Fate of Metals in Fish under Variable Sewage Input in Fish Ponds

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Abstract- Use of raw sewage in aquaculture spelling prosperity to farmers is actually a veritable threat to the environment. Metal contaminated fish grown in the sewage-fed East Kolkata Wetlands, is potentially hazardous for the consumers. Alarmed by the increase of these persistent elements, an in-depth study was carried out to find out a possible way to reduce metal body burden in fish. The first part of the study deals with the quantitative analyses of metals in several species of fish reared in the sewage fed system. In the second part, sewage input in the ponds was temporarily stopped for six months that helped us to understand the depuration pattern of the accumulated metals from fish tissues. It was observed that, copper accumulates maximally with age in the fish liver especially in the benthic species such as Cirrhinus mrigala and Oreochromis nilitica. However, its depuration rate from the organ is low. After six months, the metal is completely excreted from muscle, skin, gills and kidney. Gills of Catla catla accumulate excess of zinc. Lead and zinc show size dependent increase in muscle, liver, kidney, skin and gills of fish but are steadily depurated in the relatively uncontaminated system. The rate of cadmium uptake and release is extremely low in fish.

Index Terms- Depuration, fish, habitat, metals, wastewater, wetlands

I. Introduction

The East Kolkata Wetlands with an area of 25,000 hectares are the largest basin for natural purification of wastewater .Once occupied by the Bidyadhari River, these wetlands receive city sewage, storm water run off and effluents from thousands of industries. Underground sewers drain the untreated city sewage to several pumping stations from where it is led into open surface outfall channels networks that distributes this water into the different fish ponds. Here the recycled organic waste of the sewage provides nutrients for commercially grown fish. Aquaculture thus maintains a balanced ecologically efficient system where effluent quality comes down to an acceptable range, reducing the BOD value of the sewage water. About 308 sewage-fed ponds show a turnover rate of 15,000 million tones of fish annually where average rate of organic loading vary between 20-70 kg per hectare. Efficient utilization of sewage is therefore crucial for aquaculture production that effectively meets the challenge of unemployment and protein crisis for the ever-increasing population of this area. (Kundu et al., 2008) .

However, these wetlands receive enormous pollutants including metals along with the effluents. In the natural waters, metals remain either in particulate or in soluble form but the labile fraction are more harmful.The impact of metal pollution depends upon the, source of wastewater, duration of their input or the presence of organic and inorganic particles in the water and sediment. Bioaccumulation of metals occurring in the food chain of the aquatic system, ultimately affects the human through, consumption of contaminated fish. In fish, metal levels vary as a function of their dietary difference and body size, which is closely related to their growth and metabolism. (Moriarty et al.,1984). All these interrelated factors determine the elemental concentration in the system and their relative availability, transport and toxicity to aquatic organisms.

Bioaccumulation of metals in fish warrants our attention. The present study, firstly attempts to quantify metal level in the different tissues of four commercial important fish species with various body weights, feeding habits and habitats. This is to investigate the growth related changes in the level of metals in fish tissues. Secondly, to find out the depuration pattern of metals by the tissues, sewage input in the sewage fed ponds was temporarily stopped for a period of six months. Samples of water, soil and fish tissues were analyzed before and after the experimental period. Difference in body metal level in fish provided a picture of the amount of metal depurated by tissues, in the relatively uncontaminated environment.

As fish, forms the major food for the people in this part of the country, attempt has been taken to reduce metal body burden by reducing the sewage input of the system. This would reduce the background level of metals that allowed the fish to excrete the accumulated ones. Metal bioaccumulation depends on the difference in uptake and depuration that varies with metals and fish species (Tawari-Fufeyin and Ekaye, 2007). Fish differing in food habit and its position in the tropic level show marked variation in depuration of metals. So different species with various body weights were chosen to determine the size related depuration pattern as younger fish have higher metabolic rate than the older ones.

II. SITE OF STUDY

The study was carried out in a sewage-fed farm "The East Kolkata Fisherman's Cooperative Society", situated by the Eastern Metropolitan Bypass near the Kasba connector ($22^o30'$ N; $88^o25'$ E), West Bengal, India. The farm, a part of the East Kolkata Wetlands, covers an area of 44.55 hectares supporting about 109 sewage-fed ponds. Here five sewage fed ponds were chosen for experiment that nursed four commercially important species , such as *Labeo rohita* Cuvier (a column feeder), *Catla catla* Valenciennes (a surface planktivore) , *Cirrhinus mrigala* (benthic fish) and *Oreochromis nilotica* Oken which is an exotic omnivore , ubiquitous in the derelict waters (Jayaram,1981) . Two different body weights (50g and 800g) of all the species were chosen for analyses.

III. MATERIALS AND METHODS

Fish were regularly collected from the sewage-fed ponds for quantitative analysis of the metals. These were dissected and the different tissues like muscles, liver, kidney, gills, skin and intestine were wiped dry and weighed accurately to 1g each for acid digestion. A modified wet digestion procedure was used to prepare biological samples for the determination of copper, lead, zinc and cadmium following the procedure of Chernoff (1975). Samples of fish tissues were taken in hard glass test tubes, and 5 ml of conc. HNO_3 acid was added to one gram of the sample, and left for overnight digestion at room temperature. The mixture was placed in a hot plate at 85 ± 5^oC and 5 ml (3: 2, Conc. Sulfuric acid: Perchloric acid) was then added to it. The digestion was carried out until it turned into a pale transparent solution. The mixture was cooled and filtered through an acid soaked filter paper and was adjusted to the required volume, with distilled water. Metal levels in water and soil were determined by standard methods of APHA , (1998) and Nafde, et al., (1998) respectively. The concentrations were detected in atomic absorption spectrophotometer (Varian AA.575).

Based on total amount of the sample taken, the actual concentration was calculated, and results were expressed in μg/g. A standard reference material checked the accuracy of determination. Comparison between metal concentrations in the tissues of the fish with various body weights were performed by One Way Analysis of Variance, ANOVA (Gomez and Gomez, 1984). Statistically significant differences were observed in the mean metal values of tissues and were evaluated at $p<0.5$.

IV. RESULTS AND DICUSSION

Quantitative estimation of metals in water and soil samples is shown in Table (1) and those in fish tissues in Figs (1-8). Percentage of metals depuration from the various tissues after the experimental period, in shown in Table (2).

Accumulation of metals in fish is time-dependent but their distribution depends on the metabolic demand of the various tissues. Besides, environmental factors along with the feeding habit, habitat, age, sex and body weight of fish play key role in accumulation (Authman, 2008)**.** Thus, the accumulation in various tissues is determined by the relative rates of metal binding and release. Moreover, chronic sublethal exposure of one metal can alter the uptake and distribution of another. Besides, accumulated elements are continuously released from the body, which is part of the homeostatic regulation of the organism. Excretion of metals also depends on both environmental and biological factors of a particular species while the final metal concentration in the body is influenced by the organism's ability to regulate these toxic, lipophilic elements.

Most of the metals exert certain amount of toxic effects especially when accumulated in excess, but the essential metals play important physiological roles at lower concentration. In the freshwater system, the essential metals such as copper and zinc are added from domestic sewage while agricultural and industrial runoffs contribute quantum of non essential metals such as lead and cadmium in the environment (Ambedkar and Muniyan, 2011).

Metal concentration in the East Kolkata Wetlands were recorded to be considerably higher in both soil and water fractions due to the continuous sewage input (Table 1). These are present at high enough levels to be considered as environmental health hazard as fish is especially susceptible to waterborne chemicals that are ultimately transferred to the human food chain.

In the present experiment, when sewage input in the fish rearing ponds was stopped for six months the level of copper, lead, zinc and cadmium in the water decreased considerably. However, appreciable decrease in the sediment was not evident because metals are irreversibly bound to soil matrix with the various inorganic and organic particles (Aderinola et al., 2009). It was observed that fish grown in sewage fed ponds show marked decline in its metal body content after six months of their stay in the relatively uncontaminated environment. However, specific metals show specific depuration rates from specific fish tissues.
Liver accumulates mostly dietary metals while other tissues accumulate those taken up from gills. (Jezierska and Witeska, 2006) .Therefore, in the benthic fish (C. *mrigala* and *O. nilotica*), copper is maximally accumulated in liver that rises with chronic exposure and body weight (Fig. 2). The metal has great affinity for organic particles in the sediment, which makes it readily available to the benthic organisms through their food. However, bioavailability of sediment-associated metals can also vary greatly between different taxa. Copper level in *L. rohita* and *C. catla* are much less due to their feeding habit in the upper strata of the pond. The metal remains low in other tissues in all the species under study. (Fig. I, 2) Metallothionein which play a protective role is synthesized to sequester and detoxify metals that are either depurated or stored in the body. However, elevated levels of hepatic metalothionein are observed even after rapid

excretion (McCarter and Roch, 1983). Within the cells, metals bound to this protein are incorporated into liposome, which are excreted through an electrochemical gradient that further facilitates metal uptake in a contaminated environment. However, when the capacity of the cell exceeds, histopathological changes may appear. Depuration process however may not always recover the lesions caused by chronic metal exposure.

When sewage input in the ponds was stopped, rapid clearance of copper was observed from all the tissues excepting from the liver and intestine (Fig. 1, 2) .This suggests multiple elimination routes of the metal including urinary, branchial and faecal. Depuration rate from liver and intestine was very low especially in the benthic species due to the availability of the food borne metal from the sediment. Besides, the liver being a storage organ actively removes accumulated metals from the other tissues. According to the spill over theory, part of the accumulated metal in liver is depurated through bile, which is reabsorbed through the intestinal mucosa. Thus, intestinal mucosa has been observed to concentrate considerable amount of copper from where the excretion rate is very slow. Intestine forms the ultimate depository of metals that is taken through food hence C. *mrigala* and *O. nilotica* accumulate higher amount of copper in this organ. (Olowu et al., 2010)

Results reveal that, younger *C. mrigala* accumulates higher levels of lead, the rate of which subsequently falls with chronic exposure in a polluted system (Fig. 3, 4). In the other species, lead rises only moderately with body weight. Gills and skin of catla also store the metal where the elimination rate is very slow. Generally, lead accumulation in tissues is proportional to the ambient concentration, (Tao et al., 1999) but the rate of its depuration is dependent on the overall body concentration (Schulz-Balder, 1974). Muscles of *L. rohita* and *O nilotica* shows higher depuration rate of the metal after six months. Liver and kidney of *C. catla* , *O* . *nilitica* and *C. mrigala* tend to retain the metal. However, from the hepatic tissue bile helps in elimination of the sulfhydryl reactive metals especially lead and cadmium through the formation of metal-glutathione complexes.

Dissolved and particulate metals are usually absorbed through passive diffusion or carrier mediated transport over the gill epithelium. These are also absorbed through skin and food. Zinc accumulates maximally in the gills especially in the surface residing *C. catla*. Normoxic condition of the surface water probably pumps the available metal, which continues to accumulate in its gills (Hughes and Floss, 1978). Kidney and gills act as both storage and depuration sites. Although considerable release of zinc was observed in the fish under study (excepting younger benthic fish) yet kidney is hardly considered as an excretory organ for metals in teleost.

Zinc content in the tissues is positively correlated with body weight of *C. catla* and *L. rohita* possibly due to the breakdown of their regulatory mechanism with continuous exposure in contaminated system. Juvenile fishes maintain higher proportion of this metal from their plantonic diet (Fig. 5, 6). However, in the two benthic species, the metal rises only slightly with body weight and after six months, these show very slow depuration rate. Zinc is retained in *C. catla* even in the uncontaminated environment. Mucus in gills and skin helps in considerable removal of metals..

Cadmium concentration keeps low and constant in all the fish species under study and body concentration reflects its level in the environment (Ekeanyanwu et al., 2011). The uptake of the metal is non-linear, and is not correlated with the body weight of fish (Bohn and McElroy, 1976) .This complements our findings (Fig. 7, 8). In spite of this low bioaccumulation potential, the metal shows a high concentration factor and its average concentration in fish tissue rise above the mean international level.

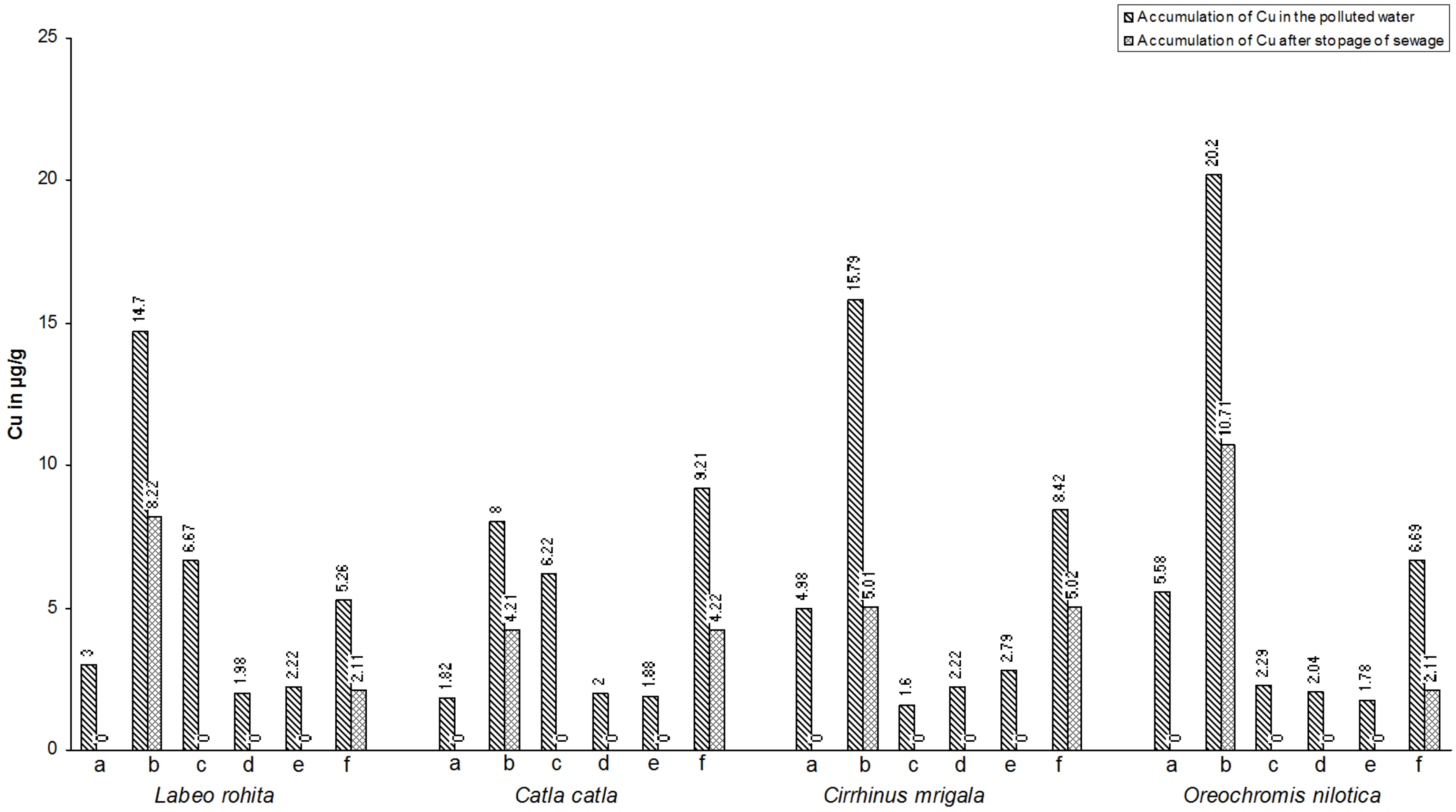
After six months, lead and zinc levels were reduced considerably than their initial value in the fish tissues while excretion rate of cadmium was low. Metal is excreted gradually in a metal free environment (Harrison and Klaverkamp, 1989). However, fish shows retention of lead and cadmium in the kidney and zinc in the gills.

In general, muscular depuration is normally higher as binding proteins are present in only low levels in these tissues. However, after six months only copper and lead levels decreased below the set international standard, in the muscle. (According to WHO standards , Maximum Permissible Limit for metals in the fish tissues are copper 20, lead 2 , zinc 45 and cadmium 0.3.) . Retention of metals in fish tissues, even at lower ambient environmental metal concentration is due to their binding in the non-exchangeable or slowly exchangeable pools of the body.

Adjusting metal uptake and depuration are the primary means of maintaining metal homeostasis that occurs naturally for essential elements. This control is less evident for non-essential ones as lead and cadmium show lower excretory rate even in the uncontaminated environment. Tolerable dietary intake of metals exceeded among population consuming large amount of fish chronically exposed to sewage effluents. Trace metal contaminated fish from sewage-fed wetlands can predispose consumers to toxicity so there is a need for constant monitoring of this stressed environment.

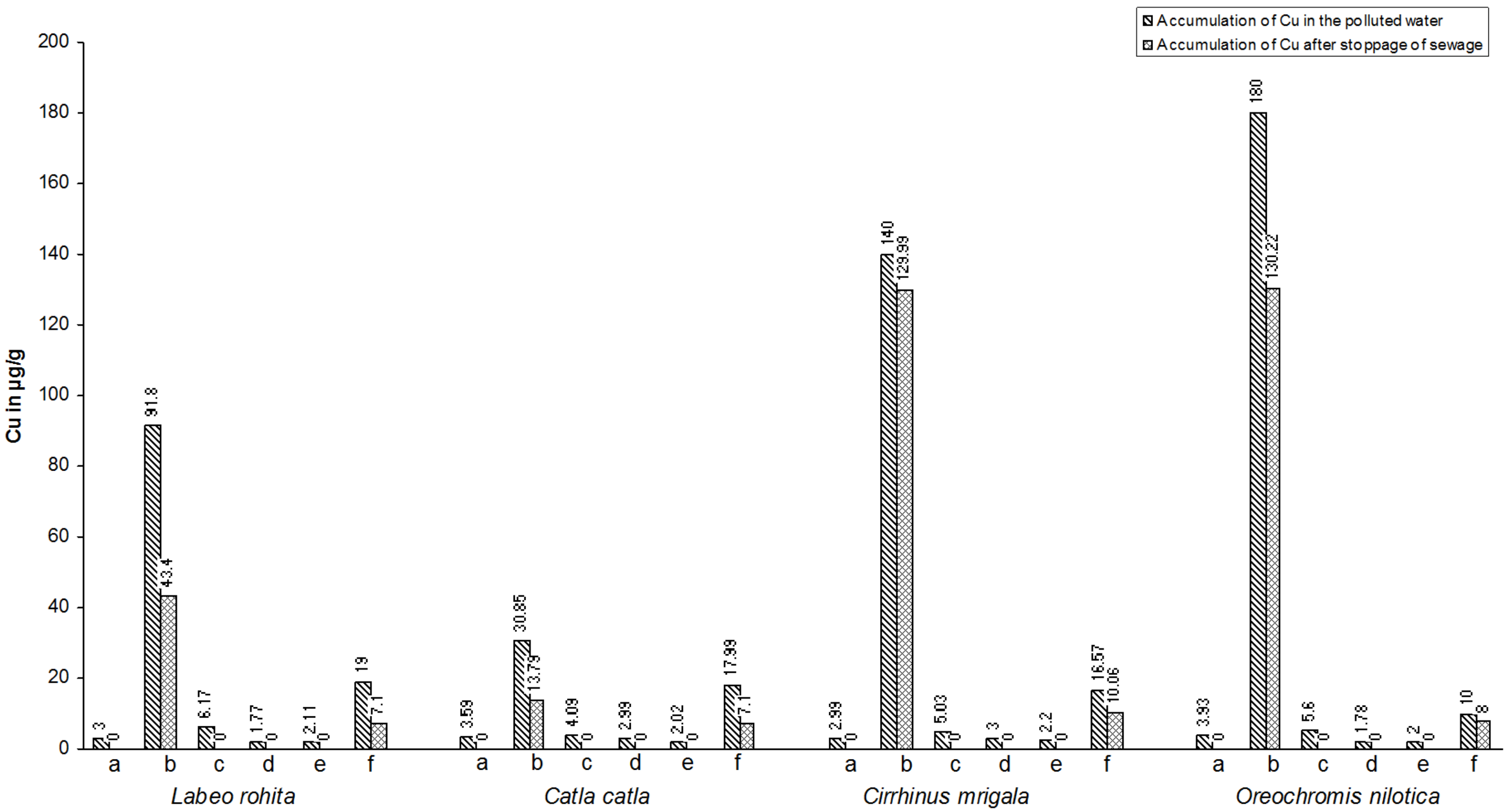
Measures to eliminate metals from commercial fish is a priority so regulation or temporary stoppage of sewage effluents in aquaculture ponds can reap positive results to reduce metal related health hazards for population that depend on fish as the major source of animal protein. In a contaminated environment the rate of metal uptake exceeds its excretion but fish exposed to polluted system, if allowed to stay in a relatively metal free environment, show a gradual decline of these xenotoxic substances.

Fig. 1 : Depuration pattern of accumulated copper in the different tissues with fish of 50 g of body weight



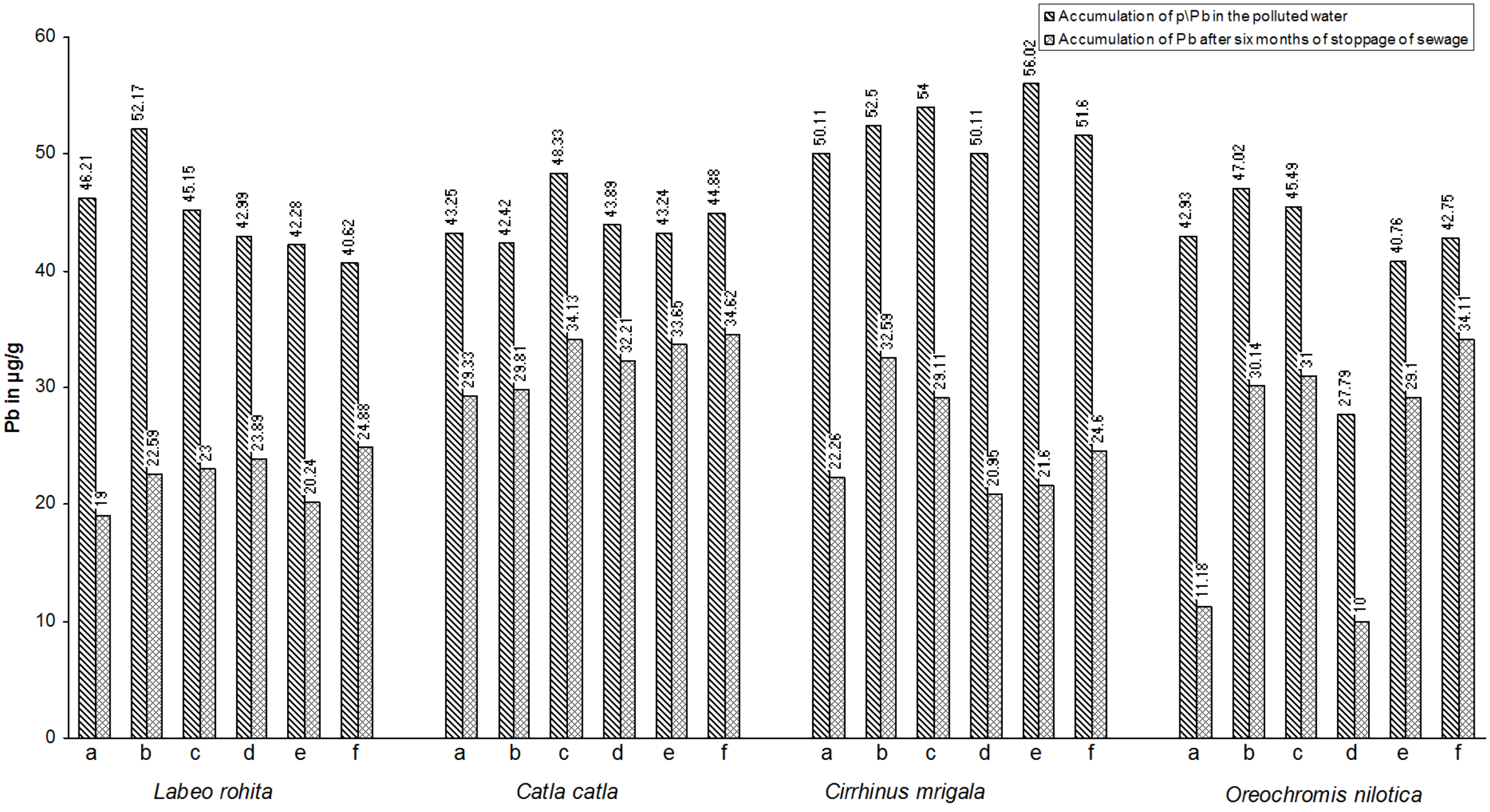
a : Muscle, b : Liver, c : Kidney, d : Gills, e : Skin, f : Intestine

Fig. 2 : Depuration pattern of accumulated copper in the different tissues with fish of 800 g of body weight



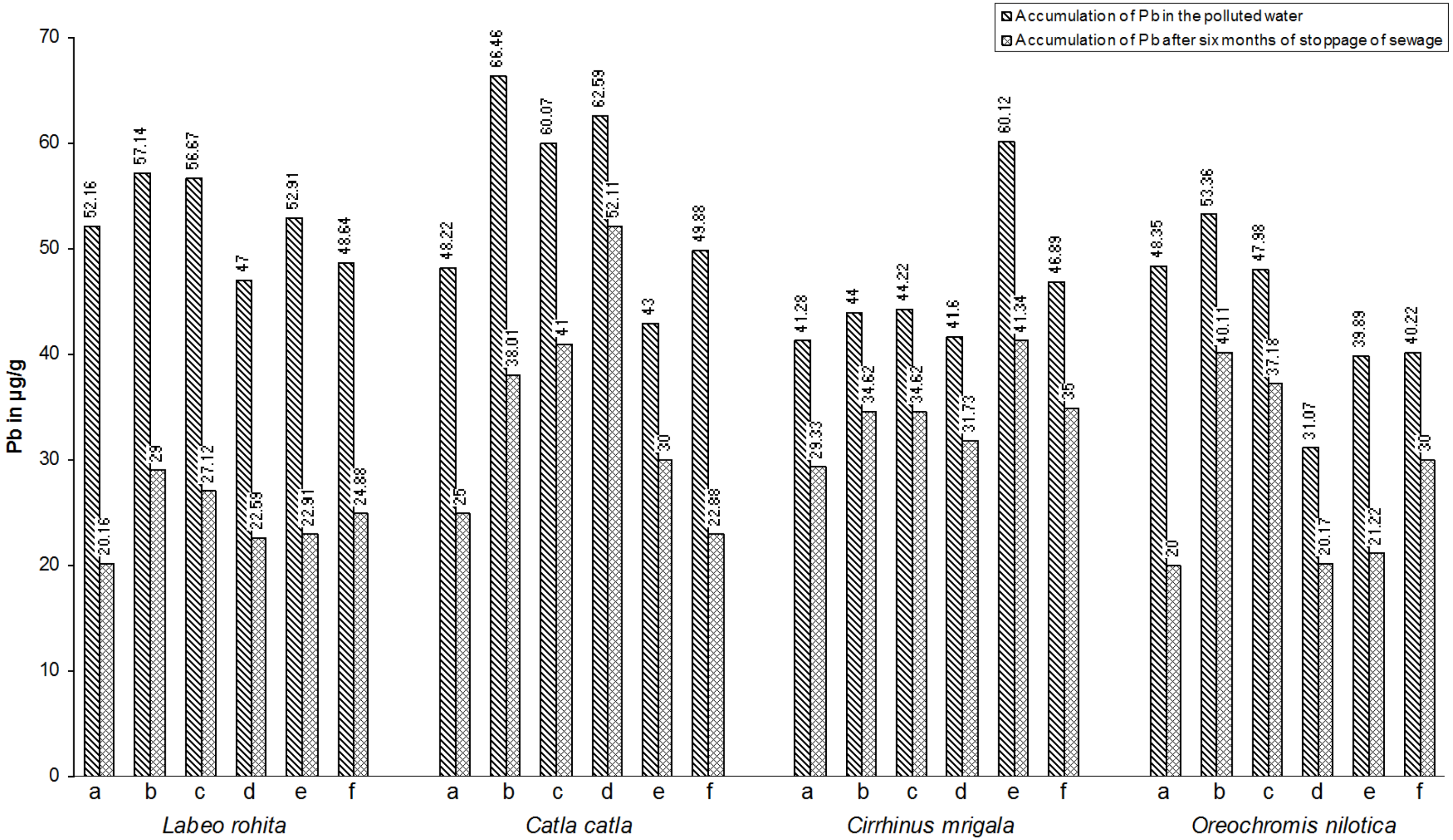
a : Muscle, b : Liver, c : Kidney, d : Gills, e : Skin, f : Intestine

Fig. 3 : Depuration pattern of accumulated lead in the different tissues with fish of 50 g of body weight



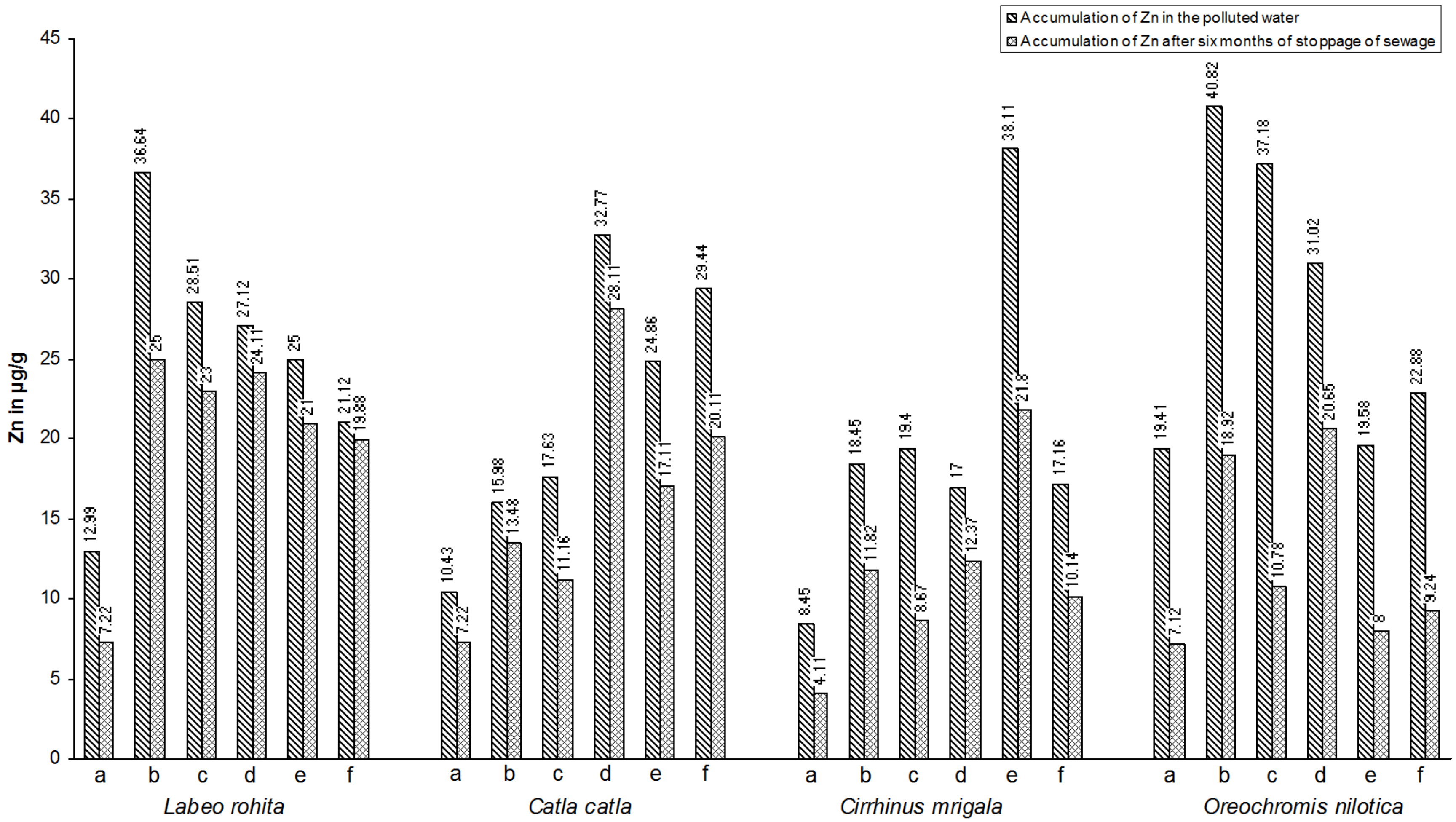
a : Muscle, b : Liver, c : Kidney, d : Gills, e : Skin, f : Intestine

Fig. 4 : Depuration pattern of accumulated lead in the different tissues with fish of 800 g of body weight



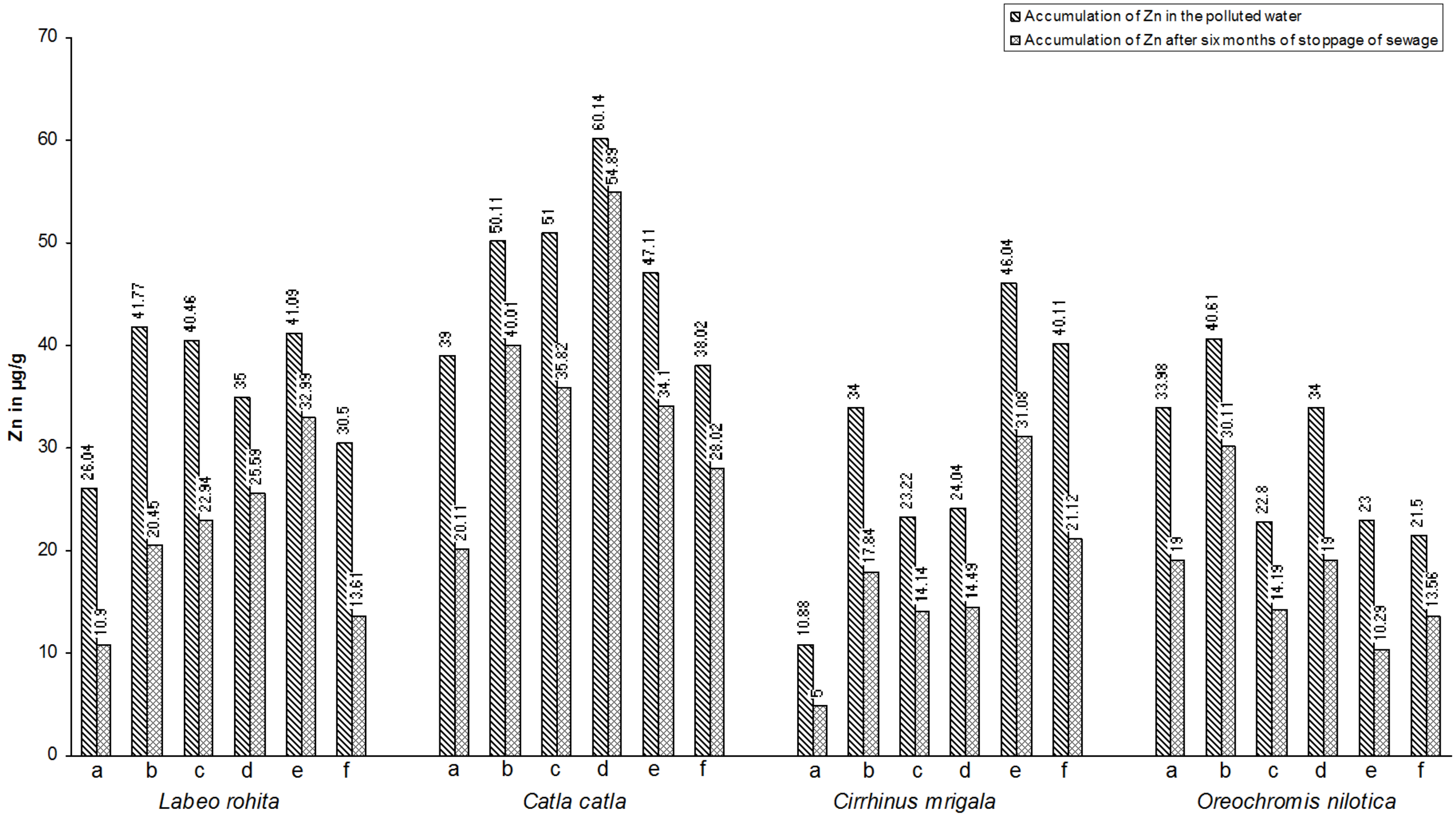
a : Muscle, b : Liver, c : Kidney, d : Gills, e : Skin, f : Intestine

Fig. 5 : Depuration pattern of accumulated zinc in the different tissues with fish of 50 g of body weight



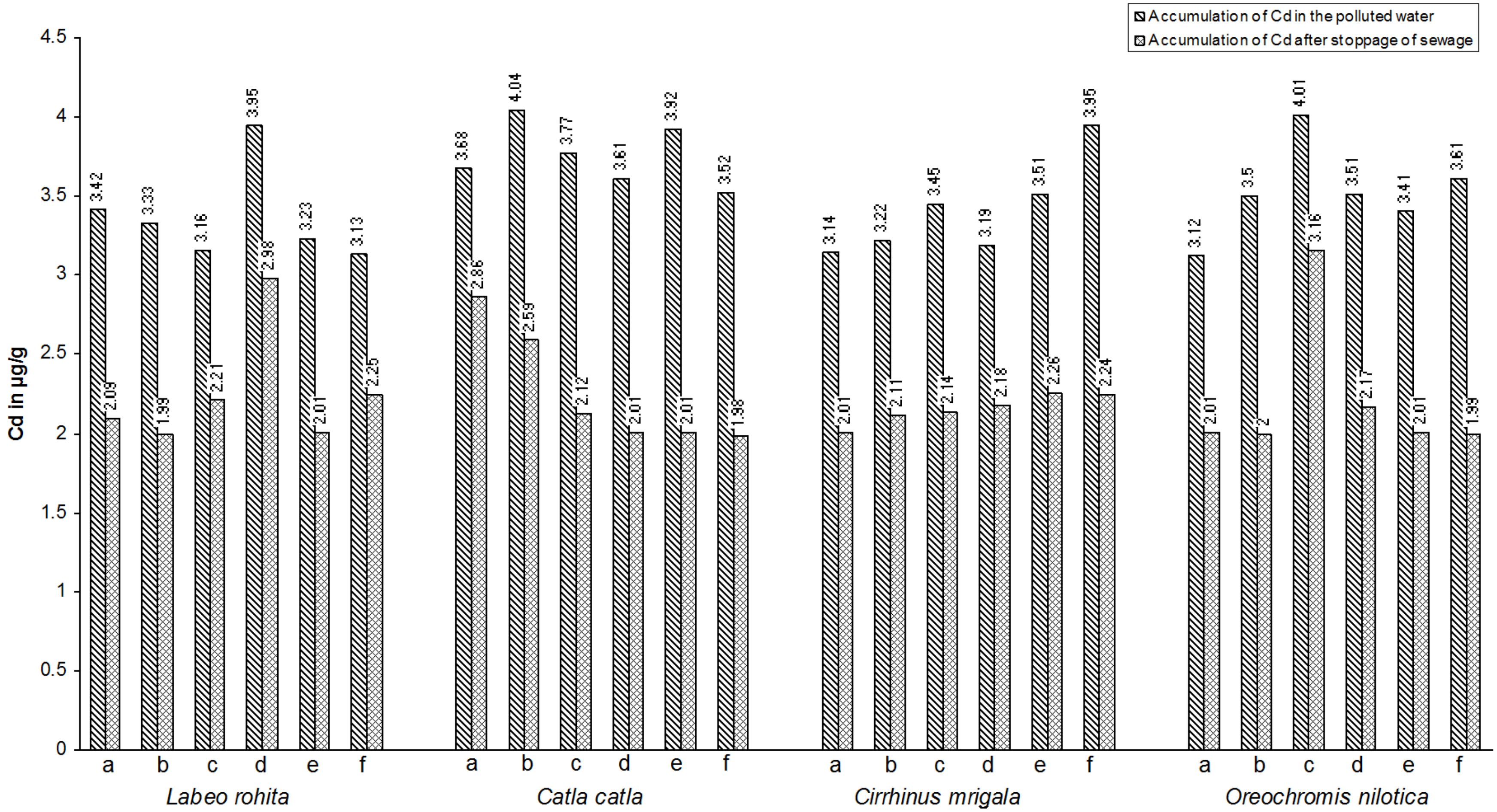
a : Muscle, b : Liver, c : Kidney, d : Gills, e : Skin, f : Intestine

Fig. 6 : Depuration pattern of accumulated zinc in the different tissues with fish of 800 g of body weight



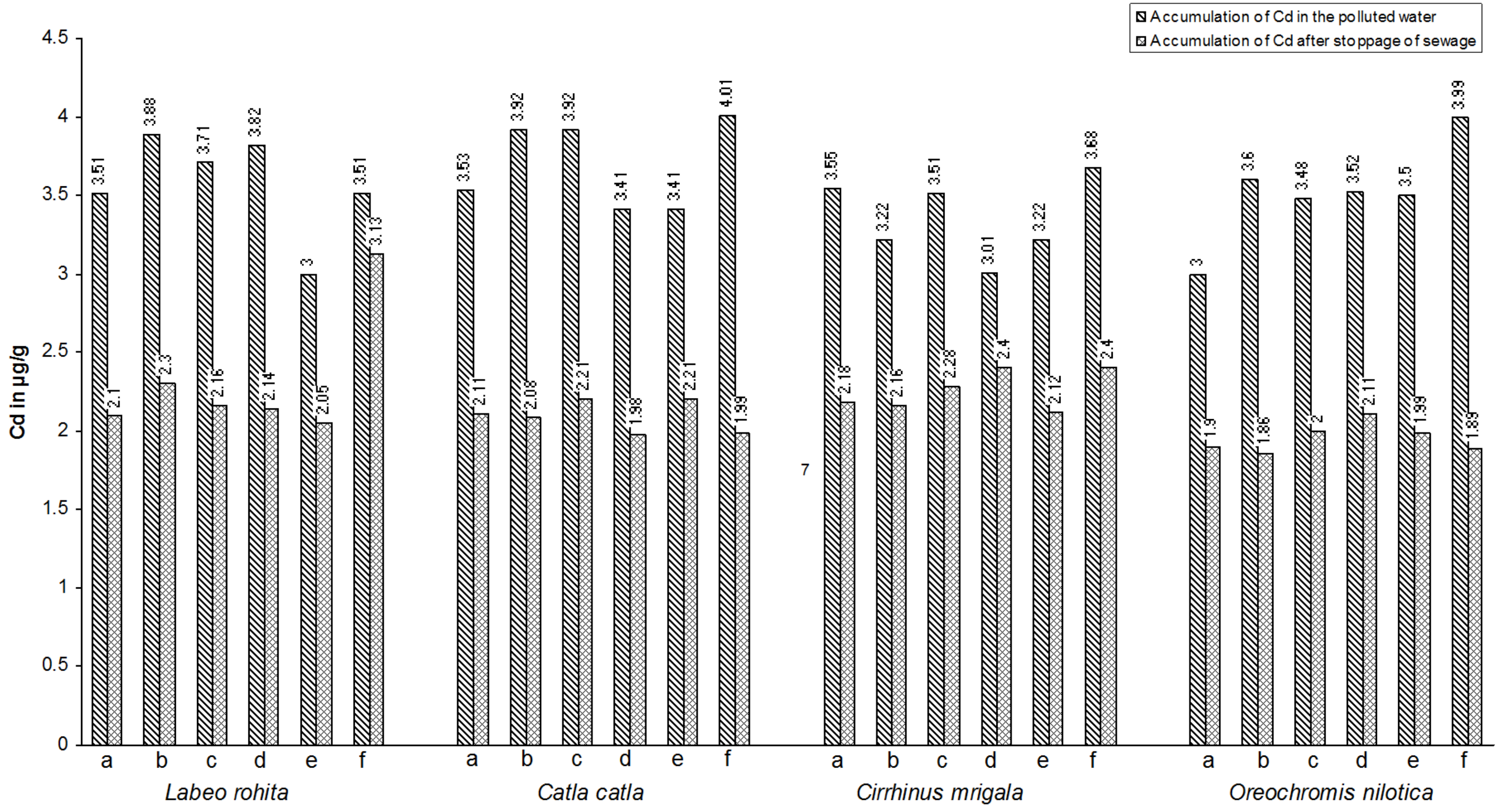
a : Muscle, b : Liver, c : Kidney, d : Gills, e : Skin, f : Intestine

Fig. 7 : Depuration pattern of accumulated cadmium in the different tissues with fish of 50 g of body weight



a : Muscle, b : Liver, c : Kidney, d : Gills, e : Skin, f : Intestine

Fig. 8 : Depuration pattern of accumulated cadmium in the different tissues with fish of 800 g of body weight



a : Muscle, b : Liver, c : Kidney, d : Gills, e : Skin, f : Intestine

Table 1: Deputation percentage of metals from the tissues of Fish

Metals	Tissues	*L. rohita 50g*	*L. rohita 800g*	*C. calta 50g*	*C. catla 800g*	*C. mrigala 50g*	*C. mrigala 800g*	*O. nilitia 50g*	*O. nilitica 800g*
Copper	Muscle	100	100	100	100	100	100	100	100
	Liver	44.08	52.73	47.38	55.30	68	7.15	46.98	27.66
	Kidney	100	100	100	100	100	100	100	100
	Gills	100	100	100	100	100	100	100	100
	Skin	100	100	100	100	100	100	100	100
	Intestine	59.88	62.63	54.18	60.53	40.38	39.29	69.00	20
Lead	Muscle	58.88	61.35	31.07	48.15	55.58	28.95	73.96	58.63
	Liver	56.70	49.25	29.73	42.81	37.92	21.32	35.90	24.83
	Kidney	49.06	52.14	29.38	31.75	46.09	21.71	31.85	22.51
	Gills	44.43	51.94	26.61	16.74	58.19	23.73	64.01	35.08
	Skin	52.13	56.70	22.18	30.23	61.44	31.24	28.61	46.80-
	Intestine	38.75	48.85	22.86	54.13	52.33	25.36	20.22	25.41
Zinc	Muscle	44.42	58.14	30.78	18.89	51.36	54.04	63.32	44.08
	Liver	31.77	51.04	11.89	20.16	35.93	47.53	53.65	25.86
	Kidney	19.33	44.51	36.70	29.76	55.31	39.10	71.00	37.76
	Gills	12.54	27.06	14.22	8.73	27.24	39.73	33.43	44.11
	Skin	16.00	19.71	31.17	27.62	42.80	32.49	59.14	55.26
	Intestine	05.80	16.89	31.69	26.30	40.91	47.34	59.61	36.93
Cadmium	Muscle	40.17	35.97	22.28	40.22	35.99	38.59	35.58	36.67
	Liver	40.72	58.26	35.89	46.94	34.47	32.92	42.86	48.33
	Kidney	41.78	30.06	43.77	43.62	37.97	35.04	21.20	42.53
	Gills	43.97	24.54	44.32	59.53	31.66	20.27	39.89	40.05
	Skin	31.67	28.48	48.72	35.19	35.61	34.16	33.14	43.14
	Intestine	10.82	28.12	43.75	50.37	35.70	34.78	44.88	52.63

Values are expressed in percentage

Table 2: Concentration of Metals in Water and Sediment

	Copper	**Lead**	**Zinc**	**Cadmium**
Fresh water *	Trace	Trace	Trace	Trace
Sewage Water *	10.97± 2.87	93.33 ± 9.39	16.44± 5.59	2.23± 0.74
Sewage Water, after six months. *	2.11 ±0.07	17.79± 1.88	3.99± 0.74	0.79±.01
Pond sediment.*	41.00± 5.00	32.00± 2.18	60.80 ±9.11	3.48± 1.11
Pond Sediment , after six months. *	37.00± 3.44	28.11± 2.99	56.00± 4.07	3.14±0.99

***Mean ± SD of 5 different observations

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