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REGULAR ARTICLE

ARSENIC-INDUCED OXIDATIVE STRESS IN FRESH WATER CATFISH *TILAPIA MOSSAMBICA*

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SUMMARY

An experiment was conducted to investigate bioaccumulation potential of arsenic and changes in oxidative stress indices in liver tissue from arsenic exposed fish *Tilapia mossambica* were used for the present investigation. The *Tilapia mossambica* were exposed to two non lethal doses of arsenic for 10 days, which induced tissue lipid peroxidation, increased the ratio of oxidized to reduced glutathione and produced excess H_2O_2 within 1–2 days of exposure. Furthermore, arsenic treatment increased the activity of antioxidant enzymes such as superoxide dismutase (SOD), and catalase but decreased glutathione reductase (GR) activity within a day of exposure, indicating the generation of oxidative stress in fish at an early stage. It is therefore concluded that peroxisomal H_2O_2 metabolizing enzymes are potential targets of arsenic toxicity in *Tilapia mossambica*.

Keywords: Arsenic, Fish, LPO, Antioxidants
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1. Introduction

In recent years, anthropogenic activities such as treatment of agricultural lands with arsenical pesticides, treating of wood using chromated copper arsenate, burning of coalin thermal power plants, and gold-mining operations have increased the environmental pervasiveness of arsenic and its release into freshwater bodies [1]. It has also been demonstrated that fish can accumulate arsenic in the liver to a moderately high level in a dose related manner [2]. Of the total arsenic thus accumulated in the liver, the proportion of inorganic arsenic was found to be higher than that of the organic forms in case of freshwater fish [3]. It is therefore conceivable that the freshwater fish have considerable risk of damage through accidental or sustained release of arsenic in freshwater. Since not much is known about the biochemical mechanism of arsenic toxicity in fish, we have attempted to develop a biomarker of arsenic exposure in *Clarias batrachus*, a freshwater air-breathing teleost. Since an early indication of toxicity could be used as a hazard assessment tool, we have also stressed the early effects of arsenic to this fish. The biochemical basis of the toxicity of arsenic has been investigated in various other model systems. It has been indicated that arsenic toxicity may be exerted through excess production of reactive oxygen species (ROS), namely superoxide (O_2d),

hydroxyl (OHd), and peroxyl (ROOd) radicals and hydrogen peroxide [4]. The ability of inorganic arsenic to bind critical thiol groups of proteins and cellular nonprotein thiols such as glutathione (GSH) is another mechanism through which arsenic could impart its toxicity. Regulation of the activity of antioxidant enzymes such as glutathione peroxidase (GPx), glutathione reductase (GR), and GSH-independent antioxidant such as superoxide dismutase (SOD) and catalase by arsenic has also been implicated in the toxic mechanism [5].

Arsenic is a toxic element for humans and it is commonly associated with serious health disruptions [6]. Total diet As studies carried out in various countries have shown that fish and shell fish are the most significant dietary source of As, accounting for nearly three quarters of total intake [7]. The concentration of As was found in environmental samples, mainly in waters, where inorganic form is predominant [8]. Arsenic exposure has been related to the appearance of some types of cancer [9]. Some of these human health effects currently observed in population of south and south eastern Asia, particularly in countries such as Bangladesh and India. Besides the direct exposure of humans to As through drinking contaminated water, the As might also be biologically available to aquatic organisms, such as fish which are used as human food thereby providing an additional source of As. Arsenic has a considerable tendency to accumulate in bottom sediments [10]. For this reason, issues related to As content in aquatic organisms and sea fish in particular, have attracted considerable attentions. The relevance of this as intake will depend on the concentration of As accumulated by the fish [11]. During recent years, serious concern has been voiced about the rapidly deteriorating state of fresh water bodies with respect to toxic metals pollution. Fishes are often at the top of the aquatic food chain and accumulate large amounts of some metals from the water [12]. Water pollution leads to fish contamination with toxic metals from many sources, e.g., industrial and domestic wastewater, natural run off and contributory rivers [13]. Fishes, living in polluted water may accumulate toxic trace metals via their food chains, they assimilate metals by ingestion of particulate material suspended in water, ion exchange of dissolved metals across lipophilic membranes, e.g., the gills, adsorption on tissue and membranes surfaces [14]. The bioaccumulation of metals is therefore, an index of the pollution status of the relevant water body [15].

Arsenic (As) is a pollutant widely distributed in nature and released into the environment through industrial processes and agricultural practices [16]. Different countries have established various As concentration limits for the protection of aquatic life [17]. Arsenic can be found in both organic and inorganic compounds with variable oxidation states. In general, for inorganic forms, arsenite (AsIII) is considered more toxic than arsenate (AsV) [18], while organic form seem to be less toxic [19]. In water, As is normally found in the pentavalent form, but upon consumption by humans and most mammalian species its metabolic conversion includes reduction of arsenate to arsenite and ultimately methylated As species. Moreover, some studies showed that GSH could be involved in the As biotransformation processes, serving as an electron donor for the reduction of arsenate to arsenite in aqueous solution and in erythrocytes [20,21]. Exposure to As can affect enzyme activities such as pyruvate dehydrogenase and α-ketoglutarate dehydrogenase [22], cell signaling and DNA repair and induce reactive oxygen species (ROS) generation in mammalians cells after very short times of exposure. This antioxidant defense system includes the tripeptide glutathione. Glutathione (GSH) and enzymes related to GSH comprise a system that maintains a reduced intracellular environment and acts as a primary defense against excessive generation of harmful ROS [23]. To protect cells against some oxidative damage by ROS, a detoxification battery has evolved in aerobic organisms [5,24]. Sakurai et al. [25] showed that when cells (TRL 1215) were exposed to monomethylarsenic acid (MMAV) at the mM range there was a significant increase in cytolethality and enhanced the production of cellular ROS parallel with a depletion of cellular

reserves of GSH. Furthermore, specific glutathione dependent methyl transferase catalyzes the methylation of iAs in mammals [26].

Fish is an important source of protein rich food for humans and has become a popular component of human diet during the last few decades in entire Indian subcontinent. This fish has been recognized as an important candidate species for commercial cultivation because of many economic criteria [27]. Natural water reservoirs are traditionally being used for aquaculture and they contribute significantly to total fish production across the globe. Unfortunately, these natural resources are getting polluted with environmental pollutants and contaminants [28]. Industrial, agriculture and communal wastewater containing alarmingly high levels of heavy metals cadmium compounds enter into different water reservoirs without their prior treatment. This has resulted in higher concentrations of heavy metal residues in many fish culture ponds in different parts of India [29]. Fishes, living in polluted water may accumulate toxic trace metals via their food chains, they assimilate metals by ingestion of particulate material suspended in water, ion exchange of dissolved metals across lipophilic membranes, e.g., the gills, adsorption on tissue and membranes surfaces. The bioaccumulation of metals is therefore, an index of the pollution status of the relevant water body. The pollution level of big fresh water lake (Manchar Lake).

Free radicals and oxidative stress have been incriminated in the pathogenesis of several toxicities including heavy metal exposure in experimental rats [30,31] and freshwater teleost, *Oreochromis mossambicus* (tilapia) [1] and goldfish (*Carassius auratus* gibelio Bloch.) Zikić et al. [32] suggesting a beneficial role of antioxidants in the alleviation of Cd toxicity in fishes. Garlic and taurine are known to have antioxidant properties in biological systems, and taurine has ameliorative effect on Cd toxicity in rats. Therefore, this investigation was aimed to study the changes in oxidative stress indices in liver tissues of *Tilapia mossambica* exposed to arsenic.

2. Materials and methods

Chemical

Heavy metal arsenic has purchased from High Media Chemicals, India Private Limited, Mumbai, India.

Experimental fishes

The fresh water fish *Tilapia mossambica* were collected from fish farm at Puthur, Tamil Nadu, India. The collected fish were acclimated to laboratory condition for 15 days [33]. They were checked thoroughly for injury and disease conditions, and only healthy fishes were used for this study. After washing with 0.01% $KMnO_4$ solution for 15 min, they were placed in nine plastic pools (500 L) containing non-chlorinated water. Prior to the start of the experiment, the fishes were acclimatized to the food and laboratory conditions with 12 h dark and 12 h light cycles, pH range of 6.95 to 7.60 and temperature ranging from 16 to 24 °C for 15 days.

Experimental design

Fishes were divided into three equal groups each comprising of 36 fishes. Each group was kept in separate plastic tanks. The first group was kept as negative control; the fishes were maintained in water containing normal water without any treatment. The fishes of two groups were exposed to a sub-lethal concentration of 0.1 ppm and 0.05 ppm concentration of Arsenic added in the water for 5 and 10 days respectively. Solutions were renewed once daily after exposure period, animals (n=20/group) were sacrificed and the gills were removed, homogenized and stored at −80 °C for further biochemical analyses. Reactive oxygen species concentration (ROS) was analyzed in fresh tissue. The following variables were measured: total intracellular GSH content, enzymes activities (catalase, glutathione reductase, glutathione-S-transferase, and glutamate cysteine ligase), lipid peroxidation.

Preparation of basal diet for fish

The basal diet for fishes was prepared as suggested by Datta and Kaviraj (2003) by mixing rice bran (25%), wheat flour (25%), mustard oil cake (22%), fish meal (26%) and mineral mixture (2%) containing copper (3.12%), cobalt (0.45%), magnesium (24.14%), iron (9.79%), iodine (1.56%), zinc (21.3%), calcium (30.0%), phosphorous (8.25%). Ingredients were mixed to form dough, passed through the sieve to prepare pellets and dried in hot air oven at 60–70 °C. Experimental fishes were fed with feed once daily and the residuals were removed after 48 h by siphoning.

Determination of LC_{50} of arsenic trioxide

The 48 h median tolerance limit or LC_{50} value was determined by straight-line graphical interpolation of the data. Briefly, the median value is derived by plotting the experimental data on semi logarithmic coordinate paper, with test concentration on the logarithmic scale and percentage of survival on the arithmetic scale. A straight line was drawn, joining two points that were above and below 50% survival rate. The point of intersection of this straight line with the 50% survival line was considered as the index of lethality or LC_{50}. In the present case, LC_{50} was found to be 84 mg/L for *C. batrachus*. The measurements based upon acute poisoning were unhelpful since they told nothing of the impact that the much lower concentrations had upon the ability of the affected organisms to undertake the responses necessary to ensure survival and, more particularly, reproduce successfully. Such responses can only be investigated with organisms not at the point of death, i.e., in truly sublethal studies. In the present investigation, therefore, the 10-d LC_{50} was determined (15 mg/L) and no mortality was recorded below 8.5 mg/L.

Estimation of lipid peroxidation

The level of lipid per oxidation in kidney tissue was estimated with the method of Nichens and Samuelson. Whole kidney tissue homogenate was prepared in Tris – HCL buffer (pH 7.5). 1 ml of the tissue homogenate was taken in a clean test tube and 2.0 ml of TBA-TCA-HCL reagent was added and then mixed thoroughly. The mixture was kept in a boiling water bath (60°C) for 15 minutes. After cooling, the mixture was centrifuged at 1000 rpm for 10 minutes and the supernatant was taken to read the absorbance of the chromophore at 535 nm against the reagent blank in a UV visible spectrophotometer (Spectronic –20, Bausch and Lamb). 1, 1′, 3, 3′ tetra methoxy propane was used to construct the standard graph.

Estimation of reduced glutathione

The glutathione (reduced) in whole kidney tissue was determined according to the method of Beutler and Kelley. The kidney was homogenized in PBS buffer solution and centrifuged at 2500 rpm for 5 minutes. 0.2 ml of the sample (supernatant) was taken n a clean test tube and 1.8 ml of EDTA solution was added. To this 3.0 ml of precipitating reagent was added and mixed thoroughly and kept for 5 minutes before centrifugation at 3000 rpm for 10 minutes. In each test tube, 2.0 ml of the filtrate was taken and to this 4.0 ml of 0.3M disodium hydrogen phosphate solution and 1.0 ml of DTNB reagent were added. The appearance of yellow colour was read at 412 nm in UV-visible spectrophotometer (Spectronic-20, Bausch and Lamb). A set of standard solution containing 20-100 μg of reduced glutathione was treated similarly.

Assay of glutathione reductase (GR)

The glutathione reductase was analysed by the method of Kenji. GR activity was measured and using the principle that GR utilizes one molecule of NADPH to catalyze the conversion of one molecule of substrate (GSSG) into two molecules of GSH. At first the liver was blanched in 150mM KCl, blotted dry, and weighed. Then a 5% liver homogenate was prepared in 50mMphosphate buffer (pH 7.4). The liver homogenate was then ultra centrifuged at 100,000g for 60 min at 4 1C to prepare the cytosol containing GR. Due to the thermolabile nature of GR, the temperature of

the assay was strictly maintained at 41C. An assay mixture was constituted with50mM phosphate buffer (pH 7.6), 1mM EDTA, 0.1mM NADPH, 1mMGSSG, and 0.1% BSA. The mixture was preincubated for 5 min at room temperature, the sample (cytosol) was added to it and mixed, and the decrease in absorbance was monitored at 340nm in a Beckman DU 640 spectrophotometer. The increase in D O.D./min was calculated using a blank, which contained all the components of the assay mixture except GSSG. The activity of the enzyme was calculated using the molar extinction coefficient of NADPH (2340 ¼ 6:22 103M1 cm1) and the results were expressed in terms of U/mg protein.

Estimation of Catalase

Catalase was assayed calorimetrically with adopting the method of Sinha. The whole kidney tissue was homogenized in phosphate buffer solution. 0.1 ml of the homogenate was taken in a test tube and 1.0 ml of phosphate buffer was added. 0.4 ml of hydrogen peroxide was added to the above mixture. After 30 and 60 seconds 2.0 ml of dichromate acetic acid reagent was added. Test tubes were kept in boiling water bath (60°C) for 10 minutes. The mixture was cooled immediately in tap water and the colour was read at 620 nm against a reagent blank in UV-visible spectrophotometer (Spectronic-20, Bausch and Lamb).20-100µ moles of H_2O_2 is used as standard

Estimation of superoxide dismutase

The activity of superoixde dismutase was assayed with the method of Kakkar. The kidney tissue was homogenized with 3 ml of 0.25 M sucrose solution and centrifuged at 10,000 rpm in cold condition for 30 minutes. The supernatant was dialysed against Tris HCL buffer (0.0025M, pH 7.4). The supernatant, thus, obtained was used as an enzyme source.

Enzyme assay

The assay mixture (2.0 ml) contained 1.2 ml of sodium pyrophosphate buffer, 0.1 ml of PMS, 0.3 ml of NBT, 0.1 ml of enzyme preparation (tissue homogenate) and 0.3 ml of water. The reaction was started by the addition of 0.2 ml of NADH solution and then it was incubated at 30°C for 90 seconds. After incubation the reaction was arrested by the addition of 1.0 ml of glacial acetic acid. The reaction mixture was stirred and shaken with 4.0 ml of n-butanol. The mixture was allowed to stand for 10 minutes and then centrifuged for 15 minutes at 3000 rpm. After centrifugation, the butanol layer was separated. The colour intensity of the chromogen was measured at 560 mm in UV visible spectrophotometer (Spectronic-20, Bausch and Lamb).Water was used as blank.

Statistical significance was evaluated using ANOVA followed by Duncan Multiple Range Test (DMRT).

3. Results

Lipid peroxides (LPO)

Table 1 shows the tissue LPO levels in liver at different observation periods. The level of LPO in liver was significantly ($P<0.05$) greater in the arsenic exposed fishes. But in the case of higher concentration, the LPO level in liver increased significantly ($P<0.05$).

Table 1. Level of lipid peroxidation and antioxidants in the liver tissue of *Tilapia mossambica* treated with arsenic

Parameters	Group-I control	Group-II 15 days	Group-III 30days
LPO (nmole/mg.of protein)	5.43 ± 0.05	5.54 ± 0.09*	5.83 ± 1.01*
GSH (nmole /mg.of protein)	320.8 ± 0.07	392.4 ± 0.52*	456.9 ± 0.69*
GST(nmole/NADPH /min / mg.of protein)	1.9 ± 0.64	1.2 ± 0.78*	1.02 ± 0.62*
CAT (Unit/mg.of protein	262.3 ± 0.90	287.4 ± 1.18*	2.98 ± 0.75*
SOD (Unit/mg.of protein	12.47 ± 0.28	14.61 ± 0.46*	16.86 ± 0.82*

Mean ± S.D of six individual observations. Significance *($p<0.05$) Group I compared with group II and III

Level of reduced glutathione

The GSH activity was significantly higher in liver in 5 ppm arsenic exposed groups till 15th and 30th day respectively, as compared to respective control levels. The subsequent to observations in low and high exposure groups revealed a comparable ($P < 0.05$) GSH activity in liver between positive and negative controls.

Level of Glutathione reductase

Table 1 shows the Glutathione reductase activity at different observation periods in different groups. The Glutathione reductase activity reduced significantly in liver with increasing duration of arsenic exposure in fishes.

Level of Catalase

The catalase activity at different observation periods in different groups. The catalase activity reduced significantly in liver with increasing duration of arsenic exposure in fishes.

Level of superoxide dismutase

Table 1 shows the superoxide dismutase activity at different observation periods in different groups. The superoxide dismutase activity reduced significantly in liver with increasing duration of arsenic exposure

4. Discussion

Arsenic is known to induce LPO in various animal and cellular models. However, arsenic toxicity studies in fish mostly dealt with chronic exposure to a low-dose arsenic and indicated that it may or may not induce LPO, depending on the species involved. In the present study, the role of GSH in antioxidant responses after As exposure was analyzed in liver tissue of *Tilapia mossambica*. Acute exposure to As is known to induce a cellular stress response in mammalian cells that involves increase in heat-shock proteins, heme-oxygenase, and GSH. The present results showed an increase in GSH levels, which can explain the absence of increased ROS concentration and of LPO levels in As exposed fishes. Other authors, in fact, have observed a significant increase in LPO content in the Indian catfish (*C. batrachus*) after exposure to low concentration of As (1–3 mM) [34]. Interestingly, other authors have observed augmented liver GSH levels after exposure of the goldfish to 200 μ of sodium arsenite. The same authors observed absence of arsenic effect of lipid peroxidation measured as thiobarbituric acid content and protein carbonyl groups, although higher levels of lipid hydroperoxides was registered in liver goldfish after 1 and 4 days of exposure. In this study no significant changes were observed for glutathione reductase (GR), which has the critical role of reducing GSSG in order to maintain high levels of intracellular GSH. In fishes exposed to low concentration of As, a decrease in GR activity [34], while Schuliga et al. [23] showed an increase of GR activity in fibroblasts, but the mechanisms by which As regulates the activity of GR in vivo remain unclear. The increments in GSH levels and GCL activity observed in this study indicate that the GSH antioxidant response to the As pro-oxidant challenge is mediated through de novo synthesis of the thiol, while the reduction rate of GSSG remained unchanged. Note that, however, the As-induced antioxidant responses can be deleterious to the organisms at long term, taking into account that GSH synthesis divert ATP from other cellular purposes and that the alteration of the redox state, even when moved to a more reduced one, can alter cellular functions. Also, the lowering of oxygen consumption observed at the highest As concentration (100 μg/L) suggested a loss of aerobic metabolism which is another potential problem in ATP generation.

The enzyme GST catalyze conjugation of GSH to electrophilic substrates and, despite these enzymes are known to be involved in As metabolism and detoxification, our results did not reveal significant change in *Danio rerio*. In a previous study, GST activities were lowered in the polychaeta *Laeonereis acuta* after one-week exposure to 500 μg As/L [35], while Sakurai et al. [25] showed that human cells exposed to

monomethylarsonic acid (MMAsV) increased cellular GST activity, and addition of a specific GST inhibitor, significantly enhanced the cytolethality of both MMAsV and arsenite. Note that, in the context of present data, the fact that GST remained unaffected indicates that the tested concentration were not deleterious since some authors proposed that GST is involved in the detoxification of endogenous molecules such as 4-hydroxykenals (membrane peroxides) and base propenals which are products of the oxidative DNA degradation. Also CAT activity was not affected in As exposed fishes, further supporting the efficacy of increased GCL and GSH in preventing additional oxidative perturbations. This is in contrast to observation in *C. batrachus* where an increase in CAT activity after As exposure [34]. These different responses in antioxidant system and oxidative damage observed in fishes and other organisms show clearly that As toxicity is dependent upon species and tissue analyzed. As metabolism and detoxification include absorption, distribution, biotransformation, and excretion factors [23] can also greatly vary in different tissues. The decrease in activities of these two enzymes can inhibit the citric acid cycle and thereby decrease the generation of reducing equivalents such NADH and NADPH, impairing ATP production [22] and oxygen reduction to form water. Moreover, As can affect NADH dehydrogenase and cytochrome oxidase. The significant decline in the activity of two enzymes would result in the inhibition of electron flow from NADPH to oxygen, augmenting the chance of ROS generation and lowering oxygen consumption. Note that at long term, a reduction in ATP synthesis capability should impair GSH synthesis, favoring an oxidative stress scenario.

Inorganic As occurs in water mostly as arsenate, whereas arsenite may exist in anaerobic water or with low dissolved oxygen. In this study, we showed that the predominant As form in water was arsenite, although under aerobic conditions, suggesting that the oxidation state of As observed in this study could be due mostly to microbial activity associated with fish. In aqueous environments, prokaryotes and eukaryotes reductively biomethylate inorganic As to dimethylarsenic (DMA) and monomethylarsonic (MMA). As a general conclusion, it can be stated that As affected the antioxidant responses in zebrafish gills in terms of GSH concentration and GCL activity, although no oxidative damage was detected. It is likely that with longer exposure times, the decrease of oxygen consumption observed here could impair ATP production, affecting GSH synthesis and triggering oxidative damage.

The higher activities of SOD and catalase in liver of different treatment groups suggested that liver is an active site for synthesis of these antioxidant enzymes. SOD and catalase activities were greater in Cd exposed non-treated fishes than in fishes without arsenic exposure, and the activity tended to decrease from day 15 onward in arsenic exposed fishes. In experimental arsenic toxicity in Nile tilapia (*Oreochromis niloticus*), Almeida et al. [36] also observed increased activities of SOD and catalase in liver and muscles. Basha and Rani [1] also noted significant elevations of SOD and catalase activities in liver and kidney from day 7 onward, and these activities were maintained until day 15 and then decreased slightly on day 30 of exposure. Increased activities of SOD and catalase in tissues might be due to the detoxification mechanisms under long term exposure of Cd to protect animals from free radicals. Basha and Rani [1] suggested that upregulation of enzyme production might be a defense mechanism, providing first line of defense against Cd toxicity before the induction of metallothionein synthesis.

In the present study, the role of GSH in antioxidant responses after As exposure was analyzed in liver tissue of *Tilapia mossambica*. Schuliga et al. [23] showed that up-regulation of GCL activity by arsenite parallels a corresponding increase in the level of GCL mRNA and also showed that gene expression of GSH-related enzymes in human cells is increased under conditions of oxidative stress. GCL activity is the rate-limiting step in the de

novo synthesis of GSH and its expression can be modulated by a number of different factors, including depleting agents, reactive oxygen and nitrogen species, cytokines, and hormones. It has been widely reported that iAs can be toxic in mammalian cells by induction of a severe burst of ROS production and that cellular GSH scavenges these ROS [25]. Acute exposure to iAs is known to induce a cellular stress response in mammalian cells that involves increase in heat-shock proteins, heme-oxygenase, and GSH.

The present results showed an increase in GSH levels, which can explain the absence of increased ROS concentration and of LPO levels in As exposed fishes. Other authors, in fact, have observed a significant increase in LPO content in the Indian catfish (*C. batrachus*) after exposure to low concentration of As (1–3 mM) [34]. Interestingly, other authors as have observed augmented liver GSH levels after exposure of the goldfish to 200µ of sodium arsenite. The same authors observed absence of arsenic effect of lipid peroxidation measured as thiobarbituric acid content and protein carbonyl groups, although higher levels of lipid hydroperoxides was registered in liver goldfish after 1 and 4 days of exposure. In this study no significant changes were observed for glutathione reductase (GR), which has the critical role of reducing GSSG in order to maintain high levels of intracellular GSH. In fishes exposed to low concentration of As, a decrease in GR activity was reported [34], while Schuliga et al. [23] showed an increase of GR activity in fibroblasts, but the mechanisms by which As regulates the activity of GR in vivo remain unclear. The increments in GSH levels and GCL activity observed in this study indicate that the GSH antioxidant response to the As pro-oxidant challenge is mediated through de novo synthesis of the thiol, while the reduction rate of GSSG remained unchanged. Note that, however, the As-induced antioxidant responses can be deleterious to the organisms at long term, taking into account that GSH synthesis divert ATP from other cellular purposes and that the alteration of the redox state, even when moved to a more reduced one, can alter cellular functions. Also, the lowering of oxygen consumption observed at the highest As concentration (100 µg/L) suggested a loss of aerobic metabolism which is another potential problem in ATP generation.

Fattorini and Regoli [37] observed remarkable accumulation of As in the branchial crown of *Sabella spallanzanii* with dimethylarsinate (DMA) as the main As metabolite, while in another polychaete species, *Arenicola marina*, As is accumulated mostly in the inorganic forms [38]. It can be stated that As affected the antioxidant responses in zebrafish gills in terms of GSH concentration and GCL activity, although no oxidative damage was detected. It is likely that with longer exposure times, the decrease of oxygen consumption observed here could impair ATP production, affecting GSH synthesis and triggering oxidate damage.

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