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Sreelekshmy SG
Department of Zoology, Fatima Mata National College, Kollam, Kerala 691001

Miranda MTP
Department of Zoology, Fatima Mata National College, Kollam, Kerala 691001.

Rajesh BR
Department of Biotechnology, Cashew Export Promotion Council of India Laboratory & Technical Division, Kollam, Kerala 691001.

Correspondence
Sreelekshmy SG
Department of Zoology, Fatima Mata National College, Kollam, Kerala 691001.

Acute toxicity of industrial effluent on the marine catfish *Arius nenga* (Hamilton, 1822)

Sreelekshmy SG, Miranda MTP, Rajesh BR

Abstract
Industrial effluents, controversially, is one of the largest and major contributors of pollution in aquatic ecosystem and affects the aquatic organisms in general and fishes in particular. Several laboratory studies were conducted to evaluate the effects of both treated and untreated effluents on fish. The studies showed wide ranges of toxicity at organismal as well as at population levels. The present work deals with the determination of acute toxicity of Kerala Minerals and Metals Limited industrial effluent on the marine catfish *Arius nenga*. The effluent was highly acidic and contains a battery of heavy metals. Static bioassays were performed on, *A. nenga* to evaluate the median lethal concentrations of industrial effluent. Mortality increased as the concentration of effluent increased and 5.8% of effluent was obtained as 24 h LC_{50}. The work eventually revealed that the KMML industrial effluent has a high level of toxicity to marine catfish. Harmful effects such as biomagnification and other aberrations may occur in man if exposed to substantial concentration of the effluent.

Keywords: *Airus nenga,* 24h LC_{50}, Acute toxicity, industrial effluent

1. Introduction
Aquatic ecosystem is exposed to a wide range of pollutants emitting from industrial plants, sewage treatment plants and drainage from agricultural and urban areas. Industrial discharge is the most important source that contaminates the aquatic environment [1]. Substances contained in the industrial effluents have been reported to be highly toxic, depending on the dose and duration of exposure [2], and they can impart serious harm to aquatic life [3]. The effect of water pollutants not only produces damage to the individual specie and populations, but also to the natural biological communities. It occurs when these pollutants are discharged directly or indirectly into aquatic system without adequate treatment to eradicate these harmful constituents [4]. Toxicity study is an essential part of ecotoxicological studies which help to find out toxicant limit and its safe concentration, so that there will be less harm to aquatic fauna. Among the several aspects of toxicity studies, the bioassay represents one of the most frequently used methods in aquatic toxicological studies. The prerequisite of determining the toxicity of substances in aquatic organisms at the lower level of the food chain has been useful and accepted for the management water quality. Microsystem assays are now becoming increasingly popular and have proved its valuable potential in the validation of bench tests. Short term bioassay data with single species are an early warning signal in predicting acute poisoning in the field. It can be used to predict the toxicities of contaminants and can also predict effects in various physico-chemical conditions. Under laboratory conditions, toxicity testing procedures (mortality studies LC_{50} estimates) may provide information regarding the harmfulness of industrial stress for aquatic animals, including fishes [5]. In the acute toxicity of contaminants in static bioassays, the use of LC_{50} has been widely recommended as a preliminary step in toxicological studies on fishes [6-12].
The acute toxicity test serve as a basement for understanding the limiting effects of various chemicals and heavy metals on organisms. Median tolerance limit or LC_{50} as it later become known, proved to be a reliable measure for the establishment of water quality criteria with the emergence of water pollution control legislation. Fish has been considered as an ideal test organism in most of the ecotoxicological studies, to examine both acute and chronic toxicity of pollutants. The wide use of fishes is most likely due to their availability and adaptability to laboratory conditions to varying degree of sensitivity to the toxic substance [13]. During fish bioassay, a great importance has been laid on its lethality. The low concentration of pollutants

may not result in fish mortality, but they can cause sublethal toxic effects on them. Several studies have been conducted in assessing the toxicity of pollutants to the aquatic biota especially fishes [14-16]. The fish selected for the present study was the marine catfish, *Arius nenga*, (Hamilton, 1822) an edible fish of Kerala. The aim of this work was to study the mortality rate and to determine the acute toxicity by using 24h LC_{50} of *A. nenga* by exposing them to different concentrations of industrial effluent, discharging directly into the coastal belt of Kollam, South west coast of Kerala.

2. Materials and methods

2.1 Collection and Laboratory Maintenance of *A. nenga.*

A. nenga were collected locally and transported to the laboratory in large aerated polythene bags containing water from the collection site and ensuring minimum stress during transportation. Healthy fishes were placed in large glass aquaria of 45 liter capacity. Prior to this, the tanks were washed with potassium permanganate solution (20ppm). After this it was filled with sea water obtained from the collection site. Before the experiment, the fishes were allowed to acclimatize for a period of two weeks. Proper aeration was provided using aquarium aerators. The temperature was maintained at 27.0 ± 2 ^{0}C, pH 7.2 ± 0.2 and dissolved oxygen (DO) 7.5 ±0.5 mg/L. The fishes were fed daily twice with chicken liver and trash fishes. Only healthy and active animals of more or less similar size (10.1±1.12 cm length) and (weight 31.4±1.90 gm) were randomly selected from the holding tanks and used for all toxicity experiments. The fish were starved for 24 hour prior to the test. This gave sufficient time for the gut to be emptied of all food and waste.

2.2 Toxicant

The toxicant selected for the study was the Kerala Minerals and Metals Limited (KMML) industrial effluent. It consists of a battery of heavy metals including iron, vanadium, zinc, lead, manganese, nickel, cobalt, cadmium, copper and chromium. pH of the effluent was highly acidic. They were directly collected from the discharge point of the KMML plant. The physico-chemical parameters (Temperature, pH, TDS and DO) and heavy metal analysis of crude effluent were carried out.

2.3 Experiment (24h LC_{50})

Generally, two types of bioassays are employed to measure the relationship between the concentration of the pollutant and biological response the static bioassay and continuous flow bioassay. In the present study the static bioassay method was employed [7]. Static bioassay conducted as a short term toxicity test. The death of the organism was the criterion used to evaluate the toxicity of industrial effluent.

Previously cleaned aquaria were washed with 20ppm of potassium permanganate and the tank was filled with 40 liters of sea water. After acclimatization, 10 active and healthy specimens of *A. nenga* were transferred into the experimental aquaria. The effluent samples were brought freshly from the discharge sites. They were then cooled at room temperature. Various concentrations of effluents were prepared by mixing with sea water. Pilot experiments were conducted to choose the mortality range between 10% and 90%. Based on the pilot experiments, the experiments were conducted to determine the toxicity in different concentrations (2%, 4%, 6%, 8% and 10%) for 24h. The control group was kept in seawater without addition of effluent. The bioassay was conducted with 10 specimens exposed per concentration per set, following standard procedures for determination of acute toxicity bioassay. The experiments were conducted under the natural photo period with no food being given to the fish. The sublethal concentrations were found out at 1/5th of the 24h LC_{50} value.

2.4 Statistical Analysis

Mortality percentage data was used in statistical analysis. The probit mortality was found out using SPSS software 16.0 and probit plotted on probit paper and the concentrations of effluent that kill 50% of test organism (LC_{50}) during 24h exposure together with 95% confidence limit was calculated by following [17].

3. Results

The physico-chemical parameters and determination of heavy metals in the crude effluent is given in Table 1

Table 1: Chemical analysis of effluent used for experiments

Parameters	Values
Temperature	30^{o}C ± 2^{o}C
TDS	110.8 ppt
pH	4.1±0.2
Dissolved oxygen	3.4 ± 0.8 mg/l
Iron	3206.3 μg/l
Vanadium	248.9 μg/l
Zinc	41.7 μg/l
Lead	39.7 μg/l
Manganese	26.9 μg/l
Nickel	23.9 μg/l
Cobalt	22.1 μg/l
Cadmium	19.8 μg/l
Copper	12.7 μg/l
Chromium	10.7 μg/l

3.1 Determination of 24h LC_{50}.

The test was conducted to evaluate the toxicity of KMML industrial effluent on *A. nenga.* During the toxicity study, the fishes showed altered behavior including signs of weakness, irregular swimming, restlessness, jerky movements, revolving, convulsions and extensions of fin rays. The schooling behavior of fishes was also disturbed. The toxicity of effluent on the fish, was assessed and LC_{50} was determined for 24h by probit analysis. No mortality was observed in the control group. The mortality was increased significantly with the concentration of effluent (Fig. 1). The percentage mortality of fish at different concentrations of effluent (2% to 10%) is shown in Table 2.

Table 2: Mortality rate of *A. nenga* exposed to different concentration of effluent

Concentration of effluent (%)	Mortality rate (%)	Total no. of fishes (n)
2	0	10
4	2	10
6	5	10
8	8	10
10	9	10

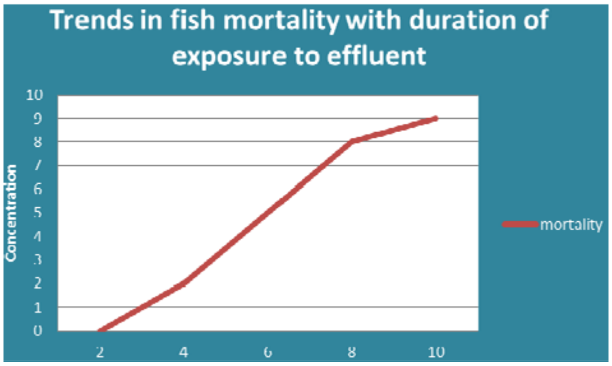

Fig 1: Mortality rate of *A. nenga* exposed to different concentrations of effluent.

During the exposure, some noticeable damage or injuries were shown among fishes especially in fishes exposed to highest concentration (8% and 10%). The injuries probably weaken the fish's resistance to toxin and consequently result in death of 50% individuals at the highest concentration exposures. This could be due to the stress and cumulative impact of heavy metals present in the effluent.

The 24h LC_{50} of KMML industrial effluent on fish was obtained by using SPSS software 16.0 (Fig. 2). The LC50 values are presented in table 3. The probit derived LC_{50} value of effluent was 5.8% for 24h and 95% upper and lower confidence limit in static test of was 4.5% and 7.0%.

Table 3: Parameter Estimates for the probit analysis of 24 hr exposure to effluent

Parameter		Estimate	Std. Error	Z	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
PROBIT[a]	concentration	5.649	1.477	3.824	.000	2.754	8.544
	Intercept	-4.325	1.199	-3.608	.000	-5.524	-3.126
a. PROBIT model: PROBIT (p) = Intercept + BX (Covariates X are transformed using the base 10.000 logarithm.)							

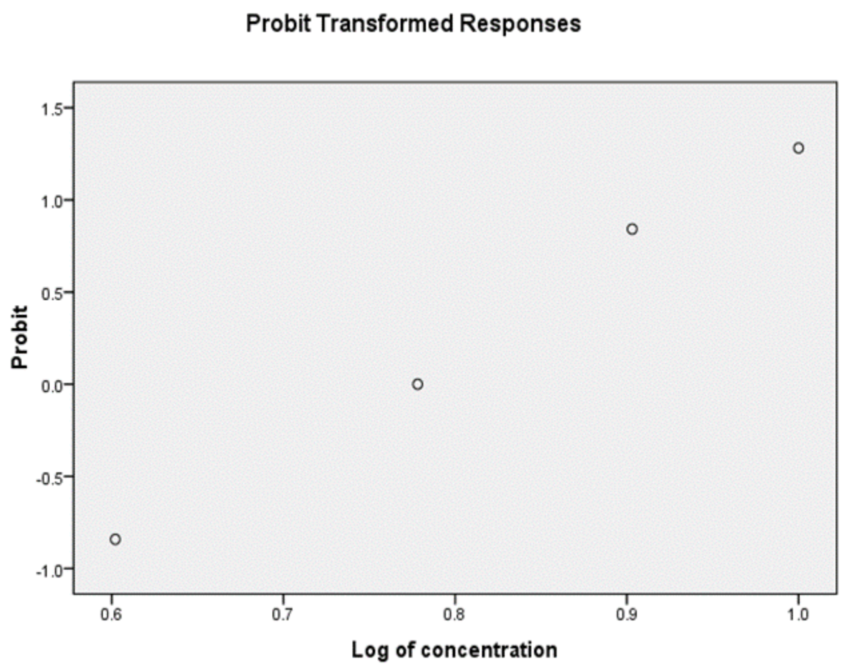

Fig 2: Probit line graph of 24h acute toxicity of effluent on *A. nenga*

4. Discussion

Acute toxicity studies provide several important benchmark in the field of environmental toxicity. Response of *A. nenga* to KMML effluent was studied and the results revealed that 24h LC_{50} was is 5.8% of the effluent and sublethal concentration was 1/5th of LC_{50} (1.2%) [18]. opinioned that, fish exposed to low concentration of toxicant do not reach the stage of fatigue, rather they quickly become adapted to the stressor. The stressful behaviour of the fish exposed to sublethal concentration of effluent in this investigation gives an indication of respiratory impairment on the gills, and this finding is in agreement with earlier reports [19-21]. According to [22], at increased lethal and sublethal concentrations, the behavioural responses of the test organisms greatly increased and later the organisms become dormant and this is a usual response of organisms in acute and sub-acute toxicity test, and the same observations were made in the present study also [20]. Reported that random deposition of effluents into an aquatic environment decrease the DO concentration leading to asphyxiation which could eventually result into organ architectural degradation such as liver dysfunction.

The effects of contaminants are usually characterized on survival, reproduction or variation in the animal. The physical, chemical and biological parameters of the environment play a vital role in manifestation of biological response to contaminants. The toxicity of a pollutant depend upon different factors such as weight [23], developmental stages [24] period of exposure of organism and temperature, pH, hardness and dissolved solids of the habitat [25]. Toxicity of the effluent mainly depend on the uptake of the effluent by the organism. The rate of uptake of substances are determined by the ratio of the permeability of body surface in contact with the medium to volume or weight of exposed animal and similar relationships persists between the rate of metabolism and weight of animal [26]. Low pH has a negative consequence on the metabolic activities of aquatic organisms [27]. Low pH concentrations are an important sign of the likely effects of effluents on the water quality as acidic pH affects alkalinity, metal solubility and water hardness. The low DO level in the effluents was due to the high load of total solid content of the effluent. This observation is in agreement with the study of [28] who observed that industrial effluents discharged into the river resulted in a depletion of DO from the point of effluent discharge and have dreadful consequences for aquatic organisms. The anthropogenic contribution of metals in water is well recognized [29] and on a broad perspective, the implications of these findings on aquatic habitats are not favorable.

In the present study the fish maintained in marine water without effluent behaved normally i.e., they were very active and movements were well coordinated. They were alert and at any site disturbance they swam faster. But in lethal and near lethal concentrations of industrial effluent they became highly irritable and highly strung. Jumping movements were observed, with profuse mucus secretion and loss of equilibrium. Examination of gills revealed significant change in their colour from dark red to brownish black. High mucus secretion over surface of gills was also observed. Some of the other behavioral changes observed in the fish exposed to effluent include abnormal opercular movement, dullness, loss of equilibrium, inability of food intake, erratic swimming, swimming at the water surface, circling movement, and gasping. Prior to death, the fish became less active or inactive, remained hanging vertically in the water or lay down on their sides at higher concentrations. Under lethal conditions the fish slowly became sluggish with short jerky movements and erratic opercular activity; finally turned upside down and died. Paralytic movements and suffocation caused by the mucus secretion over gills could be a few reasons for the death of fish at lethal concentrations. Behavioral changes observed in the present study in exposed fish, appear to be manifestation of heavy metal toxicity. Upon exposure to this, increase in surfacing and gulping of surface waters appears to be an attempt by the fish to avoid breathing in poisoned water. Similar observations have been reported in *Anabas testudineus* after exposure to monocrotophos [30]. Moreover, hypoxic condition also contributes to increase surfacing as reported by [31]. Hypoxic condition arises primarily due to damage of gills of pesticide exposed fish which hampers oxygen uptake [32]. Erratic movements and abnormal swimming are triggered by deficiency in nervous and muscular coordination which may be due to accumulation of acetylcholine in synaptic and muscular junctions [33]. It may be concluded that the results of this study are highly useful in evaluating the heavy metal toxicity by uncontrolled discharge of industrial effluent in marine environment. On the whole, with the knowledge of toxicity studies and behavioral observations it could be possible to establish limits of tolerance and susceptibility of the fish to the toxicity of heavy metals in the marine ecosystem.

5. Conclusion

Analysis of acute toxicity data demonstrates that in most cases fish are more sensitive to chemicals and thus are appropriate representative species to cover the sensitivity of aquatic vertebrates in current risk assessment procedures. The development and use of toxicity tests provide types of data regarding toxic responses of fish species, which could be more effectively used in predictive toxicology and risk assessment. In view of the importance of fish as a diet of human, it is obligatory that biological monitoring of the water and fish meant for consumption should be done regularly to ensure continuous safety of the seafood. Safe disposal of domestic sewage and industrial effluents should be practiced and where ever possible, treated to eliminate toxicity.

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