ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 11, Issue, 08, pp.5951-5955, August, 2019

DOI: https://doi.org/10.24941/ijcr.36226.08.2019

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

BIO ACCUMULATION OF HEAVY METALS AND ASSOCIATED HISTOLOGICAL CHANGES IN THE GONADS AND MORPHOLOGICAL CHANGES IN GILLS AND FINS OF LIZA PARSIA FROM ASTHAMUDI WETLAND, THE RAMSAR SITE

***Dr. Razeena Karim, L.**

Department of Zoology, Christian College, Kattakada, India

ARTICLE INFO

Article History:
Received 18th May, 2019
Received in revised form
19th June, 2019
Accepted 20th July, 2019
Published online 31st August, 2019

Key Words:
Liza parsia, Bioaccumulation,
Ashtamudi wetland, Histopathology

****Corresponding author:***
Dr. Razeena Karim, L.

ABSTRACT

The present study highlights the bioaccumulation of heavy metal and subsequent changes in the estuarine fish *Liza parsia* from Ashtamudi lake. The results of the present study on the quality of water and various aspects of *L. parsia* proved the grave situation of the lake. Morphology, biological constituents and reproductive organs of the fishes are greatly affected due to the pollutants. It will directly as well as indirectly affect the human being through the food chain. If the quantum of pollution status of the lake persists like this or increases, the quality of water, fishes and other biota of the lake will get deteriorated at a greater magnitude, becoming a serious threat to mankind.

***Citation: Dr. Razeena Karim, L.,* 2019.** "Bio accumulation of Heavy metals and associated histological changes in the gonads and morphological changes in Gills and fins of *Liza parsia* from Asthamudi wetland, the Ramsar site", *International Journal of Current Research*, 11, (08), 5951-5955

INTRODUCTION

Ashtamudi wetland, situated in the Kollam district, Kerala (Lat 8^0 59' N Long 76^0 36' E), is the second largest wetland in Kerala with a palm shaped extensive water body and eight prominent arms. Ashtamudi estuary is of extraordinary importance for its hydrological functions, biodiversity, rich fishery resources and an annual production of 23,000 tones of fish. The health of the estuary is dependent on the nature and quantity of various contaminants and potentially toxic pollutants it receives. The main contaminants are sewage, synthetic organics, petroleum hydrocarbons, pesticides, toxic heavy metals, etc. Sewage is the product of municipal drainage systems containing domestic wastes with or without the addition of discharges from industry, storm water and surface run off. Due to high toxicity to humans, heavy metals have been used as indices of pollution (Omogberale and Ogbeibu, 2005). The concentration of heavy metals in the aquatic ecosystems can be linked with effluents from industries and sewage (Ezemonye (1992). The term heavy metal is a common combined term which applies to group of metals and metalloids with an atomic density greater than $4g/cm^3$ greater than water (Duruibe *et al.*, 2007). They are also known as trace elements because they occur in minute concentrations in biological systems. The term 'heavy metal' has been used extensively to describe metals that are environmental pollutants (Walker *et al.*, 2001).

For the present study, three sites from the Ashtamudi lake were selected. Site 1, site 2 and site 3 are situated approximately 18, 7 and 5 kms respectively from the bar mouth (Neendakkara). The site 1 (Perumon), where anthropogenic influx is less, was found to be comparatively less polluted. This site is considered as reference site and it is about 11-13 kms away from other two sites. Site 2 (Kureepuzha) is located near the Muncipal waste dumping site of Kollam district which is the most polluted area adjoining Ashtamudi lake, Site 3 is located near the Neendakkara fishing harbor, where mechanized fishing trawlers are harboured. Oil spillage from mechanized boat is a major source of pollution here. The fish *Liza parsia* (Ham.) belongs to the family Mugillidae commonly known as gold spot mullet, is a catadromous fish widely distributed in the coastal waters of tropical and sub-tropical regions extending from 42^0N to 42^0S (Talwar and Jhingran, 2001), is one of the main inhabitant of Ashtamudi lake which is selected as the representative from the Ashtamudi lake. This study deals with the heavy metal analysis in water from these three sites and also the bioaccumulation of the heavy metal in the fish *Liza parsia* collected from three sites. Study also deals with the changes in the histology of gonads and also the SEM analysis were done in order to examine the external morphological changes due to the accumulation of the pollutants like heavy metals.

MATERIALS AND METHODS

Heavy metal content in water and in tissues of fishes were determined using an Atomic Absorpticon Spectrophotometer (Chemittco AAS 203). Significant differences between heavy metals in various sites were determined using One way Analysis of Varience (ANOVA) followed by multiple comparisons. For histological studies, pieces of male and female gonads were fixed in Bouins solution, dehydrated in alcohol, embedded in paraffin and sections, 5-7µ thick were prepared. Sections were stained with haemotoxylin and eosin. The slides were examined by Nikon microscope. For SEM analysis, fins and gills of the fishes were carefully taken and cut into small pieces of about 2mm diameter. 2.5% gluteraldehyde was used as the fixative. The preserved samples were then stored in refrigerator till its analysis using SEM. Gross morphological examination of fins and gills of targeted fishes were done via SEM JEOL model JSM-6390 LV.

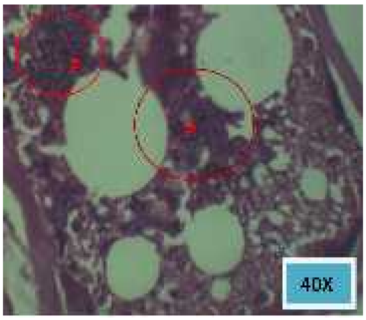
RESULTS

Heavy metals accumulation in water: Results of heavy metal analysis in surface water of Ashtamudi lake showed that Iron (Fe) showed higher values in all the three sites and the highest value was shown by site 2 (10.15mg/L) in the month of July, which is the site adjacent to waste dumping site of the Kollam district. Site 1 showed comparatively lesser values than the other two sites. Site 2 showed higher values for all the heavy metals. Results of ANOVA showed that the values of copper [$F(2, 33) = 50.103$ $p = 0.00$], lead [$F(2, 33) = 37.031$, $p = 0.00$], zinc [$F(2, 33) = 32.456$, $p = 0.00$] showed significant variation among the three sites (Table 2.3). Post hoc Multiple comparison (LSD) showed significant variation of site 2 from site 1 and 3 in case of copper. Three sites showed significant variations in case of lead, zinc. Site 1 was found to be significantly varied from site 2 and site 3 in case of iron. (Table 1).

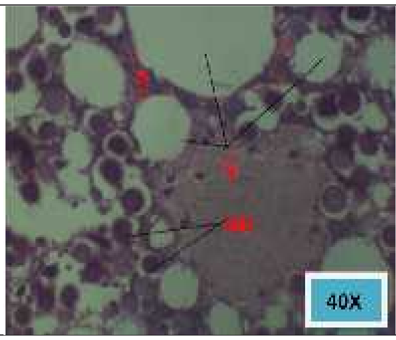
Bioaccumulation of heavy metals in tissues of fish: Analysis of Variance (One Way ANOVA) of heavy metal accumulation in the tissues of *L. parsia* comparing Sites was shown in Table 2. Iron was found to be the most abundant metal in tissue of *L. parsia*. At site 1, the concentration of iron in muscle was found to vary from 62.26 µg/gm to 84.6 µg/gm, with a mean concentration of 64.26 ± 16.8 (mean ± SD). Highest concentration of iron was noted in liver followed by ovary and muscle. When sites are compared fishes collected from site 2 were found to accumulate more iron in their tissues. One way analysis of variance (ANOVA) showed that iron accumulation in tissues [($F(2, 35) = 7.97$ $P < 0.01$) for ovary, ($F(2, 35) = 15.39$ $P<0.001$) for muscle and ($F(2, 35) = 60.01$, $P < 0.001$) for liver] were found to significantly differ among the sites. Post hoc multiple comparison (LSD) showed site 1 to significantly differ from site 2 and 3 in the accumulation of iron in the muscle and ovary, where as all the sites were found to significantly differ among each other in the accumulation of iron in liver. Zinc was found to be the second most abundant metal in tissues of the *L. parsia* from all the three sites. Highest concentrations of zinc were noted in liver followed by ovary and muscle. When sites are considered fishes collected from site 2 were found to accumulate more zinc in their tissues. One way analysis of variance (ANOVA) showed that zinc accumulation in tissues were found to significantly differ among the sites [($F(2, 35) = 4.92$ $P < 0.05$) in ovary, ($F(2, 35) = 52.37$ $P < 0.01$) in muscle and ($F(2, 35) = 4.65$, $P < 0.05$) in liver]. Post hoc multiple comparison (LSD) showed site 1 was significantly differ from site 2 and 3 in the accumulation of zinc in the muscle and liver, where as all the sites significantly differ from each other in the accumulation of zinc in ovary. Highest concentrations of copper were noticed in liver followed by ovary and muscle. When sites are compared fishes collected from site 2 were found to accumulate more copper in their tissues. One way analysis of variance (ANOVA) showed that copper accumulation in tissues were found to significantly differ among the sites [($F(2, 35) = 15.60$ $P < 0.001$) in ovary, ($F(2, 35) = 25.32$ $P < 0.001$) in muscle and ($F(2, 35) = 8.9$, $P < 0.01$) in Liver. Post hoc multiple comparisons (LSD) showed site 2 significantly differ from site 1 and 3 in the accumulation of copper in the ovary and liver, where as all the three sites significantly differ from each other in the accumulation of copper in muscle. Highest concentrations of lead were noticed in liver followed by ovary and muscle. When sites are compared fishes collected from site 2 were found to be accumulating more lead in their tissues. One way analysis of variance (ANOVA) showed that lead accumulation in liver and ovary were found to be significantly differ among sites [($F(2,35) =122.42$ $P<0.001$) in ovary and ($F(2,35) =51.07$ $P<0.001$)] in liver]. There was no significant difference in the accumulation of lead in the muscle of *L. parsia* among three sites ($F(2, 35) =0.448$ $P>0.05$) (Table 7.8). Post hoc multiple comparison (LSD) showed site 1 was significantly differ from site 2 and 3, whereas all the sites are significantly differ from each other in the accumulation of lead in liver.

Histopathological changes in gonads: From the histopathological point of view, examination of ovary and testis from the three sites showed degenerative, collapsing and necrotic changes. But the changes are more prominent at site 2 and site 3 than site1. Various histopathological changes are observed in the ovaries and testis of *L.parsia* due to the effect of the pollutants (Fig 1). The tunica albuginea is baggy and perinucleolus oocytes become atretic (Fig 1 c). Corrigation of the perinuclear ova membrane and vacuolation of the vitellogenic ova was observed and these deformed cell cause damage of membrane layers (Fig 1 a, b). In the testis, the seminiferous tubules appear with a less number of sperms indicating lack of active spermatogenesis (Plate 5.6 e). The deformed testis showed tissue necrosis, overcrowding of capillaries and vacuolated leydig cells, it also showed disrupted testicular tissue with spacious fibrous connective tissue with fibroblasts and oedema (Fig 1 5.6 f). Reduced number of oocytes with large follicular spaces and pycnotic nuclei of perinucleolus stages are observed in many ovaries of *L. parsia* in the present study, these observations coincides with those recorded by Abou Shabana *et al.* (2008). Severe damage to gonadal tissue occur due to infiltration of blood tissue and its necrosis. Contaminants present in aquatic environments are of major alarm as they can compromise reproductive success of water-dwelling organisms by severely affecting gonad (Kime, 1995) and gamete structure (Au *et al.*, 2000; Lahnsteiner *et al.*, 2004; Fitzpatrick *et al.*, 2008; Hatef *et al.*, 2010).

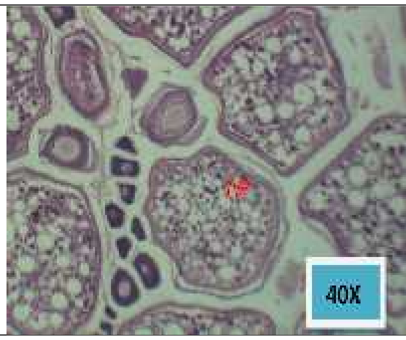
Gross morphological changes in the fin and gill of *L. parsia* due to the accumulation of Heavy metals: The gross structure of normal gills (Fig 2a) found to consist of gill arch, gill rakers and gill filaments. Arising from the gill arch are a number of gill filaments which are arranged in a single row and are equidistant from each other giving it a leaf like structure.



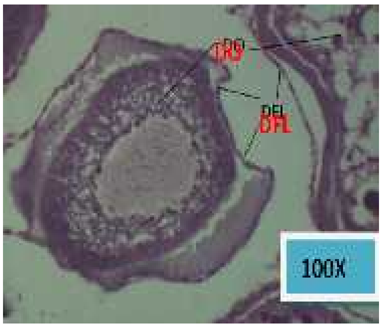
(a) Accumulation of pollutants (P) in the ovary



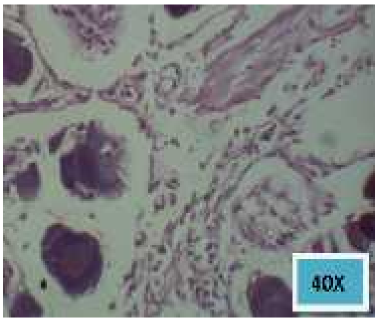
b) Oocyte in yolk vesicle showing destroyed oocytes (DO)



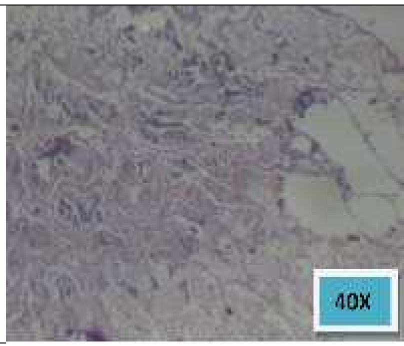
(c) Atretic perinucleus (AP) as a solid mass and atretic yolk vesicle Oocytes



(d) Destroyed Oocyte (DO) and damaged follicular lining (DFL)

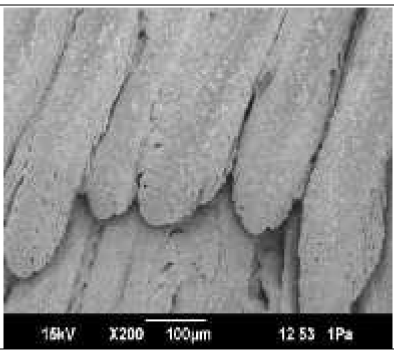


(e) Degenerative and necrotic changes in cellular elements

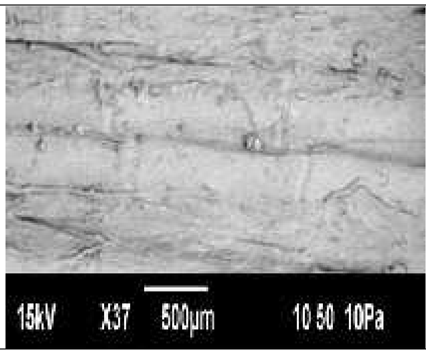


(f) Disrupted testicular tissue showing oedema

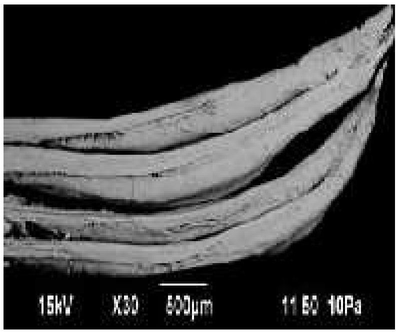
Fig 1. Histopatological changes in gonad of *L.parsia*



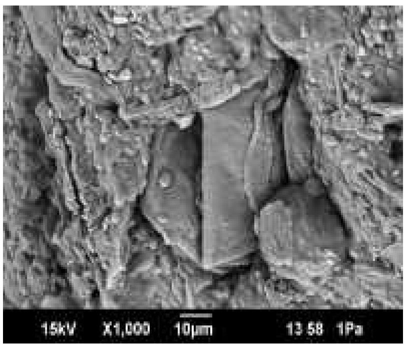
Gill of *Liza parsia* (Normal)



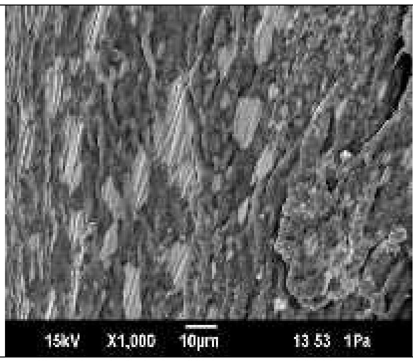
Fin of Liza parsia (Normal)

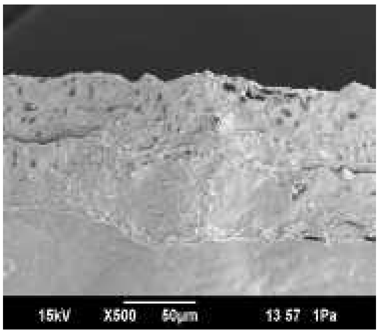


Distorted Gill of *Liza parsia* due to accumulation of pollutants



Distorted Gill of *Liza parsia* due to accumulation of pollutants





Distorted fin of *L. parsia* due to accumulation of pollutants

Fig. 2. Gross morphological changes in the gill and Fins of *L. parsia*

Table 1. Analysis of Variance (One Way ANOVA) of Heavy Metals in surface water comparing three sites of the Ashtamudi lake

Parameters	Site 1	Site 2	Site 3	F value (Comparing sites)
	Mean± SD	Mean± SD	Mean± SD	
Copper	0.02±0.11[a]	0.08±0.02[b]	0.03±0.02[a]	50.103***
Lead	0.01±0.006[a]	0.04±0.008[b]	0.02±0.005[c]	37.031***
Zinc	0.03±0.009[a]	0.10±0.032[b]	0.05±0.017[c]	32.456***
Iron	8.41±0.64[a]	9.52±0.50[b]	9.18±0.51[b]	12.828***

Table 2. Analysis of Variance (One Way ANOVA) of heavy metal accumulation in the tissues of *L. parsia* comparing Sites

Copper (μg/gm)	Site 1 Mean± SD	Site 2 Mean± SD	Site 3 Mean± SD	F value (Comparing sites)
Ovary	7.8±1.4 [a]	15.17±3.9 [b]	8.59±2.3 [a]	15.604***
Muscle	9.69±5.4 [a]	16.50±5.4 [b]	10.90±5.1 [c]	25.323***
Liver	18.81±5.8 [a]	31.18±9.3 [b]	22..39±7.2 [a]	8.997**
Lead (μg/gm)				
Ovary	2.91±1.5 [a]	19.21±3.3 [b]	3.97±2.1 [b]	122.426***
Muscle	1.45± 0.43	1.58±0.34	1.58±0.3	0.448[NS]
Liver	7.82±1.3 [a]	14.28±1.4 [b]	9.39±1.3 [c]	51.076***
Zinc (μg/gm)				
Ovary	87.55±9.2 [a]	104.16±13.9 [b]	93.94±10.1 [b]	4.928*
Muscle	18.42±0.3 [a]	26.35±2.6 [b]	21.41±1.4 [c]	52.372***
Liver	108.33±14.2 [a]	304.12±72.6 [b]	281.8±59.7 [b]	4.654*
Iron (μg/gm)				
Ovary	66.47±15.0 [a]	136.45±50.0 [b]	103.63±37.8 [b]	7.797**
Muscle	64.26±16.8 [a]	101.00±15.2 [b]	103.87±18.4 [b]	15.394***
Liver	333.01±60.7 [a]	645.65±58.5 [b]	558.53±58.9 [c]	60.001***

ns- Not Significant, **=p<.01 *** = p < .001. df = (2,35). SD- Standard Deviation, [a, b] -Means within rows with differing subscripts are significantly different, using Fisher's LSD post hoc test.

The secondary lamella is also observed to be projecting at right angles to the long axis of the filaments, each lamellae lying parallel to the adjacent lamellae. A number of gill rakers are observed to radiate from both sides of the gill arch on the opposite sides of the gill filament; the inner side of the rakers is equipped with minute projections. But the SEM analysis of present study showed the clubbing of the ends of the secondary lamellae, fusion of adjacent secondary gill lamellae, necrosis in the primary lamellae, epithelial lifting, necrosis and curling of secondary lamellae which were well marked in many samples, may be due to the exposure of fishes to pollutants particularly heavy metals (Fig 2b). Gill of fishes also showed gill epithelium disorientation, hypertrophy and hyperplasia of epithelial cells, fusion of gill lamellae, and decrease in the number of mucus cells (Fig 2c). Examination of normal fins under SEM revealed a smooth distal edge, whilst the surface of the fin consisted of alternating smooth and corrugated areas of epithelium and the smooth areas were situated over the fin rays (Fig 2 d). The surface of the fin was covered with normal epithelium. Examination of a number of sections in the present study suggested that there was a progressive thinning of the epithelium towards the distal fin. In the present study a clear cut white deposition was seen in the fins of fishes and this may be due to the combined effect of heavy metals like Cu, Zn and Cr (Fig 2e). The end of fin filaments of fishes are more affected due to heavy metal accumulation (Fig 2d).

DISCUSSION

Due to high toxicity to humans, heavy metals have been used as indices of pollution (Omogberale and Ogbeibu, 2005). The concentration of heavy metals in the aquatic ecosystems can be linked with effluents from industries and sewage (Ezemonye (1992). In the present study it was evident that the heavy metals were present in the water body of Ashtamudi Lake. Iron is the fourth most abundant element by mass in the earth's crust. In water, it occurs mainly in ferrous or ferric state (Ghulman *et al.,* 2008). Iron in surface water is generally present in ferric state.

Iron is the second most abundant metal in the earth's crust. As per WHO (2008), the iron concentration in rivers has been reported to be 0.7 mg/L. The permissible limit for iron in river water is approximately 0.5 to 1 ppm. The presence of increased amounts of heavy metals may have a direct impact on the health of humans as well as aquatic animals (Charles *et al.,* 2014). In the present study all the three sites exceeded this limit which indicates the severity of pollution in the lake. The very high value of iron in the surface water may be due to the result of mixing of untreated domestic and industrial wastes. Iron varied at different locations as well as in different season. Zinc is an essential trace element found in aquatic environment in the form of salts or organic complexes (WHO, 2008). The values of zinc are found in the range of 0.02 to 0.15mg/L in the Ashtamudi Lake. Highest value was recorded in the site 2 and lowest value at site 1. Zinc is one of the important trace elements that play a vital role in the physiological and metabolic process of many organisms. Nevertheless, higher concentrations of zinc can be toxic to the organism (Rajkovic *et al.,* 2008). It plays an important role in protein synthesis and is a metal which shows fairly low concentration in surface water due to its restricted mobility from the place of rock weathering or from the natural sources (Rajappa *et al.,* 2010). Presence of copper in the study sites may be due to the presence of industrial and domestic wastes. In the present study the site 2 and 3 exceeded this limit indicating the heavy metal pollution. The presence of lead in site 2 and 3 could be attributed to the industrial and agricultural discharge as well as from spill of leaded petrol from fishing boats. Higher levels of lead often occur in water bodies near highways and large cities due to high gasoline combustion **(**Banat *et al.,* 1998). Domestic and industrial effluents are the major sources of the observed high level of Pb, Zn, Co, Cu, and Cr (Abdo 2002). The persistence and concentrations of these trace elements in the water column and bottom sediment may reflect the concentrations found in resident aquatic organisms (through bioaccumulation), such as fish. This presents a human health concern if contaminated aquatic organisms, like fish and shellfish, are consumed. The different pollutants such as industrial and agriculture wastes, pesticides and also different types of bacteria have histopathological effects on reproductive tissues of fish gonads (Lye *et al.,* 1998), these effects may disturb the development of germ cells and may reduce the ability of fish to reproduce, while metal accumulation occurring in the testis, affects the process of spermatogenesis and suppressing sperm production (Yamaguchi *et al.,* 2007) which was well revealed in the histopathology of present study. In the present study the presence of heavy metals in the tissues of fishes from the three sites showed that the Ashtamudi lake is getting polluted by the anthropogenic activities. Fish samples from site 2 (Kureepuzha) displayed the highest metal concentrations in their tissues followed by site 3 (Kavanad). This may be due to the effect of waste dumping along the banks of Ashtamudi lake and release of oil hydrocarbons from mechanised boats. Site 1 also showed heavy metal accumulation in the tissues of *L. parsia*, but found to be comparatively lesser than other two sites. Many authors demonstrated that fish surviving at highly polluted areas accumulate higher levels of heavy metals than those surviving in less polluted areas of the same lake. (Bahnasawy, 2001; Khalil and Faragallah, 2008). The present study concludes that the Ashtamudi lake is severely polluted by several anthropogenic influx. The study throws light on the influence of pollutants on the water, well being and reproductive potentiality of fish *L. parsia.* The results of the present study on the quality of water and various aspects of L. parsia proved the grave situation of the lake. Morphology, biological constituents and reproductive organs of the fishes are greatly affected due to the pollutants. It will directly as well as indirectly affect the human being through the food chain. If the quantum of pollution status of the lake persists like this or increases, the quality of water, fishes and other biota of the lake will get deteriorated at a greater magnitude, becoming a serious threat to mankind. The pollutants were found to seriously affect the reproductive potentialities of the fish, thereby interfering with its race. The depletion and declining trend of fish population will adversely affect the fishery economy as it has commercial value and the local fisher man community being their major livelihood. Urgent and immediate steps are required to be taken to protect Ashtamudi lake, the Ramsar site from pollution.

REFERENCES

Abdo, M.H. 2002. Environmental studies on Rosetta branch and some chemical applications at the area extend from El-Kanater El-Khyria to Kafr El-Zayt city. *Ph.D. Thesis*, Faculty of Science, Ain Shams University, Egypt, 187 pp.

Bahnasawy, M. 2001. Levels of heavy metals in cat fish, *Clarias gariepinus*, from different habitats and their effects on some biochemical parameters. *Egyptian Journal of Aquatic Biology & Fish*eries, 5(1), 99-125.

Charles, M. M., Samson, O. O. and Josiah, O. 2014. Heavy Metal Bioaccumulation as Indicators of Environmental Pollution and Health Risks. *IOSR Journal of Environmental Science, Toxicology and Food Technology*, 8(2), 24-31.

Duruibe, J. O., Ogwuegbu, M.C. and Egwurugwu, J. N. 2007. Heavy metal pollution and human biotoxic effects. *International Journal of Physical Sciences*, 2,112-118.

Ezemonye LIN (1992). Heavy metal concentration in water, sediment and selected fish fauna of Warri River and its tributaries. PhD Thesis, University of Benin, Nigeria. p.175.

Ghulman, B. A., EL-Bisy, M. S. and Ali, H. 2008. Groundwater Assesement of Makkah Almokarama. *Proceedings of the 12th International Water Technology Conference*, Umm Al-Qura University, Makkah, 1515-1527.

Khalil, M. *and* Faragallah, H. 2008. The distribution of some leachable and total heavy metals in core sediments of Manzala lagoon, Egypt. *Egypt. J. Aquat. Res*, 34(1), 1-11.

Lye, C.M., Frid, C.L.J. and Gill, M.E. 1998. Seasonal reproductive health of flounder *Platichthys flesus* exposed to sewage effluent. *Mar. Ecol. Prog. Ser*, 170, 249-260.

Omoigberale, M. O. *and* Ogbeibu, A. E. 2005. Assessing the environmental impacts of oil exploration and production on the Osse River, Southern Nigeria. *African Journal of Environmental Pollution and Health*, 4(1), 27-32.

Rajkovic, M.B., Lacnjevac, C.M., Ralevic, N.R., Stojanovic, M.D., Toskovic, D.V., Pantelic, G.K., Ristic, N.M and Jovanic, S. 2008. Identification of metals (heavy and radioactive) in drinking water by indirect analysis method based on scale tests. *Sensors*, 8, 2188-2207

Talwar, P.K. *and* Jhingran, A.G. 2001. Inland fishes (Vol 2). Oxford & IBH Publishing Co. Pvt. Ltd. pp. 1158.

Walker, C.H., Hopkin, S.P., Sibly, R.M. and Peakall, DD.B. 2001. Principles of ecotoxicology,2nd Edn.Taylor and Francis Group, Fetter lane, London.

WHO 2008. Guidelines for Drinking-water Quality, 3rd edition incorporating the first and second.

Yamaguchi, Y., Kagota, S., Nakamura, K., Shinozuka, K. *and* Kunitomo M. 2000b. Inhibitory effects of water extracts from fruiting bodies of cultured *Cordyceps sinensis* on raised serum lipid peroxide levels and aortic cholesterol deposition in atherosclerotic mice. *Phytother Res*, 14, 650–2.

****