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HEAVY METAL CONTENTS IN SOME SELECTED LOCAL FRESHWATER FISH AND RELEVANT WATERS

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Concentrations of Mn, Zn, Fe, Cu, Cr, Ni, Pb, Hg, Cd and As in the edible muscle tissue of seventeen species of freshwater fish are estimated by the flame/flameless atomic absorption method. The fish have been selected on the basis of their commercial value from local freshwater lakes/streams and hatchery ponds in Punjab and NWFP. Analysis of the relevant waters in respect of these metals is also conducted to establish correlation between the heavy metal concentration in fish muscles and in water. The concentrations in fish muscle have been found to range from 0.115-11.157, 1.875-50.650, 2.805-180.550, 0.193-7.200, 0.365-13.200, 0.628-38.800, 0.765-45.316, 0.020-26.800, 0.004-1.500, 0.480-7.500 mg/kg, wet weight basis respectively for the above mentioned metals. The study shows a positive correlation between the concentrations of zinc and arsenic in the fish muscle and in water, and the distribution of metals is species-specific irrespective of area of catch.

Key words: Heavy trace metal contents in fish; Freshwater fish analysis.

INTRODUCTION

During recent years the role and importance of fish towards studying the problems of pollution of the aquatic environment arising from heavy metals have been actively recognized. Several studies related to the heavy metal distribution in the aquatic organisms have been undertaken [1,2,3,4]. It is well known that heavy metals when present beyond traces are toxic to humans. Initially they may combine with the proteins and may not cause any poisoning, but when their concentrations exceeds the tolerance limit, they become a real health concern [5,6,7,8].

The heavy metals such as Pb, As, Cd, Cr, Ni, As and Hg are well known toxic pollutents of the aquatic environment. Some other elements, such as zinc, produce acute toxicity to freshwater fish and invertebrates [9,10,11]. With increased industrialization and urbanization, a whole gamut of these pollutants have found their way to fresh water reservoirs through natural run off, thus disturbing the delicate balance of the aquatic ecosystem resulting in several irregularities in fish physiology [12]. As fish are located on the end of the aquatic foodchain, they may clearly reflect the status of water quality [13,14] and may act as an indicator of water pollution in terms of these metals. Thus heavy metal pollution resulting from the uptake, concentration and retention of the metals by fish may be monitored through fish analysis and potential health hazards for the consumer may be averted.

The study reported here embraces the above cited objectives through the following considerations. Firstly, it presents the first comparative data on the heavy metal contents in local fresh-water fish and relevant waters, and secondly, it attempts to establish a correlation between the heavy trace metal contents in the two media so that a background enrichment ratio could be defined for a pollution free area of catch. Such a study will also help define the nutritional value of local fish. The fish species included in the study (Table 1) were collected from several locations. The choice of these species was tentative as they are widely consumed and some of them are currently under study for expansion of our export market. Thus it became imperative to estimate their heavy metal contents both from a nutritional and a health hazard point of view. The heavy metal estimations were done by flame and flameless atomic absorption technique using standard analytical procedures on a Shimadzu atomic absorption spectro-photometer having automatic background correction facility.

MATERIALS AND METHODS

The water samples were collected and filtered (Whatman No. 40) in situ as per procedure described in earlier work [15]. No stabilizers were added to these samples and they were directly aspirated within 3-4 hrs. The water sampling was conducted at sites where fish was caught. Only surface waters were sampled. Fresh fish were obtained randomly from local fisherman/contractors catering fish sale at different locations (Table 1) during September, 1987 to November, 1987. The edible muscle tissue of each sample (≅ 100 g) was briefly washed with distilled water, dried under folds of filter paper, packed in small polythene bags and deep frozen at -10°. For analysis, 10.0 g muscle tissue was digested using a mixture of 5 ml, HNO_3 (65%) and 0.5 ml, $HClO_4$ (70%). Digestions were carried out in Pyrex test tubes of suitable size placed in a stainless steel rack. The rack was maintained at 120° in electric oven until the samples were clear and approximately 0.5 ml of the liquid remained. After cooling, each sample was diluted to 25.0 ml with 5% HNO_3 (v/v) prior to analysis. The digest so obtained was used for the estimation of Zn, Fe, Mn, Cu, Ni, Cr, Pb and Cd. This wet digestion procedure was preferred over the dry ashing procedure in view of the volatile nature of many of the metals being analyzed [16,17]. Arsenic was estimated through the procedure given by Santa Maria [18], while mercury was determined by the cold vapour atomic absorption technique [19]. In order to get representative results, 3-5 runs were conducted for a given water/fish sample analysis. All reagents used were of GR grade guaranteed 99.9% purity, of E. merck origin. Calibration of the instrument was checked periodically with auto-background correction throughout the investigation. WHO standard were run in parallel for intercalibration of our own standards. All data were computed on a $\mu g,^{-1}$ wet weight basis. A Shimadzu atomic absorption spectrophotometer, model AA-670, was used during the work. The correlation data were computed on a WANG personal computer using the MSTAT statistical package. The limits of detection of various heavy metals are given in Table 4.

RESULTS AND DISCUSSION

The concentrations of the heavy metals in seventeen species of freshwater fish (Table 1) are given in Table 2. The data reveal that the variability of heavy metal distribution among different species is quite distinct. The metal contents are in general species-specific and their is no significant difference between large and small fish in the levels of metals in the tissues examined. The inclusion of weight as an additional independent parameter in Table 1 is to indicate the overall dependence of heavy metals on the weight/age of the relevant fish. For instance, the Mn

Table 1. Some data on various fish species.

S. No.	Species Local name (F.A.O. name)	Location	Weight (g) ± SD	Number of fish samples *
1.	Chilwa (*Chela cachius*)	Rawal Dam	27 ± 10	5
2.	Deali (*Ophiocephalus punctatus*)	Rawal Dam	60 ± 18	7
3.	Naili (*Ompok bimaculatus*)	Rawal Dam	90 ± 26	10
4.	Saol (*Channa aruleus*)	Mangla Dam	456 ± 120	11
5.	Chidoo (*Puntius ticto*)	Rawal Dam	35 ± 12	12
6.	Tilapia (*Tilapia nilotice)*	Fish Hatchery Punjab	20 ± 9	8
7.	Gold fish (*Carassius auratus*)	Fish Hatchery Punjab	15 ± 8	6
8.	Mori (*Cirrhinus mrigala*)	ADBP Fish farm	700 ± 165	9
9.	Baam (*Mastacembelus armatus*)	Rawal Dam	170 ± 56	13
10.	Rohu (*Labeo rohita*)	Rawal Dam	900 ± 350	12
11.	Singhara (*Mystus seenghala*)	Mangla Dam	1050 ± 430	10
12.	Mahaseer (*Tor putitora*)	Mangla Dam	850 ± 310	12
13.	Gulfam (*Cypirnus carpio*)	Mangla Dam	900 ± 250	10
14.	Khagga (*Rita rita*)	Mangla Dam	1150 ± 275	9
15.	Baam (*Mastacemblus armatus*)	Tarbela Dam	720 ± 270	11
16.	Mulee (*Wallage attu*)	Tarbela Dam	3100 ± 1020	13
17.	Thaila (*Catla catla*)	Tarbela Dam	8000 ± 5700	12
18.	Singhi (*Heteropneustes fossilis*)	Tarbela Dam	850 ± 350	14

*Triplicate water samples in each case.

content of Thaila (average weight 8.0 kg) is found to be 0.225 mg/kg as compared with the level of metal at 11.157 mg/kg found in Chilwa, having a nominal average weight of 27.0 g only. Although the size of the fish acquired for analysis depended on the circumstances at the sampling sites at the time of sampling it was considered desirable to include small fish in the study to obtain enrichment information on a given heavy metal as a function of size/age of the fish.

The results show a large variability in the heavy metal concentrations in local freshwater fish. The largest variation is observed in the case of iron, with a minimum concentra-

Table 2. Heavy metal concentrations (mg/kg) in edible muscle of various fish.

S. No.	Mn	Zn	Fe	Cu	Cr	Ni	Pb	Hg	Cd	As
1.	11.157	24.600	33.514	3.229	2.771	4.384	3.043	25.442	0.242	4.857
	±3.124	±6.401	±8.367	±0.573	±0.493	±0.638	±0.560	±6.521	±0.063	±1.213
2.	5.100	6.745	9.650	1.025	1.660	1.685	2.605	1.835	0.095	5.300
	±1.203	±1.628	±2.537	±0.392	±0.452	±0.583	±0.754	±0.430	±0.032	±1.407
3.	0.750	4.780	13.030	1.201	2.350	2.780	4.820	0.020	0.053	1.752
	±0.210	±1.216	±3.185	±0.561	±0.816	±0.930	±1.016	±0.005	±0.017	±0.537
4.	0.852	6.270	14.940	0.830	1.560	1.433	5.359	0.733	0.091	5.228
	±0.219	±1.826	±3.521	±0.256	±0.418	±0.367	±1.758	±0.156	±0.030	±1.400
5.	0.960	6.720	13.540	0.650	2.315	2.740	2.430	0.062	0.050	1.356
	±0.256	±1.573	3.216	±0.173	±0.834	±0.910	±0.687	±0.021	±0.015	±0.451
6.	1.900	18.350	180.550	2.100	9.700	11.750	13.550	10.300	0.600	2.400
	±0.512	±3.756	±36.618	±0.561	±2.813	±3.154	±4.135	±3.102	±0.176	±0.650
7.	10.800	50.650	134.600	7.200	13.200	38.800	45.316	26.800	1.500	0.600
	±2.627	±13.268	±32.367	±1.731	±3.186	±9.653	±13.673	±6.701	±0.501	±0.212
8.	0.312	1.994	2.998	0.530	0.382	0.628	1.344	0.276	0.032	0.625
	±0.079	±0.413	±0.910	±0.167	±0.112	±0.169	±0.358	±0.083	±0.010	±0.217
9.	0.190	6.365	5.390	0.760	0.615	0.770	1.470	0.795	0.090	0.612
	±0.052	±1.637	±1.735	±0.210	±0.163	±0.186	±0.403	±0.257	±0.030	±0.208
10.	0.270	2.670	4.480	0.680	0.620	0.930	0.915	0.037	0.155	0.589
	±0.071	±0.730	±1.216	±0.169	±0.182	±0.276	±1.130	±0.009	±0.058	±0.180
11.	0.115	1.875	3.260	0.775	0.660	0.965	1.570	0.870	0.140	0.550
	±0.032	±0.438	±0.810	±0.258	±0.183	±0.312	±0.479	±0.251	±0.032	±0.171
12.	0.320	2.155	3.785	0.525	1.020	0.895	1.960	1.225	0.060	0.485
	±0.103	±0.551	±1.112	±0.168	±0.401	±0.218	±0.654	±0.329	±0.018	±0.113
13.	0.325	2.681	3.280	0.460	0.865	0.795	1.855	1.095	0.055	0.490
	±0.097	±0.658	±0.831	±0.152	±0.215	±0.204	±0.489	±0.318	±0.016	±0.137
14.	0.250	5.755	6.465	0.775	0.920	0.805	2.565	1.535	0.801	0.900
	±0.068	±1.352	±1.548	±0.210	±0.250	±0.235	±0.639	±0.402	±0.022	±0.301
15.	0.260	3.080	3.770	0.193	0.910	0.865	2.210	0.360	0.092	0.890
	±0.062	±0.875	±0.915	±0.051	±0.212	±0.276	±0.560	±0.125	±0.030	±0.236
16.	0.200	2.515	4.225	0.915	0.365	0.830	1.515	0.575	0.105	0.480
	±0.052	±0.673	±1.317	±0.231	±0.110	±0.226	±0.403	±0.158	±0.037	±0.140
17.	0.255	2.095	2.935	0.210	0.860	1.155	3.760	1.635	0.004	7.500
	±0.060	±0.564	±0.759	±0.052	±0.271	±0.316	±1.001	±0.493	±0.001	±1.621
18.	0.175	2.630	2.805	0.445	0.715	1.170	0.765	1.755	0.050	5.000
	±0.048	±0.715	±0.710	±0.126	±0.186	±0.322	±0.198	±0.500	±0.017	±1.345

Table 3. Concentrations (mg/l) of various metals in waters from five sampling sites.

Sampling sites	Mn	Zn	Fe	Cu	Cr	Ni	Pb	Hg	Cd	As
Rawal dam	0.013	0.012	0.031	0.028	0.025	0.002	0.032	0.924	0.007	0.030
	±0.004	±0.003	±0.007	±0.006	±0.005	±0.001	±0.007	±0.286	±0.002	±0.010
Mangla dam	0.012	0.006	0.076	0.043	0.092	0.210	0.284	0.955	0.006	0.647
	±0.004	±0.002	±0.021	±0.016	±0.026	±0.058	±0.073	±0.301	0.002	±0.156
Fish hatchery punjab	0.010	0.046	0.009	0.025	0.020	0.003	0.028	0.244	0.005	0.016
	±0.003	±0.013	±0.002	±0.004	±0.005	±0.001	±0.007	±0.068	±0.001	±0.004
ADBP fish farm	0.029	0.006	0.124	0.044	0.014	0.003	0.123	1.026	0.002	0.040
	±0.007	±0.002	±0.037	±0.004	±0.004	±0.001	±0.038	±0.301	±0.001	±0.012
Tarbela dam	0.018	0.008	0.060	0.048	0.100	0.180	0.260	0.805	0.009	0.812
	±0.005	±0.003	±0.020	±0.016	±0.027	±0.055	±0.071	±0.286	±0.003	±0.152

Table 4. Limits of detection* for various heavy metals under optimum AAS operating conditions.

Heavy metal	Limit of detection (mg/l)	Heavy metal	Limit of detection (mg/l)
Mn	0.001	Ni	0.002
Zn	0.0006	Pb	0.005
Fe	0.002	Hg	0.00004
Cu	0.001	Cd	0.0004
Cr	0.002	As	0.001

* Defined as : 2 x Standard deviation of background noise

tion of 2.805 mg/kg for Singhi and a maximum of 180.550 mg/kg for Tilapia. An other quite divergent distribution is observed in the case of zinc and lead, with extremum concentrations, 1.875-50.650 mg/kg and 0.765-45.316 mg/kg respectively. The minimum metal concentration in all the species is observed to be for cadmium, 0.004% mg/kg, and the maximum concentration for iron, 180.550 mg/kg. The results thus indicate that the iron and zinc concentrations are far more variable than those of other metals. Same fish belonging to different locations of catch show a weight independent heavy metal content. For instance, the two Baams (samples 9 and 15) belonging to Rawal Dam and Terbela Dam show a distinct negative weight dependence in the case of zinc, iron, copper, and mercury, while for other metals this behaviour is positive It may, therefore, be concluded that weight dependence of heavy trace metals is largely a phenomenon of individual fish physiology. The levels of heavy trace metals are found to be generally higher in Chilwa and Gold fish the former species is abundantly consumed in Punjab while the latter, though not popular among fish eaters, is largely employed for display in aquariums.

On the basis of US recommended daily dietary allowance and the estimated safe and adequate daily dietary intakes (EDI) for various heavy metals in 1 kg serving of fish muscle, the chromium contents in Chilwa, Naili, Chidoo, Tilapia, and Goldfish are higher than the stipulated maximum EDI level of chromium set at 2.0 ppm [4]. These fish are, therefore, unsuitable for human consumption. Similarly the estimated average mercury levels exceed the upper allowed range of 1.0 ppm [25] in about 50% of fish analyzed. In the category of non-essential heavy trace metals arsenic cadmium and lead are also found to exceed the upper safety limit of 1.0 ppm in different fish. The present study reveals that abnormally high concentrations of heavy trace metals are met with in various fish irrespective of their origin and age. In fact it may be inferred that environmental factors may influence the uptake of these metals in the fish, in agreement with an earlier study [20] on freshwater fish. The present data show good agreement in terms of Mn estimated to have a range of 0.115-11.157 mg/kg as compared with an average Mn content of 9.01 mg/kg for freshwater fish in Bolivia [21]. Also, our data on zinc (1.875-18.350 mg/kg), with the exception of species having maximum zinc content, compare well with those reported by Jenusz *et al.* [22] with a range of 6.10-20.77 mg/kg. Similarly, in the case of iron an average of 37.03 mg/kg is reported [21] in the muscle of freshwater fish. With the exception of Tilapia and Goldfish, the rest of the fish fall within this range. Similarly, the cadmium, copper and chromium levels compare well for the muscle tissue, as reported by Vanhoof *et al.* [23]. The mercury content in the muscle tissue of local freshwater fish is found to range between 0.020-1.835 mg/kg, ignoring the highest level in fish referred to earlier. This is in agreement with mercury concentration (1.33 mg/kg) reported by C.L. Ndiokwere [24] for freshwater fish. The arsenic levels reported in the present study are on the higher side of 0.42 mg/kg as reported earlier [23]. Although a direct comparison of data on the concentrations of heavy trace metals among fish belonging to different origin is not valid, it has been included to validate the results obtained. Unfortunately, comparative data on local freshwater fish is almost non-existant. ke no man-made source of heavy metal pollution is located in the vicinity of the areas of catch, the observed high concentrations of some metals in certain fish referred to above may be attributed to the food habits, uptake metabolism and habitat of the relevant fish.

In order to investigate the problem of correlation between the concentrations of heavy trace metals in fish and in relevant waters a variance study was conducted to establish a probable correlation between the concentrations of the metals in fish muscles and in relevant waters (Table 3). The MSTAT programme was run on a WANG personal computer to this effect. All metals except zinc and arsenic have shown a negative correlation between the two variables. In the case of zinc, the correlation coefficient has a value 0.812 at a probability confidence of 0.00. In the case of arsenic, the correlation has a magnitude of 0.136 and, therefore, it does not warrant a strong positive correlation between the two variables. The current analysis indicates that the accumulation patterns of zinc and arsenic are significantly different from those shown by other metals. Further studies from this angle are needed to establish this observed fact.

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