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Journal of Advanced Studies in Agricultural, Biological and Environmental Sciences (JABE)

A Peer Reviewed & Refereed, International Open Access Journal

www.jabe.in **Vol.2.Issue.1.2015 (Jan-Mar)** **E-ISSN:2394-2606**

Determination of LC50 of lead in *Cirrhinus mrigala* (Hamilton,1822) and *Ctenopharyngodon idella* (Steindachner, 1866)

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Article History

Received on:11-02-2015

Revised on:28-03-2015

Accepted on:30-03-2015

ABSTRACT

Acute toxicity studies with nutritive trace metal copper have been carried out in the exotic carp *C. carpio* [1], and in endemic carp *Labeo rohita* [2], but studies with non-nutritive trace metal lead are lacking. Hence to measure the susceptibility and survival of the two species, 96 h LC 50 tests were conducted with lead. Static bioassays were conducted on *Cirrhinus mrigala* (8±0.5 g) and *Ctenopharyngodon idella* (8.5±1 g) fingerlings, to determine the acute toxicity of lead. The experiments were designed as three replicates with five exposure groups arranged exponentially (0.06, 0.12, 0.31, 0.5 and 0.6 mg l^{-1}) for *C. mrigala,* and five concentrations (0.06, 0.18, 0.31, 0.37 and 0.5 mg l^{-1}) for *C. idella*, besides the controls. Each exposure group comprised of 10 fish each. The median lethal concentration (96-h LC_{50}) of lead was determined as 0.31 mg l^{-1} Pb for *C. mrigala* and 0.20 mg l^{-1} Pb for *C. idella* respectively. Lead proved to be more toxic to *C. idella* than *C.mrigala.* The mortality rates increased with increasing lead concentrations, in both species.

Key Words*: Cirrhinus mrigala, Ctenopharyngodon idella,* lead, acute toxicity

Introduction

Heavy metals are some of the most active polluting substances, and can cause serious impairment of circulatory, metabolic, physiological and even structural systems when high concentrations are present in aquatic environment (Shugart *et al.* [3] They constitute a major hazard because of their toxicity, and persistence in environment and thus affect the organisms (Cepanko *et. al*. [4].

Fish are good bioindicators of environmental changes, and hence are suitable for water quality studies (Loyd [5]. They are most vulnerable to heavy metals having immunosuppressive actions, as they cannot escape from their polluted aquatic ambience (Heath [6]. Heavy metal ion concentrations at mgL^{-1} level are known to be toxic, because of the irreversible inhibition of some enzymes by the heavy metal ions (Pamukoglu and Kargi [7].

Lead, a non-essential and toxic metal, is released into the aquatic environment by industrial sources such as chemical and fertilizer industries, refining of ores (Handy [8], the plating process and gasoline containing lead that leaks from fishery boats (Pascoe and Pascoe and Mattey [9]. Lead also enters in the aquatic environment through erosion and leaching from the soil, domestic and industrial waste discharges, lead-dust fallout from the atmosphere and combustion of petroleum products (Farmer [10].

Lead is a non-nutritive trace metal. It is not among the metals considered essential to the nutrition of animals or human beings. It was reported to be a cumulative toxin. Lead toxicity can affect every organ system in fish (Maria *et al*. [11]. Experiments have shown that lead is a renal and vascular poison (Calvery [12]; Fairhall

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www.jabe.in **Vol.2.Issue.1.2015 (Jan-Mar)** **E-ISSN:2394-2606**

and Miller [13] and others). Metals such as lead are known to increase susceptibility to viral and bacterial infections, increased mortality and changes in humoral and cell mediated immunity Hartung [14].

Pb II is the most stable ionic species present in the aquatic environment and gets accumulated in the aquatic organisms (Heath [6]. The primary mode of uptake of aqueous Pb 2+ in freshwater fishes is through their gills into the blood stream (Seymore [15] and during exposure, the amount of lead taken up by the fishes , have induced behavioural deficits due to disruptions in the integrative functioning of the medulla, cerebellum and optic tectum (Rademacher *et al* . [16].

The main goal in toxicity testing is to predict, with known accuracy, a concentration of a specific toxicant that will not harm an entire system (Mance [17]. Acute toxicity of lead to the fish *Catla catla*, was studied by Sajid and Muhammad [18]. Hmoud *et al*. [19] conducted a toxicity bioassay with lead acetate in *Clarias gariepinus*. Acute toxicity studies with nutritive trace metal copper have been carried out in the exotic carp *C. carpio* [1], and in endemic carps *Labeo rohita* [2], but studies with non-nutritive trace metal lead are lacking. Hence to measure the susceptibility and survival of the two species, 96 h LC 50 tests were conducted with lead.

Materials and methods

Male and female fingerlings of an endemic carp *Cirrhinus mrigala* (mrigal), and an exotic carp *Ctenopharyngodon idella* (grass carp) ranging in length from 3 ½ to 4'' and weight 8 ± 0.5g and 8.5 ± 1g respectively, were procured from a private fish farmer in Kaikaluru, Andhra Pradesh. They were acclimated at a temperature of 28 ± 2 ^{0}C and fed with rice bran and oil-cake. The lead in water and feed used for the experiments was below detectable level.

As the approximate toxicity of the test material was unknown, a range-finding test was conducted to determine the concentrations that should be used in the definitive tests American Public Health Association (APHA) [20]. The experiments were designed as three replicates in tubs containing 100 L of water. Lead nitrate was used as the lead agent. Double-distilled water was used wherever necessary, and the lead agent was of extra pure grade.

Each replicate had five exposure groups for lead as well as one control for *C. mrigala* and five exposure groups for lead as well as one control for *C. idella*. Each exposure group comprised 10 fish. Feeding was terminated 24 hours prior to initiating the tests. Mortality, if any, was only less but not more than 5% during the 48 hours immediately before conducting the test. Temperature has not varied by more than ± 2^0 C during the 96 hour test, and ± 1^0 C during any 48 hours. Mortality, if any, in a control system was never more than 10% .

To determine lethal concentration (LC_{50}), a 96 hour test with five toxicant concentrations arranged exponentially, and a control were used, according to the results of the range-finding test, adopting the static bioassay method. The experiments were designed as three replicates in the tubs containing 100 L of water. Each replicate had five exposure groups for lead as well as one control for *C. mrigala,* and five exposure groups for lead as well as one control for *C. idella.* The water quality parameters are the same as those of the range–finding tests. Feeding was terminated 24 hours prior to initiating the tests. The variation in temperature was similar to the range-finding test. Mortality, if any, in a control system, was never more than 10% .

The experiment contained five lead concentrations (0.06, 0.12, 0.31, 0.5 and 0.6 mgl^{-1}) besides the control (not containing lead) for *C. mrigala* and five lead concentrations (0.06, 0.18, 0.31, 0.37 and 0.5 mgl^{-1}) besides the control (not containing lead) for *C. idella.*

The tubs were checked daily for temperature, and dissolved oxygen as they are important factors to be monitored in toxicity experiments. Fish were not fed for one day prior to starting the experiments to the end of the 96h experiment period. Aeration was also avoided to make sure that there is no loss of toxicant

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during that period. Thus build-up of metabolic products and occurrence of high concentrations of carbon dioxide and ammonia were avoided. Death was diagnosed by lack of swimming behaviour.

The term LC_{50} is in accordance with APHA [20] which is the concentration at which 50% of test organisms survive for a specified exposure time. This term has been superseded by median lethal concentration (LC_{50}).

The LC_{50} concentration values were analysed by Probit Analysis (Finney [21]. Data on mortalities recorded in the three replicates for each concentration were pooled. Regression analysis based on probit (transformed percentage mortality) against log-dose was calculated for each metal independently, and considering these calculations for the lethal concentrations (LC_{50}), fiducial limits were determined. Statistical analysis was carried out using computer program, BIOSTAT ^{TM} package.

Table 1. 96 hr. LC_{50} values with 95% confidence limits and probit regression equations for toxicity of lead to *Cirrhinus mrigala* and *Ctenopharyngodon idella*

Name of the fish	Lower limit	96 hr. LC_{50} (mg/l)	Upper limit	Probit Regression Equation
Cirrhinus mrigala	0.2448	0.3139	0.4118	Y = 6.0683 + 2.1229 X
Ctenopharyngodon idella	0.1636	0.2041	0.2439	Y = 7.1063 + 3.0517 X

Y = Predicted Probit 6.0683 & 7.1063 = Intercept values 2.1229 & 3.0517 = Beta values X = Log_{10} value Concentration (Stimulus)

Table 2. Regression statistics for concentration values of lead and % mortality of *Cirrhinus mrigala* in different lead ion concentrations at the end of 96 h exposure experiment

LD50	0.2041	LD50 Standard Error	0.0208
LD50 LCL	0.1636	*LD50 UCL*	0.2439
Log10[LD50]	-0.6902	*Standard Error*	0.0443
Beta	3.0517	*Intercept*	7.1063
Beta Standard Error	0.4639		

Table 3. Regression statistics for concentration values of lead and % mortality of *Ctenopharyngodon idella* in different lead ion concentrations at the end of 96 h exposure experiment.

LD50	**0.3139**	LD50 Standard Error	0.0418
LD50 LCL	0.2448	LD50 UCL	0.4118
Log10[LD50]	-0.5032	Standard Error	0.0576
Beta	2.1229	Intercept	6.0683
Beta Standard Error	0.3442		

Results & Discussion

The mortality rates also increased with increasing lead concentrations, in both *C. mrigala* and *C. idella* as shown in Fig. 1 and Fig. 2 respectively. No mortality occurred in the control groups.

The concentration values causing 50% mortality at the end of 96hr. period were analysed. LC_{50} was observed at concentration of 0.23 mgl^{-1} Pb and 0.17 $mg\ l^{-1}$ Pb for *C. mrigala* and C. *idella* respectively, after 96 hr exposure. The concentration values were analysed by Probit Analysis and LC_{50} values were calculated as 0.31 mgl^{-1} Pb for *C. mrigala* and 0.20 $mg\ l^{-1}$ Pb for *C. idella* respectively. There were no significant differences ($p = > 0.05$) between the observed and calculated mortality. Fig. 3 and 4 show the dose-response relationship of *C. mrigala* and *C. idella* in different lead ion concentrations at the end of 96 hr. exposure experiment with lead. 95% confidence limits have been calculated for both *C.mrigala* and *C. idella* with lead. The calculated 96

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hr. LC_{50} values with 95 % fiducial limits, their upper and lower limits, and the probit regression equations for toxicity of lead to *C. mrigala* and *C. idella* are given in Table 1.

Fig. 1 Mortality (%) in different lead ion concentrations at the end of 96 hr. exposure experiment with *C. mrigala*

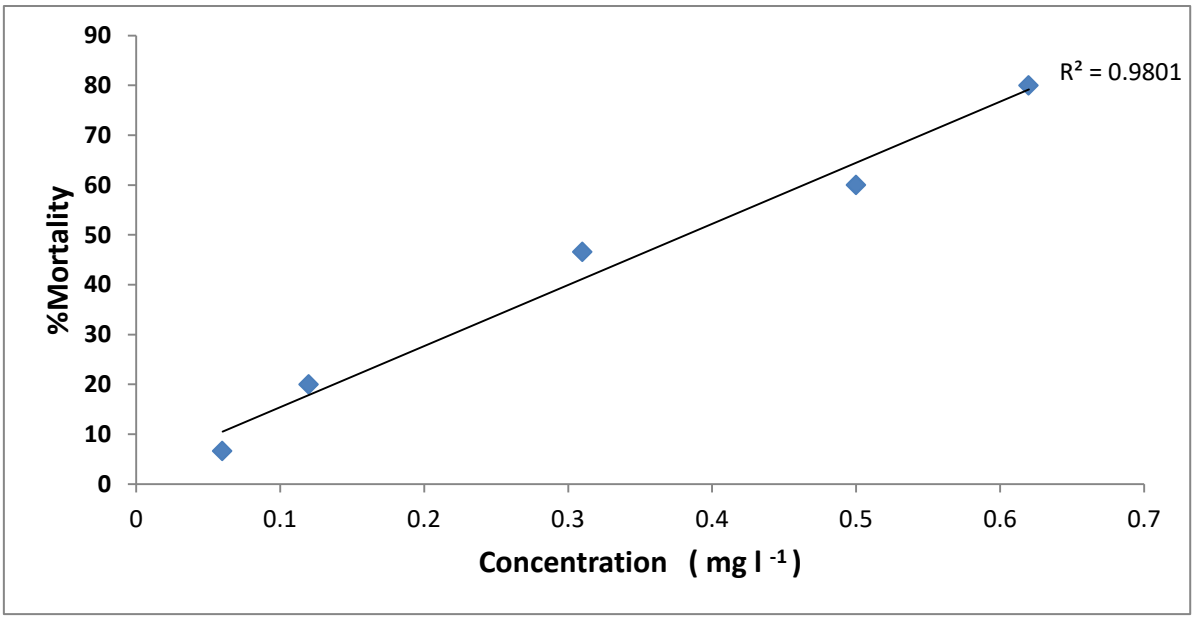

Fig. 2 Mortality (%) in different lead ion concentrations at the end of 96 hr. exposure experiment with *C. idella*

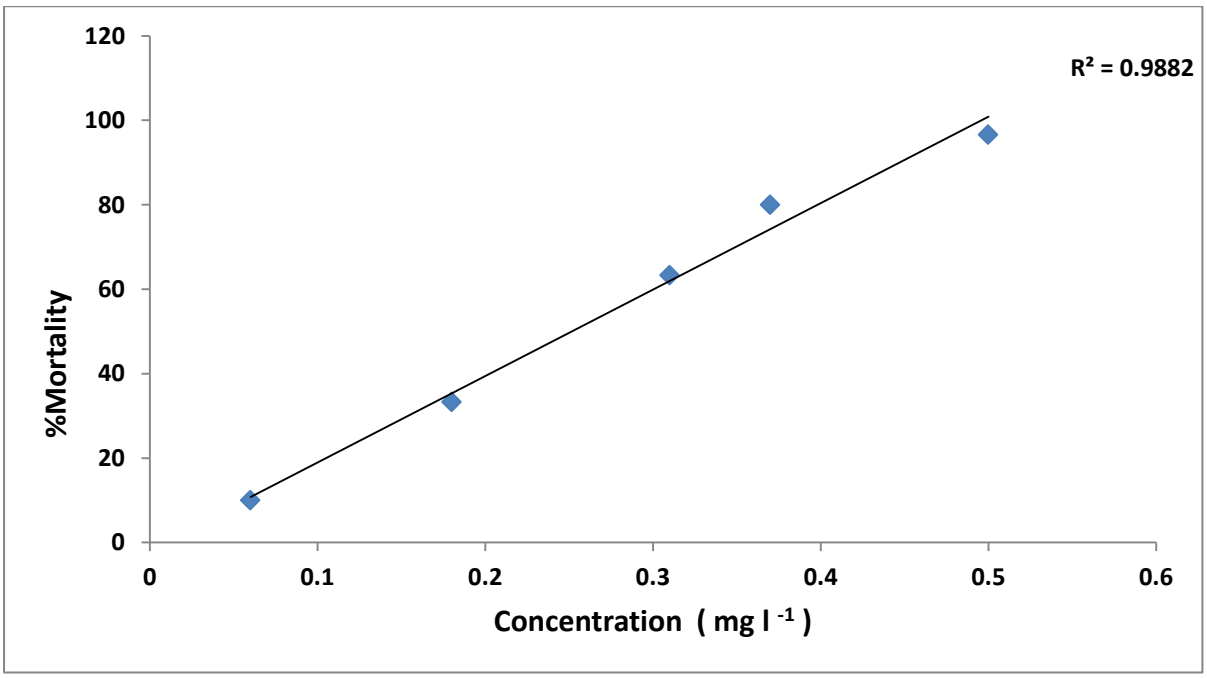

Fig. 3. Probit graph showing dose (experimental points and regression line) response (%mortality) relationship in different lead ion concentrations at the end of 96 hr. exposure experiment with *Cirrhinus mrigala*

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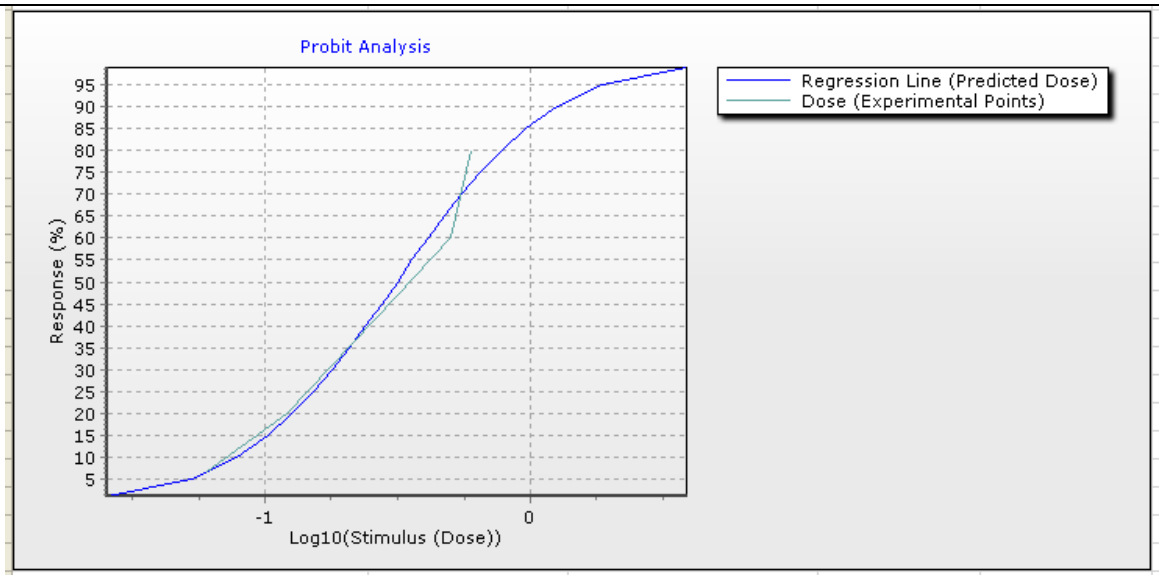
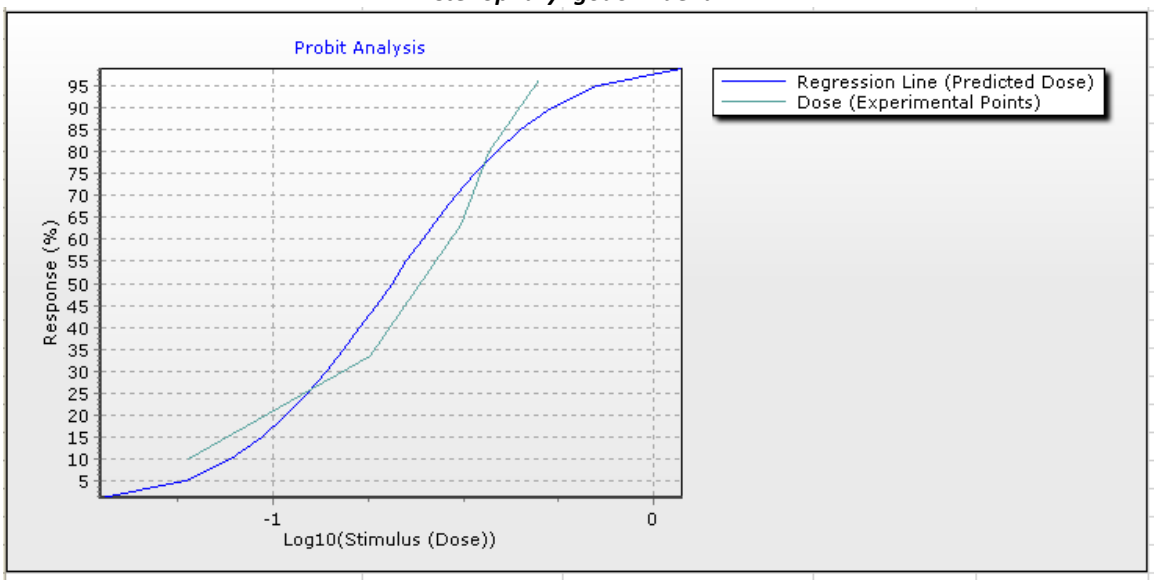


Fig. 4. Probit graph showing dose (experimental points and regression line) response (% mortality) relationship in different lead ion concentrations at the end of 96 hr. exposure experiment with *Ctenopharyngodon idella.*



Similarly , the concentration values were also analysed for LC_5, LC_{10}, LC_{25}, LC_{75} and LC_{90} by Probit Analysis. The LC_5, LC_{10}, LC_{25}, LC_{75} and LC_{90} for *C. mrigala* with lead were calculated as 0. 04 mg l^{-1} Pb, 0.07 mg l^{-1} Pb, 0.14 mg l^{-1} Pb, 0.63 mg l^{-1} Pb and 1.24 mg l^{-1} Pb and the LC_5, LC_{10}, LC_{25}, LC_{75} and LC_{90} for *C. idella* with lead were calculated as 0.05 mg l^{-1} Pb, 0.07 mg l^{-1} Pb, 0.11 mg l^{-1} Pb, 0.30 mg l^{-1} Pb and 0.48 mg l^{-1} Pb respectively. No mortality was observed at concentration 0.02 mg l^{-1} Pb for *C.mrigala* and 0.03 mg l^{-1} Pb for *C. idella* . Regression statistics for concentration values of lead and percentage mortality of *C.mrigala and C. idella* in different lead ion concentrations at the end of 96 h. exposure experiment is given in Tables 2 and 3.

Lead was less toxic to *C. mrigala* and more toxic to *C. idella*. The susceptibility of fish to a particular heavy metal is a very important factor for LC_{50} values. The fish that is highly susceptible to toxicity of one

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metal, may be less or non-susceptible to the toxicity of another metal at the same concentration of that metal (Sajid and Muhammad [19].

Similar experiments were also conducted by Rao and Manjulasree [22], Rogers et al.[23], Sajid and Muhammad [18], Adeyemo [24], Hmoud Fares [19], with *Mystus* sp. rainbow trout, *Catla catla, Clarias* sp. and *Mystus* sp. respectively. The values obtained by toxicity testing (eg., LC_{50}) are very dependent on the conditions under which tests were performed, so that interpretation of LC_{50} values needs to be done with caution (Walker *et al*., [25].

A mean LC_{50} value of 18.66 ± 1.82 mg l^{-1}, with lead chloride was determined for *Catla catla* juveniles in water having a total hardness of 101.45 mg l^{-1} by Sajid and Muhammad [18]. In the present study, 96 hr. LC_{50} values of 0.31 mgl^{-1} Pb and 0.20 mgl^{-1} Pb with lead nitrate were determined for *C. mrigala* (8 ± 0.5g) and *C. idella* (8.5 ± 1g) fingerlings in water having a total hardness of 240 mg l^{-1} respectively.

Gupta *et al*. [26] reported that the differences in acute toxicity may be due to changes in water quality and test species. Amongst fish species, considerable differences in sensitivity to lead have been reported (Salmeron *et al*. [27]. According to Demayo *et al*.[28], lead toxicity is a function of water hardness, species tested and fish age. Increased water hardness reduces lead toxicity to fish due to a significant inorganic complexation process that controls lead availability to fish (Hodson *et al*, [29]. Pickering and Henderson [30] showed that in soft water (20 mg $CaCo_3$ l^{-1}) the 96 hr. LC_{50} for *Pimephales promelas* and *Lepomis macrochirus* was 5.6 and 23.8 mg Pb l^{-1}, whereas in hard water (360 mg $CaCO_3$ l^{-1}) 96 hr. LC_{50} was 482 and 442 mg Pb l^{-1} respectively.

The results of this study indicated that mortality rate was influenced by the concentration levels of the heavy metals, as well as the kind of metals used. Besides it was found that there was a positive relationship between the mortality and concentration levels. When the concentration level increased, the mortality rate increased as well.

The present study was carried out in water with hardness ranging from 200-260 ppm. Though all the results reported above were derived from static bioassays in which lead content could vary due to absorption, adsorption and precipitation, there are differences related to water hardness, fish age etc. The result in the present study clearly reflected that the toxicity levels of lead varied depending on the experimental fish species and the hardness of water. Lead toxicity is often a function of water hardness, species tested and fish age (Demayo et al., [28].

Fish are the final trophic link of hydroecosystems which most easily accumulate pollutants (Cepanko *et. al*.[5], and cause acute and chronic effects to humans (Dogan and Yilmaz, [31] Fidan *et al*.[32].

Lead is mainly soluble in soft and slightly acidic water (Moore and Rainbow [36]. Since fish are cultured in pond waters which are soft and slightly acidic, lead that leaks from fishery boats, or which enters through erosion and leaching from the soil, or through domestic and industrial waste discharges into the aquaculture ponds, will easily dissolve in the water.

Conclusion

Bioassays serve to establish the relationship between the levels of a metal present and consequent biological effects both in controlled field studies (eg. field trials with heavy metals) and in the investigation of the biological consequences of existing / developing pollution problems in the field. Fish can be deployed along pollution gradients, so that dose–response curves can be obtained for the field as well as in the lab, and the two compared for assessing the heavy metal toxicity of aquatic environments, which aids in charting out a plan of action in the road map to environmental management.

Acknowledgements

I am thankful to the University Grants Commission for selecting me under the Faculty Development Programme (FDP) during the XI plan, and extending financial support to pursue my Ph.D.

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