from Bacillus subtilis (Sato and Kobayashi, 1998). Another system with \(\text{arsRBC}\) consisting of \(\text{arsH}\) a divergently transcribed gene was reported by Neyt et al. (1997) from Yersinia enterocolitica on its plasmid pYV which has Tn2502 reported to confer As resistance.

The \(\text{arsH}\), whose function is not known yet, is reported to encode in Y. enterocolitica an NADPH-flavin mononucleotide oxidoreductase. Its presence either in trans or cis form is essential for As resistance in Sinorhizobium meliloti and Y. enterocolitica (Neyt et al., 1997; Yang et al., 2005; Ye et al., 2007). Another gene \(\text{arsMis}\) reported to encode “an arsenite S-adenosylmethionine methyltransferase” (Qin et al., 2006). It is known to detoxify As by methylating arsenite to trimethylarsine (a volatile form). The Gram negative rods, motile strain identified as Zoogloea ULPAs1 from soils found to biotransform arsenic, this strain is able to efficiently oxidize arsenite to arsenate. As Weeger et al. (1999) point out, this strain can be a good bioremediation candidate in As dosed places due to its increased resistance to As(III) as well as other toxic metals.

Many researchers (Diorio et al., 1995; Cai et al., 1998; Sato and Kobayashi, 1998; Suzuki et al., 1998; Butcher et al., 2000; Butcher and Rawlings, 2002; Maury et al., 2003) noted that the \(\text{ars}\) genes in bacteria are carried on either chromosomes or
plasmids. Their main organized types of operons are $arsRDABC$, $arsRABC$and $arsRBC$. The $ars$ genes exist, in some cases, singly.

2.1.9 Arsenic Resistant Bacteria in Bioremediation Efforts

Potential and important biotechnological applications of metal-microbe interactions are in biobeneficiation of ores, bioleaching and/or in bioremediation of sites with metal pollution. Bioremediation is a better, cheaper and safer option than landfilling or incineration of the toxic materials.

Arsenic is a metalloid that causes harm to humans and environments. It is important to remove and reduce this pollutant from the environment through different approaches such as physical, chemical and biological. Technologies for removing arsenic from the environment should meet several basic technical criteria that include robustness, no other side effect on the environment, and the ability to sustain water supply systems for long terms and meet the quality requirement of physical, chemical and microbiological approaches (Duarte et al., 2009). Currently, there are many methods (Mahimairaja et al., 2005) for removing arsenic from the soil contaminated with arsenic, which could be divided into three categories, including physical, chemical and biological approaches.

Since most of the cases of arsenic poisoning are due to the consumption of water contaminated by arsenic, the process of cleaning up or reducing arsenic concentration in water becomes very important. Methods used in reducing arsenic levels in water are primarily divided into (i) physiochemical methods, which include filtration or coagulation sedimentation, osmosis or electrodialysis, adsorptions, and chemical precipitations and, (ii) biological methods such as phytoremediation by using aquatic plants or microbial detoxification of arsenic (Mahimairaja et al., 2005). Two important processes in the removal of arsenic from water by microorganisms are biosorption and biomethylation.

It is reported that biomethylation (by As(III) $S$-adenosylmethionine methyltransferase) is the reliable biological process for removing arsenic from aquatic media (Mahimairaja et al., 2005). Recently, Chen et al. (2013) noted that chromosomal insertion of arsenite $S$-adenosylmethionine methyltransferase (ArsM) into *Pseudomonas putida* KT2440 for potential bioremediation of arsenite from
environmental settings. The use of bioremediation to remove and mobilize arsenic from contaminated soils and aquifers could be effective and economic ways since a wide range of microorganisms have been found to be successfully degrading this pollutant from the environment. The iron oxidation biologically helps simultaneous removal of arsenic from contaminated groundwaters (Katsoyiannis and Zouboulis, 2006). Gihring and Banfield (2001) discovered some high temperature tolerant strains of *Thermus* from As-rich fluids. These can oxidize arsenite rapidly both in vitro and in vivo. In Spain, Canovas et al. (2003) isolated a filamentous fungus from the River Tinto which is highly acidic and high concentrations of toxic metals. This fungus can grow at ~15000 ppm arsenic. Whereas reference strains such as *Aspergillus nidulans* as *Saccharomyces cerevisiae* and *Escherichia coli* only withstand 20 fold lower this concentration.

Isolation from Australian gold mines and characterization of ARB are described by Santini et al. (2000). Capable of chemolithotrophic growth, some of these isolates use arsenite as electron donor and oxygen, the acceptor. Anaerobic utilization of arsenate as other electron acceptors is also known (Stolz and Oremland, 1999; Macy et al., 1996; Santini et al., 2000). Sizable As bioremediation achieved by employing ARB is reported by Chen et al. (1986).

Very slow remobilization of toxic metals (eg. Hg, Cd, As, Cu, Pb) through biogeochemical pathways leads to metal-organic complexation in marine sediments (Forstner and Wittmann, 1979; Muller et al., 2001; Gerlach, 1981; Barkay, 1987). This complexation leads to accumulation and biomagnifications via food chain, which is harmful to all life forms including humans.

Jeckel (1994) and Cavalca et al. (2013) propose different As removal steps through chemical processing on the basis of its oxidation and subsequent alkaline precipitation (Hering et al., 1997; Gupta and Chen, 1978; McNeill and Edwards, 1997; Bothe and Brown, 1999; Gregor, 2001). Disadvantages however are pollution and cost. Biological As oxidation was thus explored (Valls and Lorenzo, 2006). Starting with *Achromobacter* many bacteria capable of arsenite oxidation are collected (Green, 1918) and are followed by several, *Pseudomonas* (Turner, 1954; Turner and Legge, 1954; Ilyaletdonov and Abdrashitova, 1981) *Alcaligenes* (Osborne

Role of arsenite oxidase in detoxification is also explored (Anderson et al., 1992; Ellis et al., 2001). Adaptation to stress from metalloids and/or metals by soil flora is reported by Pennanen et al. (1996). Turpeinen et al. (2004) further reported the ARB diversity was greater in soils with elevated As, Cr and Cu concentrations.

Contaminated sites are tried with bacteria, fungi or yeast for bioremediating them (Strong and Burgess, 2008; Kumar et al., 2011). By harnessing microorganisms converting arsenite to arsenate, we can aim to clean up As contamination in the environments. The ability of marine bacteria to oxidize arsenite leading to its detoxification will be important (Oremland and Stolz, 2003). While a lot is known of genetics of bacterial resistance as well as detoxification of As in vitro, there is no certainty as yet on molecular mechanisms in microflora for in vivo trials. This study aims at understanding the fraction of marine bacteria capable of tolerance to high As concentrations, their ecology, molecular make-up for As resistance and at realizing their potential in bioremediation of As pollution.

Effluents containing loads of heavy metals are produced through uses of metals by industry. These are treated mostly chemically via ion exchange, precipitation or electrochemically (Wong et al., 2001). Chemical treatment is quite ineffective and expensive. Thus attention received for microbial bioremediation of heavy metals is recent and cognizant (Lei et al., 2000).

Physical method exhibits the simplest choice, but it was however limited to small scale operations. As Lim et al. (2014) point out, the chemical method had gained popularity by its high success rate; however, the remediation area can be exposed to other types of chemical contaminants. The usage of biological and phytoremediation methods might be the most practical methods for a small area but more research needs to be carried out especially in methylations, reduction, and oxidation using microorganisms for more effective method to remove the arsenic compound as they have a high potential application in the future.

The use of bioremediation to remove and mobilize arsenic from contaminated soils and aquifers could be an effective and economic way. This is because, a wide
range of microorganisms are found to be successfully degrading this pollutant from the environment. The sequencing of genome permits characterization of molecular basis of As reduction or oxidation. *Pantoea* sp. genome (strain IMH) draft assembly report by Tian and Jing (2014) suggests aerobic reduction of arsenate to arsenite. Presence of an intact “type III secretion system” in arsenic-gene island in the first reported draft genome-sequencing of arsenite-oxidizing bacterium, *Achromobacter arsenitoxydans* SY8 by Li et al. (2012) implies its role/s for transport of many metals and metalloids. According to Jackson and Dugas (2003), the overall of the arsenate reductase/s phylogeny implies pivotal role for *ars* gene.

### 2.1.10 Major Studies in India on Arsenic Resistant Bacteria

Within the Deltas of Ganges, Brahmaputra and Meghna are highly As contaminated aquifers (Anawar et al., 2003; Stuben et al., 2003), with over a third of Bangladesh population being at As poisoning-risk (Smith et al., 2000). The chemical disintegration, transport and deposition of As rich rocks from Himalayas enriches downstream regions. Biological alterations and solubilization of As is cause that mobilizes As subsurface. Settings favourable to As-enriched aquifers are reported from deltas of Red River (Berg et al., 2001) and Mekong River (Polya et al., 2003) in Southeast Asia. Studies by Smedley et al. (2003), Karim (2000) and Das et al. (1996) suggest that Bengal basin aquifers as well as soil contain higher As concentrations than the WHO maximum contaminant level (MCL) of 0.01 ppm (0.13 mM).

Arsenite oxidizing strains have been isolated and studied by various researchers. Mokashi and Paknikar (2002) isolated *Microbacterium lacticum* from a municipal sewage sample by enrichment culture technique which exhibited tolerance to 50 mM arsenite. Achemolithoautotroph namely *Arthrobacter* sp. 15b was identified from a sewage treatment plant site by Prasad et al. (2009). Further, Bachate et al. (2012) isolated two heterotrophic arsenite oxidizing bacteria from garden soil that were closely related to genus *Bordetella* (MIC-15 mM) and *Achromobacter* (MIC-40 mM) based on 16S rRNA gene sequencing.

### 2.1.11 Marine Environment and Arsenic

Compared to terrestrial organisms, the marine forms differ greatly in both their As tolerance/resistance levels and in their ability to deal with different forms of As.
Former type contains just ~1 ppm As in their dry mass than the latter varying from several ppm to >100 ppm (Lunde, 1977). In norm-oxic brackish and marine waters, arsenate is the dominant form. As Neff (1997) reported, toxic arsenite a potentially cancer causing species is <20% of total As.

Studies so far indicate that marine life forms do complex inorganic As to organic ligands (Kumaresan and Riyazuddin, 2001). Such ability is not shown in their terrestrial counterparts. As is present in marine organisms in water and lipid soluble forms. The former types are very stable; breakdown by metabolic or chemical processes are difficult. In marine foods As is not in arsenite form, also not easily transformed in to inorganic form (Neff, 1997), it may not be detrimental when seafood is consumed.

Archaebacterium Sulfolobus acidocaldarius strain BC (Sehlin and Lindstrom, 1992), Alcaligenes faecalis, Shewanella algae, β-proteobacteria strain UPLAs1, Alcaligenes faecalis, Comamonas terrae sp. nov, some heterotrophic bacteria (Herminiimonas arsenicoxydans) and chemolithotrophic bacteria are reported to have the ability to oxidize arsenite to a less toxic arsenate (Mahimairaja et al., 2005; Oremland and Stolz, 2005). Liao et al. (2011) reported that 11 arsenic reducing bacterial strains from seven different genera (i.e., Pseudomonas, Psychrobacter, Citrobacter, Bacillus, Bosea, Vibrio and Enterobacter) were isolated from environmental groundwater samples collected from well in Southern Yunlin County, West Central Taiwan that holds a significant impact in the biotransformation of arsenic that is present in the aquifer, and these communities of bacteria are well adapted to high arsenic concentrations that are present in the water.

Through screening selecting and examining literature/data, it is possible to come up with an insight on arriving at solutions for problems associated with metal pollution. As such, an overview of current knowledge of arsenic transportation and the ecological processes and As transformation is essential. However, the literature reviews here foused on arsenic resistant marine bacteria, their potential of As bioremediation and ARB capable of As detoxification. Detailed literature review with pertinent topic-wise aspects is in different chapters.