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A New Approach for Finding out the Bioaccumulation Rate of Heavy Metals in Tissues of *Liza parsia*

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Through field and laboratory studies, a concept has been developed for finding out the differential rate of bioaccumulation of some heavy metals such as copper, zinc and lead in different tissues of mullet *Liza parsia* (Hamilton-Buchanan). This new approach for finding out the maximum bioaccumulation employed a series of experiments, statistical and graphical interpretations and has put forth a concept for future investigations with different bio-accumulative toxicants.

Key words: Bioaccumulation, heavy metal, *Liza parsia*.

The accumulation of metal in fish is a function of uptake and excretion. Uptake is considered to be passive and involves diffusion gradients created by adsorption or binding of the metal to the tissue and cell surfaces (Bryan, 1976). The organs such as liver and kidney secrete metal binding proteins, and there are target organs for specific toxicant action and they accumulate metals to significant levels (Stagg & Shuttleworth, 1982). But each tissue has its maximum capacity of binding of heavy metals.

Let A be a toxicant and B a tissue/animal. Let the concentration of A in B be x, while the animal is in a normal environment and the concentration of A in that environment be y. If A is bioaccumulative in nature, its increase in the environment/medium may be reflected in B. The ratio of x to y is called as Bioaccumulation Factor (KB) (Buikema *et al.*, 1982). KB is independent of unit. Keeping the tissue and environment as constants, there are two variables namely x and y. The sequence $x_1/y_1.....x_n/y_n$ of x/y of at different points of time would provide trend of KB. The gradual trend is discussed as follows:

(A) Let the values in x series and y series be increasing and the rate of increase in y series is less than that of the increase in x series, KB will show increasing trend.

(B) If the values in x series and y series are increasing, and if the rate of increase in y series is more than that of in x series, KB will show decreasing trend

(C) If the values in both x and y series are decreasing and if the rate of decrease in y series is more than that of x series, then the KB will show an increasing trend.

(D) The values in x and y series show declining trend but the rate of decrease in x series is more than that of y series, then KB will decrease.

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(E) If the values in x series increase with decrease in y series, KB will increase gradually and vice versa.

Hypothetically for a particular bioaccumulant we take the relation of KB to that of the concentration in tissue i.e., x. If KB is seen increasing with increase in x, the relationship will be linear as shown in Fig.1. The plotting of concentration should be done in X-axis and KB in Y-axis. In this case the animal/tissue is accumulating more of A in relation to its availability in the medium/environment. In such a case the saturation level of the toxicant in the animal/tissue might have not reached, and the accumulation rate can be termed as "more than proportionate". If A is available in the medium at higher doses and its concentration in the tissue is increasing. But KB is decreasing. The relation will be as shown in Fig.2. That means the accumulation of A may be going on in B and the tissue/animal is not in a position to accumulate A proportionately to that available in environment. The accumulation rate can be termed as "less than proportionate".

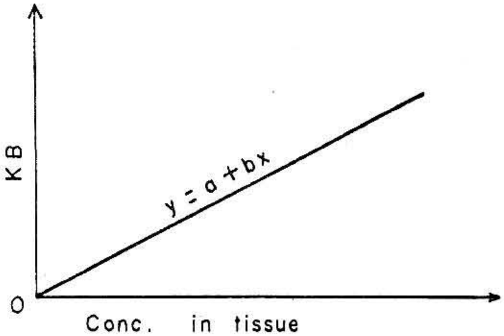

Fig. 1. Accumulation of heavy metal in fish tissue - more than proportionate.

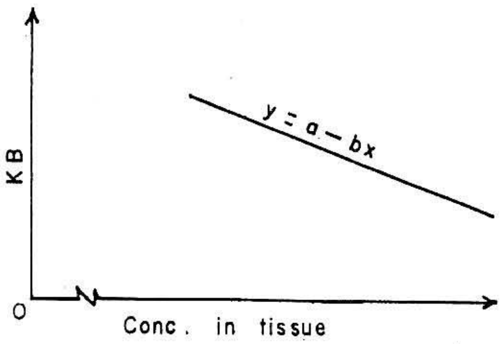

Fig. 2. Accumulation of heavy metal in fish tissue - less than proportionate.

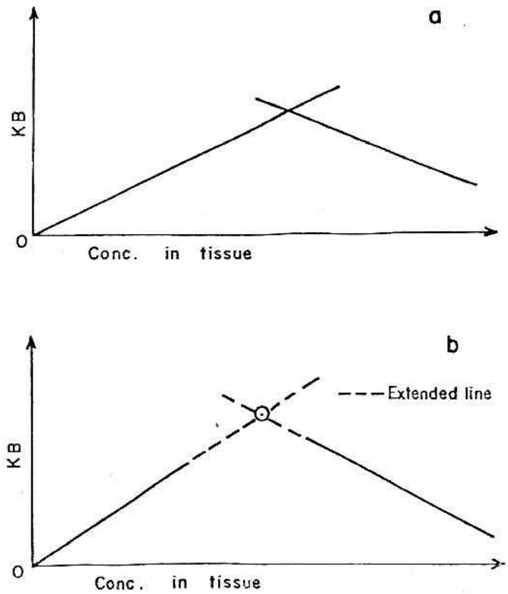

Fig. 3 a and b. Composite graph superimposing Figs. 1 and 2.

Hypothetically we can go one step more and superimpose the Fig.1 to Fig.2. The expected composite graph may be as shown in Fig.3 (a & b). From the point of intersection of both the regression lines draw a perpendicular on the X-axis as shown in Fig.4 (a & b). From X-axis the maximum accumulation of the bioaccumulant can be found out (the meeting point of the perpendicular on the X-axis). This bioaccumulation of the toxicant is the highest one in the animal/tissue in relation to is availability in environment.

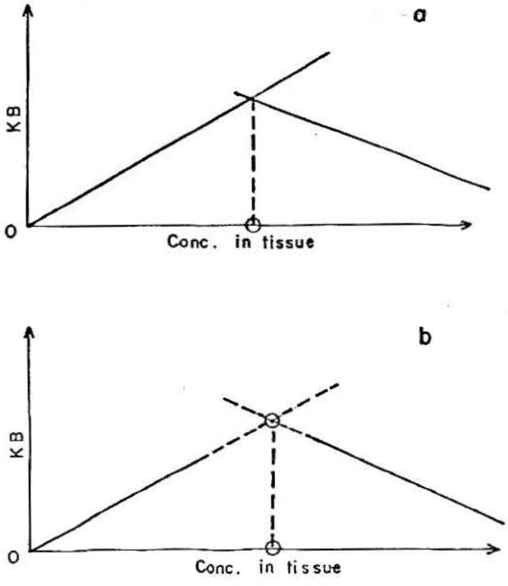

Fig. 4 a and b. Graph for finding out maximum accumulation of heavy metal in fish tissue.

The above said hypothesis was tested through experiments in the laboratory and field centres simultaneously. Mullet *Liza parsia* was used as the test animal. Heavy metals such as copper, zinc and lead which accumulate in different tissues of the fish were selected for the study. The aim was to estimate the bioaccumulation of these metals and its rate in different tissues of fish in relation to its concentration in the medium where the animals live. In this work, an attempt is also made to determine the maximum accumulation of metals using KB.

Materials and Methods

Field studies were undertaken for finding out the concentration of copper (Cu), zinc (Zn) and lead (Pb) in the tissues such as liver, gill, kidney, skin, muscle, intestine and ovary of *L. parsia* and also in the water samples from six centres in the estuarine and coastal environments both on east and west coasts of India (Table 1). The centres were Korapuzha estuary, Calicut, Cochin backwaters (both from Kerala); Tuticorin bay; Palk bay and the Gulf of Mannar, Mandapam, Ennore creek, Madras (Tamil Nadu) and Rusikulya estuary, Ganjam (Orissa). The samples were collected for

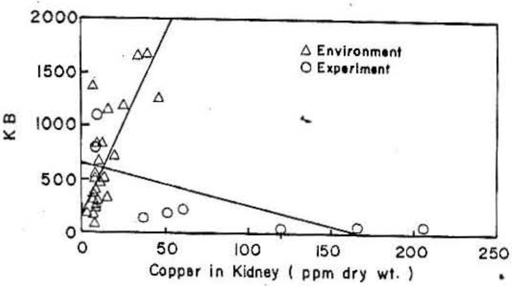

Fig. 5. Relationship between Bioaccumulation and Bioaccumulation Factor (KB) of copper in Kidney of *L. Parsia*.

Table 1. Copper, zinc and lead in water

Centres	Copper (ppb)		Zinc (ppb)		Lead (ppb)	
	Average	Range	Average	Range	Average	Range
Korapuzha estuary	6.82	1.5 - 18.5	20.22	ND - 52.5	6.67	ND - 8.82
Cochin backwaters	10.38	2.0 - 40.0	82.10	25.0 - 165.5	6.26	ND - 60.5
Tuticorin bay	5.50	1.5 - 13.0	14.14	ND - 38.5	7.46	ND - 31.0
Mandapam seawater	6.33	1.0 - 24.0	19.39	3.5 - 52.0	4.68	ND - 19.0
Ennore creek	8.92	2.5 - 33.0	38.88	9.0 - 127.0	20.74	ND - 141.5
Rusikulya estuary	3.61	ND - 10.0	19.33	1.5 - 65.0	8.46	1.0 - 39.5

ND = Not detected; Average and range with values n = 36

Table 2. Accumulation and Bioaccumulation Factor (KB) of copper in kidney, zinc in liver and lead in gills in *L. parsia*

Centres	Copper in kidney		Zinc in liver		Lead in gills	
	Accumulation (ppm dry wt.)	KB	Accumulation (ppm dry wt)	KB	Accumulation (ppm dry wt.)	KB
Korapuzha estuary	7.6 - 105.4 (35.8)	300 - 5563 (2329.7)	21.4 - 94.5 (51.7)	254 - 1506 (781.2)	2.8-47.0 (20.2)	114-961 (577.9)
Cochin backwater	ND-46.4 (12.3)	ND - 1258 (421.7)	43.7 - 331.0 (129.2)	143 - 1202 (452.3)	ND - 13.2 (5.1)	ND - 450 (185.5)
Tuticorin bay	ND - 93.1 (21.7)	ND - 9305 (1831.5)	68.3 - 244.0 (185.9)	1067 - 13303 (4737.3)	ND - 22.4 (9.9)	ND - 421 (196.1)
Mandapam area	12.3 - 43.5 (27.2)	319 -4239 (1907.0)	41.2 -173.5 (89.8)	540 - 2835 (1338.5)	ND - 13.1 (8.0)	ND - 5857 (144.5)
Ennore creek	7.5 - 34.5 (16.2)	193 - 1187 (666.1)	47.7 - 347.1 (150.9)	258 - 2364 (1125.0)	ND - 34.5 (12.3)	ND - 582 (221.4)
Rusikulya estuary	6.8 - 27.6 (12.5)	474 - 3178 (1280.5)	66.2 - 91.9 (79.8)	968 - 2671 (1417.6)	ND - 8.6 (4.8)	ND - 253 (128.4)

Mean values in parentheses; ND - not detected; Average and range with values n = 6

two years from January 1991 to December 1992, particularly during pre-monsoon, monsoon and post-monsoon in each year. The estimation of Cu, Zn and Pb in water was done as per the method of Brooks *et al.* (1967). The digestion of tissue samples was carried out as per the method of Dalziel & Baker (1983). The calculation of KB was done by the formulae of Buikema *et al.* (1982). As the composite graphs (described in a later section) could be obtained only for copper in kidney, zinc in liver and lead in gills, the mean accumulation of these metals along with their KB values in the respective tissues in different centres are given in Table 2.

In the laboratory the bioassay was done followed by the range finding bioassay method (Reish & Oshida, 1987). The two sub-lethal concentrations (1/10th and 1/100th of 96 hr LC50) of copper sulphate, zinc sulphate and lead nitrate at a ratio of 1:1:1 were selected for experiments. The level of availability of Cu, Zn and Pb from the compounds in experimental medium was calculated using the formula of Reish & Oshida (1987). The bioaccumulation and KB of

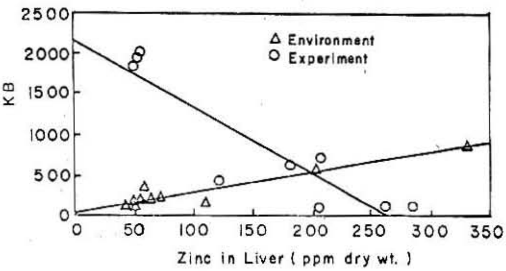

Fig. 6. Relationship between Bioaccumulation and Bioaccumulation Factor (KB) of zinc in liver of *L. parsia*.

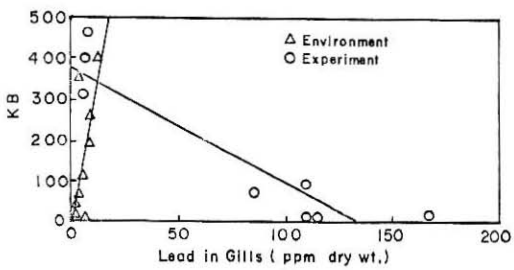

Fig. 7. Relationship between Bioaccumulation and Bioaccumulation Factor (KB) of lead in gills of *L. parsia*.

Table 3. The accumulation and Bioaccumulation Factor (KB) of copper in kidney, zinc in liver and lead in gills of *L. parsia* exposed to copper sulphate, zinc sulphate and lead nitrate in the ratio, 1:1:1

Exposure period (weeks)	Control tissue		Sub-lethal concentraton			
			1/100th 96 hr LC50		1-10th 96 hr LC50	
	Metal content (ppm, dry wt.)	KB	Metal content (ppm, dry wt)	KB	Metal content (ppm, dry wt.)	KB
Copper in kidney						
0	10.7 ± 4.9	1127 ± 513	10.7 ± 4.9	1127 ± 513	10.7 ± 4.9	1127 ± 513
1	9.3 ± 3.4	1089 ± 291	36.4 ± 2.1	127 ± 7	119.9±12.8	43 ± 5
2	7.5 ± 0.6	794 ± 49	51.0 ± 3.8	178 ± 13'	166.8 ± 7.4	58 ± 3
3	7.5 ± 0.3	795 ± 34	61.1 ± 4.5	213 ± 16	206.6 ± 6.6	72 ± 2
Zinc in liver						
0	54.7 ± 16.1	1941 ± 564	54.7 ± 16.1	1941 ± 564	54.7 ± 16.1	1941 ± 564
1	56.3 ± 3.3	2002 ± 118	121.7 ± 7.9	411 ± 27	203.8 ± 7.6	69 ± 3
2	51.1 ± 4.3	1817 ± 151	181.7 ±13.1	614 ± 44	284.3 ± 8.7	96 ± 3
3	54.0 ± 2.9	1919 ± 102	206.9 ± 12.6	699 ± 43	261.6 ±10.7	88 ± 4
Lead in gills						
0	9.4 ± 1.1	500 ± 60	9.4 ± 1.1	500 ± 60	9.4 ± 1.1	500 ± 60
1	8.6 ± 2.1	461 ± 110	85.5 ±4.4	71 ±4	109.3 ± 7.2	9 ± 0.6
2	7.4 ± 1.9	396 ± 100	84.5 ± 4.2	70 ± 4	11.8 ± 6.4	10 ± 0.5
3	5.8 ± 1.5	313 ± 80	109.3 ± 9.2	91 ± 8	167.0 ± 6.4	14 ± 0.5

± Standard deviation with n = 3; 96 hr LC50 = 106.7 ppm

Cu, Zn and Pb in different tissues of *L. parsia* were determined after 1st, 2nd and 3rd week. The accumulation and KB of copper in kidney, zinc in liver and lead in gills from different laboratory experiments (Table 3) were used for drawing composite graphs. Efforts were made to correlate the results obtained from laboratory experiments with that of field samples. The better correlated, significant relationship from the laboratory experiments (n=9) for KB and accumulation were superimposed to that from field observations (n=36) to get the composite graphs in a computer.

Table 4. Statistical analyses of regression lines in composite graphs (Figs. 5 to 7)

Heavy metal	Tissue	Env./Expt.	Correlation Coefficient	Sig./NS
Copper (Cu)	Kidney	Environment	+ 0.352	5%
Copper (Cu)	Kidney	Experiment	- 0.707	5%
Zinc (Zn)	Liver	Environment	+ 0.347	5%
Zinc (Zn)	Liver	Experiment	- 0.822	1%
Lead (Pb)	Gill	Environment	+0.196	NS
Lead (Pb)	Gill	Experiment	- 0.875	5%

Results and Discussion

Relationships could be established for pooled environmental data for bioaccumulation and KB, and similarly for the laboratory experiments. Only three composite graphs were obtained for copper in kidney (Fig.5), zinc in liver (Fig.6) and lead in gill (Fig.7). The error was seen in case of lead accumulation in tissues and its relation with KB was not significant in environment. The statistical analysis is given in Table 4. Based on the composite graphs the maximum accumulation of Cu in kidney is 24 ppm; Zn in liver, 200 ppm; and Pb in gill, 12 ppm (on dry weight basis).

The KB for different metals in different tissues of *L. parsia* showed positive relationship with the contents observed in different field centres. In the laboratory experiments the same was seen significantly negative indicating that the increase of bioaccumulation with the increase of concentration in medium will reduce KB. In the natural environment the metals are available in low amount (ppb levels). The same were added in the experimental medium in higher amount (ppm levels). When the level of metals were more in medium, the accumulation was higher in all tissues. At the same time the KB decreased. It is concluded that the increase in bioaccumulation was less than proportionate in the laboratory experiments and more than proportionate in the natural environments. It is clear from Fig.5 to7 that the tissues cannot accumulate metals beyond certain limits even though the same is available in the environment abundantly.

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