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Kinetics of Lead and bio-concentration factor (BCF) in different tissues of *Clarias batrachus* during experimental Plumbism

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Abstract:A Common Indian cat fish, *Clarias batrachus Linneaus* was treated with Lead Acetate for sub-acute and chronic exposure to the xenobiotic. The sub acute study included 3 days and 7 days exposure to different concentrations of lead acetate, while chronic study included exposure to 15 ppm lead acetate for 105 days. In all the cases tissues from Gills, Bones, Liver, Blood, Kidneys, Muscles, Skin and GI tract were removed and analyzed by AAS to find lead accumulation. The Bio-Concentration Factor (BCF) was calculated .The BCF varies from organ to organ. Additionally , X-rays photography was made on the chronic treated fishes from time to time, to see whether skeletal tissue is a target of the heavy metal or not. The Co-relation Co-efficient (r') in these tissues between Lead treatment and Lead accumulation was found to be highly significant suggesting it both as a dose-dependent and time-dependent process.

Index terms: Atomic Absorption Spectrophotometer(AAS), Bio-Concentration Factor (BCF), chronic treatment, Clarias, Kinetics, Lead accumulation, Pool, Sub acute, Target organ, X-rays.

I. INTRODUCTION

The heavy metals occupy a frontier position in IRPTC. These have been recognized and registered as potentially toxic chemicals, may be neurotoxic, haemotoxic, genotoxic or carcinogenic. The most biologically significant and most significantly studied of these heavy metals include cadmium, mercury, lead, zinc, chromium and copper. As a leading member of the heavy metal family. Lead (plumbum-pb) is extensively used and studied for its toxic nature like mercury(Hg) and cadmium(Cd). Leads toxicity comes from each part of the environment i.e. air, water and soil due to its ubiquitous uses and entry to the respective zones either naturally or artificially or both. Obtained as galena, in the earth's crust, lead has been variously used in different ways. Biologically lead has no significant role in any way, but still it is found in the human body up to 120 mg as a non-essential trace element. Its ubiquitous use has made its entry in to our body through various sources as a contaminant. The over increasing use of this metal has so heavily contaminated the environment that exposure to lead is inescapable. Estimations from various sources have shown that the primitive man had only about 2mg of lead in contrast to 120 mg today. The lead content of the Egyptian mummies was also found to be markedly less-only about 1.5 mg i.e. one-tenth of the lead load in present day (Kanwar and Sharma 1987): The average daily intake of lead by present day man is about 0.45 mg which comes mainly from food (0.22 mg to 0.4 mg) water (0.02 mg), inhalations (0.03 mg) and cigarettes (0.04 mg of one smokes 30 cigarettes a day).

In our country India, the department of Science and Technology (DST) had launched a study related to heavy metal pollution from 1982 through 1987. it has been found various states leading in lead pollution in various forms in India. Murti (1989), based on six years studies reported the lightest level of lead as follows:

Air	–	0.699 μg/m^2 (Bihar)
Water	–	0.968 μg/ml (Tamilnadu)
Vegetarian food	–	18.51 μ/g (Himachal Pradesh)
Non-vegetarian food	–	9.32 μg/g (Orissa)

As reported by Jana (1987), several brands of shampoos contain lead sulphide to give the hair a dark hue. The eye cosmetics "Kajal" used by Hindus and "Surma" by Muslims have large concentrations of lead, reported 88% lead sulphide in mascara. Face powders, pastes and liquids also contain about 67% lead.

The use of Sindoor, basically red lead by Hindu women continues till date and there is no indication of ban on its use in the near future. Sindoor poses hazards that are well documented but it continues to be considered sacred.

Its properties such as durability, corrosion resistance, easy gluing of its pieces, have made lead a perfect metal for use in batteries, cables, pigneus, petrol additives, solder and steel products and ever as pesticides. The most wide spread use of lead as in

paints. Pottery work, foundry work, welding, soldering, stained glass fabrication ceramic works continue to use lead (Oladele, 2007). Very recently the use of Lead in Calabash chalk as an antidote has been reported(Google Alert, 2013).

So far the literature is concerned as above the report on lead toxicity in aquatic ecosystem are scanty. Kinetics of lead in aquatic ecosystems and its impact on aquatic animals and their systems need to be thoroughly studied as they often remain as an essential component in the food chain. The study may have some implications in understanding the problems of lower vertebrate physiology, biochemistry, immunology environmental biotechnology and aquaculture pathology etc. Therefore, in the present study our aim is to understand some of the major problems under the guidelines of WHO (1995), as out lined below :

- To select a model fish, best for laboratory work and expose it to the soluble compound of lead i.e. lead acetate for toxicological and biochemical analysis.
- To asses the toxicity of the compound with variation of dose and period of exposure in order to select suitable doses for short-term and chronic exposure.
- To study the kinetics of lead under controlled conditions of aquaria both for short-term and chronic exposure on the model fish.

II. MATERIAL AND METHODS

2.1 Experimental design:

Large-sized (120-200 gms.) fishes were collected from culture ponds of village Deopada in Bhadrak district of Odisha and aclimatized for seven days in the laboratory aquaria as reported earlier (Rout and Naik 1996). For short-term studies, different subacute concentrations of lead acetate [Pb $(CH_3\text{-}COO)_2$], Johnson and Sons, Ltd., London, 1990] were choosen after obtaining LC_{50}(500 ppm) and LT_{50} (45 days for25pm, 40 days for 50 ppm, 37days for 75 ppm., 35 days for 100 ppm, 30days for 125ppm and 28 days for 150ppm.

For 3 days and 7 days of treatment seven fishes each were selected and kept in separate aquaria demarcated for control, 25 ppm, 50 ppm, 75 ppm, 100 ppm, 125 ppm and 150 ppm of lead acetate.

For chronic treatment 15 ppm of lead acetate was chosen because of relatively high LT_{50}(150 days), suitable for chronic studies. Eight fishes taken for experiment each in separate aquaria and eight for control. The study periods involve 1^{st}, 15^{th}, 30^{th}, 45^{th}, 60^{th}, 75^{th}, 90^{th} and 105^{th} days respectively with the control for comparison.

All the experiments were repeated ten times and the physico-chemical profiles were monitored following APHA (1980) carefully in each day during replacement of water.

2.2 AAS for hard and soft tissues.

The methods of studying tissue accumulation of lead were followed to Ferguson et. al. (1993) and ATSDR (2005) using a Perkin Elmer Atomic Absorption Spectrophotometer (MODEL - 37).

The collected bone samples were minced and oven-dried at 120°C for 6 hrs. Then desired amount of the ass was taken and digested in 5:1 Nitric and Perchloric acid. The digested material then fumed in a Fume Hood (Macro Scientific Works- MSW 167) with addition of 1 ml of cone. H_2SO_4. and the solution kept in ice-bath and filtered. The volume was made up to 100 ml with de-ionized water and kept for analysis by AAS at 510 nm.

After adequate exposure, the fishes were autopsied and the tissues like gill, liver, kidney, muscle, bone and skin were blotted with blotting paper and deep freezed after weighting. Tissues (1g wt.) were digested with concentrated Nitric acid and Perchloric acid (in 4:1 ratio. These were kept in water bath for 6-7 days until the samples were digested thoroughly and became clear.

Cool digests were filtered through What man grade 541 filter paper and made up to 10 ml with double distilled water. Entire metal analysis was done by using Perkin Elmer- 37 Atomic Absorption Spectrophotometer.

Stock lead solution: 0.1599g of Pb $(NO_3)_2$ was dissolved in about 20 ml of distilled water. 1 ml of distilled nitric acid again was added following volume make up to 100 ml with distilled water.

Standard lead solutions : The standard lead solutions were prepared of concentration 1/5, 10/ 25 and 50 μg/1 by two step dilutions stock lead solution with de-ionized distilled water containing nitric acid.

2.3 Analysis of blood samples for lead:

It was followed according to Australian standard (AS2411 - 1980) for AAS. After collection the heparinized blood samples were kept at 4°C. During analysis 3ml of thoroughly mixed blood sample were immediately dispensed to the centrifuge tube. The lead in blood was made complexes with APDC by adding 0.5 ml of 2% APDC to it and extracted into 3 ml of n-butyl acetate by proper shaking. Lead was determined in the organic phase by AAS within one hour.

Calculation was done by the help of calibration curve using standard solution.

$$\textbf{Lead content} = \frac{\text{OD of the sample}}{\text{Standard Value}} \text{ μg/gm/ml of the tissue.}$$

$$\textbf{Bio concentration factor (BFC)} = \frac{\text{Concentration of Pb in tissues}}{\text{Concentration of Pb in water}}$$

2.4. X-ray photography

To study accumulation of lead on bone X-ray photography was followed according to Yarmenenko (1988) and ATSDR (2005). The fishes were anesthetized by intramuscular injection of sodium barbiturate. Then they were taken to X-ray laboratory and X-ray was taken through ME-2085 machine at frequency 50 KG x 0.2 MA.

2.5. Statistical methods:

All the data obtained from the control and experimental fishes were statistically analyzed as follows:

2.5.1. ANOVA: One-way analysis of variance (ANOVA) for accumulation and kinetic studies, DNA, RNA and protein relations, activities of different enzymes, haematological studies and Immunological studies were performed with the respective F-values in all observations following Sanders (1994). All the ANOVA tables are separately attached by an annexure.

2.5.2. Parson's Correlation Coefficient (r) : The r- values between doses of lead acetate with lead accumulation as well as the lead accumulation and various parameters of the control groups & experimental were calculated with significance following Sanders (1994) and Chainy et. al. (2008).

III. RESULTS

The AAS analysis of different tissues displayed accumulation of lead after 3 days of exposure (Table I), 7 days of exposure (Table III) and chronic 105 days of exposure (Table V) The Bio Concentration Factor (BCF) have been worked out as in Tables II, IV and VI respectively.

After 3 days of Exposure, the accumulation was a dose-dependant response in the gills, liver, blood, kidneys and GI tract as evident from the F-values from ANOVA, while in the bones, muscles and skin there was no significant response. The correlation Co-

efficient (r) between lead accumulation and lead treatment is also significant in cases of the described organs (Figure 4). The BCF is very high (Table II & Figure 1)

After 7 days of exposure, lead accumulation in various tissues was invariably a dose-dependant response, as evident from the Table III, and Figure 5. BCF was also very high (Table IV, Figure 2) and correlation coefficient between Lead treatment and Lead accumulation is also highly significant (Figure 5).

The accumulation gradually increases with increase in period of exposure during chronic treatment(Table V). There is a +ve correlation between the period of exposure and lead accumulation in different tissues for 105 days. Accumulation steadily increases up to 60 days in the gills and then falls. A similar response is observed up to 75 days in the kidneys and muscled. In other organs there is a steady increase. A similar response is in BCF (TableVI, Figure 3) and there is a highly significant correlation coefficient (r) between the period of exposure and lead accumulation (Figure 6).

The X-ray photography of control and exposed fishes have been compared (Plate-I). Gradual deposition of deep lead- lines on the skull and vertebral bones have been depicted after 45th day, 75th day, and 105th day of chronic exposure. The X-ray results support the AAS studies on Lead accumulation in the bones.

Table I: Accumulation of lead after short-term (48 hrs) of exposure to various concentrations of lead acetate.

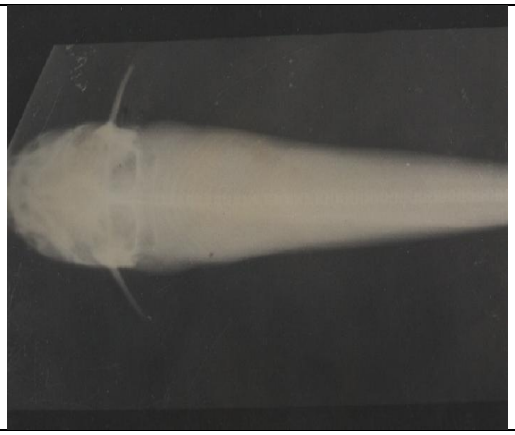
Concentration of lead acetate(ppm)	Lead content µg / gm weight of tissues							
	Gills	Bones	Liver	Blood (µg/dl)	Kidney	Muscle	Skin	GI tract
Control	0.1 ±0.001	0.2 ±0.001	0.01 ±0.001	3.8 ±0.001	0.01 ±0.001	0.01 ±0.001	0.1 ±0.001	0.01 ±0.001
25	10.22 ±0.021	0.81 ±0.012	6.83 ±0.351	12.30 ±0.269	4.54 ±0.611	1.82 ±0.082	3.02 ±0.641	2.51 ±0.001
50	15.62 ±1.232	2.19 ±0.821	8.6 ±1.152	13.01 ±0.251	5.36 ±0.731	3.63 ±0.912	5.25 ±1.231	2.63 ±0.081
75	12.14 ±2.333	3.16 ±0.981	10.36 ±2.341	13.52 ±0.512	6.21 ±1.283	4.08 ±1.114	5.53 ±1.672	4.61 ±0.061
100	13.13 ±2.461	5.68 ±1.230	11.38 ±2.152	14.13 ±0.132	8.36 ±1.391	4.45 ±1.631	5.79 ±1.231	5.23 ±0.7
125	13.57 ±2.231	5.51 ±2.381	13.13 ±1.623	13.21 ±0.213	8.59 ±1.251	5.20 ±0.291	6.23 ±1.331	5.45 ±0.8
150	15.38 ±2.110	6.21 ±1.381	14.33 ±0.231	12.69 ±0.619	8.21 ±0.631	6.31 ±0.381	7.35 ±1.161	5.62 ±0.093

⊥ P<0.005, † P<0.05

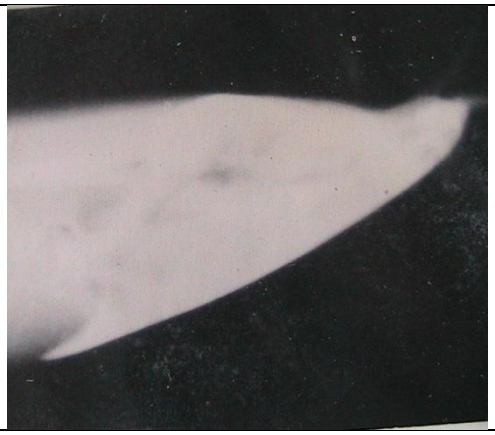
Table II: Bio concentration factor (BCF) of blood (mg / lit) in different tissues of *Clariasbatrachus* after short-term (48 hrs) of exposure:

Concentration levels	Gills	Bones	Liver	Blood	Kidney	Muscle	Skin	GI tract
25	630	50	420	760	270	110	180	0155
50	480	60	260	400	160	100	160	81
75	250	60	210	280	120	80	110	54
100	200	80	170	210	120	60	80	81
125	160	60	160	160	100	60	70	67
150	150	60	140	130	80	60	70	58

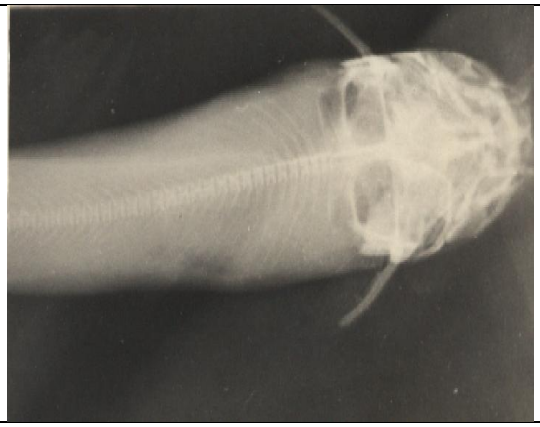
PLATE-I : X-ray Photomicrographs showing Lead Accumulation in Bone



i) Control Dorsal View

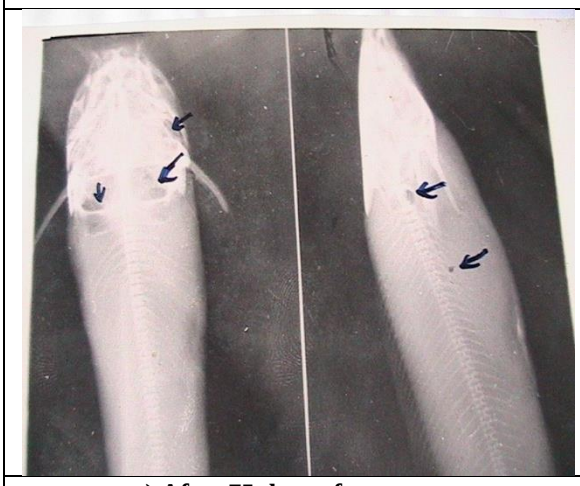


ii) Control Lateral View.

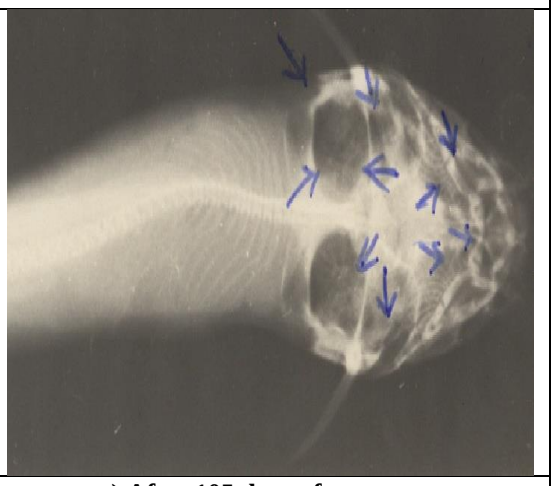


iv) After 45 days – Dorsal View

v) After 45 days- Lateral View.



v) After 75 days of treatment



v) After 105 days of treatment

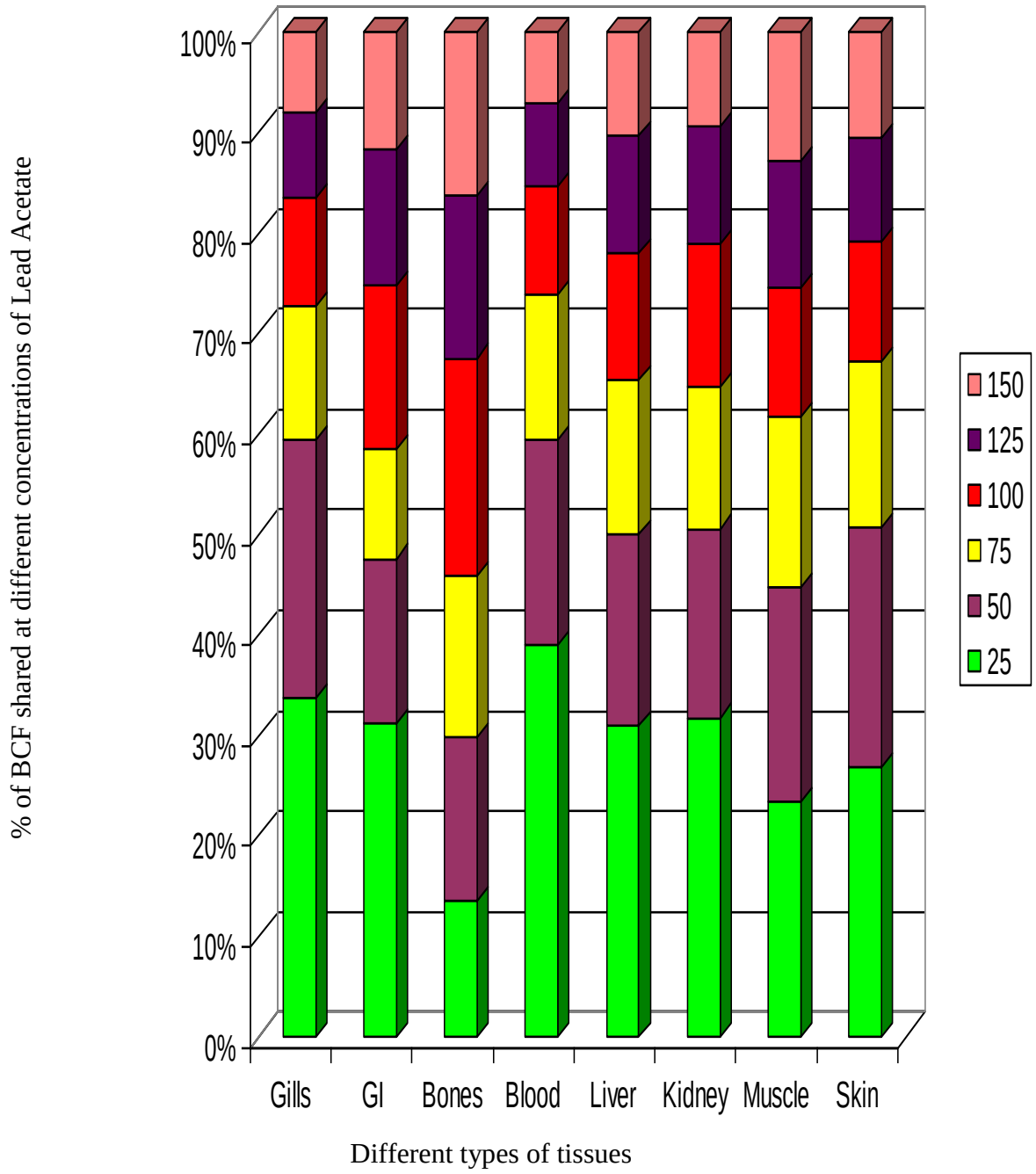


Figure 1:Bio-Concentration Factor (BCF) of Lead in different tissues after 3 days of exposure to different concentrations of Lead Acetate.

Table III: Accumulation of lead in various tissues after seven days of exposure to various concentrations of lead acetate.

Concentration of lead acetate(ppm)	Lead content µg / gm wt. on tissues							
	Gills	Bones	Liver	Blood (µg/dl)	Kidney	Muscle	Skin	GI tract
Control	0.1 ±0.001	0.2 ±0.001	0.1 ±0.001	3.7 ±0.001	0.01 ±0.001	0.1 ±0.001	0.1 ±0.001	0.01 ±0.001
25	12.46 ±1.24	5.12 ±0.938	0.72 ±0.001	13.50 ±0.544	1.01 ±0.001	2.56 ±0.001	3.58 ±0.012	6.28 ±0.08
50	15.62 ±0.924	5.56 ±0.726	0.93 ±0.001	14.56 ±0.279	1.52 ±0.001	4.23 ±0.001	7.23 ±0.312	8.69 ±0.13
75	19.69 ±0.815	9.23 ±1.291	1.59 ±0.006	15.26 ±0.716	1.69 ±0.003	4.76 ±0.002	7.82 ±1.231	10.168 ±0.113
100	24.92 ±0.741	10.13 ±0.368	2.58 ±0.007	17.19 ±0.218	2.18 ±0.001	4.31 ±0.003	9.35 ±1.114	10.25 ±0.14
125	30.13 ±0.069	12.13 ±0.694	3.62 ±0.006	18.25 ±0.125	3.18 ±1.201	4.59 ±0.231	10.12 ±1.235	11.11 ±0.115
150	38.25 ±0.192	16.52 ±0.215	5.26 ±0.123	22.21 ±0.238	3.56 ±0.126	8.39 ±0.001	10.79 ±0.115	13.12 ±0.611

⊥ P<0.005, * P<0.001, † P<0.05

Table IV: Bio concentration factor (BCF-mg / lit) of lead in different tissues after 7- days of exposure to various concentration of lead acetate.

Concentration levels	Gills	Bones	Liver	Blood	Kidney	Muscle	Skin	GI tract
25	772	317	44	837	62	158	222	389
50	484	172	28	451	47	131	224	269
75	407	190	32	315	34	98	161	210
100	386	157	40	266	33	73	145	158
125	373	150	44	226	39	56	116	137
150	395	170	54	229	36	86	111	135

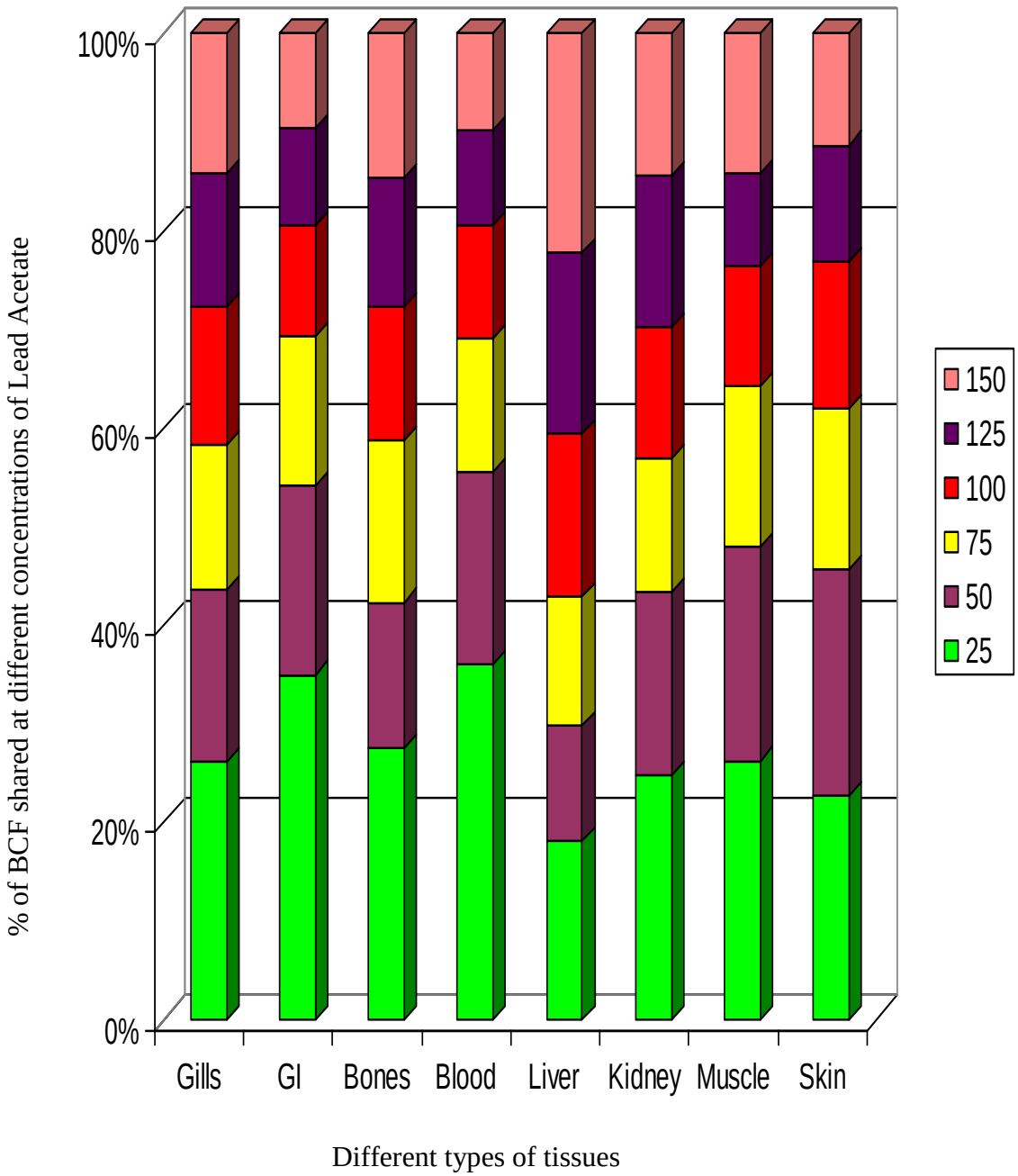


Figure 2: Bio-Concentration Factor (BCF) of Lead in different tissues after 7 days of exposure to different concentrations of Lead Acetate.

Table V:Accumulation of lead(Mean ± SEM) in various tissues of *Clariasbatrachus* during 15 ppm Chronic exposure of lead acetate and F- values obtained from ANOVA showing effectiveness of Period of Exposure.

Period of exposure (days)		Gills (µg/gm)	GI (µg/gm)	Bones (µg/gm)	Blood (µg/dl)	Liver (µg/gm)	Kidney (µg/gm)	Muscles (µg/gm)	Skin (µg/gm)
01	C	0.2 ±0.006	0.01 ±0.001	0.3 ±0.007	5.0 ±0.187	0.1 ±0.003	0.1 ±0.002	0.1 ±0.001	0.2 ±0.001
	E	5.0 ±0.051	1.56 ±0.001	0.5 ±0.041	10.11 ±0.577	3.5 ±0.211	0.1 ±0.002	0.1 ±0.001	0.8 ±0.004
15	C	0.15 ±0.001	0.01 ±0.001	0.23 ±0.002	4.83 ±0.178	0.2 ±0.001	0.08 ±0.001	0.09 ±0.001	0.3 ±0.001
	E	70.0 ±6.312	6.39 ±0.112	8.0 ±0.251	21.53 ±0.758	1.00 ±0.16	0.78 ±0.008	0.67 ±0.004	2.0 ±0.008
30	C	0.25 ±0.002	0.18 ±0.001	0.32 ±0.001	5.02 ±0.158	0.08 ±0.001	0.12 ±0.002	0.08 ±0.001	0.1 ±0.001
	E	115.0 ±8.512	0.28 ±01.132	19.53 ±3.412	41.53 ±1.561	1.35 ±0.121	1.11 ±0.008	1.56 ±0.005	6.0 ±0.052
45	C	0.23 ±0.001	0.19 ±0.012	0.26 ±0.001	4.78 ±0.129	0.06 ±0.001	0.08 ±0.001	0.09 ±0.003	0.09 ±0.001
	E	135.5 ±10.811	15.34 ±1.215	28.0 ±3.512	55.07 ±0.721	2.13 ±0.055	2.60 ±0.631	3.14 ±0.842	11.0 ±0.315
60	C	0.18 ±0.001	0.15 ±0.011	0.41 ±0.002	4.98 ±0.286	0.09 ±0.001	0.09 ±0.001	0.16 ±0.001	0.31 ±0.001
	E	161.5 ±9.856	22.39 ±2.231	38.4 ±3.151	82.25 ±1.406	3.16 ±0.691	4.81 ±0.823	3.89 ±0.235	13.0 ±2.815
75	C	0.32 ±0.002	0.12 ±0.011	0.25 ±0.002	4.85 ±0.183	0.12 ±0.001	0.12 ±0.001	0.08 ±0.001	0.26 ±0.002
	E	125.0 ±8.536	28.39 ±2.125	46.5 ±2.517	111.29 ±1.504	4.31 ±1.131	4.98 ±0.121	4.21 ±0.962	18.5 ±2.112
90	C	0.16 ±0.002	0.15 ±0.008	0.26 ±0.001	4.85 ±0.210	0.11 ±0.001	0.2 ±0.001	0.15 ±0.002	0.18 ±0.002
	E	86.0 ±5.813	35.63 ±1.129	55.621 ±3.861	123.35 ±2.508	5.23 ±1.661	3.24 ±0.151	3.51 ±0.891	25.8 ±3.512
105	C	0.23 ±0.001	0.12 ±0.001	0.35 ±0.001	4.98 ±0.613	0.10 ±0.001	0.25 ±0.002	0.09 ±0.002	0.25 ±0.002
	E	69.6 ±5.812	38.21 ±1.123	89.0 ±2.512	141.12 ±2.413	6.84 ±1.583	2.58 ±0.006	2.50 ±0.080	32.5 ±3.812
F-value	C	1.566	2.087	1.320	2.675	1.762	1.289	1.267	2.315
	E	269.987	67.54	274.147	864.154	318.076	79.324	89.567	563.254

Table VI: Bio concentration factor (BCF) of lead in various tissues of *Clariasbatrachus*during chronic 15 ppm chronic exposure of lead acetate.

Period of exposure (days)	BCF (mg / lit) in various tissues							
	Gills	GI	Bones	Blood	Liver	Kidney	Muscle	Skin
01	515	160	515	1042	360	10	10	82
15	7216	658	824	2219	103	80	64	206
30	11855	1059	2010	4281	139	114	160	618
45	13917	1582	2885	5677	219	268	323	1134
60	16649	2308	3958	8479	325	495	349	1340
75	12885	2926	4793	11473	444	513	434	1907
90	8865	3673	5731	12716	539	334	361	2659
105	717	3939	9175	14548	705	265	257	3350

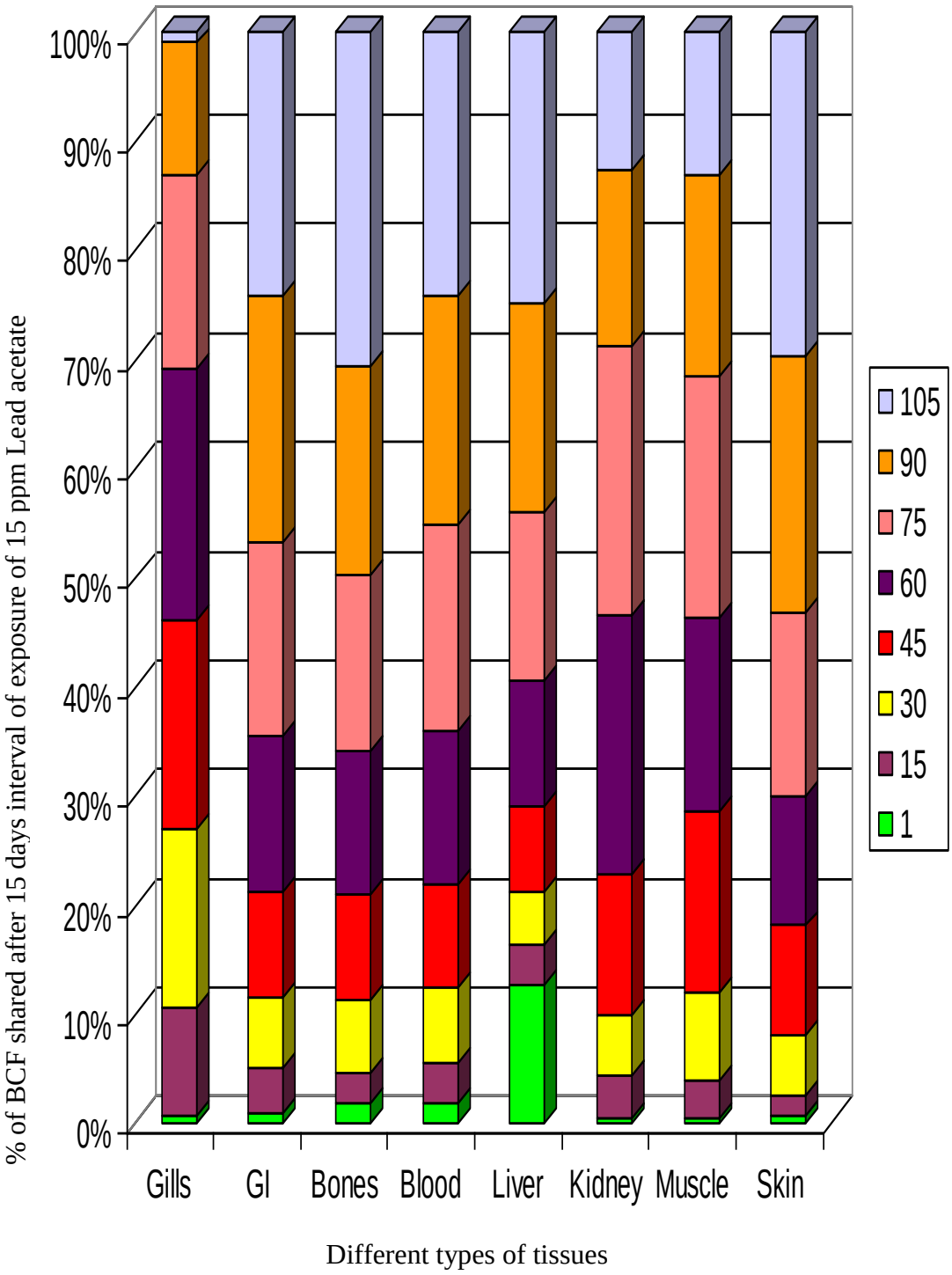


Figure 3: Bio-Concentration Factor (BCF) of Lead in different tissues during chronic 105 days of exposure 0f 15ppm Lead Acetate.

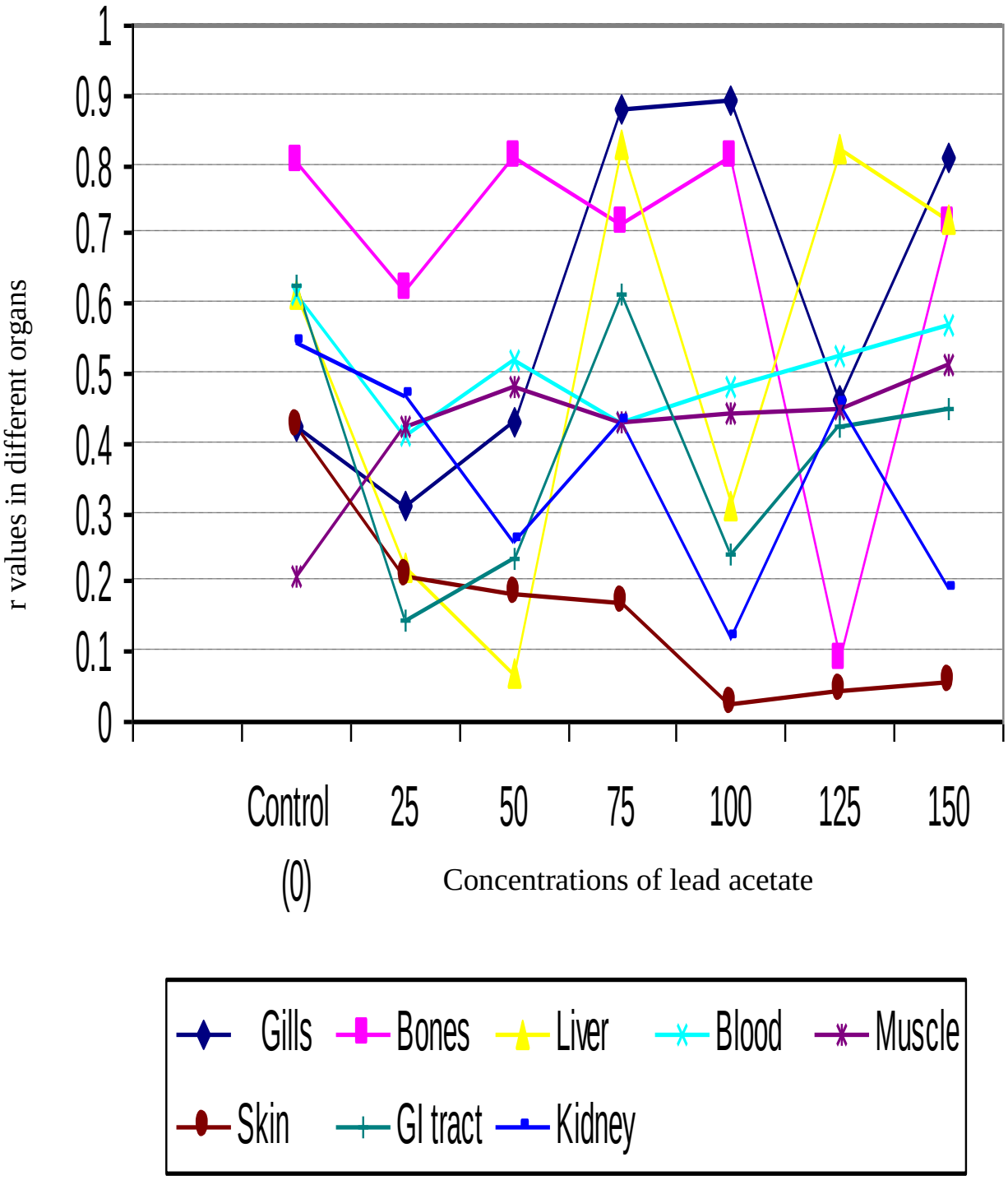


Figure 4: Co-rrelation Co-efficient ('r') in different tissues between Lead treatment and Lead accumulation in *Clariasbatrachus* after 3 days of exposure to different concentrations of Lead Acetate.

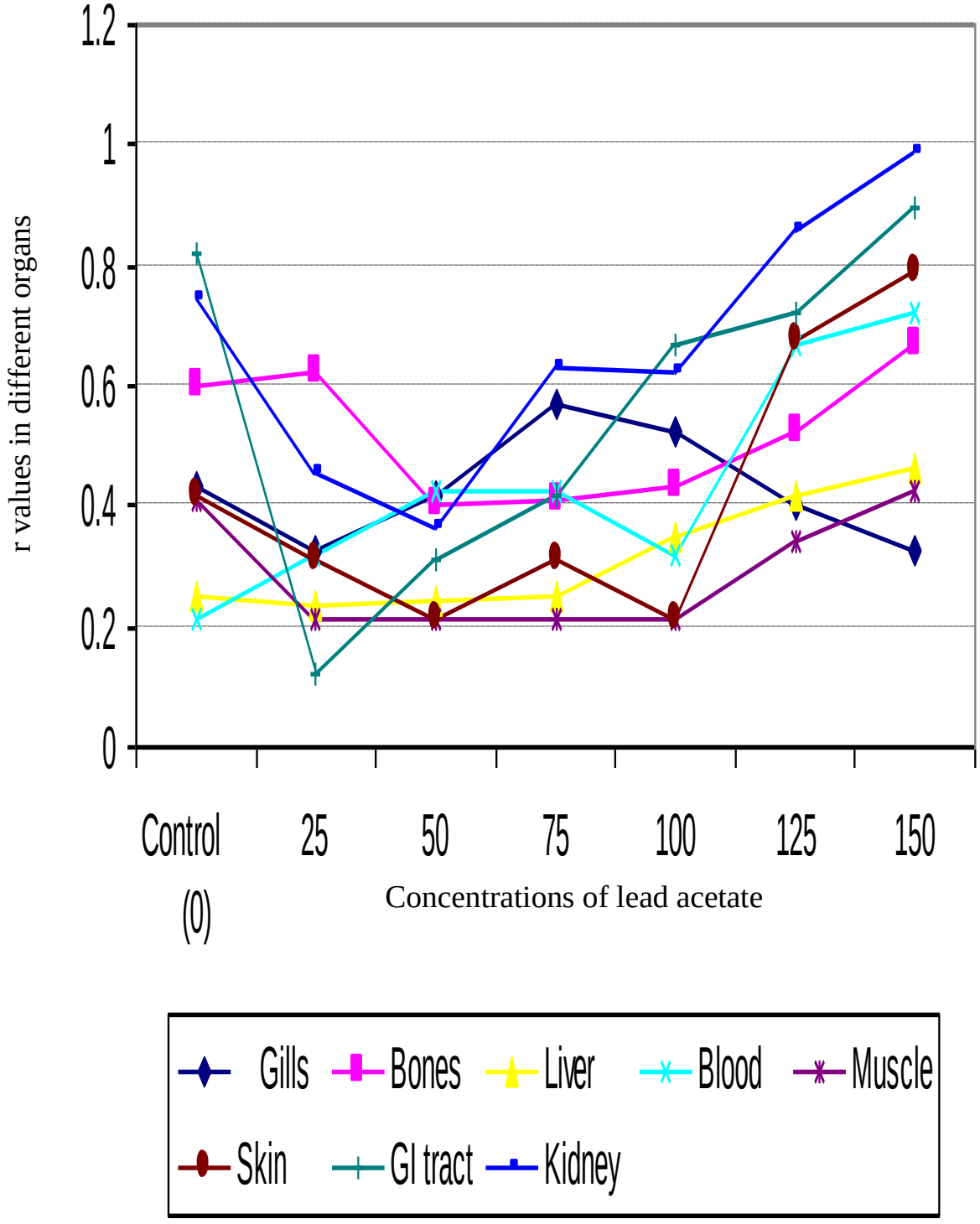


Figure 5:Co-rrelation Co-efficient('r') in different tissues between Lead treatment and Lead accumulation in Clarias*batrachus* after 7 days of exposure to different concentrations of Lead Acetate

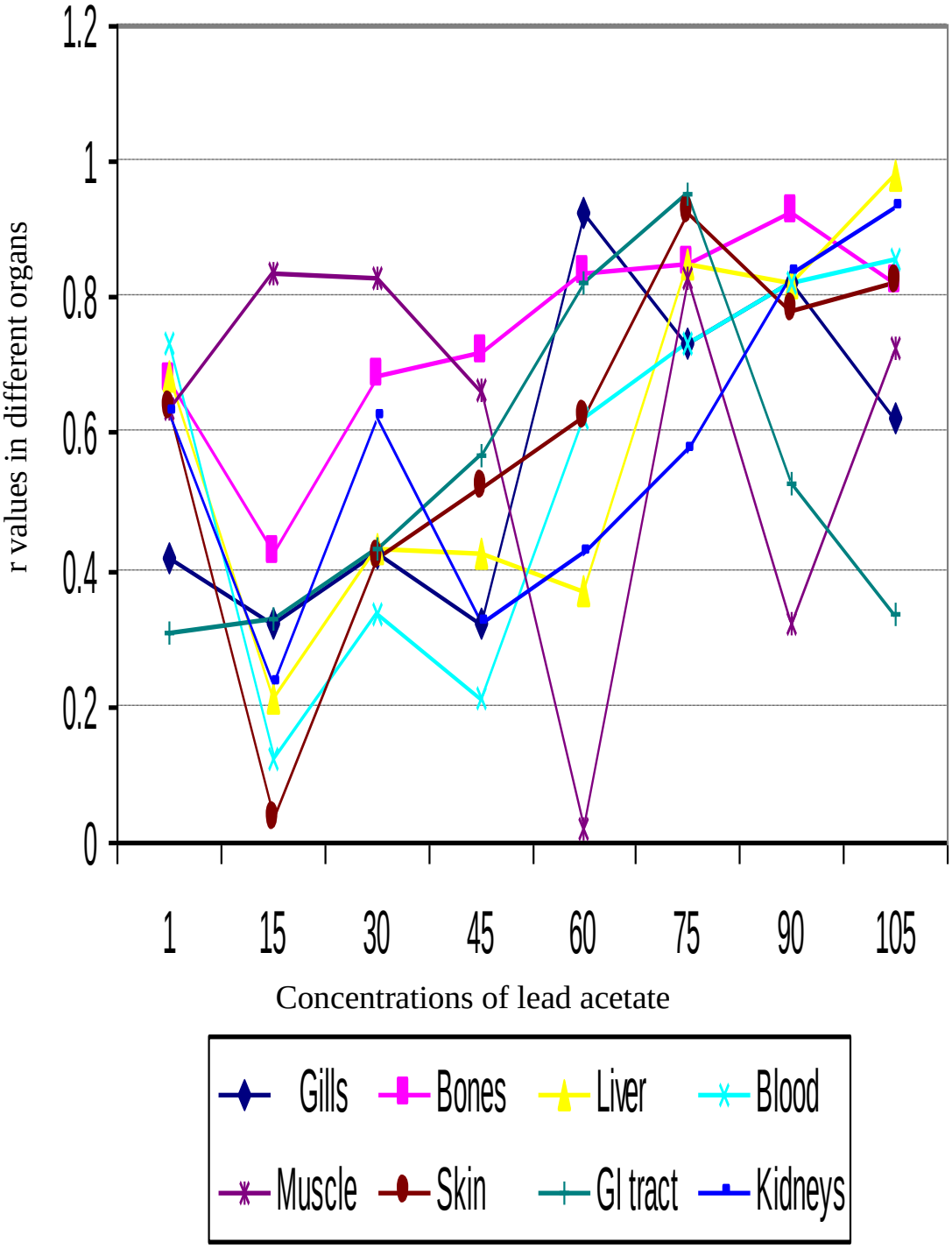


Figure 6: Co-rrelation Co-efficient in different tissues ('r') between Period of exposure and Lead accumulation in *Clariasbatrachus* during 15 ppm chronic exposure Lead Acetate for 105 days.

IV. DISCUSSIONS

The kinetics of lead can be discussed as under:

-Absorption

-Distribution

-Elimination and excretion

The absorption of lead from water to blood of the model fish *Clariasbatrachus* involves mainly three different routes, via the respiratory, dietary and dermal. The gills being major organ for respiration, has two processes, the deposition of water borne lead, on the gill lamellae and absorption and a very limited animal studies confirm that there is almost complete absorption of lead . A significantly increase in gill lead content in all the sets of subacute and chronic exposure is dose dependant as well as on the period of exposure (Sakr et.at. 2005). A decline of lead concentration after both days during chronic treatment indicates hyperplasia and necrosis of the soft gill lamellae.

In *Clariasbatrachus*, lead absorption by the gastrointestinal tract comes from the same source of water along with food. As reported earlier the food supply to the fish was live earthworm whose lead content has been analysed to be < 0.01 ppm in their muscle. Hence, the food when supplied is taken along with water of the experimental aquaria having measured lead concentration. The lead intake however increases after fasting (Abassi et.al. 1998). A dose dependent increase in lead accumulation may be a linear process that the decline in BCF from lower concentration of lead content to high concentration is equivocal with the statement that, absorption of lead in the GI tract is a saturable process. With increase in doses, lead absorption as a % of dose decrease in dietary studies. The accumulation in this case is not the highest in intestine as reported by Gupta and Bakre (1996) in case of *Pilaglobosa* But this amount certainly contributing to the absorption in to blood stream. The deposition of lead on skin is also significant. It is both dose dependent (Tables I, & III)as well as on period of exposure (Table-V). The BCF is high in chronic cases. The results however shows that the dermal absorption is minimal as on also verified earlier by Abassi et.al.(1998). Apart of from mucus secretions the skin generally lowly permeable to lead. A good amount of accumulation lead may be shared from internal uptake mechanisms.

4.2.1. The relationship of internal lead exposure to blood lead concentration:

In the fish all these three routes provide their way to lead concentration in blood. External exposures are sum of the quantities of lead consumed from all sources.

Historically, these are two lines of approach in under study the lead exposure and blood lead relationship (WHO-1995) most have been empirical and measured environmental lead and PbB levels either at one time or repeatedly with these observed correlations and with no assumptions about how lead moves inside the body, many reasonable predictions is optimum when only one source dominates. Some of these only on linear functions, while others have specified non-linearity's, especially, over very wide range of lead exposures. However when multiple sources are considered these predictive models have been less satisfactory.

Blood is the compartment in which lead is most often measured as a marker of exposure. The lead concentration with either increase in dose or period is curvilinear. A number of biological factors may explain the curvilinear relationship such as increased renal clearance with high PbB as advocated by Chamberlin (1985), distributuional non-linearities due to differences in lead finding sites in different tissues, or a sizeable pool of mobile lead in bone maintained more or less independently of uptake.

4.2.2. Distribution:

The initial distribution of lead in the body may depend upon the rate of delivery of blood to various organs. However it would appear that distribution occurs in a similar manner regardless of the route of absorption. The results from short-term subacute

and seven days subacuteexposures show that accumulation in Liver, Kidney, and bones is considerably more significant than muscle apart from blood as previous by found in other animal models. The level of significance is very high in all these tissues during chronic exposure up to 105th day. About 90% lead is stored in bone which is visible under X-ray photography. The amount of lead accumulation as in bone > Skin > Muscle > Liver > Kidney with high BCF. The results obey the studies of Rabinwitz et.al. (1976) and Aufderheide (1992) showing three different pools of biokinetic movements of lead. These three pools are blood, bone and soft tissues in human beings show distinct half-lives of lead. Blood lead is most labile with a half-life of about 36 days, bone lead is the most stable with about 27 years and lead in soft tissues has a half-live of approximately 40 days. Until recently it had been assumed that bone lead is metabolically insert and with a little heath-risk assessment. Current evidence is that bone comprises and is a target for toxicity. These factors complicate bone lead kinetics as applied to long term modelling; the toxicity of bone lead to blood is import. In the 1st day liver showed high lead content probably reflecting its role in detoxification of the xenobiotic.

Two physiological compartments appear to exist for lead in cortical and trabecular bone (ATSDR, 2005).

• the inert component stores lead for decades

• the labile component readily exchanges bone lead with the blood.

Under certain circumstances, however, this apparently inert lead will leave the bones and reenter the blood and soft tissue organs.

• Bone-to-blood lead mobilization increases during periods of pregnancy, lactation, menopause, physiologic stress, chronic disease, hyperthyroidism, kidney disease, broken bones, and advanced age, all which are exacerbated by calcium deficiency.

• Consequently, the normally inert pool poses a special risk because it is a potential endogenous source of lead that can maintain BLLs (Blood Lead Levels)long after exposure has ended.

Because lead from past exposures can accumulate in the bones (endogenous source), symptoms or health effects can also appear in the absence of significant current exposure.

• In most cases, toxic BLLs reflect a mixture of current exposure to lead and endogenous contribution from previous exposure.

• An acute high exposure to lead can lead to high short-term BLLs and cause symptoms of lead poisoning(UNEP, 2010).

• It is important that primary care physicians evaluate a patient with potential lead poisoning, examine potential current *and* past lead exposures and look for other factors that affect the bio-kinetics of lead (such as pregnancy or poor nutrition).

4.2.3. Elimination and Excretion:

In both humans and experimental animals, lead is eliminated from the body in both urine and faeces. About 85% of lead ingested lead excreted of it about 90% comprise faeces. But in our model fish, *Clariasbatrachus*. kept in the aquaria containing lead acetate, the excreted lead again reenters into the fish body. However, the significant amount of lead during subacute (short-term and 7days) exposure is accumulated reflecting retention of lead in the intertubular as (Jana 1998) reported earlier (Gennart and Lauwerys 1992) leading to intratubular spaces as it is Fanchoni syndrome.

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