Chapter - 4

Review on arsenic removal techniques and biotransformation
4.1 State of art for removal of arsenic - Chemical Techniques:

Extensive researches have been taken place in the world in this field of arsenic removal from ground water. Arsenic generally exists in the inorganic form in water, under different redox conditions. Arsenic is stable in +5, +3, −3, 0 oxidation states. The pentavalent or +5 or arsenate species are AsO_4^{3-}, HAsO_4^{2-} and H_2AsO_4^{-}. The trivalent or the arsenite species include AS(OH)_3, AS(OH)_4^{-}, ASO_2OH^{-2} and ASO_3^{3-}. The pentavelent arsenic species are predominant and stable in oxygen rich, aerobic environments, where as the trivalent arsenite species are predominant in moderately reducing anaerobic environment such as ground water (1). The pH determines the predominance of arsenate or arsenite. According to Gupta and Chen (2) the stability and predominance of arsenic species in the aquatic environment shown in table 1.

<table>
<thead>
<tr>
<th>pH</th>
<th>0-9</th>
<th>10-12</th>
<th>13</th>
<th>14</th>
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<tbody>
<tr>
<td>AS(III)</td>
<td>H_3AsO_3</td>
<td>H_2AsO_3^{-}</td>
<td>H_3AsO_3^{2-}</td>
<td>ASO_3^{3-}</td>
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<tr>
<td>pH</td>
<td>0-2</td>
<td>3-6</td>
<td>7-11</td>
<td>12-14</td>
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<tr>
<td>AS(V)</td>
<td>H_3AsO_4</td>
<td>H_2AsO_4^{-}</td>
<td>H_3AsO_4^{2-}</td>
<td>ASO_4^{3-}</td>
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Table 1: Stability and predominance of arsenic species in different pH ranges (2).

Over the years, various treatment methods have been developed to remove arsenic from water under both laboratory and field conditions. The major mode of removal of arsenic from water is by physical-chemical
treatment method. Various treatment methods are as follows:

4.1.1. adsorption-coprecipitation using iron and aluminium salts.
4.1.2. adsorption on activated alumina/activated carbon /activated bauxite.
4.1.3. reverse osmosis.
4.1.4. ion exchange
4.1.5. oxidation followed by filtration.
4.1.6. fly ash filter and chemical tablets method.

4.1.1. Adsorption-coprecipitation using iron and aluminium salts:

The adsorption and co-precipitation technique is based on the coagulation method in which arsenic will coagulated with iron or aluminium salts. It is most conventional process used for the removal of arsenic from water. United States Environmental Protection Agency (USEPA) has shown that >90% arsenic removed (0.3 mg/l) by the application of alum of pH<7, ferric chloride at pH>10.5 (3). Iron and alum coagulation method were studied for the removal of 0.1-20 mg/l As(V) from water samples (4,5). As(V) removal depends on the initial concentration of As(V). Thus, at As(V)<1.0 mg/l concentration, coagulation with 30 mg/l of either alum or ferric sulfate in ≥ 90% removal (6). If the concentration of As(V)>1.0 mg/l ferric sulfate perform better than aluminium sulfate. As(V) removal increases with an increase of pH. By using either ferric sulfate or alum @ 30mg/l 90% arsenic can be removed if the initial concentration of As(V) was adjusted at 0.05 mg/l at pH 5.0-7.0 (7). Aluminium and ferric hydroxide have been used to remove As(V) from solution by Gulledge (8). He found ferric hydroxide is more effective in removal of As(V) than aluminium hydroxide. A number of coprecipitation method have been reported for precipitation of arsenic from water. Iron(III) oxide (9,10), hydroxide and zirconium and cerium (11) are among the many coprecipitants that have been studied. Thinolide
has also been used in the coprecipitation of arsenic from sea water with 95% efficiency (9). Adsorption and co-precipitation are the major factors controlling aqueous arsenic concentrations. Phases coprecipitate with or adsorb arsenic include hydrous oxide and hydroxide of iron (12,13), aluminium (14), manganese (15,16), clay minerals (17) and organic matters (18). From laboratory analysis (19), high pH solution have a lower adsorption capacity than the more acidic solutions in using iron oxyhydroxide for removing arsenic from ground water. The lower adsorption at higher pH is due to formation of negative surface charge on ferric oxyhydroxide, which results in an electrostatic repulsion between the sorbent and the arsenic anions. Adsorption and coprecipitation of arsenic are affected due to the presence of other substances that can compete with adsorption site. It was found that phosphate, compete with arsenic for adsorption sites on iron oxide (20) and soils (21).

According to N. E. Krapf (22) Ferric hydroxide is more cost effective and efficient complex precipitant used in arsenic removing, whether arsenic present as inorganic or as methane arsonate. He suggests that pretreatment with oxidants such as $\text{H}_2\text{O}_2$, $\text{Cl}_2$, NaOCl, KMnO$_4$ and others did not significantly improve arsenic removal. The carbon arsenic bond in methane arsenic acid is highly resistant to oxidation is aqueous system. In experimental study, he added feric chloride to the water with air agitation at an iron - arsenic ratio of 7 to 1. The pH was then adjusted at 5.5 by adding NaOH and the mixture was allowed to stand and settle the ferric flocculant for overnight. In the next day the clear supernatant water was discharged. By using this process N. E. Krapf was able to achieve in reducing upto 90%-95% of arsenic from ground water.

Lauf et al., (23) removed arsenic (III) and arsenic (V) by using ferric sulfate and hydrous manganese oxide (HMO). They found ferric
sulfate has high removal efficiency for AS(V) than AS(III). The AS(V) removal of 97.5% was achieved at ferric sulfate dosage of 5mg/l at pH 8.0. Where as, the freshly formed HMO can remove <10% AS(III). HMO was formed \textit{in situ} by oxidizing Mn(II) with KMnO$_4$ in the AS(III)/AS(V) solution. This \textit{in situ} formation of HMO was believed to aid in arsenic removal by adsorption and co-precipitation. The removal of arsenic by HMO was not affected by pH and an initial concentration of 200 µg/l was reduced to 48 µg/l KMnO$_4$ oxidizes AS(III) to AS(V) first.

Edwards (24) studied the removal of AS(III) and AS(V) by using FeCl$_3$, alum and then oxidizes. AS(V) removals were independent of initial AS(V) concentration and coagulation dosage and solely dependent on pH, with maximum removals occurring at 7.0. AS(III) removals were found to be independent of pH but dependent on initial AS(III) concentration. Shen (25) found FeCl$_3$ is most effective coagulant for the removal or arsenic from ground water. An initial arsenic concentration of 1mg/l in raw water having pH 6.8 was reduced to 0.08 mg/l (92% removal) using 30 mg/l dosage of FeCl$_3$.

In Taiwan area, where arsenic contamination from ground water was happened, there was a big water treatment plant to remove arsenic from ground water before consumption. In one experiment alum Al$_2$(SO$_4$)$_3$ was added and in other experiment ferric sulfate Fe$_2$(SO$_4$)$_3$ was added and in other experiment ferric sulfate Fe$_2$(SO$_4$)$_3$. 3H$_2$O was added. In both the cases varying the removal of alum and ferric sulfate with the change of pH was done. The experimental result showed that absorption of ferric hydroxide exceeds the adsorption of aluminium hydroxide. In both the cases, increased coagulant dosage results in increased arsenic removal.

4.1.2. Adsorption on activated alumina/activated carbon/activated bauxite:

In this process activated alumina has been used as sorbant which
adsorbs arsenic on the surface and alumina can be regenerated with the alkali treatment. The basic principle for adsorption of arsenic by activated alumina is protonation and deprotonation. The particle size of the alumina is kept around 0.30-0.60 mm. The activated alumina is capable of ligand exchange process. The specific reaction of sorption process is given below:

\[ \text{Al-OH} + H^+ + H_2\text{AsO}_4 (\text{here Al-OH denotes active site}) \]

\[ \text{Al-H}_2\text{AsO}_4 + \text{H OH} \rightarrow (\text{i}) \quad (\text{Protonation of alumina}) \]

\[ \text{Al-H}_2\text{AsO}_4 + \text{OH}^- \rightarrow \text{Al-OH} + H_2\text{AsO}_4 \rightarrow (\text{ii}) \quad (\text{Deprotonation of alumina}) \]

This have been found in acidic condition. Over burden of proton occurs at that time when activated alumine adsorbs arsenic ions efficiently [from equation No. (i)]. The regeneration of activated alumina bed laden with arsenic is done by strong base like caustic soda having 4% strength at high temperature. 70% of the arsenic deposited in the bed can be regenerated with this method. Through this removal system, have some limitations like pH maintenance. This process works best within pH 4.0-8.2. Below the pH 4.0 the activated alumina dissolved in the acid and as such removal efficiency hampered and above the pH 8.2 activated alumina become cationic exchange material, but as we know, arsenic is metalloid, it normally present in anionic form rather than cationic form.

Ginocchio (26) reported that about 90% As(V) can be removed by precipitation by using flocculation/sedimentation at pH ranges between 6.5-7.0 using 5g/m³ trivalent iron flocculant admixture. The concentration of As(V) should be in the range of 0.1 to 0.4 mg/l. The adsorption capacity of activated alumina for As(V) indicates a specific loading capacity for As(V) of 10-15 mg/dm³. A concentrated caustic soda was used for the regeneration of activated alumina.
Clifford (27) suggested that the pH range 5.5 to 6.0 is best for the removal of arsenic from ground water by activated alumina. He recommended 4% NaOH can be used for the regeneration of the spent activated alumina column and a 2% H₂SO₄ solution for acid neutralization. About 50%-70% alumina can be regenerated.

Bellack (28) proposed an initial concentration of 0.06 mg/l was reduced to 0.003 mg/l using activated alumina treatment. He observed at higher arsenic level (0.5 mg/l) arsenic removal decreased initially, occasionally depending on the initial pH to an effluent conc. of 0.01 mg/l and increased thereafter. This phenomenon was attributed to the interruption in flows which resulted in enhanced exchange capacity.

Adsorption of As(V), methylarsonate and dimethylarsenate by activated alumina had also been studied (29). The affinity of the adsorption decreased in the following order:

As(V) > methylarsonate > Dimethylarsenate

Cox and Ghosh studied surface complexation of methylarsonate and dimethylarsenate by activated alumina (30). Adsorption of methylarsonates was found to decreased with increasing pH and initial arsenic concentrations. An initial conc. of 1.0 x 10⁻⁵ M dimethylarsenate showed less than 70% removals with activated alumina.

Lignite based activated carbon, with high ash content (22.1% on dry basis) effectively removed As(V) from water and removals were about 5 times more than those with low ash content. The major mechanism of arsenic removal was suggested to be a combination of ion exchange and chelation. The main mechanism was suggested to be a strong interaction between arsenic and the mineral matter on the carbon ash (31).

The rate of uptake by activated bauxite and activated carbon for
As(III) was much slower in comparison to As(V) (2). The uptake of As(V) by activated bauxite was found to be a maximum in the pH range 6.0 to 7.0. But in activated carbon, the optimum pH range is 3.0-4.0 for the uptake of As(V). High removal was observed for As(V) than As(III), and pretreatment by chlorine oxidation of arsenite to arsenate prior to adsorption was suggested.

A laboratory study carried out by Guha and Chaudhury (32) to remove As(II) from ground water using low cost materials like bituminous coal, lignite, crushed coconut shell, illite, kaolinite, rice husk, fly ash, charcoal, sand. This process reduces arsenic level below 0.05 mg/l from 1.0 mg/l for 32 hours. In this experiment further studies were recommended.

4.1.3. Reverse Osmosis:

Reverse osmosis comprises a prefilter for sediment removal and if necessary, an activated carbon filter for chlorine removal can be used.

For uses a 5 µm prefilter for removing inorganics through the reverse osmosis system and also uses activated refilter to remove chlorine. The reverse osmosis membrane used was a spiral wound polyamide film membrane. Arsenic can be removed upto 73.3% from initial concentration of 0.101 mg/l, which was pumped at pressure of 42±2 psi.

Huxstep (35) reported at high pressure (400 psi) and also at low pressure (200 psi) by reverse osmosis system inorganic arsenic contaminants can be removed from ground water. He observed that at high pressure the rate or removal can be achieved upto 91%-98% in case of As(V), whereas in case of As(III) it is upto 77%-87%. But at low pressure the rate of removal is slow in both the As(V) and As(III). Clifford (6) recommended prior to use the reverse osmosis system oxidation of As(III)...
to As(V) should done. Chang et al., (36) found microfiltration to be more effective than conventional filtration for arsenic removal.

4.1.4. Ion-Exchange:

The ion exchange system is similar in principle to the adsorption process. As arsenic occurs in anionic form and the arsenic acids are weak acids, strong base anion exchange resins in chloride form can be used as anion exchange column. Various types of resins are used in ion exchange method. I. B. Ambro (37) reported, VARION AD, VARION AT-4 and VARION AT-6 strong anion exchange resins are used for studying the ion exchange equilibrium of arsenic ion. In the chromatographic experiment the effect of arsenic concentration is depend on ion-exchange column and pH of the effluent. VARION AT-6 resin is proved to be the most suitable for removing arsenic from highly alkaline solution (37). Sandhu et al., (38) reported that the polluted water was first digested with KMnO₄ and eluted first with KMnO₄ and then eluted through chromatographic column packed with Amberlite, IRA-40, I. S. C. P., ion-exchange resin.

Removal and recovery of arsenate and arsenite anions from dilute aqueous solution were studied using a weak base chelating resin (DOW XFS-4195) in ferric ion form by ligand exchange sorption process (39). It is a macroporous chelating resin with a weak base functional group which binds As(V) or As(III) by ligand exchange (ion exchange with counteraction of Fe(III) followed by complexation of metal ions). Later on another chelating resin Chelax-100 in ferric form was used instead of XFS [Fe(III)] (40). The resing Chelax-100, has a crosslinked polystereine macroreticular lattice. Chelax-100 resin favours mild alkaline pH for removing As(III), while mild acidic pH was required for removing As(V).

4.1.5. Oxidation followed by filtration:

American Water Works Association, 1993 (41) reported the use
of manganese greensand filter can remove arsenic along with iron [Fe(II)] by oxidation-and filtration method. Chlorine and potassium permanganate were used to oxidize Fe(II), to Fe(III), Mn(II) to Mn (IV) and As(III) to As(V). An iron to arsenic ration of 10 : 1 reduces 0.26 mg/l of arsenic to less then 0.005 mg/l at a pH of 7.0. When potassium permanganate was used, the oxide coated adsorption clarifier and filter media promoted greensand effect. It has been found that higher iron to arsenic ratio improves arsenic removal.

Y. S. Shen (25) reported that arsenic removal is too some extent pH dependent. In his experiment he first tried with plain sedimentation. The raw water was placed in a beaker and settled without agitation. Removal of arsenic was progressed asymptotically - the level of arsenic remaining in the water reaching a near equilibrium level after ten days. Sedimentation apparently is capable only of removing half of the arsenic content, and this after a prolonged period. Secondly, the ion exchanger Ionac A-260 used has an exchanger layer thickening of 60 cm. Diameter of the filter tube is 2.2 cm. The rate of filter run was 320 m³/m²/day. The synthetic water containing 1.06 mg/l of arsenic was removed by thus process. But using the natural water from deep well in the area of the study and containing 0.84 mg/l was used, the result proved elimination of arsenic almost to the extent of 100%. Thirdly, various coagulants such as aluminium sulfate, lime, ferrous sulfate and ferric chloride has been used for the removal of arsenic. It was found that FeCl₃ was the best coagulant for the removal of arsenic content in the finished water sufficiently to a level below the W. H. O. standard for drinking water. So chlorine and potassium permanganate were used as oxidant. It was found that 15 mg/l of chlorine coagulating with 50 mg/l of FeCl₃ has resulted the best effect. The filtered water passed through the plastic tube with a diameter of 6 inch. Two kinds of filter medium were used: anthracite and
sand slow rather than fast filtration, can remove arsenic from water, but the filtration run is too short lasting - only five days.

4.1.6. Fly ash filter and chemical tablets method:

Based on previous conclusions and methods D. Das carried out an experiment for removing the arsenic from around water satisfactorily (19) by following fly ash and chemical tablet method. His system consist of two earthen jars, one filter candle made of fly ash and a chemical tablet. Two earthen jars placed one above the other. The fly ash candle fitted in the upper jar, now chemical tablet along with the contaminated water poured and stirred well through wooden stirrer. After an hour, the candle mouth was opened by rotating the cap of the candle. Arsenic free water starts to drop in the lower earthen jar. D. Das submitted the fly ash filter, chemical tablet and the whole filtering system for arsenic removal from ground water for "patent" formalities, under Govt. of India. He uses FeCl₃/Fe(SO₄)₃·3H₂O/Al₂(SO₄)₃·18H₂O/MnO₂·2H₂O as coprecipitation agent for both arsenite and arsenate. Oxidizing agents used by him are H₂O₂/KMnO₄/nascent chlorine/ NaOCl for conversion of arsenite to arsenate of the water.

He claims that, the chemical tablet prepared by him stabilise upto 20 months, and it is clear from his experimental results. As the filter candles are heated upto 1200°C during preparation, probably most of the metal salts are volatilized and decomposed or converted to non leachable form, so there had been no significant change in quality of the water sample found often filtration.

4.2. Bioremediation:

There are considerable studies made over past few decades on microbial removal of arsenic species and their biotransformation in the aquatic environment (42,43). Arsenical compounds are established as carcinogenic compounds. The concept of methylation is a natural detoxification process by sequential reduction/methylation involved in biotransformation of
inorganic arsenical substances. Inorganic arsenic ingested as either As\(^{3+}\) or As\(^{5+}\) state. As\(^{5+}\) or arsenate in less toxic than As\(^{3+}\) or arsenite. Before methylation arsenate is reduced to arsenite. During methylation, two subsequent steps take place; first, arsenite methylated to monomethylarsonate or MMAA and second, it further methylated to dimethylarsenate or DMAA. Both the MMAA and DMAA are less toxic than inorganic arsenicals (arsenite and arsenate) and bind less to tissues (60). The microflora in the environment posses the metabolic machinery to transform these compounds into gaseous arsines. Many fungi can transform inorganic arsenic species into trimethylarsine. While bacteria will produce anaerobically dimethylarsine. Moulds can transform sodium methylarsenate CH\(_3\)AsO(ONa)\(_2\) and sodium cacodylate (CH\(_3\))\(_2\)AsO(ONa) to trimethylarsine. Beside this several mono and dialkylarsonic acids RAsO(OH)\(_2\) and RR'AsO.OH or their sodium salts were also methylated by \textit{S. brevicaulis} to ethyldimethylarsine, n-propyldimethylarsine, allyldimethylarsine and methyl-ethyl-n-propylarsine. In this reaction the methyl group is supplied by the mould itself (44).

![Diagram](attachment:fig41.png)

**Fig. 4.1. Biological methylation of alky and dialkylarsonic acids**
Biological methylation of arsenic is supposed to be the transfer of methyl group from the organism to arsenic species, that methyl group already present in the tissues which posses the capacity of methylation.

\[ \text{AS(OH)}_3 \leftrightarrow \text{CH}_3\text{AsO(OH)}_2 \leftrightarrow (\text{CH}_3)_2\text{AsO(OH)} \leftrightarrow (\text{CH}_3)_2\text{As} \]

Fig. 4.2. Biological methylation of arsenite

From the experimental results of Lunde (45, 46, 47) it was confirmed that arsenic is present in the aquatic organism mainly in organic form. Majority of arsenic accumulated in the organisms as dimethylarsenic compounds and are mainly found in algal body. (48, 49, 50) and trimethyl arsenic compounds such as arsenobetaine were found in crustaceans.

Fig. 4.3. The conversion pathway from arsenosugar to arsenobetaine modified from Norin et al., 1983 (51).
Inorganic arsenic may undergo several biochemical transformations. The hydroxyl group of arsenic acid $\text{AsO(OH)}_3$ is replaced by $\text{CH}_3$ group to form $\text{CH}_3\text{AsO(OH)}_2$ (MMAA), $(\text{CH}_3)_2\text{AsO(OH)}$ (DMAA) and $(\text{CH}_3)_3\text{AsO(TMAA)}$ (52). These transformation usually occur biologically in the aquatic environment. First, inorganic arsenic is incorporated by autotrophic organisms such as algae and then it is transported through the food chain. During the transfer, methylation of arsenic progresses within the body of the organism. Methylation of arsenic is probably the main detoxifying process for organisms. Both the freshwater and marine organsims are capable to bioaccumulate arsenic from their aquatic environment. But in comparison to freshwater organisms are less efficient to bioaccumulate than marine species (53). However, both the organisms reduces the total arsenic concentration by biomethylation and increases the amount in the trophic level (54, 55, 56).

Hopenhayn-Rich, C. established that, exposure level of arsenic to human may lead to threshold for the carcinogenecity and saturation of methylation capacity. From his findings decrease in arsenic exposure associated with the small decrease in arsenic percentage in urine and also in the MMAA/DMAA ration (57). The relative distribution of inorganic arsenic, MMAA and DMAA in urine measures the human methylation capacity. Urinary analysis done by Hopenhyan-Rich, C., presence of MMAA and DMAA and nonmethylated arsenic shows the indicator of methylation capacity. A few biological media were used to determine the exposure to arsenic. But, blood is not a good indicator, because arsenic get cleared from the blood within a few hour, specially at low level of exposure (58, 59). Near about 60%-75% dose exreted from urine in ingestion study (60, 61, 62).

As the fresh water algae has enormous capacity to bioaccumulate and biotransform the inorganic arsenic, so they can be used in removal
process (42), which are very much resistant to arsenic. Chapman first revealed that marine organisms like Shrimp contain arsenic in high level. There is a great difference in the arsenic content of marine and terrestrial organisms. Generally, marine species contain high level of arsenic. But it has been proved that lower members of the trophic level in marine ecosystem, such as algae, accumulate and alkylate arsenic more efficiently than the terrestrial organisms (63, 64). Such algae methylate the inorganic arsenic to arsenocholine, arsenobetaine which are seem to be non/less toxic (51).

An experiment done by Maeda (55), he came to the conclusion that the arsenic compounds transformed and transported through the freshwater food chain. He investigated that the lower member of the trophic level such as Chlorella, Phormidium were allowed to grown in arsenic containing medium for 7 days, then these organisms were fed by Moina for 7 days. Moina acquired arsenic concentration but lower in amount than the Chlorella and Phormidium. Next guppy feeded on Moina and shows lowest arsenic concentration among those organisms. From this, he concluded that, almost all arsenic present in algae was in inorganic form, but 85% of arsenic was in di and trimethylated form in guppy. Occurrence of high ratio of methylated compounds in higher organisms reveal that the preferential uptake or retention of methyl arsenic compounds result to the methylation by the higher organisms. Beside this transformation of arsenic compounds, organisms of marine food chain were also studied by many reserchers (65). A blue green algae - Nostoc sp. was screened by Maeda et al., from arsenic rich environment and bioaccumulation rate of arsenic was investigated by them (43). By the bioaccumulation mechanism arsenic can be removed biologically from the aqueous phase. He proved that when arsenic polluted water was enriched with the nutrients of MMA medium, the arsenic level was found to be effectivley lowered by the Nostoc
Insensee and coworkers (66) worked on the distribution of dimethyarsenic compounds among freshwater organisms in a model ecosystem (Water - algae - snail) and (Water - diatoms - \textit{Daphnia} - fish). In his experiment, he observed that lower food chain organisms (algae and \textit{Daphnia}) bioaccumulate more dimethylarsenic compounds than did higher food chain organisms (snail and fish). The methylated compounds are less toxic to the environment and also the bioaccumulation of dimethylarsenic compounds to the lower group in huge amount indicate that it did not show a high potential to biomagnify in the environment.

Wrench and Co-Workers (67) established that biotransformation process found on three trophic levels of marine organisms (Phytoplankton -> \textit{Dunaliella marine}, Zooplankton -> \textit{Artemia salina} and Shrimp -> \textit{Lysmata seticaudata}). From the experimental result, he concluded that organic forms of arsenic in marine foods were derived from an \textit{in vivo} synthesis by primary producers and were efficiently transferred along the marine food chain. The shrimp, the highest trophic level organism of the food chain could not form organic arsenic by itself. Here, arsenate taken up from water was converted largely to arsenite. Similar results obtained from the experimental results on phytoplankton -> mussel -> Crab (68), phytoplankton -> grazer -> carnivore (69, 70) and phytoplankton -> lobster (65) system. In the three steped fresh water food chain experiment done by Maeda and Coworkers (71) (\textit{Chlorella vulgaris} -> \textit{Moina macrocarpa} -> \textit{Carassius carassius auratus} juvenile), concluded that transformation of arsenic occur via freshwater food chain. Result shows grazers and carnivores also accumulate arsenic from water and methylated a part of it. The arsenic accumulation from food decreased on order of magnitude and the biomethylation ratio of arsenic increased, successively with an elevation in the trophic level, i.e., arsenic concentrations are not magnified in the aquatic food web (55, 56). From other experimental results (56, 72-74), it
has also been observed that relative concentration of arsenic species accumulated in low amount in higher trophic level organisms, at the same time relative concentration of dimethyl arsenic and trimethyl arsenic compounds dramatically increased successively with an elevation in the trophic level.

In another experiment Maeda and Coworker (75) proved that a small part of arsenic bioaccumulated by C. vulgaris was methylated in vivo. But the quantity of arsenic methylated in the cell increased with an increase of arsenic concentration in the medium (77, 78), but the relative concentration of the methylated arsenic compounds to the total decreased from 1.8% to 0.4%. Result shows that (75) when C. vulgaris accumulated inorganic arsenic compounds from the aqueous phase, the quantity of the arsenic accumulated increased with an increse in aqueous arsenic concentrations and a part of the arsenic methylated in the cell but the quality of methylated arsenic did not increase in proportion to that of the total arsenic accumulated. The algae seemed, therefore, to have a limited methylation capacity. In this experiment he also concluded that in the growth phase of C. vulgaris, a small fraction of the arsenic accumulated in the cell was first transformed to monomethyl and dimethyl compounds during the early exponential phase and after a short period of time a fraction was transformed to trimethylarsine species. The blue green algae Phormidium sp. also shows tolerance to arsenic and accumulate arsenic with in the cell with an increase of the surrounding arsenic concentration upto 7000μg/gm. Phosphorus concentration in the medium affect the growth rate and bioaccumulation rate of the algae. There is a competition between arsenic acid and phosphoric acid for accumulation. Beyond certain level of phosphorus concentration in the medium inhibits the accumulation of arsenic (78). The arsenic was methylated upto 3.2% of the total arsenic accumulated. When the cells were transferred into the arsenic free medium,
85% of the arsenic accumulated was excreted. 58% of the excreted arsenic was in methylated form implying extensive methylation in the arsenic free medium. Excretion of arsenic also been reported from other researchers (75, 79). In the excrement, the relative concentration of methylated arsenic was found to be only in the dimethyl arsenic form. No nonmethylarsenic compound was detected in the excrement. The dimethylarsenic compound was found to be most preferable chemical form for excretion from C. vulgaris (75). This algae not only accumulate the inorganic form of arsenic but also accumulated methylated arsenical compounds from medium. The methylated arsenical compounds which were accumulated, they were further biomethylated. About half of the monomethyl arsenic compounds were transformed to dimethylarsenic species in the algal cells, but no demethylation occurred. When the cells were exposed to dimethylarsenic compounds, only DMAA was accumulated. It shows neither methylation nor demethylation occurred during dimethylarsenic accumulation. In the case of arsenobetaine accumulation, no demethylation occurred. These experimental results revealed that C. vulgaris takes up not only inorganic arsenic compounds but also methylated arsenic compounds, and the methyl arsenic compounds taken up are further biomethylated but not demethylated. Dimethyl arsenic species seemed to be the most stable arsenic form in the algal cells (75).

There is a direct effect of iron and manganese on the rate of accumulation of arsenic as shown in phosphorus (79). Experimental results established that the increase of iron concentration in the medium causes low growth rate of algae C. vulgaris but the increased concentration of iron in the medium causes increase in cellular accumulation capacity and decrease in the iron concentration in the medium causes decrease in the capacity of arsenic bioaccumulation. But, beyond certain limit of iron concentration, opposite effect was observed. The managanese concentration also acts in the same way.
Generally, arsenic accumulated in the living cells of *C. vulgaris* was found be combined with a protein having the molecular weight of about 3000 (76). This finding has been established when *C. vulgaris* cells were solvent extracted with Chloroform : methanol (2 : 1 vol./vol.), and the fractions were analyzed for arsenic determination. Analysis of the amino acids of arsenic bound protein shows that no metallothionein like protein was inductively biosynthesized in *C. vulgaris* when exposed to arsenic rich medium. But, when the same alga was exposed to other metals like zinc, cadmium produces metallothionein like protein (75, 79). So, from this it can be concluded that, though, biosynthesis of metallothionein protein is a detoxifying mechanism but in arsenic rich medium alga bypasses this mechanism rather it obeys another detoxifying mechanism such as methylation.

Lunde (46) reported that arsenic bound generally in the lipid phase when extracted from freshwater algae (*Chlorella ovalis, Phaeodactylum tricornutum* and *Oscillatoria rubescens*) using the neutron activation technique. These alga bioaccumulated arsenic in the lipid phase at concentrations of 0.5 to 5 μg/gm dry weight from enriched cultures containing 1 to 3 μg/dm³.

Accumulation of arsenate *Tetraselmis chui* - a marine alga was observed by Irgolic *et al.*, (80). This alga grow well in Instant Ocean medium at arsenate level ranging from 0.5 to 50 ppm. The lag phase increased with the arsenic concentration. In his experiment he found 5000 liters of algae cultures will produce several grams of the arsenic compounds.

The alga *Tetraselmis chui* make a complex with arsenate with the protein molecule located in the algal cell membrane prior to its chemical transformation by the biochemical apparatus of the cell (81). The arsenic
containing lipids also been isolated from *T. chui*, which binds dimethylarsine and trimethylarsine. Arsenic is incorporated in the lipid in the form of arsenobetaine, and the arslenocholine might be converted to arsenobetaine form.

Accumulation and methylation of arsenic also performed by yeast, fungi and bacteria (82). In the early nineteenth century, arsenic poisoning reported from a wall paper containing arsenical pigments such as "Scheele's green" [Cu(AsO₂)₂, CuHAsO₃] and "Paris green" [Cu(CH₃COO)₂. 3Cu(AsO₂)₂] in a musty room with a characteristic garlic odour, due to the release of volatile arsenic compounds produced by moulds on damp arsenic pigmented wall paper (83). After proper investigation and research it was identified that the chemical nature of this volatile arsenicals are methylated compounds of arsenic (83, 84).

Microbial transformation of arsenic in soil and water involve oxidation, reduction and methylation reactions. Volatile organic methylated forms are derived by methylation of arsenic from inorganic and organic arsenical compounds. Volatile forms are mainly dimethylarsine and trimethylarsine. Inorganic arsenic methylation is coupled by the methane biosynthetic pathway of methanogenic bacteria (85). This pathway proceeds by the reduction of arsenate to arsenite followed by methylation in presence of co-enzyme M (Co-M) - a lower molecular weight cofactor found in all methanogenic bacteria.

In more recent studies, three different fungal species *Candida humicola*, *Gliocladium roseum* and *Penicillium* sp. were found to be capable of converting MMAA and DMAA into trimethylarsine (82, 86). The fungal methylation pathway for the formation of trimethylarsine is:

In 1932, Thom and Raper (87) isolated from soil several strains of fungi that were active in producing trimethyl arsine. They also found that the strains were active with a wide variety of arsenicals used commercially and suggested that any arsenical could probably be acted on by fungi. Several fungi isolated from sewage (82, 86) can reduce arsenic compounds to trimethylarsine (TMAA). Mc. Bride et al. (85) reported that the dimethylarsine was mainly produced by anaerobic organisms, while trimethylarsine resulted from aerobic methylation. The following mechanism for the methylation of arsenate had been proposed by Challenger (87) and Mc. Bride et al. (85).

\[
\begin{align*}
\text{As}^{V}O_{4}^{3-} & \quad 2e^- \quad -O^- \quad \text{As}^{III}O_{3}^{2-} \\
\text{(CH}_3\text{)}_2\text{As}^{V}O & \quad 2e^- \quad -O^- \quad \text{(CH}_3\text{)}_2\text{As}^{III}O_{2}^{2-}
\end{align*}
\]

The proposed mechanism indicates that As(V) has to be reduced to As(III) before being methylated. In a recent study by Huysmans et al. (88), it was found that in the central valley of California, arsenic is present in soil at naturally high concentrations, being derived from marine sedimentary parent material of the Coastal Range. Due to intense agricultural irrigation, soluble arsenic is leached from the soil and accumulates in evaporation ponds where it may pose an environmental threat to the water fowl and wildlife. A *Penicillium* sp. isolated from evaporation pond water was found to be capable of methylating and subsequently volatilizing organic arsenic. It is apparent that a wide variety of fungi, particularly those found in soil, can methylate both organic and inorganic arsenic compounds to the highly volatile TMAA, which could thereby be lost to the air.
4.3. References


19. Das, D., Ph. D. Thesis, Arsenic species along with other metal/metalloid present and responsible for arsenic episode in ground water of West Bengal and a cheap technique to remove arsenic, thus making the ground water suitable for drinking and cooking, Jadavpur University, Calcutta, India, 1995.


