PRINT ISSN 1119-8362
Electronic ISSN 2659-1499

Full-text Available Online at
https://www.ajol.info/index.php/jasem
https://www.bioline.org.br/ja

J. Appl. Sci. Environ. Manage.
Vol. 28 (3) 771-784 March 2024

Arsenic Species Distribution and Toxicity in the Environment, Bioaccumulation, Biomethylation and Bioremediation by Microalgae: A Review

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ABSTRACT: Arsenic (As) is a noxious metalloid that has been designated a priority pollutant and is present in the environment as a consequence of both anthropogenic and natural processes. Its toxicity in environmental and biological systems depends strongly on the chemical species. Interest in arsenic and microalgae interactions is important because microalgae are at the base of the aquatic food chain, are used in animal nutrition and has potential for As phytoremediation. This paper reviewed the current information on As species distribution in the environment especially as it relates to its toxicity to microalgae as well as its bioaccumulation, biomethylation and bioremediation by microalgae using appropriate methods. Information obtained revealed that Microalgae have evolved mechanisms for dealing with As in the environment with arsBHC operon mediating the reduction and extrusion of arsenite from the cells. They accumulate large amounts of arsenic from their surroundings which could lead to toxicity, As excretion from cells, reduction, methylation or complexation with metal binding peptides like glutathione and phytochelatins. This has made them suitable as ecological indicators to give an indication of As bioavailability and also in possible applications for the process of As remediation. Microalgae are been proposed for bioremediation purposes in aquatic environment since they show a high capacity for biosorption and bioconcentration of As and most importantly since they are able to methylate inorganic As to non-toxic organic and volatile As.

DOI: https://dx.doi.org/10.4314/jasem.v28i3.18

Cite this Article as: OBUEKWE I. S; AJUZIE, C. U (2024). Arsenic Species Distribution and Toxicity in the Environment, Bioaccumulation, Biomethylation and Bioremediation by Microalgae: A Review. *J. Appl. Sci. Environ. Manage.* 28 (3) 771-784

Dates: Received: 18 January 2024; Revised: 24 February 2024; Accepted: 12 March 2024 Published: 29 March 2024

Keywords: Arsenic; microalgae; bioaccumulation; biomethylation; bioremediation; biosorption

Arsenic (As) is a naturally toxic trace element that is rather ubiquitously distributed through-out the world but it is also an important environmental pollutant (Huang and Kretzschmar, 2010). Its environmental contamination often results from natural sources, such as weathering of rocks and minerals with high As contents as well as from human activities such as mining, metal smelting, pesticide application and burning of fossil fuels (Fendorf *et al*., 2010; Rahman and Hasegawa, 2011; Silva *et al*., 2012; Ye *et al*., 2012; Pell *et al*., 2013; Al-Makishah *et al*., 2020). It poses a threat to human and ecosystem health, particularly when incorporated into food or water supplies (Assis *et al*., 2010; Mitra *et al*., 2012; Ye *et al*., 2012; Akhtar *et al*., 2013; Wang *et al*., 2014). It has been estimated that rice is the largest contributor (about 60 %) of inorganic As (iAs) ingestion through food consumption in China (Ye *et al*., 2012). Approximately 35 to 77 million people have been exposed to As through drinking water in Bangladesh alone (Ye *et al*., 2012; Rahman *et al*., 2015; William and Magpantay, 2024). Human exposure to As can lead to an various diseases including bladder, skin and lung cancers; diabetes; metabolic disorders, developmental disorders; and neurological disorders (Huang and Kretzschmar, 2010; Pisani *et al*., 2011;

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Rahman *et al*., 2015). A dose response relationship between arsenic exposure and serum vascular endothelial growth factor (VEGF; a specific marker for angiogenesis) levels was found in humans exposed to arsenic chronically in Bangladesh (Rahman *et al*., 2015). As is a redox active element that has its normal valency of 3 or 5 and generally exists in either the +3 or +5 oxidation state. Both oxidation states lead to oxyanions-Arsenate [As(V) as H_3ASO_4] and Asenite [As(III) as H_3ASO_3] (Pisani *et al*., 2011; Rahman and Hasegawa, 2011; Silva *et al*., 2012; Kumar *et al*., 2013; Rahman *et al*., 2014; Zhang *et al*., 2022). Arsenic changes chemically from volatile to insoluble forms under the influence of physicochemical and biological processes. It becomes problematic from a health perspective principally when it partitions into aqueous rather than the solid phase. Dissolved concentrations, transformation and the resulting mobility of arsenic in the environment are governed by biogeochemical processes linked to hydrologic and biological processes, causing an As biogeochemical cycle (Assis *et al*., 2010; Miyashita *et al*., 2011; Zhang *et al*., 2013). Microalgae are native to a vast array of freshwater and marine environment. They are at the base of the aquatic food chain and can accumulate large amounts of arsenic from their surroundings (Yamaoka *et al*., 1996). Freshwater and marine microalgae have been found to take up and bioaccumulate arsenate as a phosphorous analogue during normal metabolism (Murray *et al*., 2003). This has made them suitable as ecological indicators (especially for intermittent pollution), to give an indication of bioavailability and also in possible applications for the process of remediation (Murray *et al*., 2003). Photosynthetic organisms play a significant role in As geochemical cycling by methylating toxic inorganic arsenicals to less toxic organoarsenicals (Hasegawa *et al*., 2001; Sierra-Alvarez *et al*., 2005; Murray *et al*., 2003; Foster *et al*., 2008; Miyashita *et al*., 2011; Ye *et al*., 2012; Zhang *et al*., 2013). Methylation and volatilization of arsenic to organoarsenicals and volatile arsines respectively occur in various algal cultures and is thought to be a detoxification mechanism (Hasegawa *et al*., 2001; Oremland *et al*., 2004; Yin *et al*., 2011; Miyashita *et al*., 2011; Zhang *et al*., 2013). The increased concern about arsenic risk to human health is the driving force behind the study of arsenic biogeochemical cycling in the environment (Cai *et al*., 2002). In this review, the impact of As species distribution in the aquatic environment is discussed especially as it relates to its toxicity to microalgae as well as its bioaccumulation, biomethylation and bioremediation by microalgae.

Arsenic speciation and toxicity to microalgae: Anthropogenic and natural sources have contributed to the increase of arsenic concentration in ground and surface water, often to values higher than the threshold of 10 µg L^{-1} considered safe for drinking water by the World Health Organisation (WHO) (Silva *et al*., 2007; Akhtar *et al*., 2013; Rahman *et al*., 2014; Rahman *et al*., 2015). There is increasing evidence of cancer risk associated with chronic exposure to low levels As through drinking water (Huang and Kretzschmar, 2010; Rahman *et al*., 2015). Therefore, contamination of drinking water aquifers by naturally and anthropogenically occurring arsenic represents a significant environmental hazard that presently affects the health of millions of people worldwide (Kulp *et al*., 2004). Ingestion of As in drinking water is recognized as the exposure route presenting the greatest risk to humans, and dispersal of As-rich mine wastes can accelerate geochemical and microbiological reactions that release arsenic to waters (Andrade *et al*., 2008; Foster *et al*., 2011).

Arsenic toxicity in environmental and biological systems is strongly dependent on the chemical species. Its speciation has received significant attention over the last years due to its-species-dependent toxicity (Salgado *et al*., 2006; Pisani *et al*., 2011; Onnby *et al*., 2012; Wang *et al*., 2015). As speciation plays a significant role in its behavior and fate in the environment, and different As species differ greatly in their mobility, availability and toxicity to cells (Cai *et al*., 2002; Wang *et al*., 2014). The most poisonous form of arsenic for humans is arsenous acid, $As(OH)_3$, or its anion arsenite. In general, arsenite (As(III)) is more toxic than arsenate (As(V)) (Bentley and Chasteen, 2002; Kumar *et al*., 2013; Zhang *et al*., 2013), it is ten times more soluble, mobile and toxic than As(V) (Komarek *et al*., 2013; Ye *et al*., 2012; Franco *et al*., 2015; Rezende *et al*., 2015).

The inorganic forms of arsenic (As(III) and As(V)) which are usually the As species accumulated by algae are more toxic than the organic ones, monomethylarsonate [MMA(V)] and dimethylarsinate [DMA(V], which show a moderate toxicity, or arsenobetaine (AsB) and arsenocholine (AsC) which are not toxic (Beceiro-Gonzalez *et al*., 2000; Llorente-Mirandes *et al*., 2010; Pisani *et al*., 2011). However, methylarsenic(III) species [MAs(III); DMA(III)] have been reported to be more toxic and probably more reactive than inorganic As (Ye *et al*., 2012) and methylarsenic(V) species in aquatic system (Hasegawa *et al*., 2001; 2002). They are also more susceptible to oxidation than arsenite, DMA(III) is particularly thermodynamically unstable in oxic aquatic solutions (Hasegawa *et al*., 2001). *In vivo*, the toxicity of soluble inorganic and organic As species are dimethylarsenite (DMAs(III)),

monomethylarsenite (MMAs(III)) > As(III) > As(V) > dimethylarsenate (DMAs(V)), monomethylarsenate (MMAs(V)) > trimethylarsine (TMAs) and trimethylarsine oxide (TMAsO) (Wang *et al*., 2014).

Inorganic As species find their way into microbial cells using distinct routes. As(III) is transported across cell membranes by aquaglyceroporin channels and may react with critical thiols groups (-SH) that are frequently located at the active sites of enzymes and tissue proteins such as glutathione with frequent inhibition or disruption of their catalytic activities (Katsoyiannis and Zouboulis, 2004; Levy *et al*., 2005; Pisani *et al*., 2011; Miyashita *et al*., 2011; Rahman *et al*., 2014; Wang *et al*., 2014; Ghosh *et al*., 2015). It exerts its toxicity through binding to dithiols, forming arsenothiols that perturb protein function and that ultimately generate reactive oxygen species (ROS) (Paez-Espino *et al*., 2009; Sanchez-Riego *et al*., 2014). As (III) binds to the main redox buffer in the cells, glutathione (GSH) because of its high affinity for sulpur forming As(III)-GSH_2 thereby depleting its pool, thus contributing to ROS generation (Pandey *et al*., 2012; Rahman and Hassler, 2014). Oxidation of thiols such as glutathione has been shown to be a potential mechanism by which algal cell division is inhibited by metals (Levy *et al*., 2005). Levy *et al*. (2005) hypothesized that, a microalgae, *Monoraphidium arcuatum* reduction of As(V) to As(III) may have been coupled with oxidation of glutathione (GSH), ultimately resulting in inhibitory effects on cell division. This was based on low thiol cell concentrations at high As(V) concentration (0.5 mg As(V)/L) in relative to the controls without As. As(V) on the other hand, can replace phosphate in several biochemical reactions i.e arsenate can be transported across the plasma membrane via phosphate co-transport systems and once inside the cytoplasm, it competes with phosphate, for example replacing phosphate in ATP to form unstable ADP-As, and leads to the disruption of energy flows in cells thus interfering with oxidative phosphorylation and ATP biosynthesis (Pisani *et al*., 2011; Rahman *et al*., 2014; Nagy *et al*., 2014; Sanchez-Riego *et al*., 2014; Wang *et al*., 2014). As toxicity to biota may also be as a result of cell membrane damage (fluidization), inhibition of adenosine triphosphate (ATP) and enzyme activity, DNA damage as well as oxidative stress due to the generation of reactive oxygen species (ROS) (Figure 1) (Levy *et al*., 2005; Tuan *et al*., 2008; Pisani *et al*., 2011; Pandey *et al*., 2012; Rahman *et al*., 2014; Sanchez-Riego *et al*., 2014; Sun *et al*., 2015).

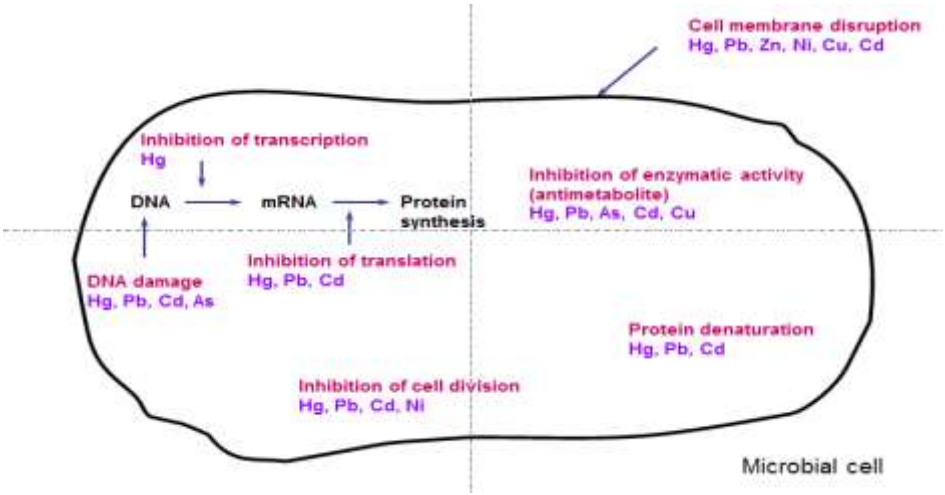

Fig 1: Mechanisms of heavy metal toxicity to microbial cell

As occurs essentially as inorganic arsenate (As(V)) and arsenite (As(III) in the environment however, it can also be methylated into organic species (mono, di, trimethylarsines or arsenosugars) by living organisms which have developed specific metabolic pathways for the transformation of As encountered in the environment (Hasegawa *et al*., 2001; Miyashita *et al*., 2011; Ye *et al*., 2012; Wang *et al*., 2014; Rahman *et al*., 2014). Most of the organoarsenic species (arsenosugars) (Figure 2; Table 1) are metabolized via the pathway for arsenic biosynthesis, which involves reduction of arsenic(V) species to arsenic (III) species followed by oxidative addition of methyl groups to the arsenic atom (Figure 3) (Hasegawa *et al*., 2001; Samal *et al*., 2004; Ye *et al*., 2012; Zhang *et al*., 2013).

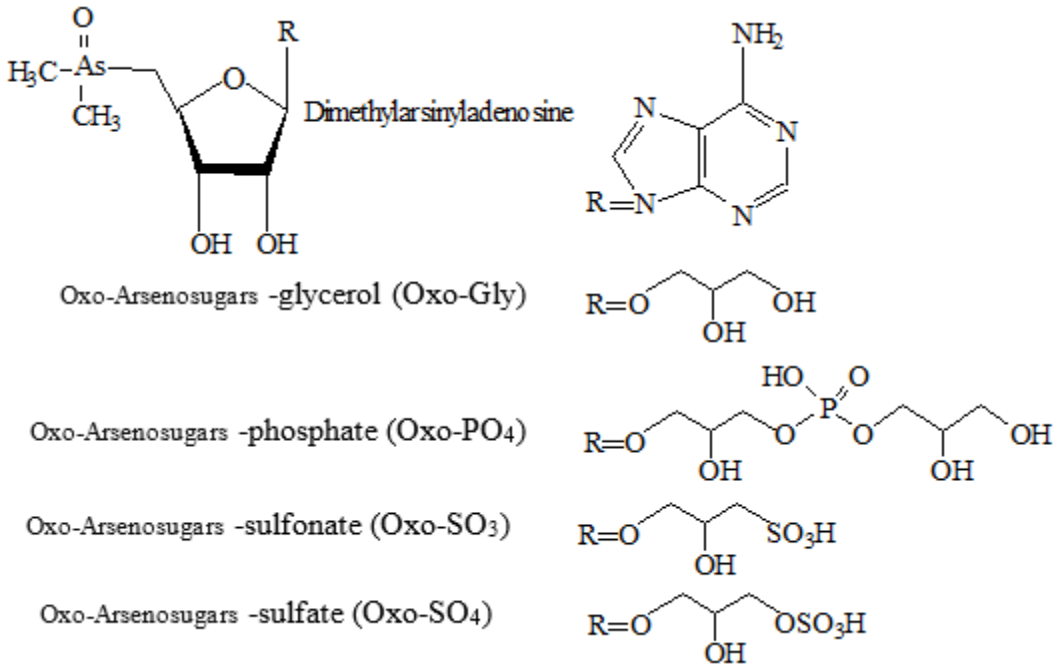

Fig 2: Oxo-Arsenosugars: Names, Abbreviations and Structures (Miyashita *et al*., 2011)

Table 1: Important inorganic, organic and biological forms of arsenic in the environment (Rahman *et al*., 2011)

Arsenic species abbreviation	Names	Formulae
	Inorganic arsenicals	
As(-III)	Arsine	AsH_3
As(III)	Arsenite (arsenious acid)	$As^{3-}(OH)_3$
As(V)	Arsenate (arsenic acid)	$H_3As^{5+}O_4$
	Methylarsenicals	
-	Methylarsine	AsH_2CH_3
-	Dimethylarsine	$AsH(CH_3)_2$
-	Trimethylarsine	$As(CH_3)_3$
MMAs(III)	Monomethylarsenite	$CH_3AS(OH)_2$
DMAs(III)	Dimethylarsenite	$(CH3)_2ASOH$
MMAs(V)	Monomethylarsenate	$CH_3AsO(OH)_2$
DMAs(V)	Dimethylarsenate	$(CH_3)_2$ $AsO(OH)$
TMAO	Trimethylarsine oxide	$AsO(CH_3)_3$
TMA$^+$	Trimethylarsine	$As^+(CH_3)_4$
	Organoarsenicals	
AsC	Arsenocholine	$(CH_3)_3As^+CH_2CH_2O$
AsB	Arsenobetaine	$(CH_3)_3As^+CH_2COO$
	Arsenosugars	
Oxo-Gly	Oxo-arsenosugar-glycerol	-
Oxo-phosphate	Oxo-arsenosugar-phosphate	-
Oxo-sulfonate	Oxo-arsenosugar-sulfonate	-
Oxo-sulphate	Oxo-arsenosugar-sulfate	-

Arsenic toxicity to microalgae could have large variations depending on As species. For example, the green microalgae (*Ostreococcus tauri*) had EC50 values of 78 and 120 µM for As(III) and As(V) respectively, indicating that this marine algae was more sensitive to As(III) than As(V) when it was grown in Keller medium Artificial Seawater (KASW) (Zhang *et al*., 2013). However, for *Chlorella sp* grown in a range of As(V) and As(III) concentrations ranging from 0.75 to 60 mg/L As(V) and 10 to 200 mg/L As(III) respectively, IC50s of 25.4 mg/L As(V)/L and 25.2 mg/L As(III)/L were observed. Thereby, almost equal toxicity of both inorganic arsenicals to *Chlorella sp* was observed (Levy *et al*., 2005). Interestingly, these authors also reported that *Monoraphidium arcuatum* was more sensitive to As(V) (IC50:0.254 mg As(V)/L) than As(III) (IC50:14.6 mg As(III)/L).

The growth of freshwater green algae (*Chlamydomonas reinhardtii* CC-125) was strongly surpressed by 0.5 - 5mM As(V) through 24 h with chlorophyll contents close to zero after 12 h (Miyashita *et al*., 2011). Speciation of As (added as 0.1mM As(V)) in algal (*Chlamydomonas reinhardtii*

CC-125) extracts for 24 h by HPLC/ICP-MS indicated that the predominant chemical form accumulating in cells was As(III) after 10 min, 1 h and 6 h, followed by As(V) after 6 h. Relatively, small amounts of methylarsonic acid (MA(V)), dimethylarsonic acid (DMA(V)), oxo-arsenosugar-glycerol (oxo-Gly) and oxo-arsenosugar-phosphate (oxo-PO_4) were also detected (Miyashita *et al*., 2011). A brown-macro alga, *Fucus serratus* exposed to arsenate at 100 µg/L developed dark spots on the fronds and the edges of the receptacles and within three weeks, the frond edges fragmented which eventually led to the death of the macro algae (Geiszinger *et al*., 2001). Arsenate uptake by the algae was larger at the highest studied concentration (100 µg/L) as compared to lower concentrations (50, 20, 0 µg/l) arsenate but there was a decrease in the uptake of As at higher concentrations (50 and 100 µg/L) with time and this coincided with the onset of toxicity in these concentrations (Geiszinger *et al*., 2001). *Anabaena* sp PCC7120 grown in 40 mM arsenate produced filament fragmentation, thickening, enlargement and vacuolation of cells, transformation of bluish green cells into yellow brown and dense pigmentation at one side of the cell within 48 - 72 h (Pandey *et al*., 2012).

Biological availability and toxicological impact of trace elements are not only directly related to their chemical speciation but also to different algal species and the phosphate concentrations in the test medium (Hasegawa *et al*., 2001; Levy *et al*., 2005; Pisani *et al*., 2011). Microalgae vary in their sensitivities to arsenic in environmental and biological systems even in a single genus. Their ability to accumulate arsenic differs with different species of microalgae (Yamaoka *et al*., 1996). A microalgae (*M. arcuatum)* was more sensitive to As(V) (IC50 of 0.254 mg As(V)/L) than *Chlorella sp* (IC50 of 25.4 mg As(V)/L) when both organisms were exposed to same As(V) concentrations (Levy *et al*., 2005). Depending on algal type, different As species and concentrations of arsenic compounds are observed (Salgado *et al*., 2006). For instance, when two different microalgae (*Dunaliella* sp and *Chattonella antique*) were exposed to different As (Na_2HAsO_4) concentrations, arsenate was predominantly the As species observed in the *Dunaliella* sp. (having more arsenate in its cell wall) as compared to *Chattonella antique* which had predominantly arsenite in its cell wall. This was thought to be as a result of differences in the organic component of the cell wall and in the physical adsorption or ion exchange at the cell surface (Yamaoka *et al*., 1996). Investigation of littoral zone algae from the Adriatic sea showed that the main arsenic in some species (*Ceramium* sp., *Cystoseira barbata* and *Polisyphonia* sp) was inorganic form (As(V)) but the dominant As species in red/green algae consisted of arsenosugar 1 with arsenosugar 3 been dominant in brown algae (Llorente-Mirandes *et al*.,2010). Four different algae species analyzed for arsenic species using a microwave-based procedure were observed to have different As species. *Chlorella vulgaris* (lyophilized Bioma-6 material) had As(III), As(V), MMA and DMA, *Sargassum fulvellum* (lyophilized Sargasso material) and *Hizikia fusiformis* (commercial product) had only one species (As(V)) while no As species was found in *Laminaria digitata* algae (commercial product) (Salgado *et al*., 2006).
Variable effects of Phosphate (P) media concentration on As toxicity to algae have been reported by many authors generally because arsenate and phosphate compete for uptake in algal cells. Extensive evidence has demonstrated that increasing external P concentration can decrease As toxicity levels by means of reducing As(V) uptake and favouring the accumulation of internal P (Wang *et al*., 2014). For example, a 10-fold increase in phosphate concentration decreased the toxicity of As(V) to *M. arcuatum* by approximately 20-fold. The concentration of phosphate in solution significantly reduced the amount of arsenic adsorbed to the surface of *M. arcuatum* and the amount of arsenic that was accumulated inside the cells (Levy *et al*., 2005). Increase in P concentration from 0.1 to 1mM also reduced As(V) uptake by 17-71% in six arsenic-resistant bacteria indicating that P and As(V) were taken up by P transporters (Ghosh *et al*., 2015). Intracellular As accumulation upon exposure to As(V) or As(III) was observed to be very high in *Microcystis aeruginosa* under phosphate depleted (-P) treatments when compared to phosphate-enriched (+P) treatments (Wang *et al*., 2014). Arsenate was converted to methylarsenicals in *Closterium aciculare* after the decrease of phosphate in the medium and the incorporation of arsenate into *C. aciculare* (Hasegawa *et al*., 2001). Fifty percent (50 %) of As(III) was methylated to trimethyarsine oxide (TMAO) within 66 h when the cells of *Cyanidioschyzon* sp was grown in phosphate free media (Qin *et al*., 2009). The specific growth rate and cellular partitioning of *M. aeruginosa* was clearly higher under +P treatments than under –P treatments when grown in 10 µM arsenate or arsenite, suggesting that phosphorus can be used to reduce arsenic toxicity (Yan *et al*., 2014). However, Yamaoka *et al*. 1996 reported increase in arsenic content of *C. antique* when the phosphate concentration was raised from 2.25 to 4.5 mg/l.

Microalgae, however, have developed several strategies to detoxify metalloids such as arsenic. These include arsenic excretion from the cell; reduction of arsenate to arsenite followed by either excretion or

complexation with glutathione (GSH) and sequestration into vacuoles (Levy *et al*., 2005; Pisani *et al*., 2011); production of other metal binding proteins such as phytochelatins (PCs); and methylation to less toxic organic forms, together with excretion (Levy *et al*., 2005; Pisani *et al*., 2011). Following As uptake, As(V) is reduced efficiently to As(III) in plant cells. As speciation in plant tissues shows that the As(III) oxidation state is prevalent, despite their common exposure to As(V) (Rahman *et al*., 2014). Since As(III) has high affinity to sulphhydryl (-SH) groups of peptides such as glutathione (GSH) and phytochelatins (PCs), the reduction of As(V) to As(III) can thus be mediated by GSH and enzymes as part of plants detoxification mechanism (Rahman *et al*., 2014). Plants are also suspected to control the production of ROS and the resulting unbalanced cellular redox status by various enzymes (e.g cysteine synthase. superoxide dismutase, catalase, glutathione peroxidase, ascorbate peroxidase) and cellular compound, for example, GSH can act as an antioxidant needed for the synthesis of metalloid chelating ligands (Bhattacharya and Pal, 2011; Rahman *et al*., 2014). An increase in the synthesis of chelators such as GSH and PCs is considered a highly effective approach to remediate metals and metalloids (Rahman *et al*., 2014).

Again, As(V) can be reduced to As(III) inside the cells through the action of ars operon (typically arsRDABC) encoded either on the chromosome or on plasmids. Arsenic reduction minimizes As(V) competition with P uptake so the cells can maintain normal growth and metabolism. However, As(III) can enter the cell through aquaporins and be methylated and immobilized in the bacterial biomass (Ghosh *et al*., 2015; Keren *et al*., 2022).

As bioaccumulation and biomethylation by microalgae: Biomethylation of As is a natural detoxification process by which living organisms reduce and add methyl group/s to As to transform inorganic toxic arsenicals to less toxic mono, di, trimethyls and non-toxic organoarsenicals (Figures 2 and 3) (Qin *et al*., 2009; Rahman *et al*., 2014). Biomethylation of As has a fundamental impact on the global biogeochemistry of this trace element including its mobility and toxicity; it is widespread in nature and has been observed in bacteria, archaea, fungi, algae, plants, animals and humans (Miyashita *et al*., 2009; Miyashita *et al*., 2011; Ye *et al*., 2012; Zhang *et al*., 2013). Green algae (*Cladosphora glomerata*) isolated from As contaminated river contained 18,000 µg/kg dry weight (DW) total arsenic, and the dominant water-soluble arsenical was oxo-arsenosugar-glycerol at a concentration of 1700 µg/kg DW (Miyashita *et al*., 2009). The authors also reported that arsenobetaine (AsB) (Table 1) was the main arsenical detected in herbivorous fish (*Plecoglossus altivelis*). An anaerobic microbial consortium from methanogenic anaerobic sludge biotransformed As(V) to As(III), MMA(V) and DMA(V) (Sierra-Alvarez *et al*., 2002). A protozoan (*Tetrahymena thermophila*) methylated arsenate when grown in modified Neff medium to form As(III), MMAs(V) and DMAs(V) (Yin *et al*., 2011).

Photosynthetic organisms may play a significant role in As geochemical cycling by methylating As to different As species, but little is known about the mechanisms of methylation (Ye *et al*., 2012). Methylated As species have been found in many photosynthetic organisms, and several arsenite-S-adenosylmethionine (SAM) methyltransferases have been characterized in cyanobacteria and algae (Yin *et al*., 2011). Microalgae are key contributors to arsenic cycling in the marine environment primarily as a food source for higher organisms, therefore, they are responsible for the greater proportion of arsenic species (As(III), MA, DMA and arsenosugars) present in marine waters (Foster *et al*., 2008). As is thought to be taken up by microalgae from seawater in the form of arsenate (As(V)) via the phosphate transport systems located in cell membranes and converted to As(III) as As(V) is known to interfere with metabolic processes associated with phosphorylation (Foster *et al*., 2008; Zhang *et al*., 2013). At longer exposure times, As(III) may be methylated to MMA, then to DMA and trimethylated arsenic species, which then diffuse into the growth medium (Bently and Chasteen, 2002; Levy *et al*., 2005).

Inorganic arsenicals have been shown to be metabolized by microalgae forming methylated arsenic species (MA, DMA) and arsenosugars (Edmonds and Francesconi, 1987; Geiszinger *et al*., 2001; Foster *et al*., 2008; Miyashita *et al*., 2011; Miyashita *et al*., 2012; Zhang *et al*., 2013). Arsenosugars are As containing ribosides and are thought to be end products of arsenate detoxification processes (Figure 2). They seem likely to be biosynthesized by algae through sequential reduction and methylation by S-adenosylmethionine (SAM) (under the control of methyltransferases) of arsenate to produce, initially, methylarsonic acid and then dimethyarsinic acid. Adenosyl group of the methylating agent is transferred to the arsenic atom then enzymatic, hydrolytic removal of adenine would be followed by formation of glycosides by reaction with available algal metabolites (Edmonds and Francesconi, 1987; Murray *et al*., 2003; Miyashita *et al*., 2011; Miyashita *et al*., 2012; Zhang *et al*., 2013).

The most common types of arsenosugars; oxo-arsenosugars, contain a chemically active dimethylarsinoyl group [$(CH_3)_2AsO$-] at the C5 position of D-ribose derivatives (Figure 2) (Miyashita *et al*., 2011; Miyashita *et al*., 2012). These oxo-arsenosugars are oxo-arsenosugar-glycerol (Oxo-Gly), oxo-arsenosugar-phosphate (Oxo-PO_4) oxo-arsenosugar-sulfonate (Oxo-SO_3) and oxo-arsenosugar-sulfate (Oxo-SO_4) (Figure 2). Oxo-arsenosugar-glycerol (Oxo-Gly) and oxo-arsenosugar-phosphate (Oxo-PO_4) can occur in almost all marine microalgae at various concentrations, and they compose the majority of arsenosugars, especially in Chlorophyta (green algae) and Rhodophyta (red algae) (Geiszinger *et al*., 2001; Miyashita *et al*., 2011).

Zhang *et al*. (2013) demonstrated the transformation of As(V) to oxo-arsenosugar phosphate (arsenosugar 2) by *Ostreococcus tauri* cells after exposure to 10 and 30 µM As(V) for 4 weeks. Similarly, *C. reinhardtii* CC-125 exposed to 0.1mM As(V) for 10 min to 24 h contained Oxo-Gly, together with Oxo-PO_4 (Miyashita *et al*., 2011). Foster *et al*. (2008) showed that As sequestration in the lipid fraction of microalgae (*Dunaliella tertiolecta* and *Phaeodactylum tricornutum*) incorporated predominantly OH-ribose, AS(V) and DMA moieties. Substantial amounts of inorganic arsenic also sequestered into vacuoles (water-soluble) and in residue fractions after exposure to 2 µg/L arsenate during microalgae exponential growth at low phosphate concentrations. The presence of a number of arsenic species in the lipid component that reflect structures of the water-soluble As species suggests that cells readily incorporate As species within lipids that may be used for membrane structures or storage products, releasing As species into the cytosol as enzymatic degradation of lipids occur. Substantial amounts of inorganic arsenic sequestered into vacuoles (water-soluble) are most likely As-PCs (arsenic-phytochelatins) while inorganic As in residue fractions is likely to be complexes with intracellular structural elements of the cells (Yamaoka *et al*., 1996; Geiszinger *et al*., 2001; Foster *et al*., 2008).

Sierra-Alvarez *et al*. (2005) conducted a batch experiment to evaluate the potential of an anaerobic microbial consortium to biologically mobilize arsenate (As(V)) adsorbed onto activated alumina (AA) a common adsorbent for treating arsenic in drinking water. The authors observed 37 % As (V) removal from activated alumina and that As (III) was the most important species in periods of high As mobilization. This mobilization was attributed to the biological reduction of As(V) to As(III) by anaerobic microbial consortia from methanogenic anaerobic sludge. Sorbed As(V) was subject to two types of biotransformation reactions: reduction to As(III); and methylation to MMA(V) and DMA(V). Hasegawa *et al*. (2001) showed a decline in arsenate concentration with increase in As(III) concentrations during the exponential growth of phytoplankton (*Closterium aciculare*) while methylarsenic species appeared at the end of exponential growth of the phytoplankton. However, As(III) decreased during the stationary phase of growth while methyarsenic (V) species (DMAA(V) and MMAA(V) increased rapidly at this phase (stationary phase), followed by a gradual increase toward the end of the experiment. Arsenate was converted to arsenite and methylarsenicals, main species were arsenite (< 0.1-27%) and DMAA (4.3-43%), and minor species were MMAA(V), DMAA(III) and MMAA(III) (Hasegawa *et al*., 2001). A unicellular eukaryotic red algae, Cyanidioschyzon sp 5508 formed trimethylarsine oxide (TMAO) and dimethylarsenate (DMAs(V)) when grown in As(III) (Qin *et al*., 2009).

Chlorella sp and *Monoraphidium arcuatum* methylated As(V) to low concentrations of MMA, DMA and phosphate arsenoriboside in the cells but not in solution (Levy *et al*., 2005). A fresh water microalgae (*Chlorella vulgaris*) when grown in different concentrations of As(V) was able to transform As(V) to As(III), DMA(V) and arsenosugars 1, 2 and 3 (Murray *et al*., 2003). *M. aeruginosa* exposed to BG 11 media without P (-P) was able to form 49 ± 5% and 40 ± 3% DMA from arsenate and arsenite respectively (Yan *et al*., 2014). Organic arsenic (DMA) was detected in microalgal cells (*Ostreococcus tauri*) after incubation for 8 d with As(III) or As(V), and small amount of oxo-arsenosugar-phosphate was also detected in same microalgal cells after exposure to 10, and 30 µM As(V) for four weeks (Zhang *et al*., 2013). Volatilization of As was significantly higher in As(III) than in the presence of As(V) when *Ostreococcus tauri* was exposed to 20 µM of these inorganic arsenicals (Zhang *et al*., 2013).

Assay of As species (supernatant) of *Chlorella vulgaris* grown in a basic solution containing As(III) with HPLC-HG-AAS revealed that *C. vulgaris* retained 50 % of As(III) in their cells and metabolized 25% of As(III) which it expelled as As (V) (Beceiro-Gonzalez *et al*., 2000).

Mechanism of As biomethylation by microalgae: Arsenic from natural and man-made sources is widely distributed contaminants of freshwater, seawater and ground water (Lopez-Maury *et al*., 2003; Fendorf *et al*., 2010; Rahman and Hasegawa, 2011; Pell *et al*., 2013). It can be taken up by microalgae to undergo

reduction, methylation and volatilization to form a variety of dissolved forms in natural waters (Hasegawa *et al*., 2001; Miyashita *et al*., 2012; Wang *et al*., 2014). Algae are thought to use the mechanism of methylation and volatilization as a method of detoxifying the inorganic arsenic species (Murray *et al*., 2003; Wang *et al*., 2014; Thomas, 2021). Arsenate is the form of As mostly found in marine water and algae take it up readily to produce a number of related water and lipid soluble arsenic compounds (Edmonds and Francesconi, 1987; Bently and Chasteen, 2002; Miyashita *et al*., 2009). Many algae in As contaminated environment contain either chromosomal or plasmid-encoded gene involved in arsenical resistance (ars genes). There are two necessary components of arsBHC operon involved in arsenic resistance in *Synechocystis*: (i) the reduction of As(V) to As(III) by a reductase enzyme (ArsC) and (ii) an As(III) expulsion pump (ArsB), which subsequently extrude As(III) (Lopez- Maury *et al*., 2003; Rahman *et al*., 2014; Yamamura and Amachi, 2014; Ghosh *et al*., 2015). ArsH has no known function but one possibility is that it works as an alternative electron carrier protein under some specific conditions (Lopez-Maury *et al*., 2003).

Arsenate is taken up by algal cells using a phosphate transport system due to its similarity to phosphate, reduced to As(III) in the cell by thiols and/or dithiols, and then excreted into the growth medium, probably by an active transport system. At longer exposure times, As(III) may be methylated to methylarsenite (MMA), then to dimethylarsenite (DMA) and trimethylated arsenic species, which then diffuse into the growth medium (Figure 3) (Bently and Chasteen, 2002; Levy *et al*., 2005). Excretion of As(III) may not keep pace with arsenic reduction, leading to accumulation of As(III) in the cells. Arsenite is known to bind strongly to thiols in plants and animals and appears only to be toxic once accumulated inside cells. Levy *et al*. 2005 observed that As(III) was not toxic to either *M. arcuatum* or *Chlorella* sp in the medium however, thiol oxidation was observed in *Chlorella* sp at both cell inhibitory and non-inhibitory As(V) concentrations (Levy *et al*., 2005). The authors thought that this indicated that As(V) reduction may be coupled with thiol oxidation, but the algae lacks the arsenite transporter to excrete As(III) into the medium. It is possible that *Chlorella* sp is able to detoxify arsenite inside the cell by sequestering it into subcellular compartments, transferring the product from cytosol into vacuoles via a specific transporter (Levy *et al*., 2005).

In As(III) dominated environments like acidic geothermal waters, the cell may first attempt to detoxify its immediate environment by converting As(III) to the less toxic As(V) oxyanion. However, the low level of inorganic phosphate in such environments would likely cause As(V) to be readily taken up by the cells via phosphate permeases. Therefore, As(III) methylation could represent an additional mechanism to rid the cell of the accumulated As(V), with the expectation that, under insitu conditions, the eventual final product would be TMA(III), a volatile gas that would leave the cell, presumably by a passive mechanism (Qin *et al*., 2009).

Arsenite-S-adenosylmethionine (SAM) is the methyl donor during As biomethylation by arsenite methyltransferase (Murray *et al*., 2003; Ye *et al*., 2012). As methyltranferase illustrates conserved motifs and cysteine residues as well as regions of appreciable variability (Ye *et al*., 2012; Wang *et al*., 2014). The conserved region is limited to a core of about 150 amino acids although the lengths of these proteins range from 248 to 400 amino acids. There are three fully conserved cysteines, corresponding to residue 48, 143 and 195 in *Synechocystis* sp PCC6803, which are probably involved in As binding, because of the affinity of the thiolate to the metalloid As(III) (Ye *et al*., 2012). It is noteworthy that Cys 143 is located within the consensus sequence of the SAM-dependent methyltransferase domain, whereas Cys 48 and 195 are approximately 15 residues upstream and downstream of this domain, respectively (Ye *et al*., 2012). It has been hypothesized that the core region with three conserved cysteines and the SAM-dependent methyltransferase domain are required for As methyltransferases. The core region is critical for methyl group transfer to As, whereas the rest of the protein is species specific, and its function needs to be investigated further (Ye *et al*., 2012). Yin *et al*. (2011) identified ArsM (As(III) S-adenosylmethionine methyltransferase) homologues in three cyanobacteria [NsarsM (*Nostoc* sp.); MsarsM (*Microcystis* sp; SsarsS (*Synechocystis* sp.)] with each gene encoding an ArsM homologue of 323 residues. ArsM genes from these cyanobacteria conferred As resistance and ability to methylate arsenic to As hypersensitive *E.coli* when they were cloned and expressed in this organism (Yin *et al*., 2011). The ars genes of cyanobacteria (*Synechocystis* sp. PCC7120) are in the form of arsBHC operon containing three genes: arsB (arsenite efflux protein), arsH unknown protein and arsC (arsenate reductase) regulated by the transcriptional repressor arsR (Pandey *et al*., 2012; Jose Huertas *et al*., 2014).

Interactions mechanisms between a biological substrate and metallic species may be taking place at the cell wall or inside the cell (cell membrane). In alga

with biological activity, there is the possibility that metallic species (As (III)) is being metabolized inside the cell, and later expelled as another arsenical species (As (V). However, with the employment of alga without biological activity As(III) could be adsorped to the cell wall, occupying phosphates- and nitrate-binding sites or transformed to another species by the functional groups present on the cell wall (Beceiro-Gonzalez *et al*., 2000).

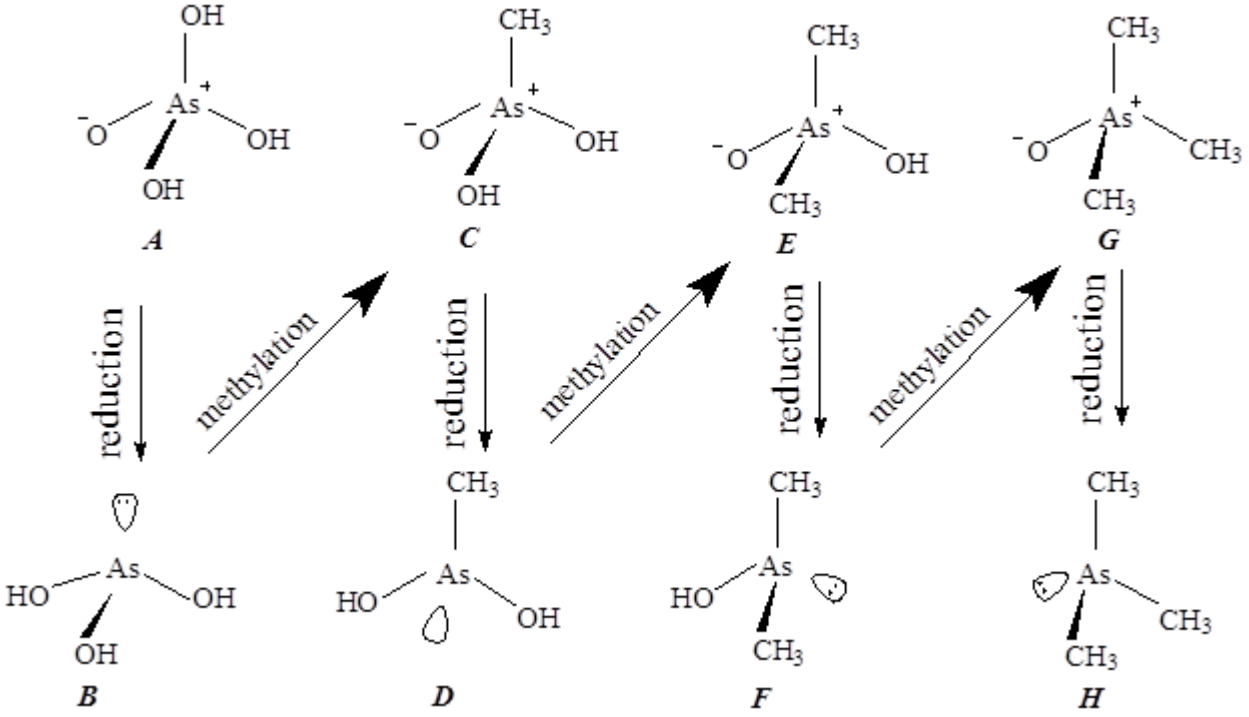

Fig 3: Challenger mechanism for the conversion of arsenate to trimethylarsine. (A) Arsenate; (B) arsenite; (C) methylarsonate; (D) methylarsonite; (E) dimethylarsinate; (F) dimethylarsinite; (G) trimethylarsine oxide; (H) trimethylarsine. The top line of structures shows the As(V) intermediates. The vertical arrows indicate the reduction reactions to the As(III) intermediates (bottom line), and the diagonal arrows indicate the methylation step by S-adenosylmethyltransferase (SAM) (Bentley and Chasteen, 2002)

The implication of arsenic bioaccumulation and biomethylation by microalgae in bioremediation of As contaminated environment. There is clearly a need to develop cost-effective technologies to remediate As polluted water since conventional physicochemical methods such as coagulation, coprecipitation, ion exchange, adsorption and membrane separation are expensive and have the problem of possible secondary pollution (Wang *et al*., 2015). The possibility of using microalgae to do this is cost-effective and environmentally-friendly and has spurred the search for resistant organisms that are capable of biotransforming As (Jiang *et al*., 2011; Franco *et al*., 2015). Microalgal activity play a key role in biogeochemical As cycling because of their ability to mediate redox transformations, chelation to intracellular cysteine-rich polypeptides and methylation of As (Jiang *et al*., 2011; Levy *et al*., 2005; Zouboulis and Katsoyiannis 2005; Rahman and Hassler, 2014). Such processes have the potential to promote As removal from contaminated soils/waters when used appropriately (Yamamura and Amachi, 2014; Franco *et al*., 2015). As toxicity varies greatly with its speciation; for example, organic forms such as MMA and arsenosugars are typically 2 - 4 orders of magnitude less toxic than inorganic forms (Jiang *et al*., 2011). Given the differences that exist between arsenic species toxicity, methods capable of converting inorganic arsenic to other, less toxic species have been subject of many investigations (Murray *et al*., 2003; Levy *et al*., 2005; Jiang *et al*., 2011).

Microalgal accumulation and biotransformation of large amounts of arsenic from their surroundings have been reported by various authors (Yamaoka *et al*., 1996; Hasegawa *et al*., 2001; Murray *et al*., 2003; Miyashita *et al*., 2012; Franco *et al*., 2015). Bioaccumulation of As(V) by three cultures of cyanobacteria (*Oscillatoria tenuisa*; *Anabena affinis; Microcystis aeruginosa*) was reported to have increased rapidly in the logarithmic phase from an initial values of $3.23x10^{-2}$ - $5.40x10^{-2}$ to $5.06x10^{-1}$-$6.73x10^{-1}$ ng/cell after growth for 10 d. This increase in As(V) concentration was dependent on concentration, been reduced at higher As(V) concentration (50 mg/L) as compared to lower

concentrations (0.05; 0.5) (Huang *et al*., 2014). Similarly, *Chlorella vulgaris* when grown between the range of 1 - 200 mg As/L was able to remove between 69 and 79% of As^{5+} present in the medium irrespective of the intial As^{5+} concentration, and GSH level increased significantly with increased concentration of As^{5+} (Jiang *et al*., 2011). A marine microalga, *Phaeodactylum tricornutum* grown in arsenate at different concentrations (between 0.1 and 1µM As) induced a prompt synthesis of phytochelatin (PC), with a maximum rate of PC formation within the first hour of exposure (Morelli *et al*., 2005). A bacterium (strain GFAJ-1) isolated from high As containing Mono Lake (California) was able to grow and assimilate AsO_4^{3-} into biomolecules including nucleic acids, proteins, and metabolites (Wolfe-Simon *et al*., 2011). *Microcystis aeruginosa* (cyanobacteria) isolated from an algal bloom contaminated with arsenic showed tolerance to varying concentrations of As(III) and As(V) (Yan et al., 2011).

As(III) oxidizers are found in various groups of bacteria and archaea isolated from As-rich environments and include both heterotrophic As(III) oxidizers (HAOs) and chemolithoautotrophic As(III) oxidizers (CAOs) (Rahman and Hassler, 2014; Yamamura and Amachi, 2014). Heterotrophic As(III) oxidation is generally considered a detoxification mechanism that converts As(III) into less toxic As(V), although it may be used as a supplementary energy source (Yin *et al*., 2012; Yamamura and Amachi, 2014; Franco *et al*., 2015). In contrast CAOs use As(III) as an electron donor during CO_2 fixation coupled with reduction of oxygen (Yamamura and Amachi, 2014). Franco *et al*. 2015 showed a microalgae (*Synechococcus* sp) that was able to oxidize As(III) to As(V) because of the dominance of As(V) observed within cells after its growth in As(III) for 30 d. Bio-oxidation of As(III) to As(V) was the predominant transformation process in algal cells in freshwater enriched with As(III) and phosphate (Wang *et al*., 2013). Since As(III) is more toxic and less adsorptive than As(V) therefore, As (III) oxidation is an important process for bioremediation of As contaminated water using microalgae as well as adsorption and coprecipitation using Al/Fe(III) minerals (Wang *et al*., 2015). Chemical oxidation of As(III) via oxygen is very slow, however, application of aerobic As(III) oxidizers can be effective remediation for removal of As from contaminated water (Yamamura *et al*., 2014).

A wide variety of bacteria known as As(V) resistance microbes (ARMs) can reduce As(V) via detoxification systems (Yamamura and Amachi, 2014). Others known as dissimilatory As(V) reducing prokaryotes (DARPs) can reduce As(V) as the terminal electron acceptor (Yamamura and Amachi. 2014). For example, *Synechococcus* sp was able reduce As(V) to As(III) and transform inorganic As species into methylated and other organic As species (Franco *et al*., 2015). Similarly, *M arcuatum* induced As(V) reduction to As(III) intracellularly (Levy et al., 2005) and *M. aeruginosa* biotransformed As(V) into reduced As species as a precursor for methylation (Wang *et al*., 2013). Since As(V) is detected as the major species of As in contaminated soils, its reduction to less adsorptive As(III) can promote As removal from solid to the aqueous phase; therefore, it might be applicable for remediation of soils. Dissimilatory As(V)-reducing prokaryotes (DARPs) are desirable agents because As(V)-resistance microbes (ARMs) can only reduce aqueous As(V) that has entered the cell (Yamamura and Amachi, 2014). A freshwater algae (*Chlorella vulgaris*) was able to biomethylate arsenate to As(III), DMA(V) and arsenosugars even at the highest concentration of 1000 mg l^{-1} (Murray *et al*., 2003). As(V) methylation to MMA, DMA and phosphate arsenoriboside by *Chlorella sp* and *M. arcuatum* was reported by Levy *et al*. (2005). The distribution of intracellular As speciation after 15 d of *Microcystis aeruginosa* exposure to As(V) or As(III) demonstrated that As(V) was the predominant species followed by As(III), DMA and MMA (Wang *et al*., 2013).

Future treatment methods for environmental pollutants need to enable factors such as low-cost, Low-energy and low-environmental impact (Onnby *et al*., 2012). Arsenic bioaccumulation and biomethylation using microalgae can be one way to achieve this since they show a high capacity for biosorption and bioconcentration of As, therefore, they are been proposed for bioremediation/phytoremediation purposes in As polluted aquatic media (Rubio *et al*., 2010).

Conclusion: Microalgae are natives and key members in a vast array of freshwater and marine environment and play important role in As cycling in the environment. Examples cited in the review demonstrate their ability to bio-accumulate large amounts of arsenic, their ability to reduce or oxidize As species, their role in As biomethylation as well as their advantages if applied for bioremediation/phytoremediation of As contaminated waters. As poses a threat to human and ecosystem health, especially when incorporated into food or water supplies, therefore, studies on solving the problem of As in the environment is extremely important. Biogenic (algal/cyanobacterial) slimes and Fe^{3+} hydroxide flocs can sequester significant amounts of As yet very few studies have been done on this. The low-cost and low-environmental impact of using

microalgae is very attractive however, the relative importance of abiotic and biotic As sequestration mechanisms need to be thoroughly investigated.

Acknowledgement: The author is grateful to the Brazilian Research Council (CNPq) and Third World Academy of Sciences (TWAS) for financial support. This study was carried out while the author was a postdoctoral fellow at Universidade Federal de Vicosa, Minas Gerais, with funding from CNPq (190130/2014-8).

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