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Acute toxicity bioassay of copper on the juveniles of Asian sea bass, *Lates calcarifer* (Bloch)

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Abstract
The present study was undertaken to investigate the acute toxicity of copper in the juveniles of Asian sea bass *Lates calcarifer* (Bloch) which is considered as an important candidate fish species for brackish and freshwater aquaculture. Experiments for the bioassay were performed in static bioassay test condition according to the standard guidelines. Median lethal concentrations (LC_{50}) were calculated for 24h, 48h, 72h and 96h by Probit analysis. The LC_{50} values for 24, 48, 72 and 96h and 95% confidence intervals for the juveniles of sea bass *L. calcarifer* showed 110.83, 93.58, 76.71 and 68.32 ppm/l for copper.

Keywords: Copper, *Lates calcarifer,* LC_{50}

1. Introduction
Various chemicals from agricultural waste and industrial effluents such as heavy metals contaminate in different water environments. Since heavy metals are not degradable and potentially toxic when bound to endogenous compounds after they enter the body [1]. Urbanization poses a significant risk to estuarine fauna, particularly fishes. Historically, *Lates calcarifer* is an economically important species supporting major fisheries and has been used extensively in toxicity testing because they are widely distributed and sensitive to environmental contaminants.

Biological toxicity testing is a relatively simple laboratory bioassay that measures the biological response of marine organisms, particularly at their highly sensitive early life stages [2]. The overall toxicity of heavy metals is commonly assessed using laboratory bioassays where organisms are exposed to contaminants [3]. Environmental stress from pollutants seems to be an important determining factor signaled by the occurrence of increase in various diseases [4]. The presence of heavy metals in the environment has increased in some areas to levels, which threatens the health of aquatic and terrestrial organisms [5]. A major challenge, therefore, is to predict the effects of contaminants on aquatic organisms and to establish toxicity criteria for acceptable levels of chemical contamination. A reason for interest in heavy metals and behavior in aquatic communities is that heavy metals may have different behavioral effects at concentrations much less, than at which they have lethal effects, suggesting that regulatory pollution limits based upon standard toxicological studies may be too high to prevent damage to aquatic communities through the sublethal behavioural effects [6].

Toxicological studies of the pollutants upon aquatic organisms are very important from the point of environmental consequences. Fishes are often forced to encounter in the highly contaminated water especially in areas where the dilution rate of waste water is low. Fish species can be used as test organism because, it is the best understood aquatic species and can be a front line indicator of suspected aquatic pollutants such as metals [7]. Acute toxicity studies can help to detect, evaluate and abatement of pollution by providing reliable estimates of safe concentration, from which water quality criteria can be derived [8]. The best method of acute toxicity testing is by the determination of LC_{50} or LD_{50}, which represents the amount of a toxicant either in the form of Lethal Concentration (LC) or Lethal Dose (LD) showing 50% kill of the population of the test animal within fixed period of time [9].

2. Materials and Methods
2.1. Experimental Fish
Healthy hatchery reared 3-month-old juvenile Asian sea bass L. calcarifer with a mean total length of 7.06±0.15 cm and a mean total weight of 10.18±0.24 g were obtained from the Sea

bass hatchery, Rajiv Gandhi Centre for Aquaculture, Thirumullaivasal near Sirkali, Nagapattinam Dist, Tamil Nadu, India. Fish samples were acclimatized for 2 weeks in a stock tank to the experimental glass aquaria (120×50×50 cm) filled with 250 l of water with a salinity of 27±2 ppt, under a natural photoperiod 12:12 h (light: dark) cycle. The water in the tanks was passed through a 1-µm filter, UV-sterilized and refilled daily. Fish were fed twice daily with commercially prepared sea bass pellet feed which contains 2.5 mg/kg of copper. They were starved for 24 h before and during experiment.

2.1 Chemicals Used
For preparation of stock solution, 3.9 g of copper ll sulphate pentahydrate ($CuSo_4$ 5 H_2O) (Merck) was dissolved in 1000 ml of double-distilled water and used as stock solution. It was stored in a clean standard flask at room temperature in the laboratory.

2.2 Experimental Procedures
A static bioassay test was performed to determine the 96-h LC_{50} of copper to *L. calcarifer*, following the Standard Methods [10]. After acclimatization period, 2 month-old fish (7.06 ± 0.15cm in length and 10.18 ± 0.24gm in weight) were transferred from the stock tank to the experimental aquaria. Ten fishes were randomly placed in each glass aquarium filled with 250 l (120x50x50 cm) of water, with loading densities of 0.64 g/l. Fishes were exposed to nominal copper concentrations (10,20,30,40,40,60,70,80,90,100 and 120 ppm/l). Each concentration was done in three replicates. Control fish were held in a similar facility without exposure to copper. The water quality characteristics were measured daily: dissolved oxygen (DO) 6.8±0.5 mg/l, temperature 28.5±0.4ºC, salinity of 27±2 ppt and pH 7.65±0.2. The criteria for death were no gill movement and no reaction to gentle prodding. Fish mortality in each aquarium was recorded at the intervals of 24, 48, 72 and 96 hrs using the method for the assessment of water quality [11]. Dead fish were immediately removed. Percent mortality was calculated and the values were transformed into the probit scale. Finney method [12] was used to carry out the probit analysis. Based on acute toxicity, four lethal concentrations were derived for 24, 48, 72 and 96 hours exposure duration, which have been used as the experimental concentration of the copper toxicants in the subsequent experiments.

3. Results
The percentage mortality of *L. calcarifer* was observed, when the fishes were exposed to different concentrations of copper as given in (Table 2). The LC_{50} value at 24 hrs of copper exposure was estimated as 110.83ppm. Lower and Upper limits of the concentrations were found to be from 103.71ppm to 118.44ppm. Acute toxicity of copper in *L. calcarifer* in 24 hrs was statistically not significant ($P>0.05$) (Fig.1). The LC_{50} value at 48 hrs of copper exposure was estimated as 93.57ppm. Lower and Upper limits of the concentrations were found to be from 85.06ppm to 102.94ppm. Acute toxicity of copper in *L. calcarifer* in 48 hrs was statistically highly significant ($P<0.05$) (Fig.2). The LC_{50} value at 72 hrs of copper exposure was estimated as 76.71ppm. Lower and Upper limits of the concentrations were found to be from 67.18ppm to 87.59ppm. Acute toxicity of copper in *L. calcarifer* in 72 hrs was statistically highly significant ($P<0.05$) (Fig.3). The LC_{50} value at 96 hrs of copper exposure was estimated as 68.32ppm. Lower and Upper limits of the concentrations were found to be from 60.95ppm to 76.57ppm. Acute toxicity of copper in *L. calcarifer* in 96 hrs was statistically highly significant ($P<0.05$) (Fig.4).

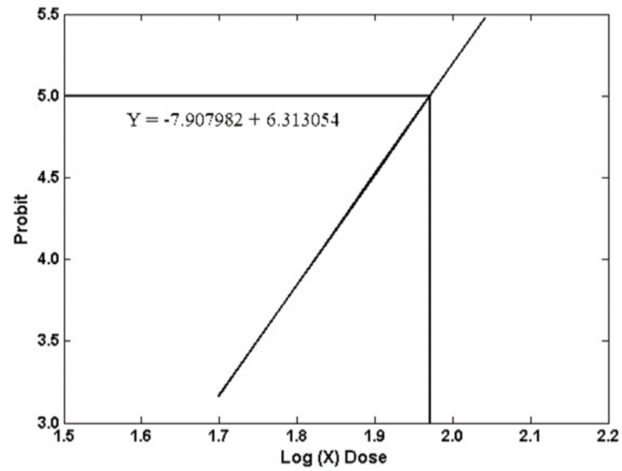

Fig 1: Response curve of copper at 24hrs in *L. calcarifer*

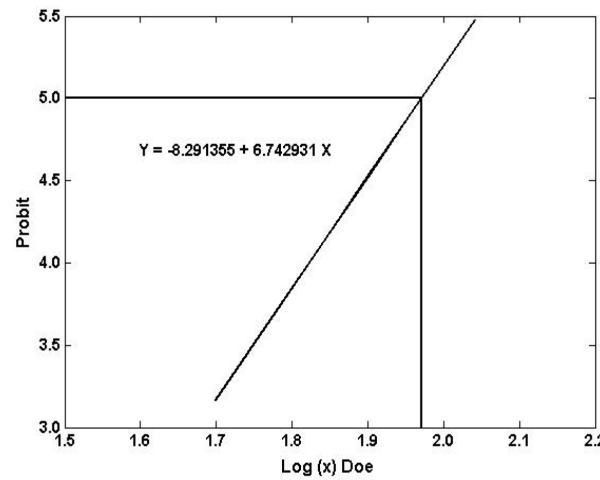

Fig 2: Response curve of copper at 48hrs in *L. calcarifer*

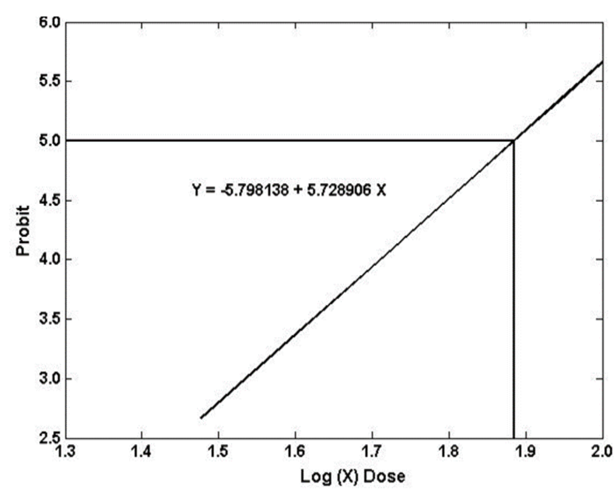

Fig 3: Response curve of copper at 72hrs in *L. calcarifer*

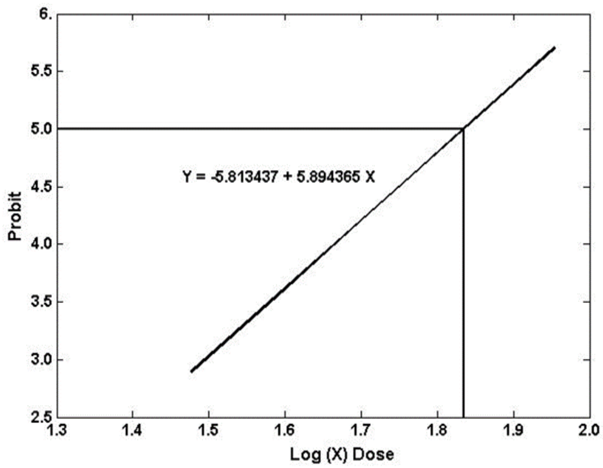

Fig 4: Response curve of copper at 96hrs in *L. calcarifer*

4. Discussion

It is evident from the results that the copper concentration has direct effect on the LC_{50} values of the Asian sea bass. Acute toxicity test is the measurement of short term lethality of a substance, by which, the concentrations of the substance proves to be lethal to the organism [13]. According to Brungs and Mount [14] the application of the LC_{50} value is the most highly related test for assessing the potential adverse effects of chemical contaminants of aquatic life.

Bryan [57] has listed a series of factors influencing toxicity of heavy metals in solution. These includes the dissolved form of metals, the presence of other metals and factors influencing the physiology and behaviour of the organisms. Copper is highly toxic to fishes and in the present investigation, the median lethal concentration (LC_{50} for 96 hrs) of copper, in *L. calcarifer* was determined to be 68.32ppm (Table 3).

It is obvious from the results that the heavy metal concentration has a direct effect on the LC_{50} values of the respective fish. LC_{50} obtained in the present study correspond to values that have been published in the literature for other species of fishes (Table 3). The differences in acute toxicity may be due to changes in water quality and test species [58].The susceptibility of fish species to a particular heavy metal is a very important factor for LC_{50} levels. Fish that are highly susceptible to the toxicity of one metal may be less or even not susceptible to the toxicity of another metal at the same level of that metal in the ecosystem. Conversely, a metal which is highly toxic to a fish species at low concentrations may be less or even non-toxic to other species at the same or even higher concentrations [59]. It is widely accepted that the stress response as a whole is characterized by physiological changes. These changes tend to be similar for stressors and could be as varied as anesthesia, flight, forced swimming, disease treatments, handling, scale loss, or rapid temperature change. Acute toxicity studies were the very first step in determining the water quality requirements of fish. These studies apparently reveal the toxicant concentrations (LC_{50}) that cause fish mortality even at short exposure. Therefore, studies demonstrating the sensitivity of xenotoxic effects of heavy metals in aquatic organisms, particularly in fish are needed. Thus, it can be concluded from the present study that the fish sea bass *L. calcarifer* was highly sensitive to copper and their mortality rate was dose dependent

Species	Element	Hours of exposure	LC_{50} (mg /l)	Reference
American eel, *Anguilla rostrata*	Cu	96	6.0	Rehwoldt *et al.,* 1972 [15]
Arctic grayling, *Thymallus arcticus*	Cu	96	0.010	Buhl and Hamilton, 1990 [16]
Atlantic salmon, *Salmo salar*	Cu	96	0.025	Carson and Carson, 1972 [17]
Atlantic salmon, *Salmo salar*	Cu	96	0.125	Wilson, 1972 [18]
Atlantic salmon, *Salmo salar*	Cu	96	0.025	Zitko *et al*., 1973 [19]
Banded killifish, *Fundulus diaphanus*	Cu	96	0.84	Rehwoldt *et al*., 1972 [15]
Black nose dace, *Rhinichthys atratulus*	Cu	96	0.32	Geckler *et al.,* 1976 [20]
Blue gill, *Lepomis macrochirus*	Cu	96	0.2	Tarzwell and Henderson, 1960 [21].
Blue gill, *Lepomis macrochirus*	Cu	96	1.25	Cairns and Scheier, 1968 [22]
Blue gill, *Lepomis macrochirus*	Cu	96	0.68	Inglis and Davis, 1972 [23]
Blue gill, *Lepomis macrochirus*	Cu	96	1.3	Blaylock *et al.,* 1985 [24]
Channel catfish, *Ictalurus punctatus*	Cu	96	0.055	Straus and Tucker, 1993 [25]
Channel catfish, *Ictalurus punctatus*	Cu	96	7.56	Birge and Black, 1979[26]
Chinook salmon, *Oncorhynchus tshawytscha*	Cu	96	0.01	Chapman and McCrady, 1977 [27]
Chinook salmon, *Oncorhynchus tshawytscha*	Cu	96	0.026	Chapman, 1978 [28]
Chinook salmon, *Oncorhynchus tshawytscha*	Cu	96	0.058	Hamilton and Buhl, 1990 [29]
Chisel mouth, *Acrocheilus alutaceus*	Cu	96	0.143	Andros and Garton, 1980 [30]
Coho salmon, *Oncorhynchus kisutch*	Cu	96	0.06-0.074	Lorz and McPherson, 1976 [31]
Coho salmon, *Oncorhynchus kisutch*	Cu	96	0.046	Chapman and Stevens, 1978 [32]
Coho salmon, *Oncorhynchus kisutch*	Cu	96	0.019-0.021	Buhl and Hamilton, 1990 [16]
Common carp, *Cyprinus carpio*	Cu	96	0.063	Khangarot *et al.,* 1983 [33]
Creek chub, *Semotilus atromaculatus*	Cu	96	0.31	Geckler *et al.,* 1976 [20]
Cutthroat trout, *Oncorhynchus clarkii lewisi*	Cu	96	0.0157	Chakoumakos *et al.,* 1979 [34]
Fathead minnow, *Pimephales promelas*	Cu	96	0.023-0.035	Pickering and Henderson, 1966 [35]
Fathead minnow, *Pimephales promelas*	Cu	96	0.47	Mount,1968 [36]
Fathead minnow, *Pimephales promelas*	Cu	96	0.075	Mount and Stephan, 1969 [37]
Fathead minnow, *Pimephales promelas*	Cu	96	0.6-0.98	Brungs *et al.,* 1976 [38]
Fathead minnow, *Pimephales promelas*	Cu	96	0.44	Geckler *et al.,* 1976 [20]
Fathead minnow, *Pimephales promelas*	Cu	96	0.46	Pickering *et al.,* 1977 [39]
Flag fish, *Jordanella floridae*	Cu	96	1.27	Fogels and Sprague, 1977 [40]
Gold fish, *Carassius auratus*	Cu	96	5.2	Birge and Black, 1979 [26]
Gold fish, *Carassius auratus*	Cu	96	0.3	Tsai and McKee, 1980 [41]
Guppy, *Lebistes reticulatus*	Cu	96	0.16	Deshmukh and Marathe,1980 [42]

Guppies, *Poecilia reticulata*	Cu	96	0.112	Chynoweth *et al.*, 1976 [43]
Guppies, *Poecilia reticulata*	Cu	96	0.764	Khangarot *et al*., 1981[44]
Largemouth bass, *Micropterus salmoides*	Cu	96	6.97	Birge and Black 1979 [26]
Mosquito fish, *Gambusia affinis*	Cu	96	0.047	Joshi and Rege, 1980 [45]
Northern squawfish, *Ptychocheilus oregonensis*	Cu	96	0.023	Andros and Garton, 1980 [30]
Orangethroat darter, *Etheostoma spectabile*	Cu	96	0.85	Geckler *et al.*, 1976 [20]
Penny fish, *Denarius abandata*	Cu	96	0.077	Williams *et al*., 1991[46]
Pink salmon, *Oncorhynchus gorbuscha*	Cu	96	0.143	Servizi and Martens, 1978 [47]
Pumpkin seed, *Cucurbita pepo*	Cu	96	1.3	Spear and Anderson, 1975 [48]
Pumpkin seed, *Cucurbita pepo*	Cu	96	1.24-1.3	Anderson and Spear, 1980 [49]
Rainbow darter, *Etheostoma caeruleum*	Cu	96	0.32	Geckler *et al.*, 1976 [20]
Rainbow trout, *Oncorhynchus mykiss*	Cu	96	0.253	Hale, 1977 [50]
Rainbow trout, *Oncorhynchus mykiss*	Cu	96	0.11	Birge and Black, 1979 [26]
Rainbow trout, *Oncorhynchus mykiss*	Cu	96	0.4	Giles and Klaverkamp,1982 [51]
Rainbow trout, *Oncorhynchus mykiss*	Cu	96	0.036	Buhl and Hamilton, 1990 [16]
Sockeye salmon, *Oncorhynchus nerka*	Cu	96	0.19	Servizi and Martens, 1978 [47]
Steelhead trout, *Oncorhynchus mykiss*	Cu	96	0.028	Chapman, 1978 [28]
Steelhead trout, *Oncorhynchus mykiss*	Cu	96	0.057	Chapman and Stevens, 1978 [32]
Steelhead trout, *Oncorhynchus mykiss*	Cu	96	0.08	Seim *et al.,* 1984 [52].
Steelhead trout, *Oncorhynchus mykiss*	Cu	96	0.0028	Cusimano *et al.*, 1986 [53]
Stone roller, *Campostoma anomalum*	Cu	96	0.29	Geckler *et al*., 1976 [20]
Striped bass, *Morone saxatilis*	Cu	96	0.62	Wellborn, 1969 [54]
Striped bass, *Morone saxatilis*	Cu	96	4	Rehwoldt *et al.,* 1972 [15]
Striped bass, *Morone saxatilis*	Cu	96	0.1	Palawski *et al.,* 1985 [55]
Striped shiner, *Luxilus chrysocephalus*	Cu	96	0.79	Geckler *et al.,* 1976 [20]
White perch, *Morone Americana*	Cu	96	6.4	Rehwoldt *et al*., 1972 [15]
Zebra fish, *Danio rerio*	Cu	96	0.149	Fogels and Sprague, 1977 [40]
Zebra fish, *Danio rerio*	Cu	96	0.24	Weinstein, 1978 [56]
Asian sea bass, *Lates calcarifer*	Cu	24	110.83ppm/l	Present study
Asian sea bass, *Lates calcarifer*	Cu	48	93.58ppm/l	Present study
Asian sea bass, *Lates calcarifer*	Cu	72	76.71ppm/l	Present study
Asian sea bass, *Lates calcarifer*	Cu	96	68.32ppm/l	Present study

Table 2: Average mortality rate of *Lates calcarifer* in different concentrations of copper during acute toxicity study

Exposure Periods (hrs)	**Total Number fishes exposed**	**Concentration of copper in water (ppm)**																			
		30		40		50		60		70		80		90		100		110		120	
		Mortality rate (number fishes and percentage of mortality)																			
		Nos	%	Nos	%	Nos	%	Nos	%	Nos	%	Nos	%	Nos	%	Nos	%	Nos	%	Nos	%
24	20	Nil	0	Nil	0	0	0	1	05	2	10	4	20	5	25	8	40	10	50	20	100
48	20	Nil	0	Nil	0	1	05	2	10	5	25	5	25	7	35	11	55	18	90	20	100
72	20	1	05	1	05	3	15	4	20	7	35	9	45	13	65	18	90	20	100	20	100
96	20	1	05	2	10	4	20	5	25	9	45	14	70	17	85	20	100	20	100	20	100

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