CHAPTER 2

LITERATURE REVIEW

2.1 Heavy metals

Heavy metals are naturally occurring elements that have a high atomic weight and a density at least 5 times greater than that of water. The heavy metals are extremely persistent in the environment; they are non-biodegradable and non-thermo degradable and thus readily accumulate to toxic levels. Soils contaminated with heavy metals have become one of major environmental problems around the world (Gratão *et al*., 2015) because the heavy metals can accumulate in the soil at toxic levels due to the long term application of wastewater (Bohn *et al*., 1985).

The contamination may occur due to the industrial expansion, mine tailing, combustion of fossil fuels, spillage of petrochemicals, and disposal of high metal waste (e.g. batteries), atmospheric deposition and agricultural practices (Liu *et al*., 2016). Agricultural practices may accumulate high levels of potentially heavy metals in soils, which may have significant consequences for the quality of plant health, soil biological processes and thus through bio magnifications enter the human body as well (Alves *et al*., 2016). The existing farming practice explores high yielding varieties and intensive cropping pattern that required higher use of external inputs such as irrigation, pesticides use and so on for more yields. It is important to understand the status and extent of soil contamination with heavy metals to make sustainable management strategies for agricultural soils. The main sources of heavy metals in agricultural soils are due to activities such as irrigation using wastewater, agricultural fertilizers, pesticides and organic manure. Monitoring of the contamination of soil with heavy metals is of interest due to their influence on ground water and surface water and also on plants, animals and humans.

Heavy metal-induced toxicity and carcinogenicity involves many mechanistic aspects, some of which are not clearly elucidated or understood. However, each metal is known to have unique features and physico-chemical properties that confer to its specific toxicological mechanisms of action.

2.2 Arsenic

Arsenic (As) is an element belonging to the group V-A, and it demonstrates characteristics of a metalloid, arsenic more easily forms anions, and its non-metal properties dominate. The major inorganic forms of arsenic include the trivalent arsenite (As (III) or As^{+3}) and the pentavalent arsenate (As (V) or As^{+5}). The organic forms are the methylated metabolites monomethylarsonic acid (MMA), dimethylarsinic acid (DMA) and trimethylarsine oxide. Environmental pollution by arsenic occurs as a result of natural phenomena such as volcanic eruptions and soil erosion, and anthropogenic activities (ATSDR, 2000). When arsenic is in an oxidation state of +5, it acts similar to phosphorus, a fact that has many implications for the way in which it reacts in soil, as well as its potential toxicity in plants. The most common oxidation states of arsenic are -3, 0, +3 and +5, arsines and metal arsines are those in which arsenic has an oxidation state of -3, and these forms are very unstable under oxidizing conditions (Moreno-Jiménez *et al*., 2012). Under aerobic conditions, the oxidation state of arsenic tends to be +5, and when this occurs at a pH between 2 and 3, arsenic acid (H_3AsO_4) is formed. When the pH rises to values between 3 and 11, this compound disassociates to $H_2AsO_4^-$ and $HAsO_4^{2-}$ (Smedley and Kinninburgh, 2002). Under anaerobic conditions, the predominant arsenic species is H_3AsO_3. Arsenic also biomethyates easily (Barán, 1995).

2.3 The use of arsenic

Several arsenic-containing compounds are produced industrially, and have been used to manufacture products with agricultural applications such as insecticides, herbicides, fungicides, algaecides, sheep dips, wood preservatives, and dye-stuffs (Tchounwou, 2014). They have also been used in veterinary medicine for the eradication of tapeworms in sheep and cattle (Tchounwou *et al*., 1999). Recently, arsenic trioxide has been approved by the Food and Drug Administration as an anticancer agent in the treatment of acute promeylocytic leukemia (Rousselot *et al*., 1999). Its therapeutic action has been attributed to the induction of programmed cell death (apoptosis) in leukemia cells (Yedjou and Tchounwou, 2007) though arsenic is a highly poisonous substance it has numerous application and has been used widely for many years.

2.4 Toxicity of arsenic compounds

Contamination with high levels of arsenic is of concern because arsenic can cause a number of human health effects. Several epidemiological studies have reported a strong association between arsenic exposure and increased risks of both carcinogenic and systemic health effects. The inorganic forms consisting mostly of arsenite and arsenate compounds are toxic to human health. Humans are exposed to arsenic primarily from air, food and water. However, Arsenic is present in water, air and soil and is absorbed by some food crops as they grow. It is not an additive or ingredient in these food crops and cannot be completely eliminated from food. Elevated arsenic level in drinking water and food is the major cause of arsenic toxicity in the world. Reports of arsenic contamination in water are available from more than 30 countries in the world (Chakraborti, *et al.*, 2002). Human health effects of chronic arsenic toxicity (CAT) are designated by the term arsenicosis which was the first coined by Mazumder (Mazumder *et al.,* 1988) and later used by WHO (WHO, 2005) to imply a chronic disease caused by prolonged exposure in humans to arsenic. Previously the condition was described as arseniasis, arsenism, arsenicism, etc. Most of the reports of chronic arsenic exposure in man focus attention on skin manifestations because of their diagnostic specificity. However, data derived from population based studies, clinical case series and reports relating to intake of inorganic arsenic in drinking water, medications or occupational and environmental exposure, show that chronic arsenic exposure adversely affects multi organ systems of human body. The symptoms of chronic arsenic toxicity (arsenicosis) are insidious in onset and are dependent on the magnitude of the dose and duration of its exposure. There is a wide variation of occurrence of symptoms in an arsenic exposed population. All members of an affected family do not show clinical symptoms, the reason for such variation of disease expression is an enigma (Mazumder, 2008)

Table 2.1 Arsenic compounds used in industry, agriculture and medicine (Kippling, 1997)

Arsenic compound	**Formula**	**Known as**	**Uses**
Arsenic	As	-	Alloying additive, Electronic devices i.e. transistor
Arsenic pentoxide	As_2O_5	Arsenic oxide Boliden salts	Chemical intermediate, Defoliant, wood preservative
Arsenic trioxide	As_2O_3	Arsenic Arsenolite White arsenic Arsenious oxide	Insecticides and fungicides Glass Chemicals Anti-fouling paints, Taxidermy, Timber preservation
Arsenic trichloride	$AsCl_3$	Butter of arsenic	Pharmaceuticals and Chemicals
Arsine	AsH_3	-	Stabilizing-selenium in trainsistors
Calcium arsenate	$Ca_3(AsO_4)_2$	-	Insecticide, Herbicide and Larvicide
Copper arsenite	$CuHAsO_3$	Scheele's green	Larvicide
Copper acetoarsenite	$3CuOAs_2O_3$ $Cu(OOCCH_3)$	Paris Green Emerald Green	Larvicide
Orpiment	As_2S_3	-	Pigment Depilatory
Lead arsenate	$PbHAsO_4$	-	Insecticide, Herbicide and Growth regulator
Sodium arsenate	Na_2HAsO_4	Wolman salts	Wood preservative, Calico printing, Insecticide, herbicides
Sodium arsenite	$NaAsO_2$	-	Herbicide, Pesticide, Corrosive inhibitor, Chemical intermediate, Fluorescent lamps
Magnesium arsenate	$Mg_3(AsO_4)_2$	Atoxyl	Trypanicide
Sodium arsanilate	$NH2C_6H_4AsO$ $(OH)(ONa)$		Pharmaceutical manufacture

Table 2.2 Arsenic species important in toxicity (Carter *et al*., 2003)

Name (formula)	Chemical form	Comment
$As(OH)_3$, arsenious acid, often called arsenite	Oxidation state, AsIII	Aqueous solution species for +III. pKa = 9.2, 12.1, 13.4.
H_3AsO_4, arsenic acid usually called arsenate	Oxidation state, AsV	Aqueous solution species for +V at pH 7.4 is -2 charged anion ($HAsO_4^{-2}$) pKa = 2.22, 6.98, 11.5.
AsH_3, arsine	Oxidation state, As(-III) Hydride (H^-)	Most reduced form of arsenic, slightly soluble in water.
CH_3–$As(O)(OH)_2$ monomethylarsonic acid, (MMAsV)	Oxidation state is +V.	Methylated metabolite of AsIII, metabolized to dimethylarsinic acid
CH_3–$As(OH)_2$ monomethylarsonous acid (MMAsIII)	Oxidation state is +III.	Reduced metabolite of MMAsV
$(CH_3)_2$–As (O)(OH) dimethylarsinic acid, (DMAsV)	Oxidation state is +V.	Reduced metabolite of MMAsV
GaAs, gallium arsenide	Oxidation state of As is 0 to -III.	Synthetic compound with no acid–base behavior

This table that the exposure to the different chemical species should be considered separately and that some chemical information would be very helpful in interpreting chemical species-specific toxicology data.

2.5 Arsenic in the Environment

Heavy metals such as arsenic essentially become contaminants in soil environments because (1) their rates of generation through manmade cycles are faster relative to natural ones, (2) they are transferred from mines to random environments where higher potentials of direct exposure occur, (3) metal concentrations in discarded products are relatively high compared to those in receiving environments, and (4) the chemical form (species) in which a metal is found in the receiving environment can render it more bioavailable (Lal *et al*. 2010). The soil arsenic contamination has become a growing risk as a result of natural mineralization, microbial activities that increase arsenic mobilization in the environment, and human activities that exacerbate arsenic contamination in soil and water supplies (Litter *et al*. 2010). Arsenic in water can enter the human food chain through edible plants. Experiments monitoring arsenic uptake by plants (rice, potato, cauliflower, onion) irrigated with contaminated water have shown that arsenic levels are highest in plant roots followed by shoots, leaves and edible parts, respectively (Dahal *et al*., 2008).

2.5.1 Arsenic in air

Approximately one-third of the global atmospheric flux of arsenic is estimated to be from natural sources (7900 tonnes per year). Volcanic activity is the most important natural contributor, followed by low-temperature volatilization, exudates from vegetation, and windblown dusts (estimated to account for nearly 24000 tonnes of arsenic emitted to the global atmosphere per year). These emissions arise from the mining and smelting of base metals, fuel combustion (e.g. waste and low-grade brown coal), and the use of arsenic-based pesticides (WHO, 2000, 2001). Arsenic is present in the air of suburban, urban, and industrial areas mainly as inorganic particulate (a variable mixture of As (III) and As (V), with the pentavalent form predominating). Methylated arsenic is assumed to be a minor component of atmospheric arsenic (WHO, 2000). Mean total arsenic concentrations in air range from 0.02-4 ng/m^3 in remote and rural areas, and from 3-200 ng/m^3 in urban areas. Much higher concentrations (> 1000 ng/m3) have been measured in the vicinity of industrial sources, such as non-ferrous metal smelters, and arsenic rich coal-burning power plants (WHO, 2001).

2.5.2 Arsenic in water

Arsenic, from both natural and anthropogenic sources, is mainly transported in the environment by water. The form and concentration of arsenic depends on several factors, including whether the water is oxygenated (for example, arsenites predominate under reducing conditions such as those found in deep well-waters), the degree of biological activity (which is associated with the conversion of inorganic arsenic to methylated arsenic acids), the type of water source (for example, open ocean seawater versus surface freshwater versus groundwater), and the proximity of the water source to arsenic-rich geological formations and other anthropogenic sources (WHO, 2000, 2001).

The concentration of arsenic in surface freshwater sources, like rivers and lakes, is typically less than 10 μg/L, although it can be as high as 5 mg/L near anthropogenic sources. Concentrations of arsenic in open ocean seawater and groundwater average 1-2 μg/L, although groundwater concentrations can be up to 3 mg/L in areas with volcanic rock and sulfide mineral deposits (WHO, 2001). The primary regions where high concentrations of arsenic have been measured in drinking-water include large areas of Bangladesh, China, West Bengal (India), and smaller areas of Argentina, Australia, Chile, Mexico, Taiwan (China), the USA, and Viet Nam. In some areas of Japan, Mexico, Thailand, Brazil, Australia, and the USA, mining, smelting and other industrial activities have contributed to elevated concentrations of arsenic in local water sources (IARC, 2004).

2.5.3 Arsenic in soils

Arsenic is a widely dispersed element in the Earth's crust and exists at an average concentration of 1.5-5 mg/kg, asking it the 52nd most abundant element (Adriano, 2001). Arsenic contaminated groundwater can pollute the soil and increase soil and plant arsenic concentrations when used for irrigation of farmlands. Although measuring the total arsenic content (As_t) in soil is undoubtedly important, it should be borne in mind that permitted levels vary throughout the world. Asian countries, including China, Peninsular Malaysia, Vietnam, India, Philippines, Indonesia, Bangladesh, Pakistan and Thailand have investigated the contamination of agricultural soils and crops by heavy metals due to their potential effects on human health and the long-term sustainability of food production in contaminated areas (Herawati *et al*., 2000; Luo and Teng, 2006; Brus *et al*., 2009; Paijitprapaporn, 1997).

In Thailand, 634 soil samples in agricultural soil that grow economic crop were collected and analyzed for arsenic. The results showed that arsenic concentration ranged from <0.005 to 64 mg/kg (Sukreeyapongse *et al*.,2009). Regarding the standard of arsenic level, it designate maximum concentration limits (MCL) less than 0.01 mg/L for drinking water, and MCL for soil as 3.9 mg/kg and 30 mg/kg for agriculture and for other usages, respectively designated by Office of National Environment Board of Thailand (National Environment Board No.8, 1994 and Phakphian, 2004).

Arsenic in the soil is important for quantifying the persistence and bioavailability of arsenic in the environment. The sorption of arsenic onto soil surfaces plays an important role in mediating the availability of arsenic in the environment. Iron, Al, and to a lesser extent Mn oxides are important soil constituents in controlling soil solution concentrations of arsenic. Soil pH has a major influence on the availability of arsenic. Arsenic is apolyprotic acid, and pH has a major influence on the valence charge of the arsenic ion in soil solution and hence on the arsenic adsorbed. Redox potential (Eh), pH, and dissolved oxygen (DO) are all important factors controlling arsenic speciation and chemistry in groundwater. Under oxidizing conditions at $pH < 2$, As(V) occurs as H_3AsO_4 in the Eh–pH diagram (Fig. 2.1; Smedley and Kinniburgh 2002). In the pH range from 2 to 14, H_3AsO_4 dissociates to $H_2AsO_4^{-}HAsO_2^{-4}$, and AsO_3^{-4}. At low Eh values, arsenic becomes dominant as $H_3AsO^0_3$. Up to pH 9, H_3AsO_3 does not dissociate; above pH 9, it appears asH_2AsO^{-3}, $HAsO_2^{-3}$, AsO_3^{-3}.

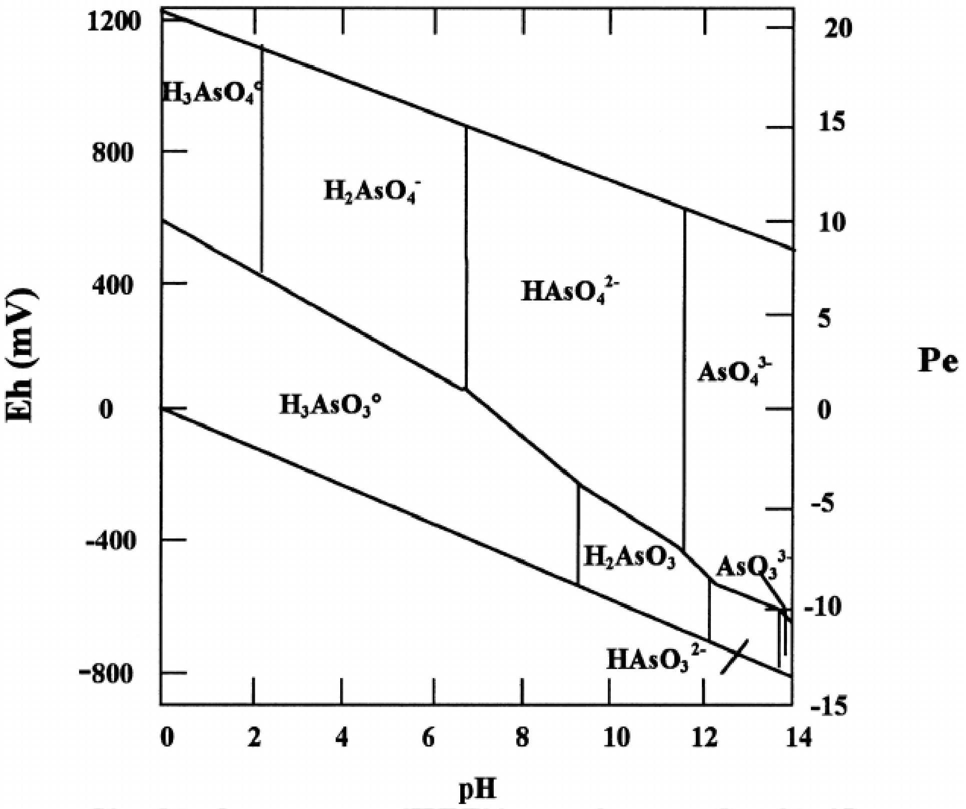

Figure. 2.1 Redox potential (Eh)–pH diagram for aqueous arsenic species in the system As-O_2-H_2O at 25°C and 1 bar total pressure. (Reprinted with permission from Smedley and Kinniburgh 2002).

2.5.4 Arsenic in plants

Plant uptake of arsenic from soils is complicated by a number of factors. In aerated soils used for crops such as wheat, maize and most vegetables, arsenic is present mainly as As(V) and, as such, is likely to be in the solid phase. Therefore, in such soils, arsenic in groundwater used for irrigation is quickly adsorbed by iron hydroxides and becomes largely unavailable to plants. In anaerobic soil conditions such as occur in flooded paddy fields, As is mainly present as As(III) and is dissolved in the soil-pore water (the soil solution) (Xu *et al*., 2008). It is thus more readily available to plant roots.

2.5.5 Toxicity to plants

There is no single level of soil arsenic that is toxic to plants. Different plant species tolerate different amounts of arsenic in soils; so do different rice cultivars. Some plants (known as hyperaccumulators) can tolerate very high levels of soil As (Ma *et al*., 2001). Various symptoms of arsenic toxicity in rice have been reported. They include delayed seedling emergence, reduced plant growth, yellowing and wilting of leaves, brown necrotic spots on older leaves and reduced grain yields (Huq *et al*., 2006). On soils contaminated by arsenical pesticides in the USA, Yan *et al*., (2005) found considerable variation in varietal resistance at high soil-As levels, ranging from virtually no yield reduction in one Chinese cultivar to 80–90% reduction in four of ten US cultivars tested.

Arsenic is non-essential and generally toxic to plants. Roots are usually the first tissue to be exposed to arsenic, where the metalloid inhibits root extension and proliferation. Upon translocation to the shoot, As cans severely inhibit plant growth by slowing or arresting expansion and biomass accumulation, as well as compromising plant reproductive capacity through losses in fertility, yield, and fruit production (reviewed by Garg and Singla, 2011). At sufficiently high concentrations, arsenic interferes with critical metabolic processes, which can lead to death. Most plants possess mechanisms to retain much of their arsenic burden in the root. However, a genotype-dependent proportion of the arsenic is translocated to the shoot and other tissues of the plant.

2.6 Microorganism and heavy metals

Microorganisms have evolved various mechanisms of metal resistance and scientists have tried to exploit genetic/metabolic basis of all such mechanisms for the production of superior strains (Adams *et al*., 2014). The chromosomal genes may be involved, bacterial resistance to heavy metals is often conferred by-products of genes simulated on plasmids rendering genetic manipulation for strain improvement easy and feasible (Silver *et al*., 2001).

The way microbes interact with metals depends in part on whether the organisms are prokaryotic and eukaryotic. Both types of microbes have the capability to bind metal ions present in the external environment at the cell surface or to transport them into the cell for various intracellular function.

The microorganisms have developed numerous mechanisms of metal resistance. At high concentrations, either metal ions can completely inhibit the microbial population by inhibiting their various metabolic activities or organisms can develop resistance or tolerance to the elevated levels of metals. In contrast, resistance is the ability of microbes to survive in higher concentration of toxic substances by detoxification mechanisms activated in direct response to the presence of the same pollutant (Ahemad, 2012). Toxic heavy metal, therefore, need to be either completely removed from the contaminated soil, transformed, or immobilized, producing much less or nontoxic species. However, in order to endure and proliferate in metal-contaminated soils, tolerance has to be present both in microbes and their associative hosts (Ahemad *et al*., 2009).

2.6.1 Arsenic resistant in microorganisms

Many bacteria have evolved mechanisms enabling them to cope with high arsenic concentrations, and even the possibility that particular bacteria may substitute arsenic for phosphorus to sustain its growth has been raised recently (Wolfe-Simon *et al*., 2010). Soil microbial transformation of a range of elements is a result of assimilatory processes in which an element is incorporated into cell biomass and/or dissimilatory processes in which transformation results in the generation of energy, oxidation/reduction and/or detoxification (Stolz and Oremland, 1999). There are several microbe species that tolerant very high level of arsenic toxicity.

2.6.2 Mechanism of arsenic resistant

Microbes transform arsenic metals via redox conversions (a detoxification process involving reductases and oxidases) of organic forms e.g. As(III) ↔ As(V). Microbial redox processes transform highly toxic arsenite to less harmful arsenate form. It was Green (1918) who first identified the microbe-mediated arsenic metabolism (arsenate reducer *Bacterium arsenoreducens* and an arsenite oxidizer *Bacillus arsenoxydans*). Interestingly, a strain of *Thermus exhibited* both As(III) oxidation and As(V) reduction mechanisms (Gihring and Banfield, 2001).

2.6.2.1 Oxidation

Bacterial oxidation of As(III) to As(V) has long been recognized. These bacteria were isolated from arsenic-impacted environments and identified as genera of *Bacillus*, *Achromobacter*, *Pseudomonas*, *Alcaligene*, *Thiobacillus*, *Thiobacillus* and *Agrobacterium* etc. CAO (chemoautotrophic arsenite oxidizer, use CO_2/HCO_3 as carbon source) bacteria oxidize arsenite to arsenate using oxygen, nitrate, or Fe^{3+} as terminal electron acceptor. HAOs (heterotrophic arsenite oxidizers) require organic carbon as energy and carbon source (Oremland and Stolz, 2005). Even though chemolithotrophic bacteria have been isolated (Santini *et al*., 2000), most of the As(III) oxidation bacteria are heterotrophic; they do not gain energy through As(III) oxidation. Several genes encoding As(III) oxidases have been cloned and characterized, and the crystal structures of the As(III) oxidases have also been studied (Ellis *et al*., 2001).

2.6.2.2 Reduction

The reduction of As(V) by bacteria has been shown via two mechanisms: dissimilatory reduction and detoxification. Dissimilatory reductions of As(V) have been carried out by microbes, either strict anaerobic or facultative anaerobic, that couple anaerobic heterotrophic growth with As(V) as the terminal electron acceptor, They oxidize a range of organic (e.g. lactate, acetate, formate and aromatics) or inorganic substrates (hydrogen and sulfide) as electron donors, resulting in the formation of As(III). The arsenic detoxification mechanisms have been investigated in various microorganisms of both anaerobes and aerobes (Niggemyer *et al*., 2001). As(V) reduction bacteria; including *Dusulfomicrobium*, *Clostridium*, *Bacillus*, *Sulfurospirillum*, *Citrobacter*, *Chrysiogenes*, *Sphingomonas*, *Pseudomonas* and *Wolinella* etc.; were identified (Oremland and Stolz, 2003).

2.6.2.3 Mobilization and Immobilization

Microorganisms can mobilize metals through autotrophic and heterotrophic leaching, chelation by microbial metabolites and siderophores, methylation, and redox transformations. Heterotrophic leaching is when microorganisms can acidify their environment by proton efflux thus leading to the acidification resulting in the release of free metal cations. Autotrophic leaching is when acidophilic bacteria retrieve CO_2 and obtain energy from the oxidation of the ferrous iron or reduced sulfate compounds, which causes solubilization of metals (Gadd, 2004).

Immobilization is a technique used to reduce the mobility of contaminants by altering the physical or chemical characteristics of the contaminant. This remediation approach can utilize microorganisms to immobilize metal contaminants. It is usually accomplished by physically restricting contact between the contaminant or by chemically altering the contaminant (Evanko and Dzombak, 1997). Chemical reagents and bacterial reagents assist with the immobilization of metal contaminants. Most sites contaminated with metals use the solidification and stabilization approach to immobilize metals.

2.6.2.4 Arsenic uptake systems

Microbes possess two types of metal uptake system. The first one is a fast unspecific transport system driven by the chemo-osmotic gradient across the membrane. The mechanism is operative for a variety of metals and is constitutively expressed (Nies, 1999). The second system is slow and highly substrate specific. It uses ATP and operates only when needed (Nies and Silver, 1995). Owing to the activity of the first system (i.e. unspecified transport). Microbes often accumulate toxic concentrations of metal ions within their cells. Structural analogs of a particular metal may fool the organism's uptake system (e.g. arsenate and phosphate).

2.6.2.5 Extrusion

The best characterized and probably the most widespread, arsenic resistance mechanism in microorganisms is the extrusion from the cells by the function of *ars* (arsenic resistance) gene system. Basically, the *ars* gene clusters consists; *ars*R-a regulatory gene, *ars*B- a gene encoding for an dedicated arsenite-specific transmembrane pump, *ars*C- a gene encoding for an arsenate reductase and *ars*A- produces an oxyanion stimulated ATPase which couple ATP hydrolysis thereby extruding arsenicals and antimonial by the action of ArsB protein.

In bacteria there are two basic mechanisms of arsenite extrusion. One is carrier-mediated efflux *via* an arsenite carrier protein, where energy is supplied by the membrane potential of the cell, and the other by an arsenite-translocating ATPase (Dey and Rosen, 1995). Two unrelated families of arsenite carriers have been identified. An arsenite-resistance membrane protein was identified in the SKIN element of *B. subtilis*, and is found in some other bacteria, archaea and fungi (Sato and Kobayashi, 1998).

Table 2.3 Examples of arsenic-resistant microbes

Organism	Reported maximum arsenic tolerance level	Reference
Escherichia coli	250 mM	Xu *et al*., 1996; Anderson and Cook, 2004
Stenotrophomonas maltophilia SA Ant 15	10 mM As(III), 20 mM As(V)	Botes *et al*., 2007
Serratia marcescens	15 mM As(III), 500 mM As(V)	Botes *et al*., 2007
Pseudomonas putida strain RS-4 and RS-5	66.7 mM As(V)	Chang *et al*., 2008
Pseudomonas spp. *Acinetobacter* sp.	1,000 mM	Nagvenkar and Ramaiah, 2010
Betaproteobacteria or Flavobacteria	100 mM	Jackson *et al*., 2005
Exiguobacterium *Aeromonas spp.*	20 mM As(III), 100 mM As(V)	Anderson and Cook, 2004
Bacillus sp., Bacillus licheniformis	10 mM	Anderson and Cook, 2004; Clausen, 2004; Bhat, 2007
Leptospirillum ferriphilum	3 mM	Tuffin *et al*., 2006
Acidithiobacillus caldus	30 mM As(III), 20 mM As(V)	Kotze *et al*., 2006
Streptomyces sp. Strain FR-008	5 mM As(III), 100 mM As(V)	Wang *et al*., 2006

Table 2.3 (continued)

Organism	Reported maximum arsenic tolerance level	Reference
Agrobacterium sp.	25 mM	Cai *et al*., 2009
Arthobacter	20 mM	Cai *et al*., 2009
Rhodococcus	20 mM	Cai *et al*., 2009
Stenotrophomonas spp.	20 mM	Cai *et al*., 2009
Acidithiobacillus ferrooxidans	20 mM As(III)	Dave *et al*., 2008
Corynebacterium glutamicum ATCC 13032	10 mM As(III), 300 mM As(V)	Ordonez *et al*., 2005
Bacterium GFAJ-1	5 mM	Wolfe-Simon *et al*., 2011

2.7 Bioremediation of arsenic-contaminated in agricultural soils

2.7.1 Biological remediation

The biological remediation includes phytoremediation, bioremediation and the combining remediation.

2.7.1.1 Phytoremediation

The phytoremediation is the use of living green plants to fix or adsorb contaminants, and cleaning the contaminants or making their risk reduction or disappearance. The phytostabilization, phytovolatilization and phytoextraction are the main three types of phytoremediation (Shen and Chen, 2000., Yao *et al*., 2012).

2.7.1.2 Bioremediation

The microorganisms cannot degrade and destroy the heavy metals, but can affect the migration and transformation through changing their physical and chemical characterizations. The remediation mechanisms include extracellular complexation, precipitation, oxidation-reduction reaction and intracellular accumulation. Microbial leaching is a simple and effective technology for extracting valuable metals from low-grade ores and mineral concentrates. Besides the industrial application for raw materials supply, microbial leaching has some potential for remediation of mining sites, treatment of mineral industrial waste products, detoxification of sewage sludge and for remediation of soils and sediments contaminated with heavy metals (Bosecker, 2001, Yao *et al*., 2012).

2.7.1.3 Combining remediation (Animal remediation)

Animal remediation is according to the characterization of some lower animals adsorbing heavy metals, degrading, migrating the heavy metals and thus removing and inhibiting their toxify (Yao *et al*., 2012).

2.7.2 Microorganisms used in bioremediation

Bioremediation technology utilizes microorganisms (bacteria, fungi, and plants) to reduce, eliminate, contain, or transform to benign product contaminants present in soil, sediment, water or air. According to EPA, bioremediation is a "treatment that uses naturally occurring organisms for disrupting hazardous substances into less toxic or nontoxic substance" (Das and Dash, 2017).

Metabolic processes of these organisms are capable of using chemical contaminants as an energy source, rendering the contaminants harmless or less toxic products in most cases. Thus, bioremediation provides an alternative tool to destroy or render the harmful contaminants through biological activity and this method is also cost effective (Kamaludeen *et al*., 2003).

2.7.3 Bioremediation arsenic by microorganisms

The microbial activity could reduce the arsenic through sorption, bio methylation, complexation, and oxidation-reduction processes. Microorganisms have evolved the biochemical mechanisms to exploit arsenic either as an electron acceptor for anaerobic respiration, or as an electron donor to support chemoautotrophic fixation of CO_2 into cell carbon (Sato and Kobayashi, 1998). The key microbes involved are categorized as dissimilatory arsenate-reducing prokaryotes from both bacterial and archaeal domains. It uses arsenate as an electron acceptor and reducing it to arsenite. Chemoautotrophic arsenite oxidizers, microorganisms use CO_2 as a carbon source and use arsenite as an electron source, oxidizing it to arsenate for energy. However, heterotrophic arsenite oxidizers use oxygen as an electron acceptor and oxidize arsenite to arsenate. Arsenate-resistant microbes reduce arsenate into arsenite, which allows the transport of toxic form from the cell (Morton and Dunnette, 1994).

Moreover, arsenic-resistant bacteria can have important applications in bioremediation strategies (Yan *et al*., 2010). Because of these reasons, the relationships between soil arsenic and bacterial function and composition are an active field of research in soil microbiology.

REFERENCE

Adams, G.O., P. Tawari-Fufeyin, E. Igelenyah and E. Odukoya. 2014. Assessment of heavy metal bioremediation potential of microbial consortia from poultry litter and spent oil contaminated site. International Journal of Environmental Bioremadiation & Biodegradation 2: 84-92.

Adriano, D.C. 2001. Trace elements in terrestrial environments. Biogeochemistry, bioavailability and risks of metals. Springer, New York.

Agency for Toxic Substances and Disease Registry (ATSDR). 2000. Toxicological Profile for Arsenic TP-92/09. Georgia: Center for Disease Control, Atlanta.

Agency for Toxic Substances and Disease Registry (ATSDR). 2007. Toxicological profile for arsenic. Atlanta, GA, USA Agency for Toxic Substances and Disease Registry (ATSDR), Public Health Service, U.S. Department of Health and Human Services.

Ahemad, M. 2012. Implications of bacterial resistance against heavy metals in bioremediation: A review. IIOABJ 3: 39-46.

Ahemad, M., M.S. Khan, A. Zaidi, and P.A. Wani. 2009. Remediation of herbicides contaminated soil using microbes. In: Microbes in sustainable agriculture, Khan MS, Zaidi A, Musarrat J, (eds.) Nova Science Publishers, New York, p. 261-284.

Alves, L.R., A.R. dos Reis and P.L. Gratão, 2016. Heavy metals in agricultural soils: from plants to our daily life (a review). Jaboticabal 44(3): 346-361.

Anderson, C.R. and G.M. Cook. 2004. Isolation and characterization of arsenate-reducing bacteria from arsenic contaminated sites in New Zealand. Current Microbiology 48: 341-347.

Barán, E.J. 1995. Química bioinorgánica. McGraw-Hill, Madrid, Spain.

Bhat, N.S. 2007. Characterization of arsenic resistant bacteria and novel gene cluster in *Bacillus* sp. CDB3. Ph. D thesis, School of biological sciences, University of Wollongong.

Bohn, H.L., B. L. McNeal and A. G. O'Connor. 1985. Soil Chemistry second edition. Wiley- Inter Science Publication, New York, USA.

Bosecker, K. 2001. Microbial leaching in environmental clean-up programmers. Hydrometallurgy 59(2-3): 245-248.

Botes, E., E. van Heerden and D. Litthauer. 2007. Hyper-resistance to arsenic to arsenic in bacteria isolated from an antimony mine in South Africa. South African Journal of Science 103: 279-281.

Brus, D., Z.B. Li, E.J.M. Temminghoffd, J. Song, G.F. Koopmans, Y. Lou and J. Japenga. 2009. Predictions of spatially averaged cadmium Contents in rice grains in the Fuyang Valley, P.R. China. Journal of Environmental Quality 38: 1126-1136.

Cai, L., G. Liu, C. Rensing and G. Wang. 2009. Genes involved in arsenic transformation and resistance associated with different levels of arsenic-contaminated soils. BMC Microbiol 9: 1-11.

Carter, D.E., H.V. Aposhianb and A.J. Gandolfia. 2003. The metabolism of inorganic arsenic oxides, gallium arsenide and arsine: a toxicochemical review. Toxicology and Applied Pharmacology 193: 309-334.

Chakraborti, D., M.M. Rahman, K. Paul, M.K. Sengupta, U.K. Chowdhury and D. Lodh. 2002. Arsenic calamity in India and Bangladesh sub-continent-whom to blame. Talanta 58: 3-22.

Chang, J.S., Y.H. Kim and K.W. Kim. 2008. The *ars* genotype characterization of arsenic-resistant bacteria from arsenic-contaminated gold-silver mines in the republic of Korea. Applied Microbiology and Biotechnology 80: 155-165.

Clausen, C.A. 2004. Improving the two-step remediation process for CCA-treated Wood: Part II. Evaluating bacterial nutrient sources. Waste Manag 24: 407-411.

Dahal, B.M., M. Fuerhacker, A. Mentler, K.B. Karki, R.R. Shrestha and W.E.H. Blum. 2008. Arsenic contamination of soils and agricultural plants through irrigation water in Nepal. Environmental Pollution 155: 157-163.

Das, S. and H.R. Dash. 2017. Handbook of metal-microbe interaction and bioremediation. Taylor & Francis. Indian.

Dave, S.R., K.H. Gupta and D.R. Tipre. 2008. Characterization of arsenic resistant and arsenopyrite oxidizing *Acidithiobacillus ferrooxidans* from Hutti gold leachate and effluents. Bioresour Technology 99: 7514-7520.

Dey, S. and B.P. Rosen. 1995. Dual mode of energy coupling by the oxyanion-translocating *Ars*B protein. Journal Bacteriology 177: 385-389.

Ellis, P.J.,T. Conrads, R. Hille and P. Kuhn. 2001. Crystal structure of the 100kDa arsenite oxidase from *Alcalingenes faecalis* in two crystal forms at 1.64 Å and 2.03 Å. Structure 9: 125-132.

Evanko, C.R. and D.A. Dzombak. 1997. Remediation of metals-contaminated soil and groundwater. Environmental Science 412: 1-45.

Gadd, G.M. 2004. Microbial influence on Metal mobility and application for bioremediation. Geoderma 122: 109-119.

Garg, N. and P. Singla. 2011. Arsenic toxicity in crop plants: physiological effects and tolerance mechanisms. Environmental Chemistry Lett 9: 303-321.

Gihring, T.M. and J.F. Banfield. 2001. Arsenite oxidation and arsenate respiration by a new *Thermus* isolate. FEMS Microbiology Letters 204: 335-340

Gihring, T.M., G.K. Druschel, R.B. McCleskey, R.J. Hamers and J.F. Banfield. 2001. Rapid arsenite oxidation by *Thermus aquaticus* and *Thermus thermophiles*: field and laboratory investigation. Environmental Science & Technology 35: 3857-3862.

Gratão, P.L., C.C. Monteiro, T.Tezotto, R.F. Carvalho, L.R. Alves, L.P. Peters and R.A. Azevedo. 2015. Cadmium stress antioxidant responses and root-to-shoot communication in grafted tomato plants. Biometals 28(5): 803-816.

Green, H.H. 1918. Description of a bacterium which oxidizes arsenite to arsenate and of one which reduces arsenate to arsenite, isolated from a cattle-dipping tank. South African Journal of Science 14: 465-467.

Herawati, N., S. Suzuki, K. Hayashi, I.F. Rivai and H. Koyama. 2000. Cadmium, Copper and Zinc Levels in Rice and Soil of Japan, Indonesia and China by Soil Type. Bulletin of Environmental Contamination and Toxicology 64: 33-39.

Huq, I.S.M., U.K. Shila and J.C. Joar. 2006. Arsenic mitigation strategy for rice, using water regime management. Land Contamination & Reclamation 14(4): 805-814.

IARC. 2004. Some drinking-water disinfectants and contaminants, including arsenic. IARC Monogr Eval Carcinog Risks Hum 84: 1-477. PMID:15645577

Jackson, C.R., K.G. Horrison and S.L. Dugas. 2005. Enumeration and characterization of culturable arsenate resistant bacteria in large estuary. Systematic and Applied Microbiology 28: 727-734.

Kamaludeen, S.P., K.R. Arunkumar, S. Avudainayagam and K. Ramasamy. 2003. Bioremediation of chromium contaminated environments. Indian Journal of Experimental Biology 41: 972-985.

Kippling, M.D. 1977. Arsenic. In: Lenihan and Flecher, editor. The chemical environment: environment and man 6: 93-120.

Kotze, A.A, M.I. Tuffin, S.M. Deane and D.Y. Rawlings. 2006. Cloning and characterization of the chromosomal arsenic resistance genes from *Acidithiobacillus caldus* and enhanced arsenic resistance on conjugal transfer of *ars* genes located on transposon TnAtcArs. Microbiology 152: 3551-3560.

Lal, R., G. Pandey, P. Sharma, K. Kumari, S. Malhotra and R. Pandey. 2010. Biochemistry of microbial degradation of hexachlorocyclohexane and prospects for bioremediation. Microbiology and Molecular Biology Reviews 74(1): 58-80.

Litter, M.I., M.E. Morgada and J. Bundschuh. 2010. Possible treatment for arsenic removal in Latin American waters for human consumption. Environmental Pollution 158: 1105-1118.

Liu, G.N., J. Wang, E.X. Zhan, J. Hou and X.H. Liu. 2016. Heavy metal speciation and risk assessment in dry land and paddy soils near mining areas at Southern China. Environmental Science and Pollution Research 23(9): 8709-8720.

Luo, Y.M. and Y. Teng. 2006. Status of soil pollution-cased degradation and countermeasures in China (in Chinese), Soil 38: 505-508.

Ma, L.Q., K.M. Komar, C. Tu, W.H. Zhang, Y. Cai and E.D. Kennelley. 2001. A fern that hyperaccumulates arsenic. Nature 409-579.

Mazumder, D.N.G. 2008. Chronic arsenic toxicity & human health, Indian Journal of Medical Research 128: 436-447.

Moreno-Jiménez, E., E. Esteban and J. M. Peñalosa. 2012. The Fate of Arsenic in Soil-Plant Systems. Reviews of Environmental Contamination and Toxicology. Madrid, Spain.

Morton, W.E. and D.A. Dunnette. 1994. Health effects of environmental arsenic. In: Nriagu, JO. Editor. Arsenic in the Environment Part II: Human Health and Ecosystem Effects. New York: John Wiley& Sons, Inc. p. 17-34.

Nagvenkar, G.S. and N. Ramaiah. 2010. Arsenite tolerance and biotransformation potential in estuarine bacteria. Ecotoxicology 19: 604-613.

Nies, D.H. 1999. Microbial heavy-metal resistance. Applied Microbiology and Biotechnology 51: 730-750.

Nies, D.H. and S. Silver. 1995. Ion efflux systems involved in bacterial metal resistances. Journal of Industrial Microbiology 14: 186-199.

Niggemyer, A.,S. Spring, E. Stackebrandt and R.F. Rosenzweig. 2001. Isolation and characterization of a novel As(V)-reducing bacterium: implications for arsenic mobilization and the genus *Desulfitobacterium*. Applied Microbiology and Biotechnology 67: 5568-5580.

Notification of the National Environment Board No.8, B.E. 2537 (1994) issued under the Enhancement and Conservation of National Environmental Quality Act B.E. 2535 (1992).

Ordonez, E., M. Letek, N. Valbuena, A.J. Gil and L.M. Mateos. 2005. Analysis of genes involved in arsenic resistance in *Corynebacterium glutamicum* ATCC 13032. Applied Microbiology and Biotechnology 71: 6206-6215.

Oremland, R.S. and J.F. Stolz. 2003. The ecology of arsenic. Science 300: 939-944.

Oremland, R.S. and J.F. Stolz. 2005. Arsenic, microbes and contaminated aquifers. Trends Microbiaology 13: 45-48.

Paijitprapaporn, A. 1997. Study and monitoring of problem on Arsenic dispersion in Ron phibun District, Nakhon Si Thammarat Province. Department of mineral Resources. Bangkok. 91 pp. (in Thai)

Phakphian, P. 2004. Heavy metal contamination in relevant to soils and staple crops in Thailand. Thai Journal of Soils and Fertilizers (Thailand), 26 (1): 31-36

Rensing, C. and B. Rosen. 2009. Heavy metals cycle (arsenic, mercury, selenium, others). In: Schaefer M(ed) Encyclopedia of microbiology. Elsevier, Oxford, pp 205-219.

Rousselot, P., S. Laboume, J.P. Marolleau, T. Larghero, M.L. Noguera, J.C. Brouet and J.P. Fermand. 1999. Arsenic trioxide and melarsoprol induce apoptosis in plasma cell lines and in plasma cells from myeloma patients. Cancer Res. 59:1041-1048.

Santin, J.M., L.I. Sly, R.D. Schnagl and J.M. Macy. 2000. A new chemolithic arsenite-oxidizing bacterium from a gold mine: phylogenetic, physiological and preliminary biochemical studies. Applied Microbiology and Biotechnology 66: 92-97.

Sato, T. and Y. Kobayashi. 1998. The *ars* operon in the skin element of Bacillus subtilis confers resistance to arsenate and arsenite. Journal Bacteriology 180: 1655-1661.

Shen, Z.G. and H.M. Chen. 2000. Bioremediation of heavy metal polluted soils. Rural Eco-Environment 16(2): 39-44.

Silver, S., L.T. Phung, J.F. Lo and A. Gupta. 2001. Toxic metal resistances: molecular biology and the potential for bioremediation. In: Nuzhat A, Qureshi FM, Khan O, Khan Y, editors. Industrial and environmental biotechnology. Wymondham, UK: Horizon Scientific Press. p. 33-41.

Smedley, P.L and D.G. Kinniburgh. 2002. A review of the source, behavior and distribution of arsenic in natural waters. Applied Geochemistry 17(5): 517-568.

Smedley, P.L. and D.G. Kinninburgh. 2002. A review of the source, behavior and distribution of arsenic is natural waters. Applied Geochemistry 17: 517-568.

Stolz, J.F., and R.S. Oremland. 1999. Bacterial respiration of arsenic and selenium. FEMS Microbiology Review 23: 615-627.

Sukreeyapongse, O., S. Panichsakpatana and H. Hansen, 2002. Transfer of heavy metals from sludge amended soil to vegetables and leachates. Paper Presented at the 17th World Congress of Soil Science (WCSS), 14th - 21st August 2002, Thailand. Symposium No 29, Paper No. 1969.

Tchounwou, P.B., J. A Centeno and A.K. Patlolla. 2014. Arsenic toxicity, mutagenesis and carcinogenesis – a health risk assessment and management approach. Molecular and Cellular Biochemistry 255: 47-55.

Tchounwou, P.B., B. Wilson and A. Ishaque. 1999. Important considerations in the development of public health advisories for arsenic and arsenic-containing compounds in drinking water. Reviews on Environmental Health 14(4): 211-229.

Tuffin, I.M., S.B. Hector, S.M. Deane and D.E. Rawling. 2006. Resistance determinants of highly arsenic-resistant strain of *Leptospirillum ferriphilum* isolated from a commercial biooxidation tank. Apply Environment Microbiology 72: 2247-2253.

Wang, S., and C.N. Mulligan. 2006. Natural attenuation processes for remediation of arsenic contaminated soils and groundwater. J Hazard Mater 138: 459-470.

WHO. 2000. Air Quality Guidelines for Europe, 2nd ed. Copenhagen: WHO Regional Publications, European Series, No. 91, 288 pp.

WHO. 2001. Arsenic and Arsenic Compounds (Environmental Health Criteria 224), 2nd ed. Geneva: World Health Organization, International Programme on Chemical Safety.

WHO. 2005. A field guide for detection, management and surveillance of arsenicosis cases. Caussy D, editor. Technical Publication No 30. New Delhi: WHO, SEARO. p. 518.

Wolfe-Simon, F., J.S. Blum, T.R. Kulp, G.W. Gordon, S.E. Hoeft, J. Pett-Ridge and R.S. Oremland. 2011. A bacterium that can grow by using arsenic instead of phosphorus. Science, 03 Jun 2011, 332(6034): 1163-1166

Xu, C., W. Shi and B.P. Rosen. 1996. The chromosomal *ars*R gene of E. coli encodes a trans-acting metalloregulatory protein. Current Issue CHC22 and CHC17 clattering have distinct biochemical properties. Journal of Biological Chemistry 271: 2427-2432.

Xu, X.Y., S.P. McGrath, A.A. Meharg and F.J. Zhao. 2008. Growing rice aerobically markedly decreases arsenic accumulation. Environmental Science & Technology 42(15): 5574-5579.

Yan, L., H.H. Yin, S. Zhang, F.F. Leng, W.B. Nan and H.Y. Li. 2010. Biosorption of inorganic and organic arsenic from aqueous solution by *Acidithiobacillus ferrooxidans* BY-3. J. Hazard. Mater 178: 209-217.

Yan, W., R.H. Dilday, T.H. Tai, J.W. Gibbons, R.W. McNew and J.N. Rutgeer. 2005. Differential response of rice germplasm to straightad Induced by Arsenic. Crop Science 45: 1223-1228.

Yao, Z., J. Li, H. Xiec and C. Yu. 2012. The 7th International Conference on Waste Management and Technology Procedia Environmental Sciences 16: 722-729.

Yedjou, G.C. and P.B. Tchounwou. 2007. In vitro cytotoxic and genotoxic effects of arsenic trioxide on humanleukemia cells using the MTT and alkaline single cell gel electrophoresis (comet) assays. Molecular and Cellular Biochemistry, 301:123-130.