

**BIOACCUMULATION OF METALS IN MUSCLE, LIVER AND GILLS OF SIX
COMMERCIAL FISH SPECIES AT ANAIKARAI DAM OF RIVER KAVERI,
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ABSTRACT: Metals are an inherent component of the environment that pose a potential hazard to human beings and animals. The consumption of fish from the polluted site may result in bioaccumulation of persistent pollutants in ultimate recipient of the food web. In the present investigation muscle tissue, gill and liver of six species of fish collected from Anaikarai dam (11°8'N latitude and 79°27'E longitude) of River Kaveri, South India were analyzed to study the metal accumulation in various tissues. The mean concentrations of Co, Cr, Mn and Zn were found to be higher in gills, but Cu, Ni and Fe were more in liver of all fishes. The mean concentrations of Cr (11.8 $\mu\text{g g}^{-1}$), Mn (4.4 $\mu\text{g g}^{-1}$) and Fe (139 $\mu\text{g g}^{-1}$) in the muscle were exceeding the permissible limit of FAO and WHO which is 1, 0.5 – 1.2 and 1.0 – 4.5 $\mu\text{g g}^{-1}$ for Cr, Mn and Fe respectively. The fish *Parastromateus niger* has shown higher concentration of zinc in all the tissues and thus it can be used as a bioindicator species for zinc pollution in aquatic environment.

Keywords: Anaikarai dam, River Kaveri, metals, muscle, gill, liver, *P. niger*

INTRODUCTION

Metal pollution from multifarious sources like effluents from industries, agricultural runoff and untreated sewage system has adverse effects on aquatic ecosystem. The metal contamination in aquatic ecosystem is yet considered to be unsafe not only for the human beings, but also for the wild organisms. Fish living in the metal contaminated waters may accumulate toxic trace metals via their food (Mendil and Uluzlu 2007). Heavy metals are taken up through different organs of fish because of the affinity between them (Karadede *et al.*, 2004). In this process, many heavy metals are concentrated at different levels in different organs of the body (Scharenberg *et al.*, 1994; Rao and Padmaja, 2000; Bervoeats *et al.*, 2001). The liver is highly active in the uptake and storage of heavy metals, hence it is a good monitor of water pollution with metals since their concentrations are proportional to those present in the environment (Dural *et al.*, 2007). Though fish livers are seldom consumed fish liver may represent a good biomonitor of metal pollution (Chaffai *et al.*, 1996). The deleterious effects of metals on aquatic ecosystem necessitate the continuous monitoring of their accumulation in key species, since it affords indication of temporal and spatial extent of the process and impact on organism's health (Kotze *et al.*, 1999). The heavy metals contamination is one of the vital factors for decline of water quality that has an obvious impact on fish diversity. Consumption of fish from the contaminated site poses a higher health risk to human. Many studies are being carried out to determine the level of metals in the fish since it is considered as one of the richest sources of protein and unsaturated omega-3 fatty acid for human (Chandrasekar *et al.*, 2003; Karadede *et al.*, 2004; Licata *et al.*, 2004; Amaraneni 2006; Calvi *et al.*, 2006; Agarwal *et al.*, 2007; Mendil and Uluzlu 2007; Ploetz *et al.*, 2007; Yang *et al.*, 2007 and Yilmaz *et al.*, 2007). River Kaveri is running through three southern states of India. The objective of the present study was to estimate the levels of metals in fishes with different feeding habits and also to find the accumulation pattern in different organs viz., muscle tissue, liver and gill of fish from Anaikarai dam of River Kaveri.

MATERIALS AND METHODS

Sample collection

Six species of fish Viz., *Channa melanosoma*, *Silurus wynaadensis*, *Mughil cephalus*, *Oreochromis mossambicus*, *Parastromateus niger* and *Scardinius erythrophthalmus* were collected from the fishermen on the spot from mukumbu (shown in the Fig- 1). The collected samples were brought to the laboratory in an ice box in cold condition and then washed with distilled water, and dissected for muscle, gill and liver and packed in polyethylene bags and stored at -20°C until analysis.

Reagents

Concentrated nitric acid, hydrogen peroxide and hydrochloric acid were of analytical grade (Qualigens Pvt Ltd., India). Ultrapure water (Elga water purification system) was used throughout the extract preparations.

Metal extraction

All the glasswares used for the analysis were cleaned by soaking overnight in 50% (v/v) Conc. nitric acid solution. USEPA methodology (3020b) was followed for the metal extraction. A Known weight of tissue samples (Muscle, Gill and Liver) were dried in a hot air oven at 100°C for overnight then ground into fine powder using agate mortar and pestle. 1g of powdered tissue samples were taken in 100 ml beaker and treated with 10 ml of 1:1 Con. HNO_3 and water at $95\pm 5^{\circ}\text{C}$ in a hot plate for 20 min and cooled. Again 5ml Con. HNO_3 was added and refluxed for 30 min. When the dark fumes subsides and the volume reduced to approximately 5ml, 2ml of ultrapure water and 3 ml of 30% H_2O_2 were added, heated until the effervescence cease. Then 10 ml of Con. HCl was added and refluxed at $95\pm 5^{\circ}\text{C}$ for about 15 min. Finally the digestate was cooled and filtered using Whatman filter paper (63 μm) and made up to 100 ml with ultrapure water in a standard flask.

Instrumentation

Flame Atomic Absorption Spectrophotometer (Perkin Elmer AA 800 model double beam mode, USA) with multielement hollow cathode lamp was used for the analysis of heavy metals (Co, Cr, Cu, Fe, Mn, Ni and Zn) present in the tissue extracts. Air-acetylene was used as fuel for flame. The wavelength (nm) and slit band width (nm) for the analysis of metals in Flame Atomic Absorbition Spectrophotometer were as follows 240.7 and 0.2 for Co ; 357.9 and 0.7 for Cr; 324.8 and 0.7 for Cu; 248.3 and 0.2 for Fe; 279.5 and 0.2 for Mn; 232.0 and 0.2 for Ni and 213.9 and 0.7 for Zn, respectively.

RESULTS AND DISCUSSION

The concentrations of Co, Cr, Cu, Fe, Mn, Ni and Zn were determined in muscle, liver and gills of six fish species and shown in the Fig 2. The concentrations in muscle tissue were Co (ND – $1.8\ \mu\text{g g}^{-1}$), Cr (ND – $4.3\ \mu\text{g g}^{-1}$), Cu (ND – $0.9\ \mu\text{g g}^{-1}$), Mn ($2 - 8.7\ \mu\text{g g}^{-1}$), Ni ($2.6 - 7.3\ \mu\text{g g}^{-1}$), Fe ($55 - 240\ \mu\text{g g}^{-1}$) and Zn ($15.3 - 41.3\ \mu\text{g g}^{-1}$). The concentration in gills were ranged to be Co (ND – $10.4\ \mu\text{g g}^{-1}$), Cr (ND – $10.3\ \mu\text{g g}^{-1}$), Cu (ND – $4.13\ \mu\text{g g}^{-1}$), Mn ($7.3 - 28.6\ \mu\text{g g}^{-1}$), Ni ($2.7 - 28.2\ \mu\text{g g}^{-1}$), Fe ($154 - 1037\ \mu\text{g g}^{-1}$) and Zn ($35.1 - 885\ \mu\text{g g}^{-1}$) and in the liver the levels were Co (ND – $2.0\ \mu\text{g g}^{-1}$), Cr (ND – $22.8\ \mu\text{g g}^{-1}$), Cu (ND – $72.0\ \mu\text{g g}^{-1}$), Mn (ND – $80\ \mu\text{g g}^{-1}$), Ni (ND – $77.7\ \mu\text{g g}^{-1}$), Fe ($505 - 3500\ \mu\text{g g}^{-1}$) and Zn ($45 - 260\ \mu\text{g g}^{-1}$). The order of metals in muscle, gill and liver samples were found to be $\text{Fe} > \text{Zn} > \text{Ni} > \text{Mn} > \text{Cr} > \text{Co} > \text{Cu}$ and $\text{Fe} > \text{Zn} > \text{Ni} > \text{Mn} > \text{Cu} > \text{Cr} > \text{Co}$, respectively.

The fish *S. erythrophthalmus* has shown higher values of Co in the muscle. The gills of *O. mossambicus* and liver of *P. niger* exhibited elevated levels of Co (Fig-2). The mean concentration of Co in the muscle, gill and liver was observed to be 0.93 , 2.41 and $0.33\ \mu\text{g g}^{-1}$. The concentration of Co observed in the gill of *O. mossambicus* ($10.4\ \mu\text{g g}^{-1}$) was nearly thirteen times higher than the value reported in the gill of *O. mossambicus* ($0.89\ \mu\text{g g}^{-1}$) from Kolleru Lake in South India (Chandrasekar et al., 2003). Co is an important element for blood pressure regulation and proper functioning of thyroid.

The highest concentrations of Cr in muscle ($4.3 \mu\text{g g}^{-1}$), gill ($10.3 \mu\text{g g}^{-1}$), and liver ($22.8 \mu\text{g g}^{-1}$) were recorded in *O. mosambicus*, *S. glanis* and *C. melanosoma* respectively (Fig- 2). Based on mean concentration of Cr the following accumulation order gill>liver>muscle was found. Liver and gills always exhibit higher concentrations of metals than muscle (Yilmaz *et al.*, 2007). Cr is an essential trace element and play an important role in insulin function (Mendil and Uluzozlu 2007). The mean concentration of Cr in muscle ($1.96 \mu\text{g g}^{-1}$) is about two times higher than the permissible limit ($1 \mu\text{g g}^{-1}$) set by FAO (1983).

The muscle tissue of *S. erythrophthalmus* has shown higher concentration ($0.9 \mu\text{g g}^{-1}$) for Cu. The gill and liver of *P. niger* and *M. cephalus* have shown the maximum concentrations which were 4.13 and $72 \mu\text{g g}^{-1}$ respectively than the other species (Fig. 2). Copper is considered to be an essential part of several enzymes and it is necessary for the synthesis of haemoglobin. In the present investigation the highest concentration of Cu in the liver tissue of *Mugil cephalus* was found to be $71.9 \mu\text{g g}^{-1}$. The mean concentration of Cu in the muscle, gill and liver were $0.3 \mu\text{g g}^{-1}$, $1.7 \mu\text{g g}^{-1}$ and $14.02 \mu\text{g g}^{-1}$ respectively. Karadede *et al.*, (2004) also observed the highest concentration of Cu in the liver tissue ($268 \mu\text{g g}^{-1}$) in mullet *Liza abu* in Ataturk Dam, Turkey. However, the concentrations in muscle, gills and liver were lower than reported by Chandrasekar *et al.*, (2003) in *Oreochromis mossambicus* from Kolleru lake, India. Except the concentration of Cu ($72 \mu\text{g g}^{-1}$) in the liver of *M. cephalus* the concentration observed in all tissues of all the fishes were well below the permissible limit of Cu proposed by FAO (1983) in fish tissue ($30 \mu\text{g g}^{-1}$).

Maximum concentration of Ni was observed in muscle ($7.3 \mu\text{g g}^{-1}$) and liver ($77.7 \mu\text{g g}^{-1}$) of *O. mosambicus*. Higher value of gills was found in *C. melanosoma* ($28.2 \mu\text{g g}^{-1}$). Ni levels in muscle, liver and gills among the species were shown in the Fig. 2. The mean concentration of Ni was high in liver ($25.76 \mu\text{g g}^{-1}$) followed by gills ($12.5 \mu\text{g g}^{-1}$) and muscle ($4.5 \mu\text{g g}^{-1}$). The concentration of Ni in the muscle and gill of mullet *M. cephalus* was 3.5 and $8.49 \mu\text{g g}^{-1}$, respectively which was higher than the range of Ni in the muscle ($0.15 - 0.37 \mu\text{g g}^{-1}$) and gills ($0.2 - 0.4 \mu\text{g g}^{-1}$) in the mullet *Liza aurata* observed by Licata *et al.*, 2003 in Lake Faro, Italy. Generally mullet feeds on the contaminated detritus in the sediment because of their feeding habit they are highly exposed to the trace metal concentrations (Kirby *et al.*, 2001). The Ni was found to be 41, 37, 87 fold higher than the levels reported in muscle ($0.18 \mu\text{g g}^{-1}$), liver ($0.5 \mu\text{g g}^{-1}$) and gills ($0.89 \mu\text{g g}^{-1}$) respectively by Chandrasekar *et al.*, 2003 in *O. mossambicus* from kolleru lake, India. The mean concentration of Ni in muscle tissue was $4.5 \mu\text{g g}^{-1}$ which is lower than the permissible limit ($10 \mu\text{g g}^{-1}$) in fish tissues set by FAO (1983).

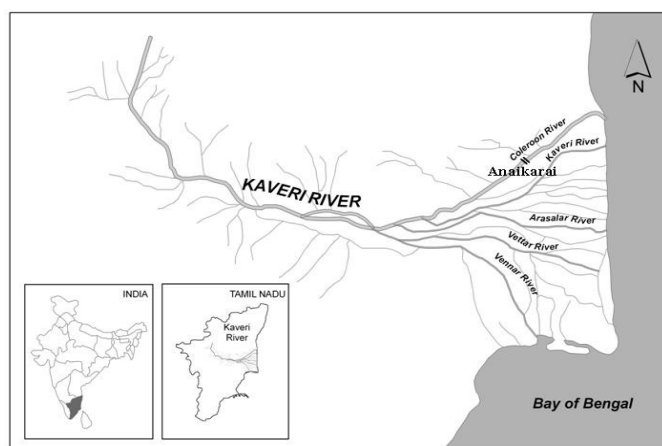


Fig:- 1 Map showing the Anaikarai Dam of River Kaveri

M. cephalus had higher concentration of Mn with $8.7 \mu\text{g g}^{-1}$ in muscle and $28.57 \mu\text{g g}^{-1}$ in gills (Fig- 2). The mean concentration of Mn was more in gill ($16.2 \mu\text{g g}^{-1}$) followed by liver ($14.3 \mu\text{g g}^{-1}$) and muscle ($4.4 \mu\text{g g}^{-1}$). Mn is recognized as an essential trace element for humans and several of their metabolic roles have been well established (Mendil and Uluzlu 2007). The levels of Mn in muscle and gill in the cat fish *Silurus glanis* was found to be 2.1 and $13.53 \mu\text{g g}^{-1}$, respectively which was higher than the concentrations reported by Olifia *et al.* 2004 in the African cat fish *Clarias gariepinus* (muscle - $1.79 \mu\text{g g}^{-1}$ and gill - $0.092 \mu\text{g g}^{-1}$) collected from a fish farm at Ibadan, Nigeria. The mean concentrations of Mn in the muscle tissue ($4.4 \mu\text{g g}^{-1}$) exceed the permissible limit of WHO (1982) which was $0.5 - 1.2 \mu\text{g g}^{-1}$.

The concentration of Fe was predominant in all the tissues viz., muscle, liver and gills (Fig- 2). Higher concentration of Fe in muscle ($240 \mu\text{g g}^{-1}$), gill ($1615 \mu\text{g g}^{-1}$) and liver ($3500 \mu\text{g g}^{-1}$) was found in *O. mossambicus*, *P. niger* and *S. erythrophthalmus* respectively. The mean concentration of Fe in the muscle, gill and liver of six fish species was observed as 139 , 713 and $1576 \mu\text{g g}^{-1}$, respectively. Fe in the muscle tissue of cat fish *S. glanis* was $153 \mu\text{g g}^{-1}$ which was slightly higher than the Fe in *S. glanis* ($117 \mu\text{g g}^{-1}$) during summer season from lakes of Tokat, Turkey (Mendil and Uluzlu 2007). The concentrations of Fe in muscle, liver and gill were observed to be few orders of magnitude more than the permissible limit set by WHO (1982) which is $1 - 4.5 \mu\text{g g}^{-1}$. The tissue accumulation of metals in aquatic animals can reflect the past exposure (Dural *et al.*, 2007). Higher concentrations of metals also found in gill tissue of fish species. Since gills are the uptake site of waterborne ions where the concentrations increase especially at the beginning of exposure, before the metal enters other parts of organism (Jezierska and Witeska 2001).

Next to Fe the Zn concentration was found more in all tissue samples (muscle, gill and liver). Zn is associated with the activities of nearly 100 enzymes involved in lipid, protein and carbohydrate and nucleic acid metabolism in all organisms (Elinder 1986). The Zinc level in muscle, gill and liver were 41 , 885 and $259 \mu\text{g g}^{-1}$ respectively of *P. niger* were higher when compared to other species (Fig- 2). The mean concentration of Zn in the muscle was $22.67 \mu\text{g g}^{-1}$, gills - $182 \mu\text{g g}^{-1}$, liver - $113 \mu\text{g g}^{-1}$. The level of Zn was found higher in gill sample than in liver and muscle. In addition to that *P. niger* has shown the presence of all metals in all tissue samples except Co in muscle and Cr in Liver. The concentration ($18.9 \mu\text{g g}^{-1}$) of Zn in *M. cephalus* in the present was lower than the level observed in the same species from Middle Eastern coast of Tunisia (Chaffai *et al.*, 1996). The concentration of Zn in the liver was ($120 \mu\text{g g}^{-1}$) for *O. mossambicus* which was higher than the concentration ($72 \mu\text{g g}^{-1}$) in *O. mossambicus* from Kolleru lake, India reported by Chandrasekar *et al.* 2003. The present investigation also showed higher level of Zn in all the tissues of cat fish *S. glanis* than the concentrations observed by Karadede *et al.*, (2004) in muscle ($10.9 \mu\text{g g}^{-1}$), liver ($20.36 \mu\text{g g}^{-1}$) and gill ($33.66 \mu\text{g g}^{-1}$) of cat fish *Silurus triostegus* from the Ataturk Dam Lake (Euphrates), Turkey. Fernandes *et al.*, 2007 also reported the higher concentrations of Zn in the gills ($114 \mu\text{g g}^{-1}$) which was 4.4 and 1.3 fold higher than the content in muscle and liver respectively. Since the muscle, gills and liver samples of *P. niger* have accumulated Zn at higher concentration it can be used as a bioindicator for Zinc pollution. The permissible limit set by FAO (1983) was $50 \mu\text{g g}^{-1}$.

Metals in different tissues were found to be in the order Co- Gills> Muscle> Liver; Cr, Mn and Zn- Gills> Liver> Muscle; Cu, Ni and Fe -Liver> Gills> Muscle. As far as fish is concerned muscle is not an active tissue in accumulating heavy metals compared to the liver and gill which are often recommended as environmental indicator organs of water pollution (Karadede *et al.*, 2004).

CONCLUSION

The study allowed the comprehensive evaluation of metals in various tissues (muscle, gill and liver) of six fish species. In the investigation, liver has showed higher concentrations of metals such as Cu, Ni and Fe and higher concentration of Co, Cr, Mn and Zn in gill. The mean concentrations of Cr, Mn and Fe in the muscle were exceeding the permissible limit of FAO (1983) and WHO (1982).

Higher concentration of Zn was observed in all the tissues of *P.niger*. Elevated concentration of metals in fish may be associated with agricultural runoff into the riverine system because of the fact that the present study area is agricultural based and cultivation is carried out throughout the year on rotational basis. To study the source of the examined contaminants, extensive localized sampling and analyses of point source of pollution would be required.

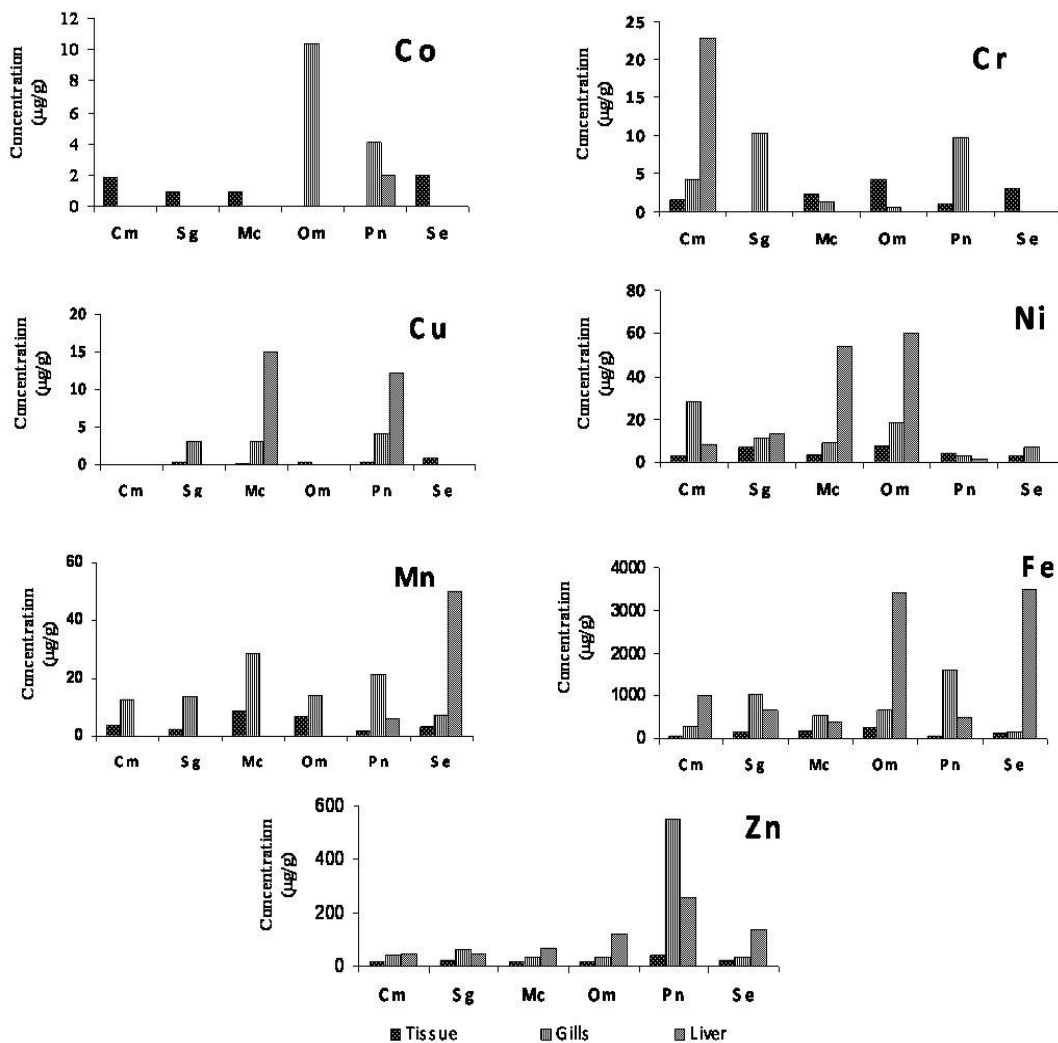


Fig:- 2 Concentration of Metals in fish Cm - *Channa melanosoma*, Sg - *Silurus glanis*, Mc - *Mugil cephalus*, Om - *Oreochromis mossambicus*, Pn - *Parastromateus niger*, Se - *Scardinius erythrophthalmus*

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