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Lead Toxicity : Bioaccumulation and histopathological study of the gill of the Teleost fish,*Anabas testudineus* (Bloch,1792) exposed to sublethal concentration of Lead nitrate

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Abstract

It was clear from the present study that,the primary gill lamellae of control fish appeared flat and leaf like bearing a central supporting axis with a row of secondary gill lamellae arranged at equal intervals and attached with the primary lamellae and free at their distal ends. Lead caused discrete pathological alterations in the gills of *Anabas testudineus* and the severity and frequency of organ lesions were found to be more prominent in fishes treated with higher sublethal concentration in short-term as well as long-term exposures. The prominent changes observed include lifting of lamellar epithelium, lamellar fusion with hyperplasia in pillar cells, haemorrhage in cartilaginous core, inter lamellar oedema and aggregation of capillaries and lamellar aneurism.Gills of fishes in the highest sublethal concentration and longest exposure showed maximum degree of degeneration with obliteration of secondary lamellae and lamellar fusion, haemorrhage and necrosis of epithelial cells.Lead accumulation in the gill tissue of the experimental fish *Anabas testudineus* was determined as per standard protocol. Lead accumulation in the gill of control fish were also determined for comparison. The accumulation rate and the BAF for lead in experimental fish tissues after 14 days and 28 days depicted significant increase from 419.209 µg/g±0.002(13.010) to 860.890µg/g±0.005(26.719).

Keywords
Lead,Sublethal Concentration,Static Renewal bioassay,LC_{50}

Introduction

Aquatic pollution is of prime concern since every kind of life invariably depends on water. Among pollutants, heavy metals are of greatest concern because upon reaching water, heavy metals deteriorate the quality of water and cause harm to both flora and fauna (Nriagu , 1998). Heavy metals are one of the principal causes of aquatic pollution and the magnitude of environmental degradation they caused is far beyond the recommended threshold limit values (Abdel-Meguid et al.2002).

Increase in population density, industrialization and agricultural practices have intensified the magnitude of contamination over the last few decades (Vutukuru, 2005). Due to rapid industrialization and unplanned urbanization, many rivers in India are experiencing severe problems of pollution. Regardless of the source of entry, water bodies are the ultimate destination and holds a variety of stressor chemicals especially heavy metals. Heavy metals are generally toxic to animals in particular and may cause even death or sub lethal pathology to organs and organ systems of aquatic animals (Wilbur, 1969). Heavy metals in the aquatic environment affect the entire aquatic biota and ultimately pose risk to fish consumers especially humans and wildlife. Certain heavy metals are necessary for specific body functions due to their nutritional value, but at higher concentrations they are toxic and detrimental to organisms.

When toxic substances accumulate in the environment and in food chains, they can greatly disrupt biological processes as suggested by Praveena and Jayantha Rao, 2013 .Fishes have the ability to uptake and concentrate metals directly from the surrounding water or indirectly from other organisms such as small fish, invertebrates and aquatic vegetation. After the absorption, metals in fish are then transported through blood stream to the organs and tissues where they are accumulated. Mode of action of chemicals varies due the diversity of toxicant and the target tissue. Some toxins exert their effect locally resulting in damage to external body surface and some others drastically affect specific organ systems. Contrary to this, there are toxins that do not cause deleterious effects on the portal or entry but they systematically affect the tissue in which they get accumulated leading to architectural changes ultimately culminating either death of the organism or making the organism less viable for its survival.

Histopathology is regarded as an ideal biomarker in toxicity studies and on the other hand it is a prominent tool for the assessment of tissue level changes in organisms. Since histological changes are manifested much earlier than other parameters, histopathological studies provide more sensitive and precise evaluation of cellular changes in target

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organs and the overall health status of the organism. When the concentration of a toxicant has been raised to a level sufficient to elicit cellular injury, sub lethal changes develop in the target tissue and they could be very well evaluated histologically as degenerative changes Histopathological studies have been employed to establish the causal relationships between contaminant, exposure and other biological responses.

Since metal ions are highly persistent and rarely been transformed, they get accumulated in various tissues causing toxicity (Gabriel et al.,2006). The correlation between metal concentration in fish organs and that of ambient medium has already been established by many researchers (Hongjun et al.,2013; Ciji and Bijoy Nandan,2014). Heavy metal pollution has been reported to be potent enough to induce irrecoverable cellular changes in aquatic animals (Moustafa and El-Sayed, 2014). Trace elements like Cd,Cr,Hg,Pb,and Co are highly toxic even at minimum level and they have no known physiological role to play (Kar,2008). The major threat from heavy metals is their persistence in aquatic systems and their tendency for bioaccumulation. Bioaccumulation is the increase in concentration of a substance in an organism manifolds over time, compared to its actual concentration in the environment. Accumulation results in the imbalance in ingestion and storage of substances against the rate at which they are metabolized or excreted.

In the present study the histopathological changes attributed by lead and the magnitude of its bioaccumulation in gill tissue have been assssed. Lead has the potential to adversely affect the human and animal health. It causes physiological, biochemical, and neurological dysfunctions in humans. Recent reports have indicated that lead can cause neurological, gastrointestinal, reproductive, circulatory, immunological, histopathological and histochemical disorders in the animals (Abdallah et al. 2010; Mobarak and Sharaf 2011). Fishes accumulate lead from the polluted environments and the metal is then distributed in their tissues. Whatever be the exposure route, heavy metals gets accumulated significantly higher in the gills, liver and kidneys.

Materials and methods

The fish selected for the present study was *Anabas testudineus*, an edible fresh water fish common in India. (www.fishbase.org). *Anabas testudineus* (Bloch, 1792) is an indigenous air breathing fish belonging to the family Anabantidae commonly found in the freshwater ponds and streams. It is an edible freshwater fish and it is consumed extensively by Keralites. *Anabas testudineus* was selected as the experimental model because of its economic importance, hardiness, availability round the year and adaptability to laboratory conditions.It is a potamodromous teleost fish which inhabit freshwater and brackish water habitats.

Toxicants selected for the experiment

The term ‘trace metals’ applies to a family of metallic elements present in living organisms in limited amounts. Trace metals are categorized into two sub-classes.Unlike essential micronutrients such as iron, manganese, copper, cobalt, magnesium and zinc,cadmium, mercury, chromium, lead etc. are non essential micronutrients without any proven biological functions and a mere presence of these metals in water is regarded as contamination.

Aanhydrous lead nitrate ($Pb(NO_3)_2$ manufactured by Merck India Limited, Mumbai has been used as the toxicant in the present study. Lead nitrate is white crystalline solid with the molecular formula, N_2O_6Pb and molecular weight 331.209g/mol.

The Experiment

The experimental design adopted for acute toxicity test was static renewal bioassay proposed by Sprague (1973). Acute toxicity tests for lead were carried out for duration of 96 hours. From the results of the range finding tests as suggested by United States Environmental Protection Agency (US EPA, 2002) for the toxicants, concentrations for definitive toxicity experiments had been chosen. The physico-chemical parameters of exposure water such as temperature, pH and dissolved oxygen were analysed as per standard protocol (APHA, 2005, Strickland and Parsons, 1972). The fishes were not fed during the tenure of the experiment. The water in the aquaria was renewed every 24 hours. From the acute lethal toxicity (96 hour LC_{50}) values for lead, a sublethal concentration of $1/5^{th}$ of 96 hour LC_{50} values of lead (32.22mg/l) was derived for the experiment.

Methodology for Bioaccumulation Studies

Fish from the control, Copper and Lead exposed groups were dissected to separate gills, liver, kidney and muscles for the determination of tissue level quantification and the resultant bioaccumulation factor. The separated

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organs were put in petri dishes for drying at 120^0C until reaching a constant weight. The organs were then placed into digestion flasks containing ultra pure concentration nitric acid and perchloric acid in the ratio 3:1.The digestion flasks were then heated to a temperature at which the entire material dissolves in the acid mixture. The resultant extract was diluted with double distilled water up to 50 ml solution. Lead accumulation were estimated using Shimadzu AA6200 Atomic Absorption Spectrophotometer and the results were expressed in µg/g dry weight. Bioaccumulation factor is derived to evaluate the magnitude of heavy metal accumulation in tissues which is the ratio of accumulation of metal in the tissues with respect to the concentration of that metal in the ambient medium. It is calculated by the formula proposed by Aboul Ezz and Abdel Razek (1991).

$$\text{Bioaccumulation Factor} = \frac{\text{Concentration of heavy metal in fish tissue in mg/Kg}}{\text{Concentration of heavy metal in water in mg/L}}$$

Methodology for Histopathological Studies

The experimental fishes were collected and acclimatized in the laboratory conditions prior to the test. Fishes exposed to $1/5^{th}$ of 96 hour LC_{50} such as 32.388mg/l for lead for 14 days and 28 days were selected for histological studies. After the completion of experimental period, fishes were killed by pithing and the gill tissue were removed and wiped thoroughly using blotting paper. The tissues were then fixed in 10 % neutral buffered formalin for 24 hours for further processing. The methodology followed for major steps in histopathological technique was adopted from Raphael (1976) and for staining as per the method suggested by Luna (1968)

Results

Histology of normal gill of control fish

The photomicrograph of the gills of control fish showed the characteristic arrangement of primary and secondary lamellae. The primary lamellae were comparatively thicker than the secondary (Figure 1.1). The primary gill lamellae appeared flat and leaf like bearing a central rod like supporting axis with a row of secondary gill lamellae on both sides. The secondary lamellae were arranged at equal intervals and attached with the primary lamellae and free at their distal ends. The normal secondary lamellar epithelium consisted of a thin single or double sheath of epithelial cells, blood vessels and a row of pilaster cells. Histology of gills in the controls of all treatments was identical and was similar to that of normal fishes. The respiratory surface in control fishes forms a barrier between the blood of fish and the surrounding water.

Histopathological changes in the gill of *Anabas testudineus* exposed to Lead

Lead caused discrete pathological alterations in the gills of *Anabas testudineus*. The severity and frequency of organ lesions were found to be more prominent in fishes treated with higher sublethal concentration (32.388mg Pb/l) in short-term (14 days) as well as long-term (28 days) exposures (Figures 1.2 and 1.3). The prominent changes observed in short term and long term exposures in the lowest sublethal concentration of lead were lifting of lamellar epithelium and decrease of interlamellar space, lamellar fusion at many loci of secondary lamellae with hyperplasia in pillar cells, haemorrhage in cartilaginous core, inter lamellar oedema and obliteration of normal lamellar architecture that led to aggregation of capillaries and lamellar aneurism at lamellar extremities

In addition to these changes, shortened and clubbed secondary gill lamellae were also detected in the gills of fishes exposed to lowest sublethal concentration (10.79 mg Pb/l) of lead. However the gills of fishes in the highest sublethalconcentration (32.388 mg Pb/l) and longest exposure (28 days) showed maximum degree of degeneration with obliteration of secondary lamellae with intensified lamellar fusion, haemorrhage, necrosis of epithelial cells and pillar cells with oedematous changes (Figure 1.3).

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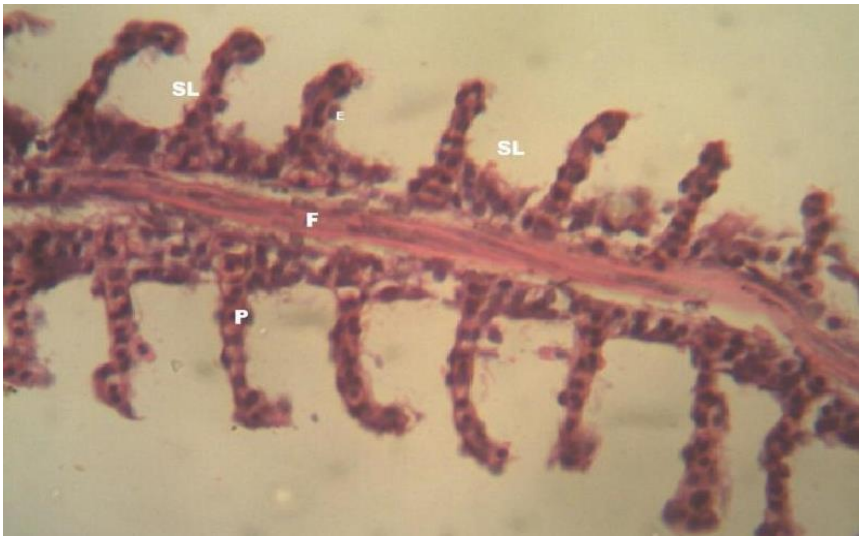

Fig.1.1: Normal histology of gill of *A. testudineus*. Secondary lamella (SL)Pillar cell (P)Primary gill filament (F)Epithelial cell (E). (H&E 40x)

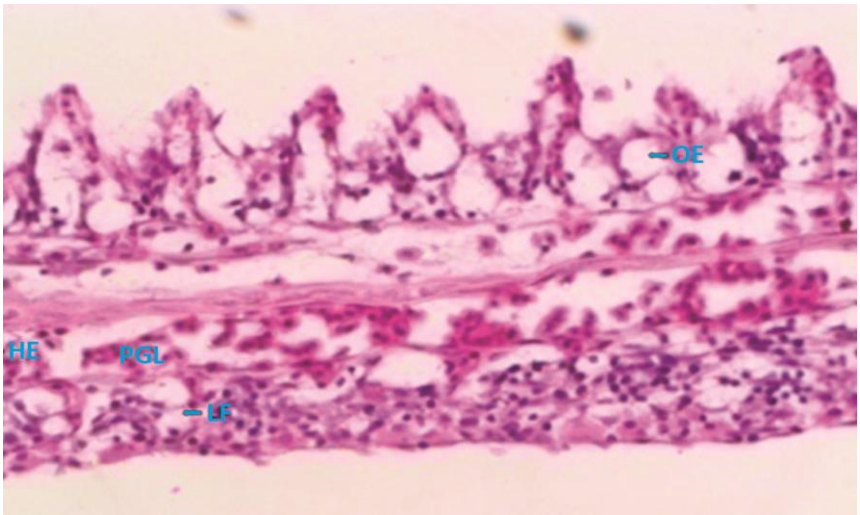

Fig.1.2: **Histopathological alterations of gill of *A. testudineus* exposed to 32.388 mg/L Lead of for 14 days.** Haemorrhage (HE) Lamellar fusion (LF) Primary gill lamella (PGL) Oedema (OE). (H&E 40x).

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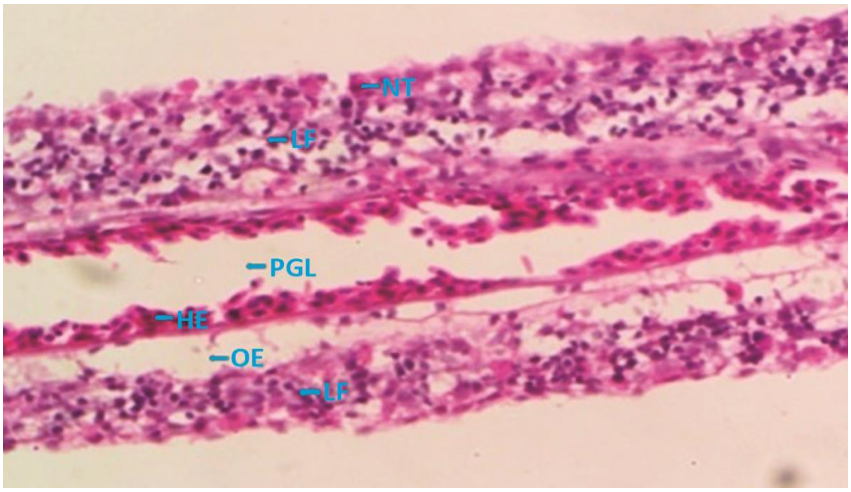

Fig.1.3: Histopathological alterations of gill of *A. testudineus* exposed to 32.388 mg/L Lead of for 28 days. Haemorrhage (HE) Lamellar fusion (LF) Primary gill lamella (PGL) Necrotic tissue (NT). Oedema (OE).(H&E 40x).

Lead accumulation in the gill tissue of the experimental fish *Anabas testudineus* was determined and the results were given in Table.1. Lead accumulation in the gill of control fish were also determined for comparison and it was 31.781µg/g±0.004 after 14 days and 38.532±0.221 after 28 days. The accumulation rate and the BAF for lead in experimental fish tissues after 14 days and 28 days depicted significant increase from 419.209 µg/g±0.002(13.010) to 860.890µg/g±0.005(26.719).

Table 1: Tissue level accumulation and bioaccumulation factor of Lead in *Anabas testudineus* exposed to sub lethal concentrations after 14days and 28 days of exposure

Duration of Exposure	Control	32.22 mg Pb/l
14 Days	31.781±0.004	419.209±0.002
Bioaccumulation factor	0.986	13.010
28 Days	38.532±0.221	860.89±0.005
Bioaccumulation factor	1.195	26.719

Discussion

Histopathology of gill of fishes exposed to various heavy metals have been extensively studied and documented by many authors. In polluted water, gills are the important route of metal uptake, and in the initial stages of exposure, metals may change the morphology of gill tissue (Campbell et al., 1999). In the present study, Anabas testudineus was selected because dgenerative studies on this fish seems to be scanty.

Fish gill is a multifunctional organ involving not only in respiration but a variety of homeostatic activities such as osmoregulation, metabolism of circulating hormones, nitrogenous waste excretion and also acid base balance. Moreover, it constitutes over 50 percent of the total surface area of fish and it is highly sensitive to waterborne chemicals (Ferguson, 1989). The respiratory system provides the most extensive interface of fish with water and is preferably the first system to be affected by dissolved pollutants (Heath, 1997). Since gills are in close contact with the ambient medium and particularly sensitive to changes in water quality, they are considered as the primary target

organ for the contaminants (Mohamed et al., 1998). Since gills are highly susceptible to even slight changes in physico-chemical parameters of water and gill filaments and lamellae provide a very large surface area for direct and continuous contact with contaminants and preferably the first system to be affected by dissolved pollutants (El Serafy, 2009). In this context gill histology is extensively useful as an index of aquatic pollution.

Bharat Bhushan Patnaik et al., (2011) in their studies on *Cyprinus carpio* emphasized that, the gill is the principal site of lead intake in fish and the interaction of lead with gill epithelium resulted in structural and functional disturbances. Oedematous changes characterized by epithelial lifting and total degeneration were detected on the 28th day in the present study agrees with similar observations in *Oncorhynchus mykiss* exposed to nickel reported by (Bharat Bhushan Patnaik et al., (2011). Lamellar fusion and hyperplasia of secondary lamellae in higher test concentrations and long exposures in the present study could be the protective mechanism to decrease the susceptibility of the gill. The damage of the secondary lamellae could be attributed to dilation and vascular congestion as observed in the gills. Lamellar fusion and hyperplasia of secondary lamellae might have induced suffocation in fish. Hyperplasia, oedema, haemorrhage, lamellar lifting from the underlying pillar cells and lamellar fusion observed in the present study corroborates the reports of Ezeonyejiaku et al.,(2011) on the fish, *Oreochromis niloticus*.

Lead mostly affects the primary and secondary lamellar epithelium of gills and also causes infiltration of erythrocytes and collapse of blood capillaries in them. Khan *et al*. (1996) reported that in African catfish exposed to sub-lethal concentration of lead results in fusion, curling and epithelial lifting of secondary lamellae and degeneration of lamellar epithelium and infiltration of erythrocytes. Kumar *et al*. (2011) found that gill lamellae undergo necrosis and epithelial lifting, vacuolation and damage of pillar cells and epithelial proliferation. Some authors reported desquamation of lamellar epithelium, hypertrophy of epithelial cells, lifting up of lamellar epithelium, intraepithelial oedema, hyperplasia and haemorrhage in the gill filament. Our results are comparable to the findings reported in these studies. The histopathological changes in the gill tissue serve as bio-markers of toxic effects of heavy metal on fish health and presence of these metals in water. It is concluded that lead is a potential toxicant to *Anabas testudineus*. Its presence in water cause severe pathological changes in the gills of fish, which results in the loss of basic histological structure and physiological function of the gills. Such condition eventually results in serious health issues for the fish.

Patel and Bahadur (2011) have studied histopathological alterations in gill and kidney of *Catlacatla* exposed to copper for three weeks and reported lamellar oedema followed by degeneration and lifting of branchial epithelium which coincides with the present study. Proliferation of epithelial cells leading to thickening of gill filaments may be the reason for lamellar fusion in toxicant exposed fishes (Figueiredo et al., 2007). Hyperplasia, hypertrophy and lamellar fusion could be defence mechanisms from the part of fish for increasing the distance between the external medium and blood to serve as a barrier to the entrance of contaminants and for reducing the effective branchial surface area which result in hypoxia and respiratory distress (Selvanathan et al., 2012). Giariet al. (2008) reported hyperplasia with lamellar fusion, telangiectasia, edema with epithelial lifting and desquamation in the gills of European sea bass (*Dicentrarchus labrax*) exposed to mercury

Assessment of bioaccumulation of heavy metals in tissues could be taken as a measure of abundance and availability of metals in the environment (Kucuksegin *et al.*, 2006) and as a warning indicator of contamination (Mansour and Sidky,2002). Heavy metal contamination deserves special interest in ecotoxicology since metals are highly persistent and can bioaccumulate and biomagnify in organisms at higher trophic levels which may lead to their extinction (Storelli et al.,2005). The persistence and non biodegradability of essential and non essential heavy metals seems to have long lasting and deleterious effects primarily on aquatic organisms and subsequently to humans, who depend to a great extent on aquatic organisms for food (Olowu et al.,2010).

Aquatic systems are the ultimate destination of contaminants and a major share of contaminants are accumulated in organisms Therefore the studies on the accumulation of heavy metals in various organs of the fish seem to be highly relevant. Gills, skin and digestive tract are potential sites of absorption of waterborne chemicals in fish. Blood transport the chemicals once absorbed to either a storage point such as the bone or liver, where they are either stored or further transported to other organs such as kidney or gills (McNicol and Scherer,1991). Generally concentrations of heavy metals are found higher in the liver and gills of fish than other tissues (Dural 2005) and hence the consumption of contaminated fish may endanger human health (Zhang *et al.*, 2007). Metals are well known in eliciting irreversible and irreparable cytotoxic, mutagenic and carcinogenic damages in animals (More *et al.*, 2003). The evaluation of toxic effects of metals on fish is meaningful in the context that they constitute an important link in food chain and their poisoning may disrupt the delicate equilibrium of the aquatic system (Firat and Kargam,2010).

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The accumulation of lead in gill were determined after 14 and 28 days of exposure in 1/5th of 96 hour LC_{50} concentration of lead. The magnitude of accumulation was found to be high in gill in the present study corroborates the findings of Ishaq *et al.*,(2011) on *Tilapia zilli* and *Clarias gariepinus*. The work of Al-Nagaawy,(2008) on the fingerlings of *Oreochromis niloticus* raised in 2 mg Cu/l polluted water after 4 weeks exposure showed copper in gill as 653.36±37.48 µg/g. However lead accumulation in gill was 1367.28±125.69µg/g on exposure to 12 mg pb/l.The high rate of accumulation in gills in the present study might be due to its close proximity to the toxicant medium as well its highly dissected structural organization occupying nearly sixty percent of the total surface area (Mayer *et al.*,1991). Jenning and Rainbow observed extraordinarily high cadmium accumulation in the gills of *Carcinus maenas* and suggested that, gills are the principal site of uptake of metals from water. The reports of high rate of accumulation of cadmium, nickel, chromium, copper and lead in gills of *Hydrocynus forskahlii* and *Hyperopisus bebeoccidentalis* from Ogun Coastal Water,Nigeria by Babatunde Murthala *et al.*,(2012) and studies of Nwani *et al.*(2010) on fishes from Afikpo, Nigeria corroborates the present finding. Shivakumar et al.,(2014) also reported high copper and lead accumulation in the gills of *Etroplus maculatus* and *Cirrhinus reba* collected from Bhadra river, Karnataka which were 1.84µg/g gill copper and 1.18µg/g gill lead. In a number of studies on fish, it has been observed that liver and gill are the most vulnerable targets of heavy metal insult due to the reason that liver is the main site of metabolism and storage of xenobiotics for detoxification by producing metallothionein (Kargin and Erdem, 1991) and the gill tissues as the interface with the environment in performing gas exchange, ion regulation, acid balance and waste excretion. The affinity of metallothionein, the metal binding protein,might be the reason for the high rate of metal accumulation in the gill of fish suggested by Nwani *et al.*,(2010) could be the reason for lead accumulation in the present study. The significantly high accumulation of metals in the gills of fish in the present study coincides with the work of Bebianno et al.,(2004),who reported that,gills are one among the major sit

es through which metal ions are absorbed since they are in direct contact with water and the thinness of its epithelium is ideal for penetration of metals. It was also reported that, the concentration of metals in gills seems to have a correlation with their availability in water where they live. (Shukla *et al.*,2007).Vidyasagar Gummadavelii et al.,(2013) studied heavy metal accumulation and subsequent BAF in *Cyprinus carpio* of Edulabad water reservoir,Andhra Pradesh and reported prominent lead accumulation and BAF in the gill.

In the study of Kayalvizhi et al.,(2013) on *Chanos chanos* of Gadiluvu River,Cudallore,the concentration of lead in gill have been reported to be 0.88 µg/l. The lead content of Gadiluvu River was exorbitantly high and that could be the probable reason for the higher rate of heavy metal accumulation in their tissues. The enhanced accumulation of lead in the gills was attributed to the mucus covering of the gills and its close contact with surrounding water. The magnitude of accumulation of lead in the gill of *Anabas testudineus* in the present study could be attributed to the concentration of lead in the ambient medium. The data

evolved from the present study could be adopted as a reference depicting the correlation between the availability of heavy metal in the ambient medium and the magnitude of tissue level accumulation of lead in *Anabas testudineus* exposed to a sublethal concentration of 1/5th of 96 hour LC_{50} after 14 and 28 days of exposure.

Since the entire aquatic population is under constant interaction with the immediate environment, any change in physico-chemical and biological constitution of the habitat can induce stress on organisms. The majority of water bodies including rivers and backwaters in Kerala are not free from the pollution stress due to the influx of effluents containing trace metals from industries, pesticides and fertilizers from agricultural sources and sewages from rural and urban areas. Since trace metal are extremely toxic, consumption of fish from polluted water may cause serious health hazards. Therefore,the study of accumulation of lead in the gill tissue of *Anabas testudineus*in this study is of paramount importance in highlighting the role of ambient metal concentration in enhancing the intensity of pollution.

Summary and Conclusion

Of the diverse types of contaminants present in aquatic ecosystems, heavy metals are considered most hazardous. Generally,metal concentrations seems to be higher in river water than sea water due to the reason that in the sea the metals are adsorbed by colloids and organic substances. Therefore freshwater organisms especially fishes in those water bodies that receive wastes from cities, industries, pulp and paper idustries, metallurgical works and agricultural fields are prone to the detrimental effects of heavy metals. The phenomenon of biomagnification of heavy metals intensify the toxic effects both directly on hydrobionts and indirectly on humans

who consumes the impacted organisms. Therefore the presence of metals and metal complexes in domestic wastes and industrial effluents are real threat to the fauna and flora of aquatic ecosystems and hence deserves immediate and timely action for the restoration of the water bodies.

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