

# DETERMINATION OF Cd, Pb, AND Zn IN FISH, WATER AND MUD FROM GHAZI GHAT OF INDUS RIVER (PUNJAB, PAKISTAN): A RELATIONSHIP BETWEEN QUANTITY OF METAL AND WEIGHT OF FISH

**Ghulam Yasin and Muhammad Younas Khokhar**

*Department of Chemistry, Bahauddin Zakariya University, Multan*

**email:** khokharmy@yahoo.com

## **Abstract**

Water, mud and fish samples were collected from the Ghazi Ghat of the river Indus (Punjab, Pakistan). Fish were dissected and their livers and muscles were analyzed. Wet digestion method was used for the digestion of fish and mud samples. All samples were analyzed for Cd<sup>2+</sup>, Pb<sup>2+</sup> and Zn<sup>2+</sup> by Flame Atomic Absorption Spectrometry (FAAS). Cadmium and Zinc were determined directly, while lead was pre-concentrated on cation-exchange resin microcolumn by Flow Injection Analysis (FIA) technique before analysis by FAAS. A relationship was found among water, mud and fish samples.

**Keywords:** Cation-exchange, flame atomic absorption spectroscopy, flow injection analysis, resin.

## **INTRODUCTION**

Rivers and lakes are very important part of our natural heritage. But the human activities contributed significantly to the pollution of water by organic as well as inorganic substances especially the heavy metals. The dust in air of crowded cities increases the concentration of toxic metals. The concentration of heavy metals in surface water is dependent on several factors: air borne contribution from long-range transport; local point sources; natural presence in bedrocks and soils; air borne contribution from soil dust; municipal and industrial discharges; agricultural run offs; acid rains; hydrocarbon products; solvents; inorganic substances including heavy metals; acid residues; pesticides and fecal bacteria are the main source of the water pollutants. As the metals are elements which do not break up into less harmful chemicals, therefore, they accumulate where they are released [Kennicott 1992].

Contaminated sediments are another significant source of pollution. They may be derived from inputs of suspended solids to which toxic substances are absorbed, such as soil particles in surface water, run-off from fields treated with pesticides. Alternatively, the natural suspended material in a water source as well as the riverbed surface can absorb chemicals and metals (Zn, Cd, Pb etc.) from water. When the suspended material settles down, the toxic material forms a reservoir;

the extent to which this can cause harm to aquatic life depends on the strength of the bond between chemicals and the particles. Thus knowledge of the distribution of heavy metals in water, sediments and fauna play a key role in detecting sources of heavy metal pollution in aquatic ecosystem [Forstner 1981]. River Indus is the biggest among five rivers that flow in Pakistan. It is water from glaciers of Karakoram and Hindu Kush. Crossing the Himalayas through a very deep gorge, it turns to the southwest and enters Pakistan. After leaving the mountainous region at Kalabagh the river enters the plains of Punjab and Sind. Before it enters the Arabian Sea, it passes along many cities like Nowshera, Dera Ismail Khan, Durya Khan, Sukkur, Tando Jam, Hyderabad, etc., and carries along with it some of the industrial and domestic water wastes.

The life of aquatic species especially fish could be greatly affected by the population wastes thrown into the river. Ghazi Ghat is a low populated area in the District Muzaffar Garh along which Indus River flows. In the present study an attempt has been made to carry out investigation about the concentration of toxic metals like cadmium, zinc and lead in fish, water, and mud in this area. Having this information, the quality of water will be judged for usage i.e. whether it is good or bad for fish. Also, the relationship between the quantity of metals and weight of fish will be discussed.

## MATERIALS AND METHODS

Through out the experimental work high purity analytical grade chemicals and de-ionized water was used. pH of de-ionized water was 6.78.

### SAMPLING OF FISH

A total of seventeen Fish samples of different varieties and sizes were collected (Table 1) randomly from the Indus River at Ghazi Ghat MuzaffarGarh in November 2001. They were healthy, mature. The fish type and size, i.e., length & weight, are mentioned in Table 1. Fish were collected using gill nets and transported to the laboratory. They were dissected with sterilized stainless steel blades. Liver and muscles of each fish was dissected and stored separately in the polyethylene airtight sterilized bottles (25ml) at 20°C for further analysis.

**Table 1:** Length & Weight of Fish caught at Ghazi Ghat, Indus River.

Sr. No.	Fish Type	Length of the Fish (cm)	Weight of the Fish (g)
1.	Mystius singala	33.0	166.95
2.	Tylapia mossambica (i)	20.0	192.50
	(ii)	19.0	133.00
	(iii)	19.0	147.10
	(iv)	19.0	140.50
3.	Notopterus notopterus	25.0	129.79
4.	Rita rita (i)	25.0	190.30
	(ii)	26.0	167.40
5.	Labeo culbaso	23.0	160.58
6.	Catla catla	44.0	1200.00
7.	Cirrhinus (i)	27.0	220.80
	(ii)	18.5	62.20
	(iii)	19.0	74.20
8.	Labeo rhita (i)	27.0	315.00
	(ii)	25.0	225.70
	(iii)	25.0	258.00

### SAMPLING OF WATER AND MUD

Three water samples were collected randomly about 20cm below the water surface to avoid the floating matter. Stoppard, acid-washed polyethylene bottles were used for sampling. Water samples were filtered with a Whatman filter paper No.41 and acidified to 1% HNO<sub>3</sub>.

Three samples of mud beneath the water surface were collected randomly with the help of scooping spoon. They were stored in Stoppard, acid-washed polyethylene jar of 1Kg and transferred to the laboratory for further analysis.

### PREPARATION OF SAMPLES FOR ANALYSIS

Wet digestion method was used for the preparation of sample solution of biological organs. Accurately weighed organs were taken in a pre-weighed china dish. For each one gram of organ, 4ml concentrated nitric acid and one ml of H<sub>2</sub>O<sub>2</sub> was added and heated to digest the biological samples. Flame heating was continued for about 55 minutes to boil off any excess of H<sub>2</sub>O<sub>2</sub>/HNO<sub>3</sub>. Whole process was repeated three times. Finally, the digested samples were dissolved in 1% HNO<sub>3</sub> and filtered. The residue was also washed with 1% HNO<sub>3</sub>. The filtrate was made up to 25ml using 1% HNO<sub>3</sub> solution.

For 5g of wet mud in pre-weighed china dish was added 20ml of conc. HNO<sub>3</sub> and 5ml of H<sub>2</sub>O<sub>2</sub> (4:1 v/v). The sample was heated to dryness using controlled and very low flame. The whole process was repeated three times. Finally, the samples were dissolved in 1% HNO<sub>3</sub>, filtered and volume was made up to 25ml in a measuring flask.

Water samples were filtered and stored in 1% HNO<sub>3</sub>. They were analyzed for Cd, Pb & Zn without further treatment.

### METHODS OF ANALYSIS FOR Cd, Zn AND Pb

The working standards were prepared by appropriate dilution of 10ppm standard solutions of metals (Cd, Zn & Pb). Cadmium and zinc standards were from 0.1 to 0.6ppm. and lead 0.04 to 0.28ppm. A matrix solution containing 5ml of 1000ppm of Na, K, Ca & Mg per 100ml of the standard solution was also added. Final volume of each standard was made up with 1% HNO<sub>3</sub>.

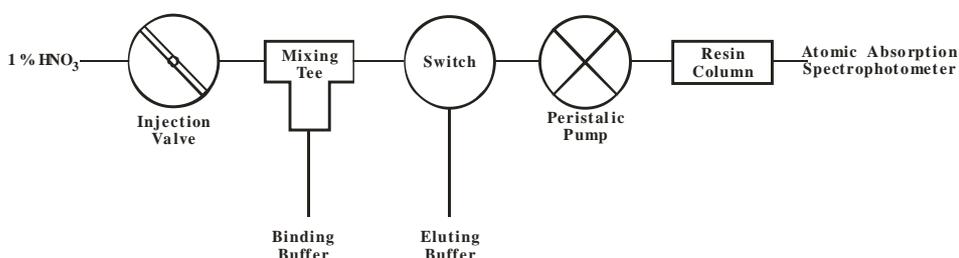


Fig. 1: Flow Injection Manifold.

Flame Atomic Absorption Spectrometry was used to determine the elements. Standards and samples for Cd and Zn were injected directly to the Flame Atomic Absorption Spectrometer. While for Pb, samples and standards were pre-concentrated by ion-exchange resin and injected automatically to the instrument through a Flow Injection manifold as shown in Fig. 1. A glass microcolumn of 0.39 ml capacity filled with cation-exchange resin and an injection valve of 2.212

ml volume were incorporated into the manifold. Binding and eluting flow rate were maintained at  $3\text{ml min}^{-1}$ . Standards and samples of lead ions in 1% nitric acid solution were mixed with borate buffer pH 9.25 in a mixing valve to obtain a pH 8.75 for binding purpose [Novikov 1990]. The binding and eluting buffers (2M  $\text{HNO}_3$ ) were changed with the help of a switch and then passed through peristaltic pump, a microcolumn and finally to the atomic absorption spectrophotometer. The output was recorded on a recorder.

## RESULTS AND DISCUSSION

The present study was carried out on the pollution of metals in water at Ghazi Ghat, river Indus. For this purpose, samples of fish, water and mud were collected from Ghazi Ghat. Fish were dissected and their livers and muscles were stored in the freezer and analyzed for Cd, Pb and Zn. Similarly water and mud samples were prepared for their trace metals analysis.

### CADMIUM ANALYSIS

Cadmium is a non-essential trace element. It is potentially toxic to wildlife and fish in water. The minimum concentration level (MCL) of cadmium in  $\text{H}_2\text{O}$  is 0.005 ppm [USEPA 1987]. Eisler [1985] found that cadmium concentration in  $\text{H}_2\text{O}$  as low as 0.8-9.9 ppb was lethal to aquatic insects, the crustaceans, and teleosts. Moore and Ramamoorthy in 1984 reported that the normal levels of cadmium in fresh water should be from 0.01 to 0.5 ppb. The cadmium concentration in the water of Ghazi Ghat at river Indus (Table 2) was found from 0.05 to 0.07 ppm, a quite high value for aquatic life. Where as the samples of mud from where the Fish were caught contain cadmium 0.45 to  $0.6\ \mu\text{g g}^{-1}$  of mud (Table 2). This indicates that  $\text{H}_2\text{O}$  of Ghazi Ghat is not appropriate for human consumption.

**Table 2:** The Concentration of Cadmium found in Water and Mud Soil.

	Cadmium		
	(i)	(ii)	(iii)
Water (ppm)	0.05	0.07	0.06
Mud Soil ( $\mu\text{g g}^{-1}$ )	0.45	0.60	0.50

The cadmium concentration in muscles and livers of Fish of different varieties at Ghazi Ghat, Indus River, was found to be very low compared to literature reported values. In *O. niloticus*, on wet weight basis cadmium is 0.43 – 0.77 ppm in liver, while it is 0.39 to 0.65 ppm in muscular tissues [Adham 2001]. Eisle [1971] determined 5.00 ppm cadmium on dry weight basis in the whole body of *Fundulus heteroclitus* which is potentially lethal. Table 3 shows that minimum concentration of cadmium in liver of *mystius singala* fish is  $1.1\ \mu\text{g g}^{-1}$  while maximum is found in *Cirrhanus*, i.e.  $4\ \mu\text{g g}^{-1}$ . Similarly cadmium concentration in muscles of Fish was found to be 0.25 to  $1\ \mu\text{g g}^{-1}$  of sample.

### ZINC ANALYSIS

Zinc is an essential trace element to life. The minimum concentration of zinc level acceptable in water is 5ppm [USEPA 1987]. The values of zinc for aquatic life and human consumer are 0.057 and 0.005 ppm zinc respectively. The values of zinc in Table 4 show undetectable level of zinc in water. The concentration of

zinc in mud soil was found to be 4.3 to 4.5  $\mu\text{g g}^{-1}$  of the sample (Table 4). Values of zinc ranging from 7 to 109  $\mu\text{g g}^{-1}$  of liver sample of Fish were found. The highest values were found in the livers of Cirrhanus and lowest in Labeo Rhita fish. In the muscles of fish zinc varies from 1.12 (Labeo rhita) to 24.88  $\mu\text{g g}^{-1}$  (Tylapia mossambica) of sample (Table 5).

**Table 3:** The Concentration of Cadmium Found in Liver and Muscles of Different Types of Fish.

Sr. No.	Fish Type	Cadmium in Liver ( $\mu\text{g g}^{-1}$ )	Cadmium in Muscle ( $\mu\text{g g}^{-1}$ )
1.	Mystius singala	1.10	0.25
2.	Tylapia mossambica (i)	0.71	0.25
	(ii)	1.80	0.50
	(iii)	1.40	0.25
	(iv)	3.00	0.62
3.	Notopterus notopterus	1.30	0.50
4.	Rita rita (i)	1.70	0.38
	(ii)	3.80	0.38
5.	Labeo culbaso	1.70	0.50
6.	Catla catla	1.50	0.50
7.	Cirrhinus (i)	1.50	0.62
	(ii)	4.00	0.38
	(iii)	5.80	1.00
8.	Labeo rhita (i)	2.20	0.50
	(ii)	1.80	0.38
	(iii)	1.80	0.62

**Table 4:** The Concentration of Zinc Found in Water and Mud Soil.

	Zinc		
	(i)	(ii)	(iii)
Water (ppb)	N.D	N.D	N.D
Mud Soil ( $\mu\text{g/g}$ )	4.30	3.90	4.50

**Table 5:** The Concentration of Zinc found in Liver and Muscles of Different Types of Fish.

Sr. No.	Fish Type	Zinc in Liver ( $\mu\text{g g}^{-1}$ )	Zinc in Muscle ( $\mu\text{g g}^{-1}$ )
1.	Mystius singala	53.06	7.12
2.	Tylapia mossambica (i)	30.34	13.12
	(ii)	42.00	24.50
	(iii)	38.93	14.00
	(iv)	91.50	24.88
3.	Notopterus notopterus	62.75	18.50
4.	Rita rita (i)	25.83	11.62
	(ii)	70.00	10.00
5.	Labeo culbaso	41.94	12.00
6.	Catla catla	63.75	15.88
7.	Cirrhinus (i)	42.75	10.87
	(ii)	109.00	12.50
	(iii)	65.00	12.00
8.	Labeo rhita (i)	43.25	11.75
	(ii)	28.25	9.25
	(iii)	7.00	1.12

## LEAD ANALYSIS

USEPA [1987] water Pb concentration is 0.015 ppm (MCL). In 1995, USEPA listed criteria for water Pb to the protection of aquatic life is 7.7 ppb and human consumer 8.50 ppb for adults. Both criteria were based on four days average

concentration. The water of Ghazi Ghat at the river Indus was found to contain Pb from 3.97 to 7.93 ppb (Table 6). In mud soil samples, Pb varies from 6.128 to 6.818  $\mu\text{g g}^{-1}$  of sample (Table 7). According to the above standards Pb level at Ghazi Ghat is non hazardous.

**Table 6:** The Concentration of Lead in Water and Mud Soil.

	Lead		
	(i)	(ii)	(iii)
Water (ppb)	7.93	4.83	4.83
Mud Soil ( $\mu\text{g g}^{-1}$ )	6.818	5.782	6.128

**Table 7:** The Concentration of Lead Found in Liver and Muscles of Different Types of Fish.

Sr. No.	Fish Type	Lead in Liver ( $\mu\text{g g}^{-1}$ )	Lead in Muscle ( $\mu\text{g g}^{-1}$ )
1.	Mystius singala	4.990	2.719
	Tylapia mossambica (i)	5.980	2.417
2.	(ii)	14.90	4.143
	(iii)	8.750	2.934
	(iv)	10.870	3.150
3.	Notopterus notopterus	8.720	3.883
4.	Rita rita (i)	8.820	3.150
	(ii)	11.220	2.502
5.	Labeo culbaso	5.850	3.322
6.	Catla catla	3.880	2.155
	Cirrhinus (i)	3.880	1.165
7.	(ii)	23.820	2.502
	(iii)	23.590	3.538
	Labeo rhita (i)	8.020	2.805
8.	(ii)	4.910	3.754
	(iii)	3.450	1.510

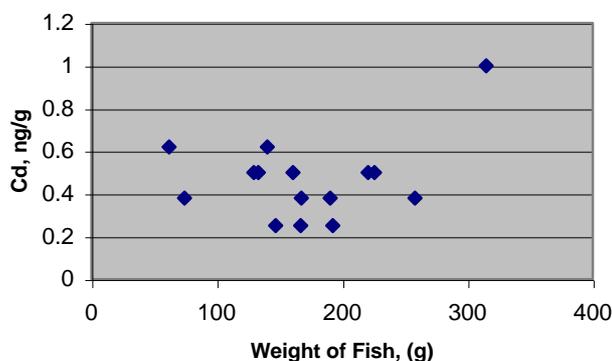
The concentration of lead in the Fish of Ghazi Ghat is quite low than literature values. The average reported concentration of Pb in the fish from New Madrid Area is 0.21ppm [Kurey 1991]. While Catostomids, Common Carp., and Blue gill on the Big River of the South eastern Missouri contained Pb 0.1- 0.8 ppm [Schmitt 1984], 3 ppm [Wiener 1984], and 0.4 ppm [Wiener 1984] respectively. According to Kurey [1991], Pb in both aquatic and terrestrial vertebrates localizes in hard tissues, such as bone and teeth. These types of tissues were not targeted for the present assays. Table 7 shows the Pb concentration in liver is higher than muscle. It was found that Pb in liver varies from 3.88 to 23.82  $\mu\text{g g}^{-1}$  of sample while it is 1.510 to 4.143  $\mu\text{g g}^{-1}$  of sample in fish muscles. Moor and Ramamoorthy [1984] reported that Lead residues in muscle tissues were only slightly lower than in specific organs. The present lead data (Table 7) is considered to be safe in Pb concentration in fish at Ghazi Ghat, Indus River, Pakistan.

## QUANTITY OF METALS VERSUS WEIGHT OF FISH

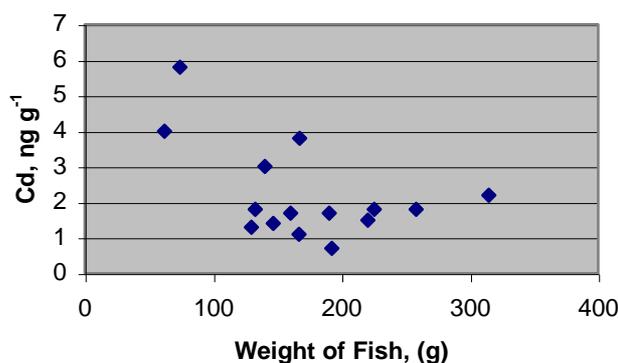
The concentration of cadmium found in muscles and liver is plotted against the weight of the fish in Figs. 2 & 3. It first increases and then decreases as the weight of the fish is increased. The values of Catla catla are not plotted as its weight was large, i.e. 1200 g. It could squeeze the other values and obscure the trend. From the diagrams, it can be safely concluded that a fish of higher weight, i.e. greater than 150 g contains less cadmium than a fish of lower weight, i.e. less than 150 g. Also, a graph between the weight of fish and concentration of zinc in

muscles and liver is plotted in Figs. 4 and 5. The trend is same as observed with cadmium, i.e. concentration of zinc is greater in lower weight of fish than higher ones. In Figs. 6 and 7 it is shown the concentration of lead in muscles and liver versus weight of fish. It is higher in lower weights of fish than larger ones. The figure shows that from 150 g to higher weights of fish contains less lead than smaller ones. Therefore, a fish of larger size has lower concentration of metals and is safe to eat.

**Fig.2 Cd in Muscles vs Wt. of Fish**

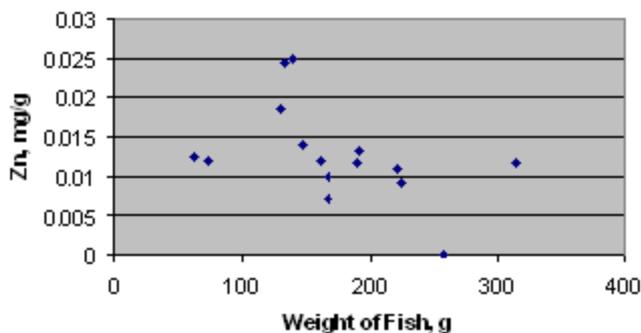
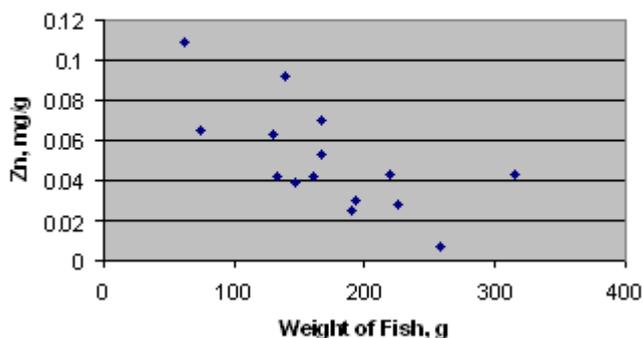
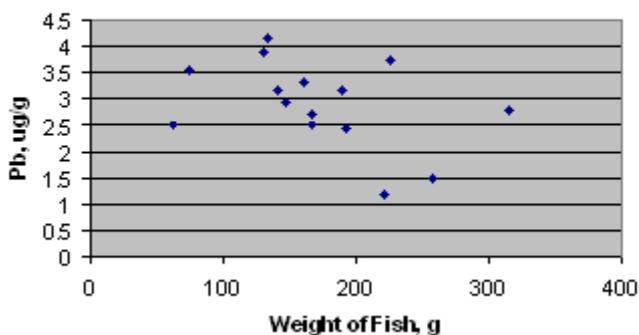


**Fig.3 Cd in Liver vs Wt. of Fish**

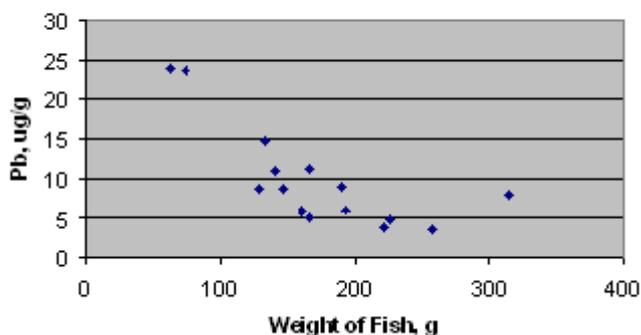


## CONCLUSIONS

From the foregoing discussion, it can be concluded that the observed value of cadmium concentration in the water of Ghazi Ghat at river Indus (Punjab, Pakistan) was found from 0.05-0.07 ppm (Table 3), which is quite high toxic value for aquatic life. The samples of mud from where Fish were caught contain cadmium 0.45 to 0.6  $\mu\text{g g}^{-1}$  of mud (Table 3). The values of zinc in water and mud (Table 5) were lower than MCL level [USEPA 1987]. The concentration of lead in water and mud were quite low (Table 6) and it poses absolutely no problem to the aquatic life and human drinking. Lead values found were much lower than MCL values [USEPA 1987].

**Fig. 4 Zn in Muscle vs Wt. of Fish, g****Fig. 5 Zn in Liver vs Wt of Fish, g****Fig. 6 Pb in Muscles vs Wt. of Fish, g**

The cadmium, zinc and Lead concentration in muscles and livers of a variety of fish of Indus River at Ghazi Ghat were found to be very low. Generally, it can be concluded that, cadmium, zinc and Pb concentration in water, mud and Fish were quite lower than the MCL values except cadmium in water, which appears to be misfit. Reasonably, it can be said that water and Fish of Ghazi Ghat are suitable to aquatic life and human consumption.

**Fig. 7 Pb in Liver vs Wt. of Fish, g**

A plot of Cadmium, zinc and lead in the muscles and livers of Fish, versus weight of the fish shows that in smaller fish, i.e. smaller than 150 g, quantities of these metals are higher. While in larger size Fish, quantities of these metals are lower and constant. This suggests that Fish of large size are suitable for human consumption.

## References

- Adham, K.G. (2001) *Pak. J. Biol. Sci.*, 4(12), 1550.
- Eisler, R. (1971) "Cadmium poisoning in *Fundulus heteroclitus* (Pisces: Cyprinodontidae) and other marine organisms" *J. Fish. Res. Board Can.*, **28**, 1225-1234.
- Eisler, R. (1985) "Cadmium hazards to fish, wildlife, and invertebrates: a synoptic review", *U. S. Fish Wild. Serv. Biol. Rep.*, **85** (1.2), 46.
- Forstner, U.Z., Wittmann, G.T.M. (1981) "Metal Pollution in the Aquatic Environment, Springer-Verlag, Berlin.
- Kennicott II. M.C., Wade, T.L. and Presley, B.J. (1992) Texas A&M University Assessment of Sediment Contamination in Casco Bay. Casco Bay Estuary Project, p.113 and appendices.
- Kurey, W.J. (1991) New Madrid refuges Contaminants Report, U. S. Fish Wild. Serv, 6950-H Americana Parkway, Reynoldsburg, Ohio 43068, 614-469-6923.
- Moore, J.W. and Ramamoorthy, S. (1984) "Heavy Metals in Natural Water – Applied Monitoring and Impact Assessment", Springer-Verlag, New York.
- Novikov, E.A., Pigum, S.H., Zolotov, L.K., Yu, A. (1990) *Analyt. Chem. Acta*, **230**, 157.
- Schmitt, C.J., Dwyer, F.J. and Finger, S.E. (1984) "Bioavailability of Pb and Zn from mine tailings as indicated by erythrocyte e-aminolevulinic acid dehydratase (ALA-D) activity in suckers (Pisces: Catostomidae)", *Can. J. Fisher. and Aquatic Sci.*, **41**, 1030 – 1040.

USEPA (1987) "Quality Criteria for Water", EPA Publication 440/5-86-001, U.S. Govt. Prin. Office, Washington, D.C.

Wiener, J.G., Jackson, G.A., May, T.W. and Cole, B.P. (1984) "Longitudinal distribution of trace elements, (As, Cd, Cr, Hg, Pb & Se) in Fish and sediments in the upper Mississippi River", In: J.G. Weiner, R.V. Anderson and D.R. McConville (Eds), *Contaminants in the upper Mississippi River*, Butterworth Publ. Stoneham.