4.1 Introduction

Toxicological investigations have become unavoidable due to the hasty progression of industrialization and modern technical development, which in due course cause pollution. Heavy metals are being released into the water bodies from various industries as effluents and waste water and initiate deleterious effects on aquatic organisms. Research on toxicity is very indispensable to evaluate the quality of water which is consumed by community. When the accumulation in the animal body surpasses definite level, this also reveals the influence of these pollutants on various systems of the body. If the toxicants are not detected and removed from the water bodies, it will definitely affect the aquatic communities triggering various alarming effects on biochemical and enzymatic pathways, when they enter into the body of the organism.

The toxicity tests are necessary in water pollution evaluation because chemical and physical measurements alone are not sufficient to assess potential effects on aquatic biota. The importance of experimental exposure of fish to industrial waste for envisaging its potential impairment to aquatic ecology has been supported and established already (Sprague, 1969). In supplement, it is an important step to detect the intensities of toxicants to be used in the experimental studies of the accumulation and effect.
of these toxicants to the marine organisms. There are many studies concern with the toxicity of cadmium on vertebrates and invertebrates (Rasmussen et al., 2000, Adami et al, 2002 and Filipovic et al., 2003).

The decision can only be made after the fish acute toxicity as fish form an important place in aquatic food chain that; whether certain xenobiotic is precarious for the aquatic system and the food chain. The development of ecotoxicity measurement techniques has become an absolute necessity because of the fact that in many industrialised parts of the world, use of contaminating chemicals is elevating (Brandao et al., 1992).

Toxicity of heavy metal to aquatic organisms has been studied extremely by numerous researchers (Khallaf et al., 2003; Filho et al., 2004; Kalpaxis et al., 2004; Shaw et al., 2006). The manifestation of heavy metals in the environment has been greater than before in some areas to levels, which jeopardize the well-being of aquatic and terrestrial organisms including human (Honda et al., 2008). Therefore, it is a key challenging task to envisage the effects of contaminants on aquatic organisms and to institute toxicity standards for tolerable levels of chemical contamination (Bat et al., 2001). A reason for curiosity in heavy metals and behaviour in aquatic communities is that; heavy metals may have dissimilar behavioural effects at concentrations much smaller; compared to which they have lethal effects. This is suggesting that regulatory pollution limits based upon standard toxicological studies may be too high to avert damage to aquatic communities through the sublethal behavioural effects (Klaschka, 2008). Hence, in the
present study the acute toxicity tests were conducted to study the impact of cadmium on Nile Tilapia.

4.2 Acute Toxicity Tests

Acute exposure is well-defined as exposure to a toxicant for a short period; usually less than 24 hr. Toxicity tests designed to explore adverse chemical effects subsequent to short-lived exposures are valuable in classifying toxic agents, protecting workers, and safeguarding the community against accidental chemical discharge. The use of acute toxicity tests for evaluating the potential vulnerability of chemical contaminants to aquatic organisms is well documented. Static acute toxicity tests provide quick and (within limits) reproducible dose-response graphs for approximating toxic effects of chemicals on aquatic organisms. Usually acute toxicity tests are used to derive estimates of the exposure concentration causing 50% mortality (LC$_{50}$) to test organisms for the duration of a specified period of time. The application of the LC$_{50}$ has increased recognition amongst toxicologists and is by and large the most decidedly rated test for assessing potential adverse effects of chemical contaminants to aquatic life (Brungs et al., 1978).

Large amount of literature is available dealing with the acute toxicity of chemicals to fresh water organisms. But still, there is observed noteworthy disparity in response depending upon the selection of species, environment of study and some other parameters. The use of standardized methodology greatly reduces variations in the obtained results for a typical investigation undertaken.
The objective of acute toxicity assessments is to determine the toxic potential of a test chemical subsequent to a single exposure. A single dose of the chemical under examination is administered to groups of laboratory animals which are then held and observed for a defined period to measure hostile consequences of exposure.

4.2.1 Significance and Use

- An acute toxicity test is undertaken to obtain information regarding the instantaneous effects on test organisms of a short-term exposure to a test material under unambiguous experimental conditions. An acute toxicity test does not deliver data about whether hindered effects will occur. Although, a post-exposure observation period, with appropriate feeding, if necessary, possibly will serve such information.

- Outcomes of acute toxicity tests could be employed to envisage acute effects expected to take place on aquatic organisms in field situations as a result of exposure under analogous conditions, excluding that (i) motile organisms might avoid exposure when possible, and (ii) toxicity to benthic organisms possibly will be reliant on absorption or settling of the test material onto the substrate.

- Results of acute tests could be utilized to associate the acute sensitivities of different species and the acute toxicities of diverse test materials, and to investigate the effects of numerous environmental factors on outcomes of such tests.

- Results of acute toxicity tests may be of much concern when judging the threats of materials or water quality criteria for aquatic organisms.
Results of acute toxicity tests might be useful for studying the biological availability of, and structure-activity relationships between, test animal and test materials.

Results of acute toxicity tests depend on the temperature, composition of the water used for dilution, condition of the test organisms, exposure technique, and other factors.

Information provided by various toxicity tests can be used in the management of water pollution:

a) To estimate the environmental effects of a waste
b) To compare different toxicants among tested animals
c) To regulate the amount of discharge of pollutants (Buikema et al., 1982).

An acute toxicity test can **effortlessly** evaluate the effects of pollutants at elevated concentrations **with ease** and can associate the toxicity of various toxicants quickly. Heavy metals have been well-thought-out to be severe pollutants bringing their toxic effects on aquatic fauna since long time (De Mayo et al., 1979; USEPA, 1980; Mance, 1984). Copper, cadmium, and mercury are particularly very toxic (Arthur et al., 1970; De Mayo et al., 1979; Nriagu, 1980; Ingersoll et al., 1982; Nebeker et al., 1984).

Acute procedures for the assessment of lethal toxicity permit us to establish quickly the effects of contaminants on the test organisms. Mortality is used as a criterion to the ultimate response of an organism to the toxic effect of a specific toxicant (Kai Sun et al., 1995; Kazlauskiene et al., 1999). Based on
such acute toxicity tests, the sensitivity of various organisms and their developmental stages to contaminants are equated (Kazlauskiene et al., 1997; Hussain et al., 2011).

### 4.3 Materials and Methods

We followed OECD guidelines for testing of chemicals for our test. We carefully chose to carry out 96 hour static acute toxicity test. We first carried out range finding tests (limit tests) with cadmium concentrations of 1 mg/L, 5 mg/L, 10 mg/L and 20 mg/L cadmium to establish the concentrations of test media, for 96 hours and observed the response. No mortality was recorded in 1 mg/L cadmium exposure. This gave 100% confidence limit. On other hand, no fish could survive for 96 hours in concentrations 10 mg/L and 20 mg/L. With help of these extreme data we determined to accomplish acute toxicity tests at concentrations from 1 mg/L to 5 mg/L. i.e. from 100% confidence level to half of 100% mortality level.

Accordingly ten acclimated specimens were placed in each exposure media with cadmium concentration 0 mg/L (Control), 1 mg/L, 2 mg/L, 3 mg/L, 4 mg/L and 5 mg/L, for 96 hours and the mortality or responsiveness were recorded at 1, 2, 4, 6, 12, 24, 36, 48, 72 and 96 hours after commencement of exposure. From the recorded information; using Probit Analysis Finney Method [Log-normal Distribution] as well as Least squares method [Normal Distribution]; we calculated various parameters including LC$_{50}$, Lower and Upper Confidence Limits, NOEC (No observed Effect Concentration), LC$_{100}$ (concentration at which all specimens dies). Confidence limits (p = 0.95) for the calculated LC$_{50}$ values are determined.
using standard procedures representing acute toxicity of cadmium concerning *Oreochromis niloticus*. Same experiment was repeated again. The physiochemical parameters of the test solution fluctuated slightly during the bioassays within tolerance range as suggested by Mackereth, (1963), but were not thought to. Therefore, they are not expected to have affected fish mortality since they were within tolerance range as suggested by Mackereth, (1963). This agrees with the findings of Adigun, (2005).

### 4.4 Results:

#### 4.4.1 Experiment conditions

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test Substance</td>
<td>CdCl$_2$·2H$_2$O</td>
<td></td>
</tr>
<tr>
<td>Test fish</td>
<td><em>Oreochromis niloticus</em> (Nile Tilapia)</td>
<td></td>
</tr>
<tr>
<td>Test type</td>
<td>Static 96 hour Acute toxicity test</td>
<td></td>
</tr>
<tr>
<td>Water Quality</td>
<td>Temperature: 30°C ± 2°C</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TDS: 80± 20 mg/L</td>
<td></td>
</tr>
<tr>
<td></td>
<td>pH: 6.2± 0.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Aeration: Continuous aeration was provided</td>
<td></td>
</tr>
<tr>
<td>Concentrations used</td>
<td>0mg/L (Control), 1mg/L, 2mg/L, 3mg/L, 4mg/L and 5mg/L</td>
<td></td>
</tr>
<tr>
<td>No. of fish in each tank</td>
<td>10</td>
<td></td>
</tr>
</tbody>
</table>
Average Mass of specimen | 36.5248 gm | 36.401 gm

Table 4.1: Acute toxicity Experiment conditions

4.4.2 Experiment Results

During the experimental period, the swimming behaviors of *Oreochromis niloticus* were observed after being exposed to cadmium to provide an indicator of sublethal toxicity and subsequent mortality. Behavioral changes are the most sensitive indication of potential toxic effects. Optomotor responses are very useful in evaluating the behavioral changes of fish (Richmonds et al., 1992).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
<th>Mean Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mortality in control group</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>NOEC (concentration at which no mortality detected)</td>
<td>0.9062 mg/L</td>
<td>1.4972 mg/L</td>
<td>1.2017 mg/L</td>
</tr>
<tr>
<td>LC$_{50}$ *</td>
<td>3.5095 mg/L</td>
<td>3.3528 mg/L</td>
<td>3.4312 mg/L</td>
</tr>
<tr>
<td>LC$_{50}$ Lower confidence Limit *</td>
<td>2.7166 mg/L</td>
<td>2.7555 mg/L</td>
<td>2.7361 mg/L</td>
</tr>
<tr>
<td>LC$_{50}$ Upper confidence Limit *</td>
<td>4.9494 mg/L</td>
<td>4.0045 mg/L</td>
<td>4.477 mg/L</td>
</tr>
<tr>
<td>LC$_{50}$ **</td>
<td>3.7089 mg/L</td>
<td>3.4856 mg/L</td>
<td>3.5973 mg/L</td>
</tr>
<tr>
<td>LC$_{50}$ Lower confidence Limit**</td>
<td>2.8509 mg/L</td>
<td>2.9560 mg/L</td>
<td>2.9035 mg/L</td>
</tr>
<tr>
<td>LC$_{50}$ Upper confidence Limit**</td>
<td>4.5668 mg/L</td>
<td>4.0152 mg/L</td>
<td>4.291 mg/L</td>
</tr>
<tr>
<td>LC$_{100}$** (concentration at which mortality detected)</td>
<td>6.5543 mg/L</td>
<td>5.2420 mg/L</td>
<td>5.8982 mg/L</td>
</tr>
</tbody>
</table>
Table 4.2: Acute toxicity Experiment results

<p>| | | |</p>
<table>
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* Results obtained using Probit Analysis - Finney Method [Lognormal Distribution]

** Results obtained using Probit Analysis - Least squares [Normal Distribution]

4.4.3 The change in behavioral patterns

- The theoretical spontaneous response in the control group was zero.
- Abnormal swimming and the fish tended to gather at the surface. Speed in movement of the fish decreased.
- Swimming disorders such as vertical and downward manner increased. The swimming posture changed from normal to head up swimming.
- Vertical and downward swimming patterns, suspending motionless on water surface and swimming around its own axis behavioral changes were observed. Thereafter, most fish became hypoactive and remained at the bottom of the container.
- Abnormal swimming behaviour increased. Spiral swimming due to the loss in equilibrium and the fish were observed to hit the aquarium walls and each other.
- The fish were observed to have breathing difficulties and tried to breathe air from the surface and their motility slowed down. Vertical and downward swimming patterns were observed. Swimming sideways and on the dorsal fin was also observed.
- Swimming disorders and loss of balance increased. Vertical and downward swimming patterns and gathering around the ventilation filter were observed.
The fish were observed to make sudden movements, display loss of balance and swimming disorders. Fish capsized in water and became motionless.

The fish display loss of balance and swimming disorders as soon as the reagent was added. There were swimming problems and the fish were observed to have breathing difficulties and gather around the ventilation filter.

The fish were observed to have breathing difficulties. Initially fish sank down to the bottom and became motionless. Dead fish were rapidly removed and recorded.

Finally, the fish convulsed and consequently died.

The restlessness, loss of balance, erratic swimming, respiratory distress, vertical movement and death, upon exposure to acute concentrations of Cadmium Chloride in this investigation is in agreement with the earlier reported works of Oti (2002), Oshode et al., (2008) and Ezike et al., (2008).

4.5 Discussion

Mucus accumulation was observed on the body surface and gill filament of dead fish during the present study. This might be as a result of increase in the activity of mucus cells due to subsequent exposure to pollutants. This also agrees with the reports of Ayuba et al., (2005) and Omitoyin (2007).

Many researchers have accepted that cadmium uptake in freshwater fish occurs mainly via gills (Winner et al., 1986). There is also some evidence that indicates that after exposure to cadmium, fish gills were structurally damaged.
and hypoplasia occurred, including high mucus excretion (Part et al., 1981). This implies point towards the fact that the gills have a large effectively irrigated area serving as the main uptake site for cadmium.

The LC$_{50}$ value varies for same toxicant from species to species of fish as well as for same fish from toxicant to toxicant.

Our study lead us to values 3.5095 mg/L and 3.3528 mg/L using Probit Analysis - Finney Method [Lognormal Distribution] and 3.7089 mg/L and 3.4856 mg/L using Probit Analysis - Least squares [Normal Distribution]. From this study we could conclude the average of all LC$_{50}$ values (using both methods) to be 3.5142 mg/L. Spehar (1976) reported that the 96 hours LC$_{50}$ of Cd for flag fish, Jordanella floridæ, was 2.5mg /L. El-Moselhy (2001) stated that toxicity of Cd to Mugil seheli decreased with increasing the exposure time and the recording LC$_{50}$ values were -12.34 mg/L, -8.92 mg/L, 6.01 mg/L and 3.45 mg/L for 24, 48, 72 and 96 hours, respectively. Gaikwad (1989) reported the 96h LC$_{50}$ values of copper for fish Etroplus maculatus to be 1.83 ppm for fish Etroplus maculatus reported by Gaikwad (1989). Bryan (1976) reported 96 hours LC$_{50}$ values of about 0.3 to 50 mg Cd/L. While 96H LC$_{50}$ of Cu ranged from 0.2 to 3 mg/L for various marine fish and crustaceans (Bryan 1976). The species type, chemical structure of metal compound, and the conditions of the experiment (water temperature, salinity, oxygen content and pH) etc. may cause variation in the LC$_{50}$ values for the same metal.
Though the organisms survive the initial attack of toxins/pollutants because of their protective adaptations, the injuries caused by the progressive exposure even in small doses will get manifested at later stages when the organism's resistance weakens due to ageing. Also, the condition and response of the test organism to the amount of metal penetrating into its body, the degree of retention and the rate of excretion influence the toxic effect of heavy metal.

Environmental factors including water temperature, hardness, and pH can also affect the acute toxicity and fish tolerance. Waiwood et al. (1978) reported that toxicity increases at lower levels of water hardness.

4.6 References


evaluation of translation efficiency in *Mytilus galloprovincialis* cells, Environmental research, 94, pp 211-220.


