Toxicology of arsenic in fish and aquatic systems

Toxicological profile of arsenic in aquatic biota- fish: A review

Bibha Kumari[1*], Vikas Kumar[2], Amit K. Sinha[3], Jawaid Ahsan[4] , A. K.Ghosh[1], Hanping Wang[5], Gudrun De Boeck[6]

[1]Department of Environment and Water Management, A.N. College, Patna, Bihar, 800013, India.

[2] Division of Aquaculture, College of Aquaculture, Food Science and Sustainable System, 103 Athletic Drive, Athletic Road, Kentucky State University, Frankfurt, KY 40601, USA.

[3]Aquaculture/Fisheries Center, University of Arkansas at Pine Bluff, 1200 North University Drive, Pine Bluff, AR 71601, USA

[4] P.G. Department of Zoology, A. N. College, Patna, Bihar, 800013, India.

[5] Ohio Center for Aquaculture Research and Development, The Ohio State University, South Centers, Piketon, 45661-OH, USA.

[6]Laboratory for Ecophysiology, Biochemistry and Toxicology, Department of Biology, University of Antwerp, Groenenborgerlaan 171, 2020 Antwerp, Belgium.

[*] **Corresponding author**: Bibha Kumari, E-mail address: bibhak136@gmail.com

Mob: +919430465396

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Abstract

Arsenic (As) is found in many natural waters including seawater, warm springs, ground water, rivers, and lakes. In aquatic environments, As occurs as a mixture of arsenate and arsenite, with arsenate usually predominating. The unrestricted application of As pesticides, industrial activities, and mining operations are leading to the global occurrence of soluble As above permissible levels (0.010 mg/L). Continuous exposure of freshwater organisms including fish to low concentrations of As may result in bioaccumulation in which liver and kidney having high accumulation in most of the cases, altering many physiological and biochemical activities like hyperglycemia and depletion of enzymatic activities. The other effects of As on fish health include various mechanisms of acute and chronic toxicity, as well as genetic and immune system dysfunction.

Thus, toxicological aspects of arsenic have mainly been discussed in connection with their environmental persistence and the ability of arsenic to induce a variety of adverse effects in aquatic bio-systems, particularly in fish. The incorporation of toxicological analysis into evaluation of arsenic exposure and its concentration/dose–response effects improves our ability to appraise the range of possible exposure scenarios and environmental risk to fish and humans who consume arsenic contaminated fish. Therefore, taking into account the toxicological importance of arsenic, this review focuses on: arsenic chemistry; occurrence of arsenic in aquatic system; transformation and metabolism of arsenic; bioaccumulation and bioconcentration; behavioral changes; acute and other effects like biochemical, immunotoxic; cyto-genotoxic effects on fish.

Keywords: Arsenic; Transformation and metabolism; Acute toxicity; Behavioral and biochemical changes; Hemato and immunological effects

Introduction

Arsenic (As) is a metalloid element which is widespread in the aquatic environment due to natural and anthropogenic processes. It is an important and ubiquitous environmental contaminant, whose risk of poisoning in humans is a public health issue worldwide (Chowdhury et al., 1999; Rossman 2003). Its toxicities have been shown in various organisms, and the data suggest that the inorganic forms of As exhibit the highest toxicity level, while organo-arsenicals are usually less toxic (Duker et al., 2005). Among those, the trivalent inorganic As seems to be the form ultimately responsible for most of the toxic effects of the semimetal (Rasmussen and Menzel 1997; Kovendan 2013). Most affected by As are those involved in absorption, accumulation, and/or excretion, i.e. the gastrointestinal tract, circulatory system, skin, liver, and kidney. It affects the hematological, biochemical and iono-regulatory parameters of organisms and particularly fish in aquatic media and alterations of these parameters can be useful in environmental bio monitoring of As contamination (Lavanya et al., 2011). Fish are usually considered as an organism of choice for assessing the effects of environmental pollution on aquatic ecosystems (Gernhöfer et al., 2001; Gaim et al 2015). They are continuously exposed to it through their gills, skin and by the intake of As - contaminated food. Fish have long been used as sentinels for bio-monitoring of aquatic environmental pollutants and are good indicators of As toxicity (Tisler et al., 2002). They are ideal organisms to work with in toxicogenomics studies due to the strong power of fish models to establish biomarkers of exposure (Das et al., 2012). Fish appear to have evolved different mechanisms for biotransformation of As to less toxic forms, which are then readily excreted (Bears et al., 2006). The high level of As concentration i.e more than permissible limit, in an aquatic ecosystem affects various physiological systems such as growth, reproduction, ion regulation, smoltification, gene expression, immune function, enzyme activities, and histopathology of fish (Pedlar et al., 2002 a & b; Datta et al., 2009) (**Figure 1**).

As contaminated fish consumption could result in As exposure to humans and lead to adverse health effects (Kar et al., 2011). As could evoke a series of molecular events involved in oxidative stress, iron homeostasis, lipid metabolism disorder, and carcinogenesis (Xu et al., 2013).

Based on previous knowledge, this is the review that deals with the toxicological profile of As in aquatic biota- fish. This review mainly emphasizes about As, its chemical nature and occurrence in the environment, evaluation of the adverse effects of its compounds on the different organs and the behavioral, physiological, and metabolic changes in the fish. Knowledge of the physiological action of the toxicant helps to predict important sub-lethal effects. Analyses of biochemistry, hematology, genotoxicology and histopathology of organs may be used to determine the mode of action of the toxicant.

1. Arsenic Chemistry

As exhibits a broad range of chemical reactivity with an ability to form alloys and covalent bonds with other elements. At normal temperatures, the inorganic As compounds are solids and are unlikely to volatile. The water solubility of these compounds varies from quite soluble to practically insoluble **(Table 1).** Some organic As compounds are gases or liquids (having low boiling points) at ambient temperatures. Except for the organic arsenic-acids, they are not readily water soluble (ATSDR 1997). Chemically As compounds are of two types- inorganic and organic As (**Table 2**). Inorganic As is divided into two types: trivalent (AsIII) and pentavalent (AsV). The most important compounds of As are arsenic-oxide. Although As is sometimes found native in the nature (**Table 3**), its main economic source is the mineral arsenopyrite.

As precipitates readily in oxidation-reduction, methylation-demethylation, and acid-base reactions. The most common oxidation states for As are +5 as arsenates V: the most

stable inorganic As oxy-compounds; +3 as arsenites III, and most organo-As compounds, and under reducing conditions, arsenite As III is the dominant form and-3 as arsenides: usually alloy-like inter metallic compounds. The arsenate form is less toxic than arsenite in both *vivo* and in *vitro* (Cervantes et al., 1994). In water, As is usually found in the form of arsenate or arsenite. The most important commercial compound, As III oxide, is produced as a by-product in the smelting of copper and lead ores (Abernathy et al., 1999).

2. Occurrence of arsenic in aquatic systems

The most common source of elevated As concentrations in the environment is attributable to anthropogenic activities. Mining activities have contributed to the contamination of soil and water primarily. However, other anthropogenic activities using As, such as agriculture, forestry, and industry have also contaminated soil and water at a localized scale (Smith et al., 2003). As in soil can be transported by wind or water; as runoff, or may leach into the soil from arseniferous rocks, mining waste, processing plants and industrial waste. Many As compounds are present in the aquifer; it may be released in ionic form into the soil under oxidizing conditions. Rainwater or snowmelt may leach soluble forms into surface water or groundwater, and soil microorganisms may reduce a small amount to volatile forms: arsines (Turpeinen et al., 1999). As would be present in well-drained soils as $H_2AsO_4^-$ in acidic or $HAsO4^{2-}$ in alkaline conditions. Oxidation, reduction, adsorption, dissolution, precipitation, and volatilization of As reactions commonly occur in soil. Water is the major means of transport of As under natural conditions. Studies examining the form of As in water supplies have largely reported only arsenate and arsenite in varying ratios (Hughes et al., 2011). The arsenate/arsenite ratio is not only dependent on the source of water but also redox conditions in the supply. It has generally been assumed that surface waters, including the sea, are "self-

purifying" with respect to As, i.e., As is removed from the solution by precipitation with sediments. As V, which is the stable oxidation state in oxygen-containing waters, can be reduced to As III **(Figure 2)** in anoxic or reducing systems (Oremland et al., 2000). The proportion of As III, however, is low in these waters and even in anoxic interstitial waters; complete reduction of As to As III has not been observed. As III released to oxygenated waters can be re-oxidized to As V within a time scale of days.

Generally, four types of As compounds are present in water. These are arsenite, arsenate, monomethyl arsonic acid, and dimethyl arsinic acid. Arsenate in both fresh water and salt water is metabolized to methylated compounds. Mono-, di-, and trimethyl arsenicals have been isolated from both fresh water and salt water. Concentrations of As in open sea water are typically less than 2 µg/L (Ng, 2005) but in freshwater, the variation is in the range of 0.15–0.45 µg /L (Bissen and Frimmel, 2003a,b). However, there have been a number of reports of higher than usual concentrations in seawater at the river outflows because of dissolved As and sediment load.

One of the sources of naturally occurring As may be geothermal aquifers, where rocks with As salts are impregnated (Smedley and Kinniburgh, 2002). High concentrations in surface water and ground water of up to 100–5000 µg/L can be found in areas of sulfide mineralization and mining (Smedley et al., 1996). Inorganic As is found in the groundwater used as drinking water in several parts of the world (Ahmed et al., 2011) and it is also readily used for aquaculture practices for various purposes. 35 mg As/L and 25.7 mg As/L have been reported in areas associated with hydrothermal activity in Japan (Tanaka 1990).

The most common source of As contamination in ground water is the mobilization of naturally occurring As on sediments. In nature, As-bearing minerals undergo oxidation and release As to water. The "pyrite oxidation" hypothesis is therefore unlikely to be a major

process, and the "oxy-hydroxide reduction" hypothesis (Acharyya et al., 1999) is probably the main cause of As contamination in groundwater naturally. According to Acharyya et al., (1999), the increase in phosphate concentration could have promoted the growth of sediment biota and the adsorption of As from sediments, and the combined microbiological and chemical process might have increased the mobility of As .

3. Transformation and metabolism of arsenic

Most environmental transformations of As appear to occur in the soil, sediments, plants and animals, and in zones of biological activity in the oceans. Three major modes of biotransformation of As species have been found to occur in the environment: redox transformation between arsenite and arsenate, the reduction and methylation of As, and the biosynthesis of organo-arsenic compounds. There is biogeochemical cycling of compounds formed by these processes. According to (Allen 2002) the bio-methylated forms of As are subject to oxidation and bacterial demethylation back to inorganic forms. As in water can undergo a complex series of transformations, including redox reactions, ligand exchange and biotransformation. the factors, which affect the processes in water, include the Eh, pH, metal sulfide and sulfide ion concentrations, iron concentrations, temperature, salinity, and distribution and composition of biota. In aquatic environments, several species of microorganisms make As biologically available to organisms (Duker et al., 2005). Seasonal variations in temperature and water levels could have strong effects on As concentration and speciation in water due to changes in microbial uptake (Drewniak and Sklodowska 2013). Aquatic microorganisms may reduce the arsenate to arsenite, as well as methylate arsenate to its mono- or dimethylated forms. Bacterial populations have been shown to be associated with both oxidation and reduction of As. Degradation of As species has even been shown in bacterial symbionts of marine mussels (Jenkins et al., 2003).

As is distributed and stored in all tissues of the animal body and is metabolized for elimination by two sequential processes. The first process is the oxidation/reduction reactions that interconvert arsenate to arsenite. Glutathione was shown to form complexes with As and mediate the reduction of arsenate to arsenite. These glutathione complexes can be eliminated in the bile and a positive correlation was found between As levels in bile and glutathione (Patrick 2003) Selenium is also able to complex with glutathione and As to form a compound that is also excreted through the bile. Another possible mechanism for As detoxification is binding with unidentified proteins. These proteins appear to be primary, along with glutathione and possibly selenium, in the removal of As (Aposhian et al., 1999). The second process is methylation, which occurs mainly in the liver, requires s-adenosyhmethionine and possibly other methyl donors: choline, cysteine, and glutathione reduced lipoic acid, to produce mono methylarsonic acid: MMA and dimethylarsinic acid: DMA (**Figure 3**). Several studies have shown that s-adenosyhmethionine: SAMe, is actually essential for the methylation of As and low methionine intakes, and can inhibit As methylation in animals (Patrick 2003). Both mono methylarsonic acid and dimethylarsinic acid have been found in human urine and are considered products of metabolization. Because dimethylarsinic acid is cleared from cells more rapidly than mono methylarsonic acid or inorganic As, and methylation reduces the amount of As retained in tissues by increasing the water solubility of arsenite. Other researchers disagree because mono methylarsonic acid may be the most toxic intracellular form of As due to its ability to induce enzyme inhibition, oxidative stress, and DNA damage (National Academy of Sciences 2001). Therefore, methylation may simply be a way of bio-transforming As rather than detoxifying it (Aposhian et al., 1999). The amount of As exposure appears to be the most important factor affecting As methylation; the higher the chronic exposure, the lower the individual's ability to methylate mono methylarsonic acid to dimethylarsinic acid .

4. Effects of arsenic in fish

4.1 Factors affecting toxicity of arsenic

In the environment, the degree of toxicity of As is dependent on the form, e.g. inorganic or organic and the oxidation state of the As (Agusa et al., 2008). Toxic and other effects of arsenicals to aquatic life are significantly modified by numerous biological and abiotic factors (Sanders 1986) also. Water temperature, pH, Eh, organic content, phosphate concentration, suspended solids, and presence of other substances and toxicants, as well as As speciation, and duration of exposure. . Temperature was shown to alter the toxicity of As. In fish, tolerance of As appears to increase with temperature, McGeachy and Dixon (1989) investigated the effects of temperature on the chronic toxicity of sodium arsenate to fingerling rainbow trout (*Oncorhynchus mykiss*) when exposed at 5°C for 77 days to sodium arsenate concentrations of 36 mg /L in water, whereas in invertebrates the opposite is true. Bryant et al., (1985) studied at three temperatures (5, 10, 15°C) and a range of salinities (5 to 35%, in 5% increments), at time intervals up to 384 h on *Corophium volutator* and *Macoma balthica.* Median survival time decreased as As temperature and concentration of As increased, but salinity changes had no significant effect. When analyzing for As exposure, it is useful to distinguish between As species, since they differ in their origin and toxicity (Orloff et al., 2009). In fish, As usually exists in two oxidation states, methylated species, arsenosugars and arsenolipids, which vary in their toxicity and the combination of these two states in tissues of fish lead to pathophysiological effects (Wrobel et al., 2002).

Arsenic compounds commonly present in the fish are presented in **Figure** 4.

4.2 Effect of waterborne and diet-borne arsenic exposure

Waterborne and diet-borne metal exposures on aquatic organisms response will require attention on several levels, including bio-availability, bio-regulation, bioaccumulation, and detoxification in individual species (Fairbrother et al., 2007; Menzie et al., 2009).Waterborne As significantly accumulated in specific organs, e.g., gill, liver, and intestine and manipulated growth toxicity to fish (Liao et al., 2004; Tsai and Liao 2006). Erickson et al., (2011) demonstrated that the diet-borne exposure is more important than waterborne exposure for growth effects when the oligochaete diet and the fish are exposed to the same arsenic concentration. Tsai et al., (2012) found that temporal trends of health rates and growth trajectories of exposed fish in different treatments decreased with increasing time and waterborne As, revealing concentration-specific patterns. Rankin and Dixon (1994) pointed out that freshwater fish could immediately reduce their feeding in response to both waterborne and diet-borne As exposure. Dietary uptake could be the primary route for As bioaccumulation in fish (Zhang et al., 2011;2012), and the corresponding contributions of waterborne and dietary uptakes were related to the bio-concentration factor of the prey and the ingestion rate of fish.

4.3 Bioaccumulation and bioconcentration

Chemicals released into the environment, including As, often accumulate most rapidly in aquatic habitats where they enter the biota and they are subsequently transferred to higher trophic levels, and in many cases, eventually to humans. As taken up by aquatic life and accumulated in the body of the organisms primarily occurs in various organic forms, with a low percentage accumulated in the more toxic inorganic form. The accumulation of different forms of As mostly depends on the analyzed species and tissue (Fattorini et al., 2004). Among aquatic organisms, fish are appropriate bio-indicators of bioaccumulation. The

accumulation and distribution patterns of toxic metals in fish tissues depend on their rates of uptake and elimination, and may cause various physiological defects and mortality when accumulation reaches a substantially high level (Kalay and Canli 2000). As bioaccumulation is sensitive to the environmental setting (marine, estuarine, and freshwater); may occur differently in different fish species; could be influenced by trophic status within aquatic food webs and may not be constant across all exposure concentrations, pathways, and routes of uptake (McGeer et al., 2003).

With its high toxicity, along with bioaccumulation in freshwater fish, As in industrial waste is a toxicant that should be given more consideration in aquatic toxicology. Many authors have reported that the accumulation of As is higher in marine fish when compared to freshwater fish due to lower concentrations of As in freshwater environments (Gaim et al., 2015). Williams et al., (2006) studies on As bioaccumulation in freshwater fish and reported the ratio of bio concentration factor and bioaccumulation factors ranging from 0.1 to 3097L/Kg. Many investigators studied on accumulation of arsenic in fish's organs (**Table 5**) and they found high accumulation of arsenic occurs in liver and gills (Allen and Rana 2004; Bears et al., 2006; Hamdi et al., 2009)) than the other part of the fish. As does not readily bio-concentrate in aquatic species. It is typically water-soluble and does not combine with proteins. Planktivorous fish are more likely to concentrate As than omnivorous or piscivorous fish (USEPA 1999). Robinson et al., (1995) observed no evidence of As uptake or accumulation from water in both rainbow and brown trout. Many species of fish that live in As polluted water contain As in the range of 1 - 10µg/g. Donohue and Abernathy (1999) reported that total As in marine fish, shellfish, and freshwater fish tissues ranged from 0.19 to 65, from 0.2 to 125.9, and from 0.007 to 1.46 µg/g dry wt, respectively. Koch et al., (2001) demonstrated that total As in freshwater fish ranged from 0.28 to 3.1 µg/g dry wt for whitefish (*Coregonus clupeaformis*), from 0.98 to 1.24 µg/g dry wt for sucker (*Catostomus*

commersoni), from 0.46 to 0.85 µg/g dry wt for walleye (*Stizostedion vitreum*), and from 1.30 to 1.40 µg/g dry wt for pike (*Esox lucius*). They had taken the samples from Back Bay, Great Slave Lake, near Yellowknife, Canada. Cornejo-Ponce et al., (2011) also found high As concentration in the fish obtained from the Atacama Desert in the north of Chile.

4.4 Acute toxicity:

Knowledge of the acute toxicity of a heavy metal like As helps in predicting and preventing acute damage to aquatic life in receiving waters. In addition, this information is useful to regulate toxic waste discharges (Vutukuru 2003). Most of the data on the effects of As on fish are based on acute toxicity tests that measure fish mortality over 96 h. Some studies have also examined sub-lethal effects such as growth, avoidance behavior, and fertilization/hatching (Kumar and Benerjee 2012b). Acute toxicity of As to fish reveals that the differences in the 96h-LC_{50} values between fish species can be attributed to the complicated metal-induced changes in the physiology and survival of aquatic organisms under metallic stress. Such changes differ from organic and inorganic As, the species of fish, and from one experimental condition to another. Zebrafish treated with 5-15 mg/L As V usually died within the first 48 h of treatment and thereafter surviving fish appeared to be able to tolerate subsequent As exposure with minimal mortality in the next 48 h (Lam et al., 2006). These observations suggest that the As concentrations used in the study produced toxicity and fish would either succumb or recover from it with increased tolerance to As. Fish that recovered from initial As exposure appeared to show better tolerance to subsequent exposure (Lam et Al., 2006; Kumari and Ghosh 2012b). Increased tolerance to As following initial exposure was reported at both cellular and organism levels and are attributed to activated adaptive responses such as detoxification and expulsion mechanisms (Liu et al., 2001; Tamas and Wysocki 2001).

Toxicity of inorganic As - LC50 to fish are presented in **Table 4.**

4.5 Behavioral changes

Quantifiable behavioral changes in chemically exposed fish provide novel information that cannot be gained from traditional toxicological methods, including short-term and sub-lethal exposure effects, mechanism of effect, interaction with environmental variables, and the potential for mortality (Saglio and Trajasse 1998). Behavior provides a unique perspective linking with the physiology and ecology of an organism and its environment: (Little and Brewer 2001). A minute amount of some toxicants can have the ability to cause abnormal behavior in fish through impaired perceptive acuity. Exposure with sodium arsenate caused various abnormal behaviors such as erratic movement, rapid movement of the opercula, jumping out of the test media, lateral swimming, and loss of equilibrium (Mosammat et al., 2008; Akter et al., 2008; Baldissarelli et al., 2012). The first visible reactions of the treated fish were observed within a few-minutes of exposure, especially at higher concentrations; 2.250 mg/L of sodium arsenate. However, the fish exposed to lower concentrations i.e., below 0.08 mg/L of sodium arsenate, showed no or little behavioral changes depending on the concentrations in the exposure media. The abnormal behaviors were caused by neurotoxic effects and by the irritation to the sensory system. Jumping and back and forth movements indicate avoidance reactions of the fish to As. Excessive secretion of mucus was probably due to irritation of the skin due to direct contact with the sodium arsenate. Lateral swimming and loss of equilibrium may be due to the impairment of the nervous system (Sinha and Kumar 1992). The avoidance threshold for golden shiner; *Notemigonus crysoleucas* was observed at 28 µg As III/L in flow-through tests (Hartwell et al., 1989).

4.6 Effects on organs

As compounds show toxicity in many organs of body such as skin, kidney, liver, lung, muscles, gastro intestinal tract, etc. (Pedlar et al., 2002; Roy and Bhattacharya, 2006). Among these, liver and kidneys are vital organs in vertebrates which perform detoxification mechanism, protein synthesis and excretion of nitrogenous waste and homeostatic functions respectively. Acute and sub acute effects of As may involve many organ systems including the respiratory, gastrointestinal, cardiovascular, nervous, and hematopoietic systems especially during long-term exposure. As accumulation in tissues will be a function of the uptake and clearance rates of individual organs. Sub-lethal effects including anemia, gallbladder inflammation, and liver degeneration, were observed at aquatic concentrations of 9.64 mg/L and dietary concentrations of 43.1-60 μg/g (Cockell et al., 1992; Rankin and Dixon 1994). In lake white fish (*Coregonus clupeaformi*) and in lake trout (*Salvelinus namaycush*) fed with dietary arsenate, histo-pathological alterations including sloughing of the epithelium, edema, and fibrosis of the sub-mucosal tissues in gallbladders were found (Pedlar et al., 2002b). Fish exposed to As for a longer period in the laboratory or in natural As contaminated habitats exhibited histo-pathological lesions in the liver, gallbladder, and kidney, which in turn affect the hepatic and renal functions (Pedlar et al., 2002b; Roy and Bhattacharya 2006). Details of organism effects are explained as follow:

4.6.1 Skin

Skin is the first physical barrier to protect the body from damage by chemical hazards in the environment. Numerous skin changes occur because of long-term As exposure. Singh and Banerjee (2008) have analyzed the toxic impact of sub-lethal concentration; 1 mg/L of an As salt, disodium arsenate heptahydrate to the skin of *Clarias batrachus* Linn. It included wear and tear; sloughing, hyperplasia of mucous cells and club cells, along with severe

degenerative changes. A thick layer of slime on the skin surface secreted by the mucus cells is an effort to protect the skin from the toxic stress of the As salt (Kumar and Banerjee 2012b). Secretion of the copious amount of slime is perhaps a common phenomenon in the skin and other respiratory organs of many other air-breathing fish, when exposed to heavy metal salts (Chandra and Banerjee 2003). The sulphated mucin is known to bind As molecules, perhaps to keep the toxicant away from the surface of the skin, at least temporarily (Singh and banerjee 2008). Metals bind to proteins in biological systems by their histidine and cysteine residues; histidine binding through its imidazole nitrogen and cysteine through its thiol groups (Venugopal 2013). It was found that the mucus covering the skin is mainly composed of glycoproteins that have electronegative charges at neutral pH. However, the protective role played by the mucus coating did not last long, perhaps due to extensive loss and the altered nature of the slime following prolonged exposure. This led to wear & tear and sloughing of the superficial cells. Continuation of exposure often led to further destruction, followed by uncontrolled regeneration of the epidermis, causing significant alteration in its histo-morphology and cellular architecture. Chronic exposure of metals leads to multisystem diseases, with most obvious consequence of As being skin lesions (Rossman, 2003). Dermatological changes are a common feature and are the key criteria for initial clinical diagnosis of As toxicity in any organisms. However hypo n hyper-pigmentation has been observed by Kumari et al., (2013) when *Heteropneustis. fossilis* were exposed to As.

4.6.2 Gills

Gills carry out three main functions viz. gas exchange, ion regulation, and excretion of metabolic waste products. Thus, gills can fulfill a vital role in the overall protection against harmful substances by acting as a first barrier and thus lowering the total uptake of toxic molecules by other organs (Mdgela et al., 2006). Due to the constant contact with the external

environment, gills are the first targets of water borne pollutants. Respiratory distress is one of the early symptoms of toxicant poisoning. A high rate of absorption of As through gills also makes fish a vulnerable target of its toxicity. The As concentration found in gill tissue reflects the direct contact of the gills with As-contaminated water. The majority of studies on As toxicity in freshwater fish have examined the effects associated with uptake of waterborne As across the gills and dietary uptake by benthic-feeding fish (Pedlar and Klaverkamp 2002). Fish exposed to As exhibited anxiety in breathing due to the clogging of gills by coagulated mucous and suffered direct damage of As ions to blood vessels, resulting in vascular collapse in the gills and anoxia (Mondal and Samanta, 2015). Desquamation, necrosis, the lifting of the lamellar epithelium, oedema, aneurism, hyper-plasia of epithelial cells, and fusion of the secondary lamellae were observed in the gills after exposure to sodium arsenite on *Oreochromis mossambicus* (Ahmed et al., 2013). Injuries in gill tissues reported due to As exposure may reduce the oxygen consumption and disrupt the osmoregulatory function of the fish. Hence, damage in gills may impair their respiratory efficacy, leading to altered metabolic processes required for the growth and development of fish.

4.6.3 Muscles

The muscles provide the power for swimming and constitute up to 80% of the fish itself. The muscles are arranged in multiple directions (myomeres) that allow the fish to move in any direction. Muscle is the most commonly consumed portion of fish and contributes most to the mass of fish (Palaniappan and Vijayasundaram 2009). Accumulation of As in the muscles was the least in all experimental groups when compared to other soft tissues. Because there is no direct contact of toxicants with muscle tissues, it is not an active site with detoxification and hence, As is not transported from other tissues to muscles. Nevarez et al., (2011) found positive correlation of As content in muscles of Channel catfish*; Ictalurus punctatus and*

Green sunfish; *Lepomis cyanellus).* Maher et al., (1999) also suggested the least quantum of As in the muscle of *Mugil cephalus.* For muscle tissue, Cossa et al., (1992) found a significant increase in heavy metal concentrations with increasing fish age. Higher concentrations of metals were found in younger fish and this generally reflects the short residence time of these metals within the fish, combined with the higher rate of metabolism compared to older organisms. While continuous exposure to As may alter the size of muscle fibers (D'Amico et al., 2014). As exposure during embryogenesis can initiate to molecular changes that appear to lead to aberrant muscle formation (Gaworecki et al., 2012). Whereas Begum et al., (2013) observed when exposed with 7 and 20 mg/L of As to *Heteropneustis fossilis*, degeneration in muscle bundles accompanied with focal areas of necrosis as well as atrophy and vacuolar degeneration. In an investigation by Lewis et al., (2012), that there was not any stability of As speciation in fish muscle samples under different storage and sample preparation conditions.

4.6.4 Liver

Liver is the major organ involved in the regulation of metabolic functions. Most of the biotransformation of inorganic As takes place in the liver (Rossman 2003). As is actively metabolized in the liver and has the tendency to accumulate here as well as reported in different teleosts such as rainbow trout (Cockell et al., 1991), Japanese medaka, and *Tilapia mossambica* (Suhendrayatna et al., 2002b). Datta et al., (2007) studied the hepatocellular alterations induced by sub-lethal concentrations (0.50µg/L) of As in Indian catfish; *Clarias batrachus* Linn. Sub-lethal As exposure altered serum aspartate aminotransferase and alkaline phosphatase levels and brought about significant changes in different serum biochemical parameters. As exposure reduced total hepatocyte protein content and suppressed the proliferation of hepatocytes in a time-dependent manner. Lam et al., (2006)

and Li et al., (2016) found metabolic and histopathological liver when the zebra fish exposed with arsenic. Extreme degenerative changes in the hepatopancreas of *Channa punctatus* were found to occur within 48 h of exposure to the non-lethal levels of As (Roy and Bhattacharya 2006). The degenerative changes were characterized by vacuolation and apoptosis of the hepatocyte, pycnosis in many of the necrotic cells, peliosis hepatis in the tissue (Ahmed et al., 2013). Other liver alterations, including fatty infiltration, central or focal necrosis, cirrhosis, cytoplasmic vacuolation, nonspecific autolytic changes, hemosiderin granules, necrotic and fibrous bodies, and nuclear and cytoplasm As inclusions, have been observed in green sunfish; *Lepomis cyanellus* exposed to aqueous concentrations of sodium arsenate (Sorensen 1991). Ahmed at al. (2013) observed abnormal behavior and altered histopathology of liver demonstrated the severe adverse effects to exposure of As in *Oreochromis mossambicus*. As is highly toxic for *Oreochromis mossambicus* and the toxicity increases with increasing chemical concentration of exposure time. Carlson et al., (2013) have demonstrated that exposure of zebrafish to low dose sodium arsenite (50 µg/L) resulted in gender-specific responses of the liver proteome.

4.6.5 Gastrointestinal Tract

The major route of dietary As exposure and uptake is through the gastrointestinal tract. Once absorbed by the gastro-intestinal tract, As is distributed to organs throughout the body via the circulatory system. Dietary exposure to As was shown to disrupt the mucosal lining of the gastro-intestinal tract of lake whitefish, which causes mucosal sloughing followed by increased mucosal production (Pedlar et al., 2002). Acute As exposure may cause gastrointestinal tract disorder. The gastrointestinal effects are seen acutely after As ingestion; however, gastrointestinal effects may also occur after heavy exposure by other routes. The fundamental gastrointestinal lesion appears to be increased permeability of the

small blood vessels, leading to fluid loss and hypotension (Haque and Roy 2012). High concentration of As; 20mg/L, exposure showed damaged serosa, disorganized and consequent fussion of mucosa, degeneration and edema between the intestinal submucosa and lamina propria (Begum et al., 2013) The exposure of different species of As occurred on the fish species [lake whitefish; *Coregonus clupeaformis*, walleye; *Stizostedion vitreum*, northern pike; Esox *lucius*, white sucker; *Catostomus commersoni* and longnose sucker; *Catostomus catostomus,* the concentrations of total As and most As species were highest in the gastrointestinal tract compared to concentrations in muscle and liver (Rosemond et al., 2008). Chauncey et al., (1988) studied the effects arsenicals on tyrosine absorption by the winter flounder *Pseudopleuronectes americanus* using isolated intestinal strips mounted in Ussing chambers, and their results indicated that Na^+-dependent tyrosine uptake is inhibited by arsenicals.

4.6.6 Kidney

Kidney is the major route of As excretion, as well as a major site of conversion of pentavalent As. Renal histopathological changes in freshwater teleosts were significant in various fish species observed in As exposed rainbow trout (Kotsanis and Iliopoulou-Georgudaki 1999) and lake white fish and lake trout (Pedlar et al., 2002 b). Roy and Bhattacharya (2006) found during their investigation that 1/20 and 1/10 LC_{50} doses of As_2O_3 resulted in discrete pathology in the trunk kidney, the effects being highly significant at both treatments. In both 1/20 (3.8 mg/L) and 1/10 LC_{50} (7.6 mg/L) of As treatment it was evident that on the very first day there was shrinkage in the glomerulus with a resultant increase in Bowman's space followed by enlargement of the glomerulus and normalization of Bowman's space during the last phase. Increase in Bowman's space suggests an increase in the filtration rate and

consequently in urine volume. Allen et al., (2004) stressed that the adaptive response exhibited by As treated fish during long-term exposure involved reduced glutathione.

4.6.7 Brain

The brain is believed to be particularly vulnerable to As due to its high oxygen consumption rate and high level of polyunsaturated fatty acids and relatively high rate of oxygen free radical generation without commensurable level of As. The significant differences in absorbance intensities between the control and As intoxicated brain tissues reflect an alteration on the major biochemical constituents, such as lipids, proteins, and nucleic acids of the brain tissues of *Labeo rohita* due to As intoxication (Palaniappan and Vijayasundaram, 2008). The reduction in the protein content after As exposure may be due to reduced protein synthesis and due to the higher affinity of metal compounds toward different amino acid residues of proteins, which is considered as the premier biochemical parameter for early indication of stress. The decreased quantity of protein may also be due to its conversion to amino acid residues in order to increase the amino acid pool. As is also responsible for alteration in behavioral parameters and brain endonucleotidases activities (Baldissarelli et al., 2012).

4.6.8 Gonads

Fish reproduction was considered a reliable indicator of endocrine disruption in aquatic systems by chemical compounds including As. A previous monitoring research in the Mekong Delta of Vietnam revealed a negative correlation between gonad development and accumulation of As in catfish, *Pangasianodon hypophthalmus* (Yamaguchi et al., 2007). Testicular degeneration was observed in freshwater perch exposed to arsenite. Fritzie et al., (2009) demonstrated that a low dose (0.1 µg/L) of As may inhibit 11-ketotestosterone (KT)

synthesis via suppression of steroidogenic enzyme activities such as 3-β-hydroxysteroid dehydrogenase, which may continuously inhibit spermatogenesis. Furthermore, exposure of testis to high concentration of As 100 µg/L produced reactive oxygen species; ROS, in testis, which consequently cause apoptosis of germ cells, especially after induction of spermatogenesis by human chorionic gonadotropin; hCG, via 11-KT synthesis. These findings suggest that a low dose of As inhibits spermatogenesis via suppression of steroidogenic enzyme activity and expression while a high dose of this compound induces oxidative stress-mediated germ cell apoptosis. Ovarian degeneration was observed in freshwater perch exposed to arsenite.

4.7 Biochemical and physiological changes

In general, the presence of toxicants in aquatic media exerts its effect at the cellular or molecular level, which results in significant changes in biochemical parameters. Among these, the blood glucose level was used as an indicator of environmental stress and reflected the changes in carbohydrate metabolism under hypoxia and stress conditions. When Indian cat fish; *Clarias batrachus* was exposed to sub-lethal concentration of As; 0.50 µM, significant changes in different serum biochemical parameters along with cytological changes was observed (Datta et al., 2007).

4.7.1 Carbohydrate

Carbohydrates represent the principal and immediate energy precursors of fish exposed to stress conditions while protein is spared. The influence of metal stressors on carbohydrate metabolism of fish includes alterations in glucose, glycogen, and lactic acid content. Among these, the blood glucose level was used as an indicator of environmental stress and reflected the changes in carbohydrate metabolism under hypoxia and stress conditions. The findings of

Garg et al., (2008) on the three Indian major carps *Labeo rohita, Cirrhinus mrigala,* and *Catla catla* exposed to sub-lethal doses of toxic metals, including As, indirectly suggest the hyperglycemic effect of As. They reported extensive glycogenolysis consequent to the exposure of As that would positively increases the blood glucose level. A similar study made on *Tilapia Zillii* and *Mugil capito* by Mohamed and Nahed (2008) indicated the hyperglycemic effect of metallic pollutant in fish. When *Channa punctatus* was exposed to different concentrations of pollutants including As, the exposure caused hyperglycemia during both short-term and long-term exposure (Bhattacharya et al., 1987). The investigators found that pollutants in single treatment and in a mixture both caused depletion of hepatic glycogen profile in *Channa punctatus*. They noticed hepatic glycogenolysis both in short-term as well as long-term treatment. The hepatic and cerebral glycogenolysis also observed by Kumari et al., (2012) when the fish were exposed to different doses of As. Shobha Rani et al., (2000) evaluated the effect of sodium arsenite on glucose and glycogen levels in various tissues subjected to sub-lethal concentration for a period ranging from 24 h to 96 h and found depletion in glycogen level in various tissues of *Tilapia mossambica*. Kumari and Ahsan, (2011a) observed that when *Clarias batrachus* were exposed to various As concentrations for six consecutive days glycogenolysis was caused in muscle. Significant differences in average muscle glycogen content were found in the treated male and female fish, in which higher concentrations were more glycogenolytic than the lower concentrations. After 96 h of treatment with As, less depletion of muscle glycogen content was recorded in both sexes of fish. Kumari and Ahsan (2011b) and Kumari et al., (2015) noticed during their investigation that acute exposure of arsenic- tri-oxide produced hyperglycemia in both sexes of *Clarias batrachus* in which females were more reactive than males. The hyperglycemia reported in fish may be due to altered insulin secreting capacity of pancreatic β-cells. As interferes with transcriptional factors involved in insulin-related gene expression resulting in a decline in

insulin production, leading to hyperglycemia (Ana et al., 2006). Thus, an elevation of the blood glucose level during sub-lethal treatment might be due to gluconeogenesis to provide energy for the increased metabolic demands imposed by As stress or due to impaired insulin secretion. During acute treatment, the significant reduction in plasma glucose levels might be due to hypoxic conditions caused by As, leading to an excess utilization of stored carbohydrates.

4.7.2 Protein

As and its metabolites can cause toxicity by directly attacking thiol or phosphate groups resulting in impaired proteins, or indirectly, through the generation of reactive oxygen species and free radicals causing oxidative stress damage to both proteins and DNA (Lam et al., 2006). The high toxicity of arsenite: AsIII, results from its greater affinity with the sulfhydryl: SH, groups of biomolecules, whereas arsenate: AsV, does not directly bind to the SH group to exert its toxic effects (Suzuki et al., 2008). Inside a cell, arsenite binds with SH groups present in proteins and arsenate interferes with phosphorylation reactions (Andrew et al., 2003). Xenobiotics are known to induce the synthesis of heat shock proteins/stress proteins (Mukhopadhyay et al., 2003). Arsenicals are also known to induce a number of major stress protein families, including heat shock proteins: HSPs, both in vitro and *in vivo* in a variety of model systems. In most of the cases, the induction of stress proteins depends on the capacity of the arsenical to reach the target, its valence, and the type of exposure. Arsenite is the most potent inducer of most of the hsps in several organs and systems with a rapid dose dependent response to acute exposure to arsenite (Papaconstantinou et al., 2003). In fish, heat shock protein 70s have been sequenced in different species including rainbow trout (Zafarullah et al., 1992), medaka (Arai et al., 1995), zebrafish (Graser et al., 1996), and tilapia (Molina et al., 2000). As also induced expression of heat shock proteins 70 in the liver,

gills, olfactory rosette, and skin of zebra fish (Blechinger 2002). The pattern of expression for heat shoak protein 70 was dose-dependent and tissue specific. Exposure to elevated concentrations of As induces the synthesis of stress proteins in various cell types, which exert a protective role on cell survival (Agarwal et al., 2009). Although in cell culture, heat shock protein 7 is induced in trout. During acute and sub-lethal treatment the decrease in plasma protein may be due to liver cirrhosis or nephrosis or might be due to alteration in enzymatic activity involved in protein biosynthesis (Yousef et al., 2008; Palaniappan and Vijayasundaram 2009). Induction of metallothionein: MT, by arsenicals appears to be regulated at the transcription level, because As III and As V increased the metallothionein-I and metallothionein-II mRNA levels, with As III being more effective (Kreppel et al., 1993**).** Channel catfish (*Ictalurus punctatus*) were treated with monosodium methyl arsonate: MSMA, sodium arsenite, and sodium arsenate exposed to 0.01, 0.1, and 1.0 mg/L of each compound for 1 week resulted in dose dependent induction of hepatic metallothionein , with significant induction occurring in fish exposed to 1.0 mg/L of monosodium methyl arsonate and sodium arsenite. As induced significant expression on metallothionein activity can be a useful biomarker in environmental bio monitoring of As contamination (Kovendan et al., 2013). A proteomic approach was employed by Carlson et al., (2013) to investigate As induced alteration in the zebra fish liver proteome following a 7-day exposure to 50 µg/L sodium arsenite. Over 740 unique proteins were identified, with fewer than 2% showing differential expression. These findings indicate that protein expression is altered following As exposure.

4.7.3 Enzymes and Oxidative Stress

Enzymes are biochemical macromolecules that control metabolic processes of organisms. Effects of As exposure on the enzyme activities of certain fish have been studied. As is

absorbed via the gills and is capable of causing disturbance to the antioxidant system and affect the antioxidant responses by increasing glutamate cysteine ligase:GCL, activity and glutathione:GSH, levels (Lima et al., 2009). In gills, antioxidant responses to a pro-oxidant challenge of As were mostly modulated through a significant induction of glucose-6-phosphate dehydrogenase: G6PDH, in organisms exposed to arsenate. There is evidence that glucose-6-phosphate dehydrogenase , a key enzyme of the pentose phosphate pathway, has an important role in antioxidant systems since the generated nicotinamide adenine dinucleotide phosphate: NADPH, is an essential element for the H_2O_2-scavenging pathway of cells (Morales et al., 2004) and for glutathione metabolism (Bagnyukova et al., 2007). Humtsoe et al., (2007) studied the effect of sodium arsenate on the activity level of the enzymes of muscle and liver of juvenile rohu carp, *Labeo rohita* found a significant depletion in enzyme activities, especially of acid phosphatase, alkaline phosphatase glutamate - pyruvate transaminase and glutamate - oxaloacetate transaminases, thereby indicating that As causes disturbances in body metabolism. Bhattacharya and Bhattacharya (2007), while studying the effect of two non-lethal doses of As on Indian cat fish, *Clarias batrachus* exposed to As for 10 days noticed an increase in the activity of antioxidant enzymes such As glutathione peroxidase:GPx, superoxide dismutase: SOD and catalase. However, there was a noticed decrease in glutathione reductase: GR, activity within a day of exposure, indicating the oxidative stress in fish at an early stage. It is due to excess H_2O_2 production, meaning that peroxisomal metabolizing enzymes are potential targets of As toxicity in *Clarias batrachus*. As toxicity is associated with the formation of reactive oxygen species, this may cause severe injury or damage to the nervous system (Patlolla et al., 2005). Enzyme activities are considered as sensitive biochemical indicators and widely used to assess health of the organism in aquatic toxicology (Gul et al., 2004). Among the array of enzymes used, the aspartate aminotransferase: AST, and alanine aminotransferase: ALT, are widely used to

detect the tissue damage caused by the toxicants (Jung et al., 2003). Datta et al., (2007) observed elevated levels of aspartate aminotransferase and alanine aminotransferase in *Clarias batrachus* exposed to As at sub-lethal concentration. Selamoglu Talas et al., (2012) also observed effects on *Cyprinus carpio.* It acts by catalyzing the transfer of amino group of the aspartic acid to α – ketoglutaric acid, to form oxaloacetic acid and glutamic acid, where as alanine aminotransferase acts by catalyzing the transfer of the amino group from alanine to a ketoglutaric acid to form pyruvic acid and glutamic acid. These enzymes are the strategic link between carbohydrate and protein metabolism and play an important role in the utilization of amino acids for the oxidation and/or for gluconeogenesis (Rodwell 1988). As can inhibit the activities of many enzymes, especially those involved in the cellular glucose uptake, gluconeogenesis, fatty acid oxidation, and production of glutathione due to its SH group binding capability (Pal and Chatterjee 2004). Several soluble enzymes of blood serum have been considered As indicators of hepatic dysfunction and damage. Among the array of enzymes used the aspartate aminotransferase and alanine aminotransferase are widely used to detect tissue damage caused by the toxicants (Jung et al., 2003). Humtsoe et al., (2007) reported significant decrease in liver aspartate aminotransferase and alanine aminotransferase in *Labeo rohita* exposed to As, which reflects significant decrease in structure and function of cell organelles like endoplasmic reticulum and the membrane transport system. They also suggested that arsenate, which resembles phosphate is used by cells for energy and signaling. By displacing phosphate in enzymes or signaling proteins, As can block energy production and normal cell signaling (Dartmouth Toxic Metals Research 2005). Enzyme delta aminolevulinic acid dehydratase; ALAD, levels were measured to reflect the interruption in hemoglobin synthesis and changes in the erythrocyte. Aminolevulinic acid dehydratase activity in the blood was reduced 62 % by As exposure. 2,3-dimercaptosuccinic acid: DMSA, alone or with N-acetylcysteine was able to restore aminolevulinic acid dehydratase levels to

those of controls: not exposed to As ; only the combination of both was able to restore RBC glutathione levels. In this study the level of acute As exposure was significantly higher (100,000 µg/L) than chronic human exposure (Patrick 2003).

4.7.4 Lipid

Lipids play key roles in diverse physiological functions and processes in organisms. Enhanced lipolysis and lipogenesis in brown adipocytes and stimulation of peripheral glucose uptake result in higher oxidative and glycolytic processes after As exposure (Minokoshi et al., 1988). Arsenic- tri-oxide affected the weight and fat content of rainbow trout. Fish exposed to 1.0 mg As/L then fish lost dry weight but the wet weight remained unchanged (Cockell and Hilton, 1988). This change in weight may be explained by a disruption in osmoregulation due to resultant kidney damage, causing water to be retained in the body. However, when exposed to 6.0 mg As/L, the wet weight also decreased. As affects the synthesis of lipids necessary for energy reserves and the development of eggs. In channel catfish exposed to arsenate, arsenite, and monosodium methyl arsonate up to 1.0mg/L for 7 days, hepatic lipid peroxidation: LPO, levels and thiobarbituric acid reactive substances: TBARS, were not significantly changed (Schlenk et al., 1997). Similarly, lipid peroxidation levels in plasma were not significantly affected in lake whitefish exposed to up to 100 µgAs/g food as sodium arsenate for 10, 30, and 64 days, although there was a general decrease in exposed fish (Pedlar et al., 2002b). It seemed that lipid peroxidation status in response to As exposure was dependent on exposure duration. In climbing perch *Anabas testudineus* exposed to As at the 1.5 mg/L level, lipid peroxidation was significantly induced at day 2, but the induction at day 30 at the 0.75 mg/L As level was not significant (Das et al., 1998). The As effect on lipid peroxidation was also dependent on exposure route and was species-specific. Bagnyukova et al., (2007) have observed augmented liver glutathione levels after exposure of

goldfish to 200 µg of sodium arsenite. The same authors observed absence of lipid peroxidation measured As thiobarbituric acid content and protein carbonyl groups in liver of goldfish, although higher levels of lipid hydroperoxides were registered after 1 and 4 days of exposure. Gonzalez et al., (2010) observed on *Fundulus heteroclitus* that As exposure may impair cholesterol homeostasis, which could have adverse effects on organismal health.

4.8 Hematological changes

The hematopoietic system is also affected by both short- and long-term As exposure. Hematological profiles of fish are widely used to monitor the environmental pollution in aquatic ecosystems (Carvalho and Fernandes 2006). Various blood characteristics of fish have been utilized to measure the responses of sub-lethal effects. The analyses of blood parameters (hematological and biochemical) of fish exposed to a toxicant are important in diagnosing the structural and functional status of the animal (Adhikari et al., 2004). Low levels of As exposure may cause decreased production of red and white blood cells (Abernathy et al., 2003). Datta et al., (2009) stated that chronic exposure of As affected the structure of head kidney characterized by loss in leucocyte number. As may cause oxidative stress in the liver of fish and brings about alterations in hematological parameters (Bhattacharya and Bhattacharya, 2007). Many researchers (Cockell et al., 1991; Pedlar et al., 2002a; Chokkalingam et al., 2010) have observed an anemic condition of the fish during acute and sub-lethal treatment, which resulted in low level of hemoglobin: Hb, in arsenate treated fish. Inhibition of erythropoiesis due to As toxicity by its action on membrane may form another possible reason. The decreased number of red blood cells: RBC, in fish due to toxicant exposure was reported by Allin and Wilson (2000) and Chowdhury et al., (2004). Tripathi et al., (2003) reported a decrease level of hemoglobin and packed cell volume in *Clarias batrachus* exposed to waterborne As. Kotsanis et al., (2000) reported significant

decrease in white blood cells count in *Oncorhynchus mykiss* exposed to As due to decrease in number of lymphocytes. It is well known that the changes in leucocytes counts after exposure to pollutants may be associated to a decrease in nonspecific immunity of the fish. Leucocytes are involved in the regulation of immunological function in many organisms and the increase in white blood cells in stressed animals indicates a protective response to stress (Witeska, 2004). The decrease in white blood cells count during acute and sub-lethal treatment; after 20th day, may be due to the extended toxic effect of As in kidney tissue, which is the primary site of hematopoiesis, provoking immune-suppression. Another possible reason may be due to inhibition of white blood cells maturation and the release from tissue reservoirs by the action of As. Chronic exposure of As affected the structure of head kidney characterized by loss in leucocytes number (Datta et al., 2009). The appearances of an atypical lymphocyte population accompanied by development of reticular tissues, edematous growth, and decreased leucocyte counts in kidney and spleen suggest that As probably affects the process of lymphopoiesis and blast formation in *Clarias batrachus.* Another possible reason may be due to inhibition of white blood cell maturation and the release from tissue reservoirs by the action of As. Selamoglu Talas et al., (2012) also found during their investigation that granulocyte, erythrocyte, hemoglobin, hematocrit values were decreased in *Cyprinus carpio*, by the use of As in comparison to control group.

4.9 Immunotoxic effects

As is immunotoxic, exhibiting its effects on various immune responses, such as reducing delayed hypersensitivity reactions, modulation of co-receptor expression and release of lymphokines, inhibiting mitogen activated T cell proliferation and increasing free intracellular Ca^{2+} production (Goytia-Acevedo et al., 2003). Head kidney: HK, spleen, and

thymus comprise the main immune competent organs in fish (Jorgensen et al., 2003; Fishelson 2005).

The head kidney macrophages: HKM, are important for the initiation of fish innate immunity and As-induced macrophage death is bound to affect the immune status of the exposed fish. To the best of our knowledge, this is the first report of *in vivo* the head kidney macrophages death to be induced by micromolar concentration of As in fish. As accumulates in , liver and kidney of fish, and it can interfere with the fish immune system by suppressing antibody and cytokine production (Ghosh et al., 2007). Sub-lethal As exposure affects the functional arms of both innate and acquired immune system in fish rendering them immuno-compromised and susceptible to pathogens (Datta et al., 2009; Guardiola et al., 2013; Banerjee et al., 2015). The effects of ecotoxicants on innate immunity may be more significant because adaptive immunity is developmentally delayed in fish (Alexander and Ingram 1992). In As contaminated groundwater Clark and Raven (2004) found that the effects of As on the innate immune system, including the ability to mount an adequate respiratory-burst response. In the zebra fish model, system expressed essential antiviral genes and produce sufficient levels of Tumor necrosis factor: TNFα, within the concentration range of As (Hermann and Kim 2005). In fish, As has a profound influence on the immune system with the two immunologically important organs; Head kidney and spleen, are responding to the toxic effects of this xenobiotic in a differential manner, and it induced a decline in T lymphoctes and B lymphoctes cell responses both in the head kidney and spleen but its effects appear to be more pronounced on the B cells (Ghosh et al., 2006). Exposure of fish to various concentrations of As also affected the phagocytic potential of macrophages and helped in the dissemination and persistence of viral and bacterial pathogens into distant host tissues (Ghosh et al., 2007; Nayak et al., 2007). While Banerjee et al., (2015) observed that As has a generalized immune-suppressive effect leading to down regulation of both T helper

cells I and T helper cells 2 cytokines; besides, it led to up regulation of the HSP genes indicating As -induced cellular stress when exposed to *Labeo rohita.*

4.10 Cyto-Genotoxic effects

As is known as a potential sulph hydryl-reactive compound that aggregates a number of proteins of cell surface (Hossain et al., 2000) Like other oxygen radical-producing stressors, As induces nitric oxide production at the level of transcriptional activation along with induction of poly adenosine diphosphate:ADP, ribosylation, nicotinamide adenine dinucleotide: NAD depletion, DNA strand breaks, and formation of micronuclei (Bernstam and Nriagu 2000; Kumari and Ghosh 2012a). Aggregation of cellular proteins, production of reactive oxygen species, and activation of protein tyrosine kinases by As might be together or individually involved in the process of cell death (Hossain et al., 2000). Toxic effects of inorganic As also include denaturing of cellular enzymes and altering gene regulation (Abernathy et al., 1999). Ahmed et al., (2008) found that high concentrations of As caused cell death in large numbers; where low As concentrations induced a lower rate of cell death in liver cells. Selvaraj et al., (2012) found during their study that As_2O_3 induces cytotoxicity via apoptosis and necrosis mediated cell death depends on exposure dose and time. Fish cell lines may serve as the sensitive surrogates for whole fish to study the cytotoxicity of other As compounds. Arsenite might induce apoptosis due to induction of oxidative stress in JF cells, while in TO-2 fish cell lines it disturbs the cell cycle without induction of (Wang et al., 2004). However, Abernathy et al., (1999) suggested that As could disrupt cell division by deranging the spindle apparatus. It induces large deletion mutations (Hei ei al., 1998), chromosome damage and aneuploidy, and also causes micronucleus formation, DNA–protein cross-linking, and sister chromatid exchange (Huang et al., 2004). Ramirez and García (2005) reported duration and dose dependent increase in the induction of micronuclei in gill cells of

zebra fish, *Danio rerio* in response to As. It is known to inhibit DNA repair (Brochmoller et al., 2000) and even to exacerbate the effects of other mutagenic agents (Abernathy et al., 1999), thereby increasing susceptibility to multiple diseases (Duker et al., 2005). Many differentially-expressed genes encoding proteins involved in heat shock proteins, DNA damage/repair, antioxidant activity, hypoxia induction, iron homeostasis, As metabolism and ubiquity-dependent protein degradation were identified, suggesting strongly that DNA and protein damage as a result of As metabolism and oxidative stress caused major cellular injury. Sodium arsenite was demonstrated to induce chromosome aberrations in different cell types. The aberrations found in many studies were chromatid gaps, fragmentations, endo-reduplications, and chromosomal breaks (Nordenson and Beckman, 1991). Huang et al., (1995) found that As significantly delays mitotic division inhibits assembly of the mitotic spindle and induction of chromosome endo-reduplication. Chou et al., (2001) also documented that As exposure causes chromosomal abnormalities, with a preponderance of end-to-end fusions. These chromosomal end fusions suggested that telomerase activity might be inhibited by As.

There are also reports on As inhibiting cellular metabolism, affecting mitochondrial respiration and synthesis of adenosine triphosphate: ATP, (Abernathy et al., 1999). The toxic effect on cellular respiration occurs because As binds to lipoic acid in the mitochondria and inhibits pyruvate dehydrogenase. The resulting uncoupling of mitochondrial oxidative phosphorylation leads to increased production of hydrogen peroxide

It is clear evidence that As can disrupt gene expression, particularly through its effects on signal transduction (Abernathy et al., 1999). At low concentrations, As was shown to affect the DNA-binding capabilities of transcription factors NFkB i.e.'nuclear factor kappa-light-chain-enhancer of activated B cells', and: AP-1 i.e activator protein 1, leading to increased gene expression and stimulation of cell proliferation (Chen et al., 2000). However,

at high concentrations, As may lower NFκB activation, inhibit cell proliferation, and induce apoptosis (Wei et al., 2005). The repair inhibition may be a basic mechanism for the co-mutagenecity and presumably the co-carcinogenicity of As. Exposure of As may cause DNA hypomethylation due to continuous methyl depletion, facilitating aberrant gene expression. Comparisons of chromosome aberration frequencies induced by tri- and pentavalent As indicate that the trivalent forms are more potent and genotoxic than the pentavalent forms (Barrett et al., 1989), Several investigators suggested that As can disrupt cell division by deranging the spindle apparatus (Abernathy et al., 1999). As induces large deletion mutations (Hei et al., 1998), chromosome damage, and aneuploidy (Abernathy et al., 1999), and causes micronucleus formation (Hazarika et al., 2012; Kumar et al., 2013), DNA–protein cross-linking, and sister chromatid exchange (Huang et al., 2004). Wang et al., (2004) demonstrated that As mediated DNA-fragmentation cell cycle arrest in two fish lines. Fin cells of *Therapon jarbua* and ovary cells of *Tilapia* that might involve oxidative stress as a causative factor were studied. Oliveria et al., (2005) demonstrated the significant genotoxic effects of As bioaccumulation in zebra fish gill cells. Oliveria et al., (2005) found in the genotoxic effect in fish by an increase in micronucleus frequency with time, which was greater in fish treated with the positive control than in fish treated with As, accompanied in both cases, by other nuclear abnormalities as well. With both treatments, As promoted cell turnover at sub-lethal concentrations, with an increase in micronucleus frequency. Yadav and Trivedi (2009) found that the gradual increase in micronuclei frequency up to 96 h as 15 ± 1.414, and then decreased gradually after 168 h as13.33 ± 0.816, exposure of As_2O_3 in *Channa punctatus*. Lam et al., (2006) examined the liver transcriptome changes that occur following As exposure at selected time points with the aim of understanding the transcriptome kinetics of As-induced adaptive responses *in vivo* in the zebra fish liver. The analysis demonstrates an increase of transcriptional activity associated with metabolism,

especially biosyntheses and transporter activities localizing in membrane, cytoplasm, and endoplasmic reticulum, while transcriptional activity associated with catabolism, energy derivation, response to stress remained significant throughout the study. Some of these findings were further supported by histo-pathological evidence that may be used as reference for phenotype-anchoring points for certain classes of genes in future studies. Although As-induced oxidative stress is a known genotoxic event, this is the first description of the relevance of major transcriptional changes associated with biological networks that provide both global and specific information of a coordinated adaptive response to acute As toxicity in the liver. The genotoxicity of As occurs due to generation of reactive oxygen species and the inhibition of DNA repair.

DNA damage is an important mechanism of toxicity for a variety of pollutants, and therefore, is often used as an indicator of pollutant effects in eco-toxicological studies. As induced DNA damage in gill, liver, and blood tissues examined by Ahmed et al., (2012) when the fish were exposed by different concentration of As. Datta et al., (2007) investigated that DNA from unexposed and As-exposed (14- and 30-day exposed) liver cells exhibited a distinct ladder indicating maximum damage. Development of the DNA ladder is considered to be a hallmark of apoptosis (Janicke et al., 1998). Wang et al., (2004) also demonstrated As-mediated DNA-fragmentation and cell cycle arrest in two fish lines, that might involve oxidative stress as a causative factor.

Conclusions

As is a widespread environmental contaminant, which enters the aquatic ecosystem from both; natural and anthropogenic sources. Its effects on fish health include various mechanisms of acute and chronic toxicity, including enzymatic, genetic, and immune system failure. Many studies revealed high concentrations of As in liver and kidney in comparison to

the other organs, which disrupts the normal metabolism. It is clear that exposure to As alters normal biological functions, resulting in the direct initiation of disease or, at least, predisposition of an organism to it. Understanding the toxicological effects of As in the aquatic environment is important to mitigate its deleterious effects on aquatic health, particularly in fish and the organisms including human who consumes fish. Regular monitoring of arsenic levels and their associated health effects in aquatic organisms: fish, may not only provide insight into overall aquatic health but may also act as a sentinel for potential impacts on food chain.

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Figures

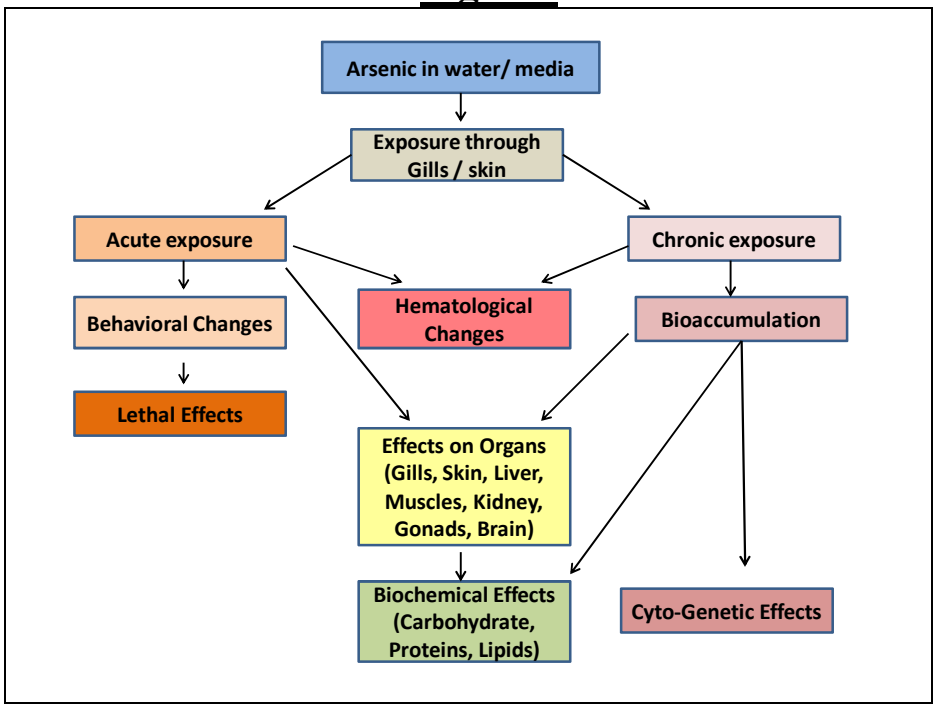

Fig 1: Arsenic in water with acute and chronic exposure through skin / gills and their toxicological pathway in fish.

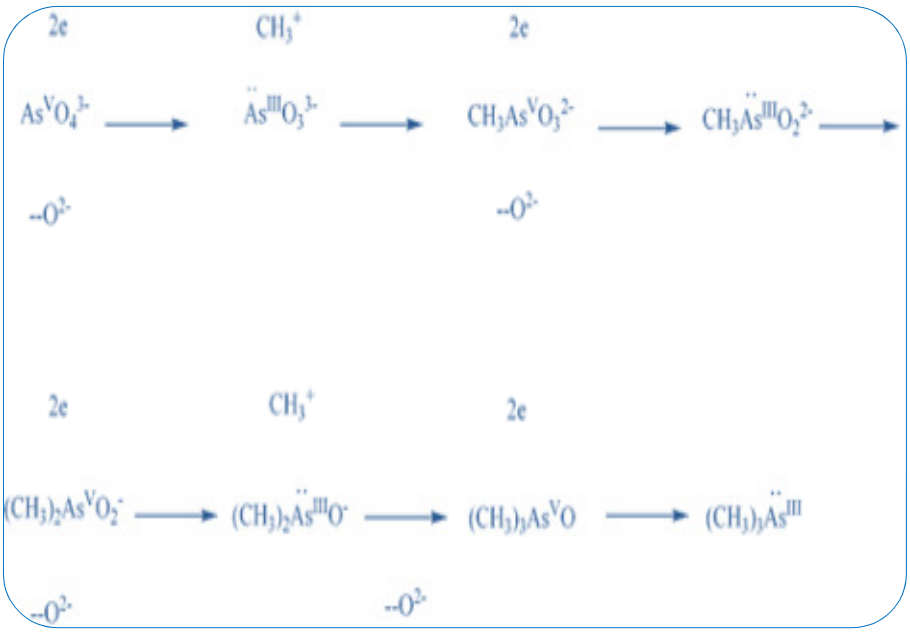

Fig 2: Suggested mechanism for the reduction of As V to As III. As V, which is the stable oxidation state in oxygen-containing waters, can be reduced to As III in anoxic or reducing systems

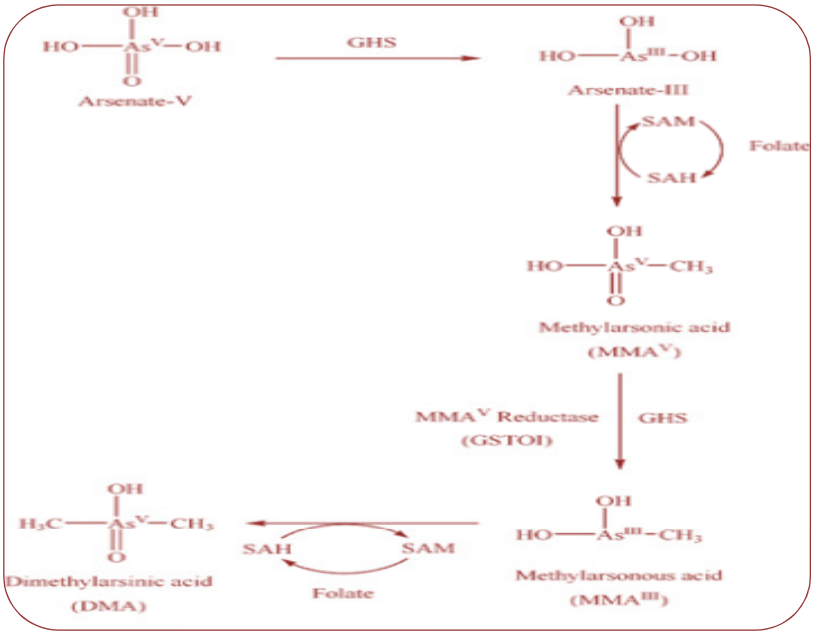

Fig 3: Process for the metabolization of arsenic in tissues of the animal. (GHS: Glutathione; SAM: S-adenosyhmethionine; SAH: S-adenosylhomocysteine; GSTO1: Glutathione S-transferase Omega 1)

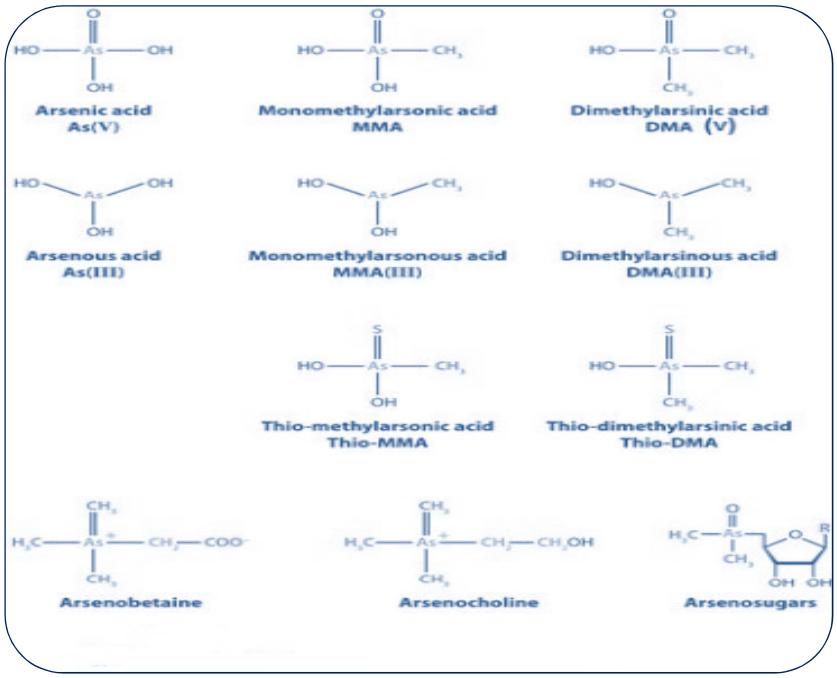

Fig 4: Arsenic compounds commonly found in marine and fresh water fish. Usually they are methylated arsenic, arsenosugars and arsenolipids.

Tables

Table 1: Physical and chemical properties of arsenic and its compounds.

S.N	Chemical Name	Molecular weight	Oxidation State	Physical state	Water Solubility
1	Arsenic	74.92	0	Solid	Insoluble
2	Arsenic acid	141.95	+5	Solid	Freely soluble
3	Arsenic tri-oxide	197.82	+3	Solid	Slightly soluble
4	Arsenic penta-oxide	229.84	+5	Amorphous solid	Freely soluble
5	Sodium arsenate	85.91	+5	Solid	Very soluble
6	Arsine	85.91	+3	Solid	Freely soluble
		77.93	+3	Gas	Slightly soluble
7	Dimethylarsinic acid	138.01	+5	Solid	Soluble
8	Methanearsonic acid	139.98	+5	Solid	Freely soluble
9	Sodium dimethyl arsenate	159.98	+5	Solid	Readily soluble
10	Sodium methane arsonate	161.96	+5	Solid	Soluble

Table 2: Chemical Identity of arsenic and selected arsenic compounds

Chemical Name	Synonyms	Formula
Inorganic Arsenic compound		
Arsenic	Arsenic -75, metallic As, As black, colloidal As	As
Arsenic acid Ortho Arsenic	acid	$H3AsO_4$
Arsenic tri-oxide	Arsenic oxide, Arsenous acid anhydride, white Arsenic, Arsenolite	As_2O_3
Arsenic pentaoxide	Arsenic (V) oxide, Arsenic acid anhydride, diAs pentoxide	As_2O_5
Sodium arsenate	Disodium arsenate, sodium biarsenate	Na_2HAsO_4
Arsine	Arsenic hydride, Arsenic trihydride	AsH_3
Organic As compound		
Arsenobetaine	Fish Arsenic	$(CH_3)_3As+CH_2CO_2$
Dimethylarsine acid	Cacodylic acid, Dimethylarsinic acid (DMA, DMAV)	$(CH_3)_2HAsO_2$

Methanearsonic acid	Methyl Arsenic acid, Monomethylarsonic acid, (MMA, MMAIII)	$CH_3H_2AsO_3$
Methylarsine	Methylarsine	CH_3AsH_2
Dimethylarsine	Dimethylarsine	$(CH_3)_2AsH$
Sodium methane arsonate	MSMA	CH3NaHAsO
Sodium dimethyl arsenate 3	Sodium cacodylate	$(CH_3)_2NaAsO_2$
Trimethylarsine	Gosio gas	$(CH_3)_3As$
Arsenic trimethyl, (4-aminophenyl)-arsonic acid	arsanilic acid, *p*-aminobenzene-arsonic acid	$C_6H_8AsNO_3$

Table 3: Arsenic compounds and minerals found in nature and their chemical formula

S.N.	Arsenic compounds/minerals	Chemical formula
1	Arsenic-oxide (AsIII)	As_2O_3
2	White arsenic	As_2O_3
3	Yellow sulfide orpiment	As_2S_3
4	Red realgar (Sulfure)	As_4S_4
5	Paris Green (Copper)	$3Cu(AsO_2)_2$
6	Calcium arsenate (Calsium)	$Ca_3(AsO_4)_2$
7	Lead hydrogen arsenate (Lead)	$PbHAsO_4$
8	Arsenic-mimetite (Lead)	$Pb_5(AsO_4)_3Cl$
9	Erythrite (Cobalt)	$Co_3(AsO_4)_2 * 8H_2O$
10	Cobaltite (Cobalt)	CoAsS
11	Skutterudite (Cobalt)	$CoAs_3$

Table 4: Toxicity of inorganic arsenic (LC50) to fish. (arranged in alphabetically by fish species) [NS = not stated]

S.N	Fish species	Size/age	As species	Duration (h)	LC50, mg As/L	References
1	*Anabus testudinus*	adult	Sodium arsenate	96	18.21	Akter et al., (2008)
2	*Apeltes quadracus*	NS	Arsenite	NS	15 n	EPA (1985)
3	*Catla catla*	adult	Sodium arsenate	96	43.78	Kavitha et al., (2010)
4	*Catla catla*	fingerlings	Arsenic tri-oxide	96	20.41	Lavanya et al., (2011)
5	*Catla catla*	adult	Arsenic tri-oxide	96	10.16	Kausar and Javed (2014)
6	*Channa punctatus*	fingerlings	Arsenic tri-oxide	96	10.8	Shukla et al., (1987)
7	*Channa punctatus*	adult	Sodium arsenate	96	42	Das et al., (2012)
8	*Chanos chanos*	fingerlings	Arsenic	96	7.29	Chou et al., (2006)
9	*Chelon labrosus*	juvenile	Arsenite	96	27.3	Taylor et al., (1985)
10	*Clarias batrachus*	adult	Arsenic tri-oxide	48	84	Bhattacharya and Bhattacharya (2007)
11	*Clarias gariepenus*	adult	Arseneous chloride	96	89	NAH Abdel Hamid (2009)
12	*Cirrhina mrigala*	adult	Arsenic tri-oxide	96	24.5	Kausar and Javed (2014)
13	*Ctenopharyngodon idella*	adult	Arsenic tri-oxide	96	22.17	Kausar and Javed (2014)
14	*Cyprinus carpio*	adult	Arsenic tri-oxide	96	32	Kovendan et al., (2013)
15	*Cyprino donvariegatus*	NS	Arsenite	NS	12.7	EPA (1985)
16	*Danio rerio*	juvenile	Arsenate	96	43	Liu et al., (2008)
17	*Fundulus similis*	juvenile	Arsenic tri-oxide	48	>30	Mayer (1987)
18	*Labeo rohita*	juvenile	Arsenic tri-oxide	96	28.3	Vutukuru et al., (2007)
19	*Labeo rohita*	adult	Arsenic tri-oxide	96	30	Kausar and Javed (2014)
20	*Lepomis macrochirus*	juvenile	Arsenite	96	17.3	Mayer and Ellersieck (1986)
21	*Limanda limanda*	adult	Arsenite	96	28.5	Taylor et al., (1985)
22	*Morone saxatilis*	juvenile	Arsenate	96	10.3	Dwyer et al., (1992)
23	*Notemigonus crysoleucas*	NS	Arsenite	96	12.5	Hartwell et al., (1989)
24	*Oreochromis mosambicus*	adult	Arsenic	96	28.68	Akter et al.,(2008)
25	*Oncorhynchus mykiss*	juvenile	Arsenite	96	91	Buhl and Hamilton (1991)
26	*Oncorhynchus shawytscha*	juvenile	Arsenic penta-oxide	96	21.4	Hamilton and Buhl (1990)

27	*Oryzias latipes*	fingerlings	Arsenic III	7d	14.6	Suhendrayatna et al., (2002a)
28	*Pimephales promelas*	juvenile	Arsenite	96	12.6	Spehar and Fiandt (1986)
29	*Thymallus arcticus*	juvenile	Arsenic penta-oxide	96	5.5	Buhl and Hamilton (1990)
30	*Xyrauchen texanus*	fry	Arsenate	96	17.8	Hamilton and Buhl (1997)

Table 5: Accumulation of arsenic in fish organs. (Arranges in alphabetically by fish species.)

S.N.	Fish Species	Accumulation of As in fish organs	References
1	*Clarias batrachus*	Liver > gills > blood > skin > brain	Kumar and Benerjee 2012a.
2	*Clarias batrachus*	Gills > blood > muscles > skin > brain	Kumar and Benerjee 2012a.
3	*Clarias gariepinus*	Muscles > Liver > gills > bone > gut > fins.	Tyokumbur et al., 2014.
4	Common carp	Liver > kidney > intestine > gonads > skin	Jabeen et al., 2012
5	Common carp	Intestine>bone>gill>liver>muscle>brain	Han et al., (2012)
6	Common carp	Kidney > liver > fins > scales	Kausar and Javed 2014.
7	*Coregonus clupeaformis*	Pyloric caeca> liver>intestine> stomach	Pedlar and Klaverkamp (2002)
8	*Danio rerio*	Liver> gills > muscles> heart>intestine	Hamdi et al., 2009
9	*Heteropneustis fossilis*	Liver > intestine > muscles.	Begum et al., (2013)
10	*Labeo rohita*	Liver > muscles.	Pazhanisamy et al., 2007
11	*Oreoc mykiss*	Muscle > liver > spleen.	Čelechovská et al., (2012)
12	*Tilapia zilli*	Muscle > liver > gut > gills > bone > fins	Tyokumbur et al., 2014.
13	*Tilapia mossambicus*	Gills > skin > muscles	Thakur and Mhatre 2015