Journal of Aquatic Biology & Fisheries | 2022 | 10(1) | pp. 90-95
ISSN 2321–340X
OPEN ACCESS
http://www.jabf.in/
University of Kerala, INDIA

Influence of heavy metal pollution on the antioxidant enzyme activity in *Anabas testudineus* and the recovery responses in pollution free water

Asha Raj, K.R.[1*] and Joseph, M.L.[2]

[1]*Department of Zoology, Christ College, Irinjalakuda, Thrissur, Kerala, India*
[2] *Department of Zoology, St. Albert's College, Ernakulam, Kerala, India*
**E.mail: drasharaj2016@gmail.com*

ABSTRACT

Heavy metal (lead, nickel, zinc, arsenic and cadmium) pollution bioaccumulation in biomass of *Anabas testudineus* from two different stations of Periyar river at Ernakulam district (Station I- Eloor Industrial area, Station II- Irumbanam, Ernakulam) during three seasons (Premonsoon, Monsoon and Postmonsoon) of two year period (2012- 2014) was analysed. The antioxidant enzyme activity (Viz., Catalase, Superoxide dismutase and Glutathione peroxidase) in liver, gills and muscle was also estimated. The recovery responses were studied in fishes kept in aquaria maintained at controlled laboratory conditions for 30 days. The antioxidant parameters showeda variation from the reference under the influence of pollution at the same time the parameters showed a tendency to come back to the normal when the fish from polluted water were kept under pollution free water, showing the recovery potentiality of Periyar river.

ARTICLE HISTORY

Received on: 29-03-2022
Revised on: 27-10-2022
Accepted on: 04-12-2022

KEYWORDS

Heavy metal,
Hepatic biochemical,
Season, Periyar

1. Introduction

Periyar river is the longest river of the state (PWD, 1974) is very importantas far as the central Kerala is concerned (Joseph, 1984). However it is contaminated with a number of hazardous chemicals released from the industries located on the banks of river (Sobha and Anish, 2003). Heavy metals also come under this category of hazardous pollutants (Joseph, 1984). Even though the metals are needed for the proper functioning of biological systems (Singh et al., 2011) its elevated levels causes stress to the inhabitants (Da Silva et al., 2001) because it shows a tendency to bio accumulate (USEPA, 1991) and induces many deleterious changes. Oxidative stress is one among these effects, so the analysis of antioxidant enzyme levels in fish give a picture about the pollution stress over them. The recovery responses shown by the inhabitants while transferring them to unpolluted waters is an indication of the recovery potential of the habitat itself. So, efficient strategies of bioremadiationcan reclaim the river.

2. Materials and Methods

The Study areas include a stagnant water body located in an unpolluted area of Cherthala, Alappuzha district (Control station) is nolonger influenced by the pollutnatsbcause the area is free from industries and reamin isolated from other water bodeis. Based on specific geographical features and anthropogenic activities, two sampling locations (Station I and Station II) were selected in Periyar river. One of the sampling area flowing through the Eloor industrial Estate (Station I). The other one, Station II is located at Irumbanam. Compared to the control site the Station I and Station II are heavily polluted because of the indisriminateindustrialdiscahrges from industries located in the banks. Three separate fish sampleswere collected from the two stations. Station one is a portion of Periyar river passing through the Eloor- Kalamassery, the study area, is 1.5 Km upstream to Eloor ferry is between 10°08'54.46"N latitudes and 76°28'51.66"E east longitudes. The study area is located downstream of industrial belt well known for large- and small- scale industries. The major industries include FACT, TCC, IRE, BZL, etc and are on the banks of the Periyar river (Sobhaand Anish, 2003). Station two is located at Irumbanam, Trippunitura. It is considered as a site located at Chithrapuzha, a tributary of Periyar river. A station in between 9°59'00.1"N latitude and 76°20'44.6"E longitude was selected for the material collection. Factory out lets of BPCL, FACT, IOCL etc were near to this site.

The entire study period was divided into 3 seasons viz., Premonsoon (February-May), Monsoon (south west monsoon June-September) and Post monsoon (October-January). Fish samples were collected at 3 seasons during the last week of the last month of each season. Fish sample (*Anabas testudineus*) were collected using Cast net with the help of local fishermen. All the sample materials were taken to the laboratory without delay. Samples of fish coming under similar size range (10±1 cm)were selected from the samples from each station (Control, Station I and Station II) during the study period. The collected materials were prepared variously.

On reaching the laboratory the fish werecategorized in two groups; one group was introduced into aquarium that has been set in laboratory conditions to carry out the recovery studies. The second group of fish were anesthetized and dissected to collect the organs (liver, gills and muscle). The organ samples were processed (APHA 1995) for heavy metal analysis and antioxidant enzyme assay. The processed biomass sample were sent to STIC (Sophisticated Test and Instrumentation Centre, Kochi, Ernakulam) for the analysis of selected heavy metal (Pb, Ni, Zn, As, Cd) concentration in it using ICP-AES system.

The organ samples were weighed and 5% homogenate were prepared in Tris HCL buffer solution (pH 7.5) using a glass homogenizer for liver and motor and pestle for gills and muscle. Both the equipment was kept in ice cold condition to minimize the enzyme activity. The prepared homogenate was then centrifuged at 3000 rpm for 10 minutes in a

cooling centrifuge (Remi) calibrated at 4^0C. The activity of Catalase, Superoxide Dismutase and Glutathione Peroxidase in the prepared tissue homogenates were carried out using the method suggested by Sinha 1972, Das et al, 2000 and Rotruck et al, 1973 respectively.

Group of fish transferred from polluted waters to clean water under laboratory conditions were maintained under well aerated and well fed laboratory conditions for 30 days in order to analyses the rate of removal of heavy metals from the biomass and the change in the concentration of antioxidants as a part of recovery from the pollutant induced stress. On the 31st day the fish were sacrificed for the liver, gills and muscle. The tissue sample was processed for heavy metal analysis and antioxidant enzyme activity as explained in the previous sessions.

The statistical analysis was carried out using the SPSS software 20.0 Package. One-way analysis of variance (ANOVA) was carried out to compare the concentration of each heavy metal in biomass (liver, gills and muscle) in three stations (control, Station I and Station II) during three seasons (premonsoon, monsoon and postmonsoon). If significant differences were revealed by the ANOVA test, Tukey's test was used to further elucidate which season and station were significantly different. t-test was carried out for the comparison of bio accumulated concentration of different heavy metals in different organs in each station before and recovery for 30 days. Significance level (P value) was set at 0.05 in all tests.

One-way analysis of variance (ANOVA) was also carried out to compare each antioxidant enzyme parameters in each organ in fish from three stations (control, Station I and Station II) during three seasons (premonsoon, monsoon and postmonsoon). If significant differences were revealed by the ANOVA test, Tukey's test was used to further elucidate the enzyme activity in which season and station were significantly different. t-test was carried out for the comparison of altered enzyme activity in each organs in fish collected from each station before and recovery for 30 days. Significance level (P value) was set at 0.05 in all tests. Correlation analysis was also carried out to know the relationship between enzymes activities in each organ of fish collected from three stations.

3. Results and Discussion

Heavy metal concentration in all the samples (liver, gills and muscle)always followed the order Zn > Pb > Cd > Ni (Table 1). Liver and gills commonly showed more tendency to accumulate heavy metals than muscle (Table 1). Highest

Table 1. Seasonal variation of heavy metal in Biomass and their influence on Antioxidant enzyme activity in *Anabas testudineus* collected from different stations

Season	Organ	Station	Pb	Ni	Zn	Cd	CAT	SOD	GPx
Pre monsoon	Liver	Control	0.11±0.01	BDL	0.92±0.08	0.007±0.003	10.67±1.15	0.39±0.04	4.63±0.37
		Station I	0.12± 0.01	0.01±0.001	1.17±0.11	0.01±0.001	4.01±0.46	0.61±0.06	5.67±0.53
		Station II	0.15±0.01	0.01±0.001	1 ±0.1	0.013±0.001	3.13±0.39	1.32±0.14	6.33±0.62
	Gill	Control	0.13±0.01	0.01±0.001	0.66±0.05	0.005±0.001	3.56±0.36	0.93±0.06	2.16±0.29
		Station I	0.17± 0.01	0.02±0.001	0.82±0.07	0.009±0.001	3.03±0.43	0.72±0.03	4.94±0.51
		Station II	0.16±0.01	0.02±0.001	0.67±0.06	0.008±0.002	0.95±0.13	0.71±0.07	3.61±0.34
	Muscle	Control	0.05±0.004	0.01±0.001	0.37±0.03	0.005±0.001	0.67±0.14	0.54±0.05	2.88±0.36
		Station I	0.08±0.006	0.01±0.002	0.65±0.06	0.007±0.001	1.65±0.12	0.36±0.02	5.42±0.61
		Station II	0.15±0.01	0.02±0.001	0.42±0.03	0.008±0.001	3.53±0.31	0.52±0.02	4.21±0.43
Monsoon	Liver	Control	0.06±0.005	BDL	0.52±0.05	BDL	9.87±0.89	0.41±0.04	4.54±0.51
		Station I	0.08±0.007	BDL	1.32±0.1	0.011±0.001	5.65±0.47	0.54±0.06	4.99±0.43
		Station II	0.14±0.01	0.01±0.001	0.81±0.07	0.039±0.003	3.45±0.42	0.87±0.09	6.43±0.68
	Gill	Control	0.09±0.01	0.011±0.001	0.54±0.05	0.004±0.001	3.01±0.29	0.91±0.08	2.02±0.2
		Station I	0.14±0.01	0.016±0.001	1.01±0.1	0.011±0.001	2.58±0.26	0.49±0.05	3.98±0.32
		Station II	0.14±0.01	0.02±0.001	0.87±0.07	0.006±0.001	1.22±0.35	0.73±0.07	6.47±0.34
	Muscle	Control	BDL	BDL	0.31±0.03	BDL	0.54±0.06	0.58±0.06	2.53±0.27
		Station I	0.1±0.01	0.011±0.001	0.7±0.06	0.006±0.001	2.01±0.19	0.47±0.03	4.68±0.48
		Station II	0.16±0.01	0.024±0.002	0.54±0.04	0.008±0.001	1.48±0.15	0.36±0.04	7.99±0.36
Post monsoon	Liver	Control	0.06±0.006	BDL	0.65±0.05	0.006±0.0002	11.26±1.28	0.28±0.03	4.89±0.47
		Station I	0.09±0.008	BDL	2.26±0.2	0.01±0.001	7.98±0.82	0.49±0.05	5.02±0.48
		Station II	0.19±0.01	0.01±0.001	0.89±0.07	0.011±0.001	9.83±0.91	1.13±0.14	5.01±0.53
	Gill	Control	0.15±0.01	0.014±0.001	0.76±0.06	0.007±0.002	2.99±0.31	0.86±0.09	1.08±.22
		Station I	0.16±0.01	0.019±0.001	1.31±0.1	0.01±0.001	2.21±0.21	0.58±0.03	2.68±0.26
		Station II	0.18±0.01	0.02±0.001	0.9±0.08	0.015±0.001	1.8±0.2	0.85±0.08	8.03±0.38
	Muscle	Control	0.09±0.008	0.01±0.001	0.35±0.03	BDL	0.51±0.05	0.49±0.04	3.39±0.34
		Station I	0.12±0.01	BDL	0.52±0.05	0.005±0.001	1.76±0.09	0.41±0.04	6.24±0.52
		Station II	0.26±0.02	0.02±0.001	0.47±0.03	0.014±0.001	1.04±0.18	0.51±0.05	7.99±0.36

BDL- Below Detectable Level

heavy metal accumulation tendency in liver and gills were supported by Vinodhini and Narayanan (2008); Murugan et al (2008), Vincent and Ambrose (1994) etc. During recovery studies all the bioaccumulated heavy metals except cadmium showed a tendency to recover significantly (Table 2). Long biological half-life and slow rate of elimination may be the reason behind the insignificant recovery with respect to cadmium. ATSDR (2000b) published similar findings related to cadmium.

Heavy metals constitute a core group of aquatic pollutants (Uysal et al., 2008) with oxidative potential. So the elevated levels of metals induce oxidative stress by generating highly reactive oxygen species (ROS) which can oxidize proteins, lipids and nucleic acids leads to damage in cell structure or even cell death (Tripathi and Gaur, 2004). Antioxidant defence system, includes both low molecular weight free-radical scavengers and a complex enzyme array (Petrulea et al, 2012) like glutathione reductase (GR), glutathione peroxidase (GPx), catalase (CAT), superoxide dismutase (SOD). The enzyme activity in 5% homogenate of liver, gill and muscle showed that the Catalase in liver and Gills, Superoxide dismutase in gill and muscle were decreased and Catalase in muscle, Superoxide dismutase in liver, Glutathione peroxidase in liver, gills and muscle were elevated as a response of pollution (Table 1 and Fig. 1-9).

Usually, higher SOD and CAT activities indicate there are more radicals need to be reacted (Chien et al. 2003); (Ross et al. 2001). So, according to Jiang et al. (2013), the enhanced activities of both SOD and CAT as shown here at low metal concentrations may enable fish to maintain health by scavenging the radicals produced. The present observationof elevated levels of Catalase in muscle, Superoxide dismutase in liver, Glutathione peroxidase in liver, gills and musclecan be substatiated with the last statement of Chien et al. 2003; Ross et al. 2001 and Jiang et al. (2013). This increase may be an adaptive mechanism ensuring the organism survival, while it will occur only in a certain extent of metal concentration. At higher concentrations, SOD and CAT activities in organs decreased. The reason for this decrease may be that metals like copper took the place of essential metals located in the active center of the enzyme or it combined with functional groups located on the enzyme molecules, such as the hydroxyl group, peptidyl, and hydrosulfide groups (Muhlia-Almazán and García-Carreño 2002), and hence decreased enzymatic activities. This may be the reason behind the observation of decreased Catalase in liver and Gills, Superoxide dismutase in gill because the liver and gill are facing the issue of higher rate of bioaccumulation and the related inactivationof antioxidant enzyme activity. This observation is substantiated further by the explanations like, many enzymes involved in the antioxidant defense process may be inactivated by the excess of oxidants, and this oxidant may be its own substrate (Modesto & Martinez 2010). Superoxide dismutase (SOD), for example, can be inactivated by hydrogen peroxide, catalase by the superoxide anion, and glutathione-S-transferase (GST) is easily inactivated by oxidants in general (Lushchak and Bagnyukova, 2006). Winston and Di Giulio (1991) reported

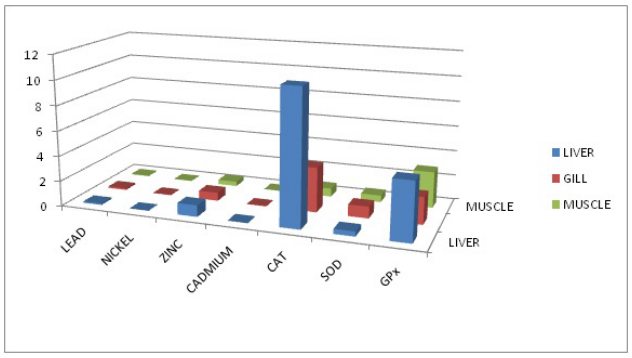

Fig. 1. Influence of premonsoon season on antioxidant enzyme activity in *Anabas testudineus* from control

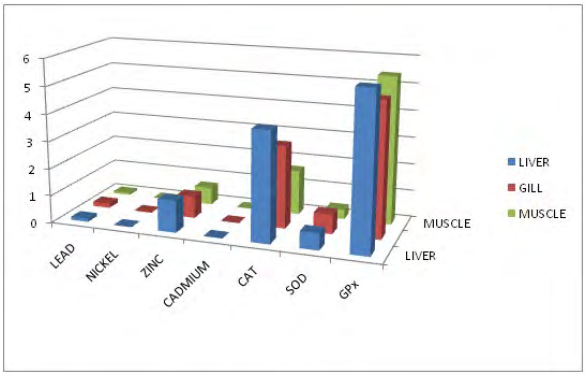

Fig. 2. Influence of heavy metal and premonsoon season on antioxidant enzyme activity in *Anabas testudineus* from station I

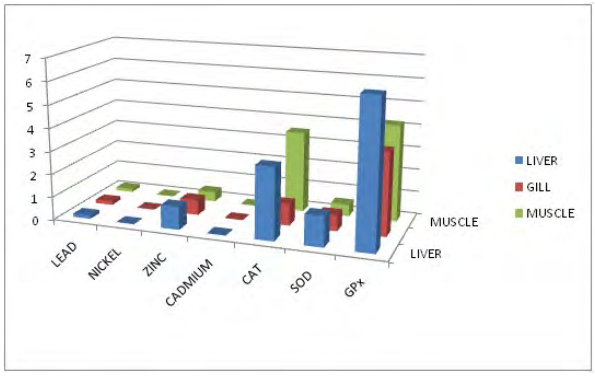

Fig. 3. Influence of heavy metal and premonsoon season on antioxidant enzyme activity in *Anabas testudineus* from station II

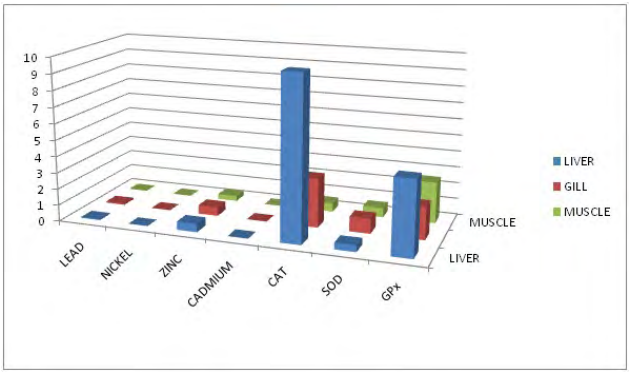

Fig. 4. Influence of monsoon season on antioxidant enzyme activity in *Anabas testudineus* from control station

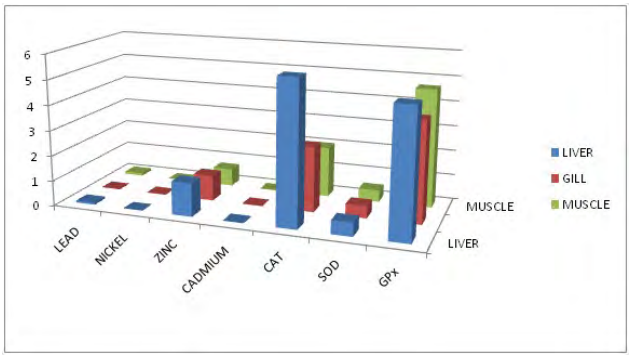

Fig. 5. Influence of heavy metal and monsoon season on antioxidant enzyme activity in *Anabas testudineus* from station I.

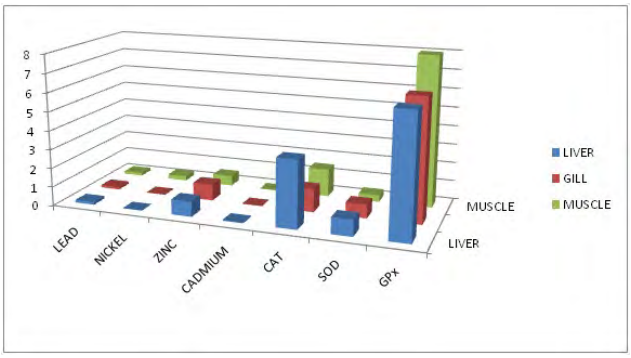

Fig. 6. Influence of heavy metal and monsoon season on antioxidant enzyme activity in *Anabas testudineus* from station II.

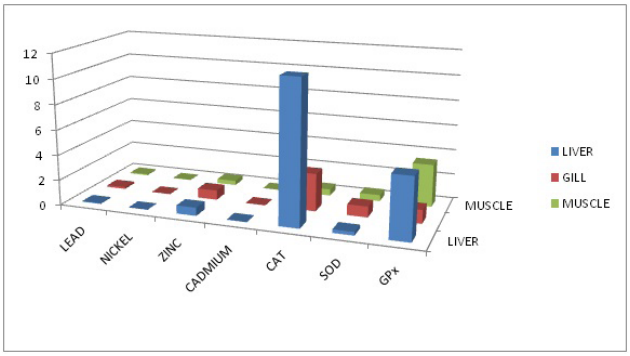

Fig. 7. Influence of postmonsoon season on antioxidant enzyme activity in *Anabas testudineus* from control station

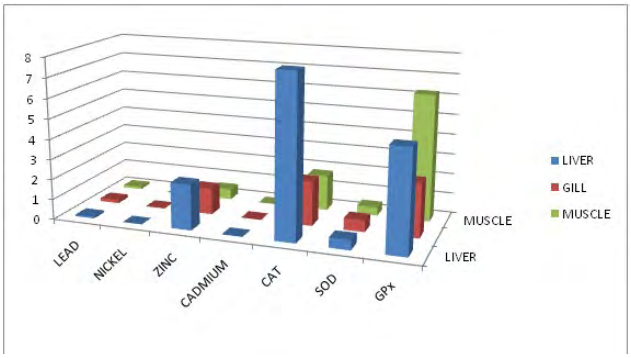

Fig. 8. Influence of heavy metal and postmonsoon season on antioxidant enzyme activity in *Anabas testudineus* from station I.

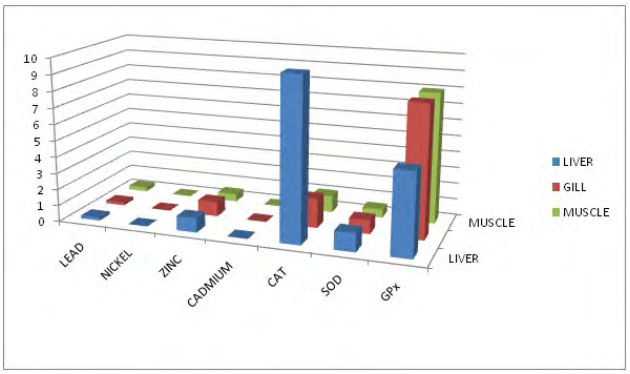

Fig. 9. Influence of heavy metal and postmonsoon season on antioxidant enzyme activity in *Anabas testudineus* from station II.

that a prooxidant condition elicited by the presence of contaminants triggering an increase in the activity of this antioxidant enzyme, as an adaptive response. Ventura et al. (2002), Stoliar and Lushchak (2012) published a similar trend with catalase enzyme. The stressed environment induced generation of ROS so the level of SOD increased. The increase in SOD activity indicates that more protein is required to protect cells against superoxide radicals. Heath (1995), Yilmaz et al (2006), Otto and Moon (1996) also reported SOD increase in liver. Modesto and Martinez (2010) reported the SOD inhibition by the transitory accumulation of hydrogen peroxide in fish liver. This can also be considered here because in liver and gill due to the elevated levels of bioaccumulated heavy metals oxidative stress may increases the concentration of hydrogen peroxide and inturn the inhibition of SOD. The SOD catalyzes the dismutation of the superoxide anion radical to water and hydrogen peroxide, which afterwards is detoxified by CAT. Therefore, a simultaneous activity induction of SOD and CAT is usually an expected response. However, this relation is not always observed and it is known to be species dependent (Ferreira et al. 2005). CAT and SOD increases as an adaptive response to chronic exposure but may get decreased when the toxicants may overwhelm antioxidant defense. This tendency was also experienced during the result analysis of the present study.

GPx is important in preventing lipid peroxidation in biomembranes (Van der Oost et al. 2003) with increase on oxidative stress (Van der Oost et al. 1996). That may the reason behind the elvated levels of GPx in this observation. Such an increase was reported by DiGiulio et al (1995) in response to pollution.

Here, one thing has to be noticed that more than a direct influence of season on enzyme kinetics but the variation in enzyme kinetics induced by seasonal variation varitiaon in heavy metal concentratrtionis more evidient and that variations is directly reflected in the levels of antioxidant enzyme activity as expalined earlier.

The antioxidant enzyme parameters along with the heavy metals accumulated in the organs recovered and registered near to control conditions during the recovery period (Table 2). This may the reversal of the conditions described above and that showed the recovery potentialof the downstream of Periyar river.

Table 2. Variation of heavy metal in Biomass and their influence on Antioxidant enzyme activity in field and recovery responses shown by *Anabas testudineus* collected from different stations during different seasons

Season	Organ	Station	Pb	Ni	Zn	Cd	CAT	SOD	GPx
Pre monsoon	Liver	Station I (field)	0.12± 0.01	0.01±0.001	1.17±0.11	0.01±0.001	4.01±0.46	0.61±0.06	5.67±0.53
		Station I (Recovery)	0.11±0.01	BDL	0.73±0.06	0.006±0.001	5.88±0.65	0.55±0.07	3.67±0.37
		Station II (field)	0.15±0.01	0.01±0.001	1±0.1	0.013±0.001	3.13±0.39	1.32±0.14	6.33±0.62
		Station II (Recovery)	0.13±0.02	0.01±0.001	0.84±0.07	0.012±0.001	5.09±0.26	1.06±0.11	4.95±0.43
	Gill	Station I (field)	0.17± 0.01	0.02±0.001	0.82±0.07	0.009±0.001	3.03±0.43	0.72±0.03	0.72±0.03
		Station I (Recovery)	0.14±0.007	0.01±0.001	0.47±0.04	0.009±0.001	3.48±0.32	1.05±0.02	1.05±0.02
		Station II (field)	0.16±0.01	0.02±0.001	0.67±0.06	0.008±0.002	0.95±0.13	0.71±0.07	0.71±0.07
		Station II (Recovery)	0.11±0.01	0.01±0.001	0.47±0.04	0.004±0.001	1.44±0.14	0.86±0.08	0.86±0.08
	Muscle	Station I (field)	0.08±0.006	0.01±0.001	0.65±0.06	0.007±0.001	1.65±0.22	0.36±0.02	0.36±0.02
		Station I (Recovery)	0.06±0.005	0.01±0.001	0.37±0.03	BDL	0.78±0.08	0.47±0.03	0.47±0.03
		Station II (field)	0.15±0.01	0.02±0.001	0.42±0.03	0.008±0.001	3.53±0.31	0.52±0.02	0.52±0.02
		Station II (Recovery)	0.14±0.01	0.01±0.001	0.4±0.03	0.005±0.001	1.32±0.19	0.57±0.06	0.57±0.06
Monsoon	Liver	Station I (field)	0.08±0.012	BDL	1.32±0.1	0.011±0.001	5.65±0.47	0.54±0.06	4.99±0.43
		Station I (Recovery)	0.07±0.01	BDL	0.66±0.06	BDL	8.37±0.86	0.49±0.05	4.40±0.41
		Station II (field)	0.14±0.01	0.01±0.001	0.81±0.07	0.039±0.003	3.45±0.42	0.87±0.09	6.43±0.68
		Station II (Recovery)	0.12±0.01	BDL	0.77±0.06	0.037±0.002	6.43±0.67	0.47±0.05	5.17±0.4
	Gill	Station I (field)	0.14±0.01	0.016±0.001	1.01±0.1	0.011±0.001	2.58±0.26	0.49±0.05	0.49±0.05
		Station I (Recovery)	0.12±0.001	BDL	0.83±0.07	0.009±0.001	2.98±0.21	0.87±0.07	0.87±0.07
		Station II (field)	0.14±0.01	0.02±0.001	0.87±0.07	0.006±0.001	1.22±0.15	0.73±0.07	0.73±0.07
		Station II (Recovery)	0.13±0.01	0.02±0.001	0.83±0.08	0.005±0.002	2.86±0.25	0.98±0.1	0.98±0.1
	Muscle	Station I (field)	0.1±0.01	0.011±0.001	0.7±0.06	0.006±0.001	2.01±0.19	0.47±0.03	0.47±0.03
		Station I (Recovery)	0.09±0.008	0.01±0.001	0.41±0.04	0.005±0.001	0.68±0.13	0.51±0.04	0.51±0.04
		Station II (field)	0.16±0.01	0.024±0.002	0.54±0.04	0.008±0.001	1.48±0.15	0.36±0.04	0.36±0.04
		Station II (Recovery)	0.14±0.01	0.022±0.002	0.49±0.04	0.006±0.001	0.68±0.07	0.48±0.05	0.48±0.05
Post monsoon	Liver	Station I (field)	0.09±0.014	BDL	2.26±0.2	0.01±0.001	7.98±0.82	0.49±0.05	5.02±0.48
		Station I (Recovery)	0.07±0.011	BDL	0.7±0.06	0.009±0.001	9.79±0.95	0.42±0.05	4.57±0.42
		Station II (field)	0.19±0.01	0.01±0.001	0.89±0.07	0.011±0.001	9.83±0.91	1.13±0.14	5.01±0.53
		Station II (Recovery)	0.18±0.01	BDL	0.91±0.08	0.011±0.001	10.73±1.34	0.97±0.09	5.32±0.7
	Gill	Station I (field)	0.16±0.01	0.019±0.001	1.31±0.1	0.01±0.001	2.21±0.21	0.58±0.03	0.58±0.03
		Station I (Recovery)	0.11±0.01	0.01±0.001	0.79±0.06	0.008±0.002	2.69±0.27	0.92±0.08	0.92±0.08
		Station II (field)	0.18±0.01	0.02±0.001	0.91±0.08	0.015±0.001	1.8±0.2	0.85±0.08	0.85±0.08
		Station II (Recovery)	0.15±0.1	0.02±0.001	0.57±0.04	0.008±0.001	2.76±0.13	0.93±0.08	0.93±0.08
	Muscle	Station I (field)	0.12±0.01	BDL	0.52±0.05	0.005±0.001	1.76±0.09	0.41±0.04	0.41±0.04
		Station I (Recovery)	0.1±0.01	BDL	0.43±0.02	BDL	0.41±0.05	0.46±0.05	0.46±0.05
		Station II (field)	0.26±0.02	0.02±0.001	0.47±0.03	0.014±0.001	1.04±0.18	0.51±0.05	0.51±0.05
		Station II (Recovery)	0.11±0.01	0.01±0.001	0.32±0.04	0.007±0.001	0.78±0.14	0.61±0.06	0.61±0.06

4. Conclusion

From these observations it can be concluded that the selected stations of Periyar river at Ernakulam distirct are contaminated with heavy metals and the absorbed heavy metals get accumulated in the biomass and that influencedthe antioxidant mechanisms harmfully. This observation can be extended to human who is in the level of secondary consumer. However these changes were reversed when the fishes were shifted to pollution free water indicating the recovery potential of Periyar river. Efficient mitigation measures can save a dying river from anirreparable damage.

Acknowledgements

The investigators are indebted to University Grants Commission for the financial assistance.

5. References

American Public Health Association. 1995.Standard Methods forthe Examination of Water and Wastewater, 19thedn. ed. Eaton,A.D., Clesceri, L.S. and Greenberg, A.E. Vol. 9, pp. 53–74,American Public Health Association (APHA), American WaterWorks Association (AWWA), Water Environment Federation(WEF), Washington, D.C.

ATSDR 2000b: Case Studies in environmental medicine: polychlorinated biphenyl (PCB) toxicity. ATSDR Publication No.: ATSDR-HE-CS-2003-0001. Atlanta, GA: Agency for Toxic Substances and Disease Registry, Public Health Service, US Department of Health and Human Services.

Chien, Y., Pan, C. & Hunter, H. 2003. The resistance to physical stresses by Penaeus monodon juveniles fed diets supplemented with astaxanthin. Aquaculture, 216, pp.177–191; doi:10.1016/S0044-8486(02)00056-X.

Das, B. and Mukherjee, S. 2000. A histopathological study of carp (Labeo rohita) exposed to hexachlorocyclohexane. Veterinarski Arch., 70(4), pp.169–180.

Da Silva, J.F., Garden, S.J. and Pinto, A.C. 2001. The chemistry of isatins: a review from 1975 to 1999. Journal of the Brazilian Chemical Society, 12, pp.273-324.

DiGiulio, R. et al. 1995. Biochemical mechanisms: metabolism, adaptation, and toxicity. In Rand GM (Editor), Fundamentals of Aquatic Toxicology: Effects, Environmental Fate, and Risk Assessment. Taylor & Francis, London. 250p

Heath, A.G., 1995. Water pollution and fish physiology. CRC. Press. Inc. Boca Raton, Florida, 359pp.

Jiang, H. et al. 2013. Changes of superoxide dismutase and catalase activities in crucian carp(*Carassius auratus* exposed to copper and recovery response. Life Science Journal, 10(1), pp.907–910.

Joseph, M.L., 1984. The Declining trend of Biodiversity and Fish Production in consequence of pollution in the Lower Reaches of Periyar River. *Status report on river Periyar, Cochin University of Science and Technology, Cochin, India*, pp.51-82.

Lushchak, V.I. and Bagnyukova, T.V. 2006. Effects of different environmental oxygen levels on free radical processes in fish. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology, 144(3), pp.283-289.

Modesto, K. A.and Martinez, C.B.R. 2010. Roundup® causes oxidative stress in liver and inhibits acetylcholinesterase in muscle and brain of the fish Prochilodus lineatus. Chemosphere, 78(3), pp.294–299.

Muhlia-Almazán, A. and García-Carreño, F.L. 2002. Influence of molting and starvation on the synthesis of proteolytic enzymes in the midgut gland of the white shrimp Penaeus vannamei. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology, 133(3), pp.383-394.

Murugan et al. 2008. Bioaccu- mulation patterns of zinc in fresh water fish Channa punctatus (Bloch.) after chro- nic exposure. Turkish journal of Fisheries and Aquatic Sciences, 8, pp.55– 59.

Otto, D.M.D. and Moon, T.W. 1996. Phase I and II Enzymes and Antioxidant Responses in Different Tissues of Brown Bullheads from Relatively Polluted and Non-polluted Systems. Archives Environmental Contamination Toxicology, 31, pp.141–147.

Petrulea, M., Muresan, A. and Duncea, I. 2012. Oxidative stress and antioxidant status in hypo-and hyperthyroidism. Antioxidant enzyme, 8, pp.197-236.

Kerala PWD. 1974. Water Resources of Kerala, Kerala PWD. Overton, I.C., Smith, D.M., Dalton,

J., Barchiesi, S., Acreman, M.C., Stromberg, J.C. and Kirby, J.M. (2014) Implementing environmental flows in integrated water resources management and the ecosystem approach. Hydrology Science Journal, v.59, pp.860877.

Ross, S. et al., 2001. Physiological (antioxidant) responses of estuarine fishes to variability in dissolved oxygen. Comparative Biochemistry and Phisiology, 130C, pp.289–303.

Rotruck, J.T. et al. 1973. Selenium: biochemical role as a component of glutathione peroxidase. Science Wash, 179, pp.588–590.

Sinha, K.A.1972. Colorimetric assay of catalase. Analytical biochemistry, 47, pp.3889 – 3894.

Singh, J. and Kalamdhad, A.S., 2011. Effects of heavy metals on soil, plants, human health and aquatic life. International journal of Research in Chemistry and Environment, 1(2), pp.15-21.

Sobha, V. and Anish, M. 2003. Imprints of environmental pollution on laterite/clay and groundwater of Eloor-Kalamassery industrial belt, kerala state, India. Environmental Geology, 44, pp. 914–918.

Stoliar, O. and Lushchak, V. 2012. Environmental Pollution and Oxidative Stress in Fish. In Oxidative stress- Environmental Induction and Dietary Antioxidants. pp. 131–166.

Tripathi, B. and Gaur, J. 2004. Relationship between copper and zinc induced oxidative stress and proline accumulation in Scenedesmus sp. Planta, 219, pp.397– 404.

Uysal, K., Emre, Y. and Kose, E. 2008. The determination of heavy metal accumulation ratios in muscle, skin and gills of some migratory fish species by inductively coupled plasma-optical emission spectrometry (ICP-OES) in Beymelek Lagoon (Antalya Turkey). Microchemistry Journal, 90, pp.67–70.

Van der Oost, R. et al. 1996. Biomonitoring of Aquatic Pollution with Feral Eel (Anguilla anguilla). II. Biomarkers: Pollution Induced Biochemical Responses. Aquatic Toxicology, 36, pp.189–222.

Van der Oost, R., Beyer, J. and Vermeulen, N.P.E. 2003. Fish bioaccumulation and biomarkers in environmental risk assessment: A review. Environmental Toxicology and Pharmacology, 13, pp.57–149.

Ventura, E.C., Gaelzer, L.R., Zanette, J., Marques, M.R.F. and Bainy, A.C.D. 2002. Biochemical indicators of contaminant exposure in spotted pigfish (Orthopristisruber) caught at three bays of Rio de Janeiro coast. Marine environmental research, 54(3-5), pp.775-779.

Vincent, S. and Ambrose, T. 1994. Uptake of heavy metals, cadmium and chromium in tissues of Indian major carp. Catla catla (Ham). Indian Journal of Environmental Health, 36, pp.200–204.

Vinodhini, R. and Narayanan, M. 2008. Bioaccumulation of heavy metals in organs of fresh water fish Cyprinus carpio (Common carp). International Journal of Environmental Science & Technology, 5(2), pp.179–182.

Winston, G.W. and Di Giulio, R.T.1991. Prooxidant and antioxidant mechanisms in aquatic organisms. Aquatic Toxicology, 19, pp.137–161.

Yilmaz, H.R. et al. 2006. An Investigation of Antioxidant Enzymes Activities in Liver of Cyprinus carpio Taken from Different Stations in the Karakaya Dam Lake. International Journal, 1(1), pp.1–6.