International Journal of Academic Research and Development
ISSN: 2455-4197
Impact Factor: RJIF 5.22
www.academicsjournal.com
Volume 3; Special Issue 1; February 2018; Page No. 16-21

Behavioural and biochemical changes in *Channa punctatus* Exposed to Arsenic and its Possible Revival with Turmeric

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Abstract
Arsenic is a moderately abundant element on the earth's crust released into the environment through both geogenic processes and anthropogenic disturbances. A high concentration of arsenic has been found in many areas of Assam which is a great matter of concern. The freshwater bodies are the main source of storage of arsenic and the rising levels of arsenicals in aquatic ecosystem and their effect on the aquatic organisms has now been recognized as a serious environmental threat. In fishes, arsenic (As) is absorbed via the gills and through contaminated food which is capable of causing disturbance to the antioxidant system. Arsenic can exist both in organic or inorganic form in nature. In general inorganic arsenicals are more toxic than organoarsenicals. Arsenic binds with sulfhydryl groups and disrupts sulfhydryl containing enzymes. Since fishes respond to toxicants in a similar way as higher vertebrates including human, hence *Channa punctatus* were taken as a laboratory animal model in the present study. *Channa punctatus* were exposed to 4.6 mg l^{-1} of sodium arsenite ($NaAsO_2$). Behavioural manifestations were recorded in a definite interval after being exposed to $NaAsO_2$. Enzymes are biochemical macromolecule which control metabolic processes of organisms. Since a slight variation in enzyme activities would affect the organism, hence by estimating the enzyme activities in an organism, metabolic disturbances in its body can be easily found. Significant alterations in the total protein and in the marker enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were observed in arsenic treated fishes. Arsenic treated fishes were then exposed to turmeric extract (1 mg l^{-1}) and significant revival from arsenicosis has been observed.

Keywords: $NaAsO_2$, AST, ALT, sulfhydryl groups, organoarsenicals, marker enzymes

Introduction
Arsenic is an element which is present at low concentrations in air, soil and water. Arsenic, a non-essential trace element, a potent toxin, mutagen and xenobiotic metalloid has recently appeared as a major pollutant of drinking water in several districts of Assam, West Bengal, Tamilnadu and Andhra Pradesh. At present, one of the most worldwide environmental problems is that the drinking water has been polluted by arsenic. Since aquatic environment is the ultimate sink for all pollutants, aquatic toxicity testing has became an integral part of the process of environmental hazard evaluation of the toxic chemicals. Fish, as a living bio indicator organism, play an increasingly important role in monitoring of water pollution since they respond with great sensitivity to changes in the aquatic environment.

Contamination of natural water system with heavy metal is found to be at concentrations below those that cause mortality, but those low levels of toxicity may be enough to interfere with the normal functioning of the body. Fish behaviour is one of the ideal parameter for studying the sublethal impacts (Moss, 1998; Scott and Sloman, 2004; Weis and Candelmo, 2012) [15, 18, 22]. Behaviour is affected by various environmental toxicants (Scott and Sloman, 2004) [14].

The use of biochemical measurements in organisms as indicators of pollution, give information about the adaptive or deleterious responses in organism exposed to a certain amount of chemicals. Such analysis provides early warning signals before other toxicological points, including death are evident (Livingstone, 1998) [11]. Understanding of the protein components of cell becomes necessary in the light of the radical changes taking place in protein profiles during pesticide intoxication. The physiological and biochemical alterations observed in an animal under any physiological stress can be correlated with the structural and functional changes of cellular proteins (Lehninger, 2004; Harper, 2003) [10, 5]. Aminotransferases mobilise the aminoacids into carbohydrate and lipid metabolism. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are two aminotransferases, mainly involved in the inter-conversion of important compounds such as pyruvate, oxaloacetate, α-ketoglutarate and aminoacids thus bringing the protein and carbohydrate metabolism on one hand and alanine, aspartic acid and glutamic acid on the other (Moore, 1964; Knox and Greengard, 1965) [14, 7].

Recently a number of organic forms of antioxidant molecules have been widely studied to explore its preventive capacity and its probable use in natural therapeutics. One of the most sought after natural antioxidant molecule bearer is turmeric (*Curcuma longa*). Turmeric is composed of three curcuminoids namely curcumin, demethoxycurcumin and bisdemethoxycurcumin and also volatile oils like tumerone, atlantone, zingiberone as well as sugars, proteins and resins (Al-Suhaimi *et al*., 2011; Jager R *et al*., 2014) [1, 6]. Curcumin can scavenge reactive oxygen species and thus initiates an antioxidant response (Trujillo J. *et al*., 2013) [21]. The phenolic groups present in the curcumin are reported to have strong

antioxidant and anti-inflammatory activity while the ketonic group and double bonds in it are found to have metal chelating property (Dinkova-Kostova and Talalay, 1999; Suzuki *et al*., 2005) [4, 20].

Thus the aim of the present study was to investigate the behavioural and biochemical changes in freshwater teleost, *Channa punctatus* (Bloch) exposed to arsenic and to assess the effect of turmeric in the revival of arsenic induced toxicity with the following objectives:

- To study the behavioural responses in the arsenic exposed fish.
- To study some selected biochemical parameters of arsenic exposed fish.
- To study the possible revival of arsenic induced behavioural and biochemical changes by using turmeric.

Materials and methods

Experimental fish specimen and chemicals

For the present study *Channa punctatus* was selected. *Channa punctatus* is a commonly available freshwater fish in India. Live adult fish specimens of *Channa punctatus* were collected from different markets of Guwahati. They weighed approximately 65-70gm each, having average length of 16 to 18 cm. The fishes were acclimatized for 15 days in glass aquaria (75X30X60 cm) containing tap water. The physicochemical characteristics of test water like temperature, pH, dissolved oxygen, carbon dioxide concentration, and hardness were monitored frequently following standard procedure (APHA, 2005) [2]. Fish were fed daily during the acclimatization period with commercial dry feed pellets. The feeding was discontinued 24 hours prior to exposure. The water of the aquarium was changed daily to remove waste products that have accumulated in the medium.

Sodium arsenite ($NaAsO_2$), molecular weight: 129.91, Loba Chemie (Ltd.) was used for the experiment. One gram of sodium arsenite was dissolved in 100 ml of distilled water to get an aliquot. To get the required concentrations of arsenic, test concentration was prepared by diluting the aliquot. Water quality of the test solution was maintained according to the standard procedures (APHA, 2005) [2]. The control set of fishes were kept in the experimental water without the addition of sodium arsenite, but kept all other conditions constant.

Turmeric rhizome was collected from the local market and was grinded to get the powder. 1% stock solution of turmeric in water was used to maintain the desired concentration.

The physico-chemical properties like temperature, dissolved oxygen, pH, total hardness, carbon dioxide concentration and ammonia concentration of the tap water were monitored throughout the acclimatisation period and the trial periods according to the standard methods (APHA, 2005) [2]. The water quality parameters did not fluctuate markedly throughout the acclimatization period and during the experimental trials and it remained within the normal range.

Determination of 96 h LC_{50} for Arsenic

The 96-h median tolerance limit (96-h LC_{50}) for sodium arsenite was determined with definitive test in semi-static condition in laboratory as per standard methods (APHA, 2005) [2]. The experiment was carried out in 50 l glass aquariua (75X30X60 cm) containing tap water. Test solution was renewed once in 24 hours. A set of 10 acclimatized fishes were exposed to each of the sodium arsenite concentrations along with control for each concentration. Mortality was recorded at logarithmic time interval that is 24, 48, 72 and 96 hours. The 96-h LC_{50} of sodium arsenite in the present study was found to be 45.96 mg l^{-1} for *C. punctatus* with lower confidence limit of 41.89mg l^{-1} and upper confidence limit of 50.42 mg l^{-1}.

Experimental design

The acclimatized fishes were grouped into four experimental groups each consisting of 10 fish. The experimental groups were categorized as follows:

- Group 1: Control group without any treatment.
- Group 2: Fishes were subjected to sublethal concentration of 4.6 mg l^{-1} sodium arsenite ($1/10^{th}$ 96 hr LC_{50}).
- Group 3: Fishes were subjected to aquous turmeric extract (1 mg l^{-1}).
- Group 4**:** Fishes were subjected to aquous turmeric extract (1 mg l^{-1}) + Sodium arsenite ($1/10^{th}$ of 96 hr LC_{50}) after 96 hours of sodium arsenite exposure.

Behavioural responses in fish of sodium arsenite exposed group, turmeric control group, arsenic and turmeric treated group and in control group were observed daily and recorded after 24 hour, 48 hour, 72 hour and 96 hour.

The fishes were at first anesthesized with MS222 (Ethyl 3-aminobenzoate methanesulfonate) and then from each fish gill, liver and muscles were secluded. The tissues were blotted and weighed. Then the tissues were homogenized in cold distilled water using glass homogenizer. The tissue homogenates were centrifuged twice (4000 rpm) for 5min.The tissue supernatants were separated to be used for the determination of enzymes activities and metabolites contents.

Estimation of total protein content

Total protein content was estimated by the modified method of Lowry *et al.*, (1951) [12]. 5% homogenates of gill, muscle and brain and 2% homogenates of liver and kidney were prepared in 5% trichloroacetic acid and centrifuged at 3000 rpm for 10 minutes. The supernatant was discarded. The suspended protein residue was dissolved in 1 ml of 1N NaOH. From this 0.2 ml of the extract was taken into the test tube and 5 ml of alkaline copper solution (50 ml of 2% Na2CO3 and 1ml of 0.5% CuSO4. 5H2O in 1% sodium potassium tartrate) was added. The contents were mixed well and allowed to stand for 10 minutes. To this 0.5 ml of 50% folin phenol reagent (diluted with distilled water in 1:1 ratio) was added. After 30 minutes, the optical density was measured at 540 nm in a spectrophotometer against a blank. The values were expressed as mg g^{-1} wet weight of the tissue.

Estimation of aminotransferases activity

The activity of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined by the method of Reitman and Frankel (1957). The selected tissues were homogenized in 5% ice-cold 0.25 M sucrose solution. The

supernatants were used for the analysis of the enzyme activities.

Estimation of ALT activity

The reaction mixture of 1.5 ml contains 1 ml phosphate buffer (pH 7.4), 0.1 ml of L- alanine, 0.1 ml of α-ketoglutarate and 0.3 ml of supernatant as enzyme source. The contents were incubated at 370 C for 30 minutes. The reaction was stopped by the addition of 1 ml of 2, 4- dinitrophenyl hydrazine solutions. After 20 minutes, 10 ml of 0.4 N sodium hydroxide was added and the colour developed was read at 545 nm in a spectrophotometer.

Estimation of AST activity

The reaction mixture of 1.5 ml contains: 1 ml of phosphate buffer (pH 7.4), 0.1 ml of L-aspartate (L-Aspartic acid), 0.1 ml of α-ketoglutaric acid and 0.3 ml of supernatant as enzyme source. The reaction mixture was incubated at 370 C for 30 minutes. The reaction was stopped by adding 1 ml of 2, 4-dinitrophenyl hydrazine solution prepared in 0.1 N HCl and was allowed to stand for 20 minutes at room temperature. The rest of the details were the same as for alanine aminotransferase.

Results and discussion

Behavioural Studies

In the present study, fish in control group were found to behave actively with normal swimming behaviour. But when they were exposed to sublethal concentration of sodium arsenite during the initial 24 hour they showed faster movement, restlessness, rapid opercular movements, hyper excitability and the tendency of getting away from the arsenic contaminated water. Throughout the exposure time, the frequency of aggressive behaviours like nudge and nip were found to be increased. However, an important observation was that the fishes were marked with increased body dispigmentation along with profuse mucus secretion all over the body, more profusely in the gill region with an increased exposure time (Table 1). With the end of exposure period, the fish showed a struggle for breathing and a low swimming performance. When the fishes were exposed to turmeric treated water, they behaved almost same as the control group (Table 2). In the other experimental set up, as soon as the fishes were released into the turmeric treated water post 96 hours of sodium arsenite exposure, the test fishes showed a sign of relaxation. The opercular movements, nudge, nip, S jerk and mucous secretion were found in a decreasing trend (Table 3).

Table 1: Behavioural responses of *Channa punctatus* exposed to sodium arsenite at different exposure time.

Exposure periods	Behavioural Responses					
	Opercular movement	Nudge	Nip	S jerk	Dispigmentation	Mucous Secretion
Control	+	_	_	_	_	_
24hr	++	++	++	++	_	_
48hr	++	++	++	+	+	+
72hr	++	++	++	+	+	++
96hr	++	++	++	+	++	++

– = None ; + = mild effect; ++ = Modetrate effect

Table 2: Behavioural responses of *Channa punctatus* exposed to turmeric at different exposure time.

Exposure periods	Behavioural Responses					
	Opercular movement	Nudge	Nip	S jerk	Dispigmentation	Mucous Secretion
Control	+	_	_	_	_	_
24hr	++	+	+	_	_	_
48hr	+	+	+	_	_	_
72hr	+	+	+	_	_	_
96hr	+	+	+	_	_	_

– = None ; + = mild effect ; ++ = Modetrate effect

Table 3: Behavioural responses of *Channa punctatus* exposed to arsenic and turmeric treatment at different exposure time.

Exposure periods	Behavioural Responses					
	Opercular movement	Nudge	Nip	S jerk	Dispigmentation	Mucous Secretion
Control	+	_	_	_	_	_
24hr	++	++	++	+	++	++
48hr	+	+	+	_	++	++
72hr	+	+	+	_	++	+
96hr	+	_	_	_	+	+

– = None ; + = mild effect ; ++ = Modetrate effect

During the initial exposure to higher concentration of sodium arsenite (24 hr. exposure) the fish showed characteristic avoidance behaviour like rapid and erratic swimming with jerky movements and hyper-excitability for which enzymatic and ionic disturbances in blood and tissues may be responsible (Larsson *et al.,* 1981) [8]. The increase in the frequency of surface visit indicated that fish adaptivity changed towards aerial respiration to get more oxygen. Arsenic toxicity also leads to the precipitation of mucus on the gills which indicate the hyperactivity of the mucus glands, which is a defensive

mechanism of the fish to protect the body from adverse environment. Secretion of mucus all over the body might be because of protection against toxicants through epidermis. Sluggishness observed at the end of exposure periods may be because of loss of energy resulted due to erratic swimming, jumping and restlessness. The increased opercular activity in the test fishes might be due to stressful toxic environment along with sensory stimulus to increase the opercular movement for proper ventilation of gills to cope up with hypoxia (Lata *et al*., 2001) [9]. When the fishes were kept in turmeric treated water, they showed almost similar behaviour like that of the control group because turmeric has no damaging effect on the body. Since turmeric has antioxidant property, thus it can protect the body from the damaging effect of arsenic. When the fishes were subjected to arsenic and turmeric treated water post 96 hours of arsenic exposure, the mucus secretion was reduced and thus precipitation of mucus on the gills also reduced which might be because of the protective effect of turmeric. The abnormal behaviours of the fishes were also found to be reduced which might be because of the antioxidant property of turmeric that prevented the neurotoxic effects of arsenic.

Biochemical Studies

1) Total Protein

The results of the present study indicate that arsenic intoxication induced in decrease level of protein content in gill, liver and muscle of the fresh-water fish *Channa punctatus.* But the protein level were almost maintained like that of control when the fishes were treated with turmeric control. Whereas the protein level were increased when the fishes were treated with turmeric after arsenic exposure. The results are summerised in Table 2.1.

Table 2.1: Change in the specific activity levels of Total Protein content (mgg^{-1} wet tissue) in different tissues of fish Channa punctatus exposed to sublethal concentrations of sodium arsenite (1/10th LC50) and turmeric extract.

Tissue	Control	Treated with Arsenic			Turmeric Control			Arsenic + Turmeric		
Time stamp→		96 HRS	10 Days	15 days	96 HRS	10 Days	15 days	96 HRS	10 Days	15 days
Liver	76.09	58.64	51.54	49.3	75.14	75.12	74.99	61.86	67.32	70.66
	±0.30	±0.38 a	±0.40 a	±0.33 a	±0.30	±0.26	±0.11.	±0.26 b	±0.51 b	±0.47 b
Muscle	93.32	69.01	60.97	53.72	92.65	92.21	92.13	59.85	73.91	82.63
	±0.16	±0.70 a	±0.50 a	±0.26 a	±0.19	±0.53	±0.45	±0.21 b	±0.21 b	±0.13
Gill	60.02	44.31	36.61	31.11	59.29	59.21	59.16	49.78	52.12	56.36
	±0.45	±0.19 a	±0.42 a	±0.35 a	±0.58	±0.44	±0.45	±0.84 b	±0.78 b	±0.77 b

Data represented as Mean ±SE, n=5. aP<0.05 versus normal control, bP<0.05 versus sodium arsenite treated group.

2) Amino transferases

Aspartate Amino Transferase (AST) and Alanine Amino Transferase (ALT) activity

The changes in the levels of aspartate amino transferases (AST) and alanine amino transferases (ALT) were studied in different tissues like liver, muscle and gill in the test fish *Channa punctatus* exposed to sublethal concentrations of sodium arsenite and then to turmeric extract. The values are expressed as IUl^{-1}.

Aspartate Amino Transferase (AST)

The results of the present study indicate that arsenic intoxication induced increase activity of AST in gill, liver and muscle of the fresh-water fish *Channa punctatus.* But the AST activites were almost maintained like that of control when the fishes were treated with turmeric control. Whereas the AST activities showed a decreasing trend towards control values when the fishes were treated with turmeric after arsenic exposure. The calculated values of AST with standard error were given in Table 2.2.

Table 2.2: Change in the specific activity levels of Aspartate Amino Transferase (AST) (IUl^{-1}) in different tissues of fish Channa punctatus exposed to sublethal concentrations of sodium arsenite (1/10th LC50) and turmeric extract.

Tissue	Control ↓	Treated with Arsenic			Turmeric Control			Arsenic + Turmeric		
Time stamp→		96 HRS	10 Days	15 days	96 HRS	10 Days	15 days	96 HRS	10 Days	15 days
Liver	4.13±0.44	5.66±0.50 a	5.74±0.52 a	5.92±0.54 a	4.19±0.48	4.24±0.20	4.23±0.30	5.13±0.45 b	4.66±0.43 b	4.45±0.34 b
Muscle	3.79±0.25	5.79±0.36 a	6.06±0.52 a	6.81±0.60 a	3.81±0.53	3.84±0.44	3.85±0.39	4.92±0.50 b	4.58±0.48 b	4.22±0.46 b
Gill	4.41±0.16	6.12±0.4 a	6.86±0.45 a	6.97±0.46 a	4.56±0.37	4.53±0.40	4.48±0.27	5.66±0.24 b	5.01±0.21 b	4.79±0.39 b

Data represented as Mean ±SE, n=5. aP<0.05 versus normal control, bP<0.05 versus sodium arsenite treated group.

Alanine Amino Transferase (ALT)

The results of the present study indicate that arsenic intoxication induced increased activity of ALT in gill, liver and muscle of the fresh-water fish *Channa punctatus.* But the ALT activites were almost maintained like that of control when the fishes were treated with turmeric control. Whereas the ALT activities showed a decreasing trend towards control values when the fishes were treated with turmeric after arsenic exposure. The calculated values of ALT with standard error were given in Table 2.3.

Table 2.3: Change in the specific activity levels of Alanine Amino Transferase (ALT) (IUl^{-1}) in different tissues of fish Channa punctatus exposed to sublethal concentrations of sodium arsenite (1/10th LC50) and turmeric extract.

Tissue	Control ↓	Treated with Arsenic			Turmeric Control			Arsenic + Turmeric		
Time stamp→		96 HRS	10 Days	15 days	96 HRS	10 Days	15 days	96 HRS	10 Days	15 days
Liver	10.79±0.19	14.60±0.09 a	15.96±0.16 a	17.65±0.32 a	10.95±0.19	10.88±0.30	10.87±0.27	13.21±0.40 b	12.43±0.20 b	11.99±0.2 b
Muscle	5.53±0.14	9.21±0.35 a	9.76±0.14 a	10.23±0.38 a	5.59±0.34	5.69±0.23	5.70±0.51	7.98±0.1 b	6.93±0.36 b	6.11±0.2 b
Gill	12.72±0.52	19.04±0.26 a	21.16±0.23 a	23.08±0.56 a	12.95±0.31	12.82±0.37	12.78±0.45	16.87±0.20 b	14.98±0.20 b	13.92±0.25 b

Data represented as mean ±SE, n=5. aP<0.05 versus normal control, bP<0.05 versus sodium arsenite treated group.

Protein is the body builder. The decrease in protein level observed in the arsenic treated fish in the present study may be due to their degradation and also due to their possible utilization for metabolic purposes. The other reason for the loss of proteins might be due to rejection of damaged cellular components of the gills resulted due to the contact of different toxicants, including an arsenic salt (Singh and Banerjee., 2008) [19].

Since the heavy metal stress was known to induce significant change in protein metabolism, it is likely that the aminotransferases were also considerably affected. Increased activities of AST and ALT in different tissues of fish suggest either increased operation of transamination or increased synthesis of amino acids from other sources like glucose or fatty acids during Sodium arsenite intoxication. The AST and ALT are liver specific enzymes and they are more sensitive measure of hepatotoxicity and histophathalogic changes and can be assessed within a shorter time (Balint *et al*., 1997) [3]. The increase in AST and ALT indicate the tissue damages in liver, kidney and gill (Rajyasree and Neeraja, 1989; Oluah, 1999) [16, 13].

Conclusion

Arsenic a xenobiotic metalloid has recently appeared as a major pollutant of water in several districts of Assam, West Bengal, Tamilnadu and Andhra Pradesh. Since fishes respond to toxicants in a similar way as higher vertebrates including human, hence *Channa punctatus* were taken as a laboratory animal model in the present study to investigate the toxicological impact of arsenic. In the present study, interest was being given to the traditional Indian ethnomedicine turmeric *(Curcuma longa)* which has a strong antioxidant and free radical scavenging properties. Because of these properties, turmeric might have resulted the revival of arsenicosis in *Channa punctatus.* Thus turmeric can be used effectively for the revival of arsenicosis in fish.

Acknowledgement

I express my heartiest gratitude to my supervisor Dr. Mamata Goswami, Associate Professor, Department of Zoology, Cotton University, for her constant guidance and advice throughout this research work. I am also grateful to Prof. Dr. Jatin Kalita, Head Department of Zoology, Gauhati University for granting me necessary permissions. I would also like to thank all the faculty members of Department of Zoology, Gauhati University for giving necessary suggestions whenever required during my period of research work.

References

1. Al-Suhaimi EA, Al-Riziza NA, Al-Essa RA. Physiological and therapeutical roles of ginger and turmeric on endocrine functions. Am. J Chin. Med., 2011; 39:215-231.
2. APHA. American Public Health Association, American Water Works Association, Water Pollution Control Federation. Standard Methods for the Examination of Water and Waste water, 21st Edition, Washington, DC, USA, 2005.
3. Balint T, Ferenczy J, Katai F, Kiss I, Kraczer L, Kufcsak O. Similarities and differences between the massive eel *Anguilla anguilla* L. devastations that occurred in lake Ablation in 1991 and 1995. Ecotoxicol. Environ. Saf., 1997; 37:17-23.
4. Dinkova-Kostova AT, Talalay P. Relation of structure of curcumin analogs to their potencies as inducers of Phase 2 detoxification enzymes. Carcinogenesis, 1999; 20:911-914.
5. Harper HA. In: Harper's Review of Biochemistry eds. DW. Martin, P.A. Mayes and V.W. Rodwell. Lange Medical Publications, Marusen, Asia Singapore, 2003.
6. Jager R, Lowery RP, Calvanese AV, Joy JM, Purpura M, Wilson JM. Comparative absorption of curcumin formulations. Nutr. J., 2014; 13:11.
7. Knox WE, Greengard O. Advances in Enzyme regulation Weber G. ed., Pergmon Press, New York, 1965; 3:247.
8. Larsson A, Bengtsson BE, Haux C. Disturbed ion balance in flounder, *Platichthys fiesus* L. exposed to sublethal levels of cadmium. Aquat. Taxicol. 1981; 1(1):19-35.
9. Lata S, Gopal K, Singh NN. Toxicological evaluations and morphological studies in a catfish, Clarias batrachus exposed to carbaryl and carbofuran. J Ecophysiol. Occup. Hlth., 2001; 1:121-130.
10. Lehninger AL. In: Biochemistry, Kalyani Publications Ludhiana, New Delhi, 2004.
11. Livingstone DR. The fate of organic xenobiotics in aquatic ecosystems: quantitative and qualitative differences in biotransformation by invertebrates and fish. Comp. Biochem. Physiol Mol. Integ. Physiol. 1998; 120(1):43-49.
12. Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ. Protein measurement with Folin phenol reagent. J. Biol. Chem., 1951; 193:265-275.
13. Oluah NS. Plasma aspartate aminotransferase activity in the catfish *Clarias albopunctatus* exposed to sublethal zinc and mercury. Bull. Environ. Contam. Toxicol., 1999; 63:343-349.
14. Moore JA. In: Physiology of Amphibia. Academic Press, New York, 1964.
15. Moss B. Ecology of Freshwaters, 3rd ed. Blackwell Science, Oxford, 1998.
16. Rajyasree M, Neeraja P. Aspartate and alanine aminotransferase activities in fish tissue subcellular

fractionation on exposure to ambient urea. Indian J Fish., 1989; 36:88-91.
17. Reitman S, Frankel S. Calorimetric method for the determination of serum glutamic oxaloacetic and glutamic-piruvic transaminases. Amer. J Clin. Pathol., 1957; 28:65-63.
18. Scott GR, Sloman KA. The effects of environmental pollutants on complex fish behaviour: integrating behavioural and physiological indicators of toxicity. Aquat. Toxicol., 2004; 68:369-392.
19. Singh AK, Banerjee TK. Toxic effects of sodium arsenate Na2 HASO4 7H20 on the skin epidermis of air – breathing catfish *Clarias batrachus* L. Veterinarski Arhiv. 2008; 78(1):73-88.
20. Suzuki M, Nakamura T, Iyoki S, Fujiwara A, Watanabe Y, Mohri K. Elucidation of anti-allergic activities of curcumin-related compounds with a special reference to their anti-oxidative activities. Biol. Pharm. Bull., 2005; 28:1438-43.
21. Trujillo J, Chirino YI, Molina-Jijon E, Anderica-Romero AC, Tapia E, Pedraza-Chaverri J. Renoprotective effect of the antioxidant curcumin: Recent findings. Redox Biol. 2013; 1:448-456.
22. Weis JS, Candelmo A. Pollutants and fish predator/prey behavior: a review of laboratory and field approaches. Curr. Zool., 2012; 58:9-20.