Toxic Stress of Nickel on African Catfish, Clarias gariepinus Fingerlings

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Citation

I Ololade, O Oginni. *Toxic Stress of Nickel on African Catfish, Clarias gariepinus Fingerlings*. The Internet Journal of Veterinary Medicine. 2008 Volume 6 Number 1.

Abstract

Toxic stress in Clarias gariepinus' after a 96-hr exposure to nickel using the seismic bioassay test was investigated. The experimental fish used were Clarias gariepinus fingerlings. The fish were exposed to different concentrations (0, 4, 6,8,10 and 12mg/l) of nickel sulphate after a range – finding test using a screening procedure. The mortality rate increased with increased concentration of toxicants. The 96-hr median lethal concentration (96-hr LC50) was 8.87mg Ni/L using the logarithmic method with dose-mortality regression line y = 174.74x – 97.711. The dissolved oxygen decreased at higher levels of toxicants. The obtained data showed that a short-term exposures to high levels of nickel induced stress reactions in C.gariepinus. Some adaptive changes prevailed, preparing the organism to an increased energy expense, while other changes indicated a considerable immunosuppressive effect of stress. Thus, the short term exposure induced persistent stress on the fish which may render them more susceptible to diseases.

INTRODUCTION

The fact that nickel is a natural element in the earth's makeup must be a factor in assessing its ability to harm the environment. Substances synthesized and released by humans represent a far more serious challenge for ecological systems than do natural substances simply because synthetic substances are new additions to the environment and organisms may not have developed coping mechanisms for them. It is important to note that simply being natural does not necessarily protect the environment from high additional fluxes of such substances from human activities. The ability of organisms to withstand some increase and the threshold point where toxic effects occur are the subjects of ecotoxicology. Nickel can do no harm (or benefit) to an organism unless the organism absorbs the divalent nickel ion into its body or the nickel ion is bound strongly enough to a membrane (fish gills) so that the membrane cannot function properly. Though, aquatic ecotoxicity testing has shown that $NiSO_4.6H_2O$ and $NiCl_2.6H_2O$ fall into the "harmful" classification which when abnormally high in concentration, can become toxic and can disturb the homeostasis of an animal (Farkas et al. 2002; Javed 2003). The concept of bioavailability of metals was recently discussed by a number of international workshops. The consensus report of one such workshop stated that the "Effects of metals on ecosystems are related to metal bioavailability and not to the total metal concentration" (ICME, 1998).

It follows logically from this recognition that the total concentration of nickel in an environmental setting (aquatic or terrestrial) is not correlated with nickel's biological effects. Only the bioavailable portion of the nickel is relevant for ecotoxicity. While a condition might be constructed in a laboratory where nickel in a given medium is 100% bioavailable, it is virtually impossible to have such a situation under any naturally occurring conditions. In the natural environment there exists a complex group of reactants which are able to bind nickel into complex ions, precipitate nickel into insoluble compounds, adsorb nickel strongly to their surfaces, all of which remove the bioavailable nickel divalent ion from the medium by converting the nickel into another form which is not bioavailable.

The aquatic environment where fish and other aquatic organisms live is subjected to different types of pollutants which enter water bodies through industrial, domestic and agricultural discharge systems thereby introducing stress to living creatures. Stress is a general and non-specific response to any factor disturbing homeostasis. Stress reaction involves various physiological changes including alteration in blood composition and immune mechanisms (Svoboda 2001). It has also been linked as one major factor

of disease outbreaks, low productivity and mortality in aquaculture (Rottman et al., 1992). Other toxic endpoints include decreased growth, mobility and reproductive effects (Allen, 1995). Stress in fish may be induced by various abiotic environmental factors (changes in water temperature, pH, O_2 concentration and pollution). Changes in environmental quality can therefore be a major determinant of year-class strength and eventually the long-term dynamics of many fish populations (Rose et al., 1993). Bioassay technique has been the cornerstone of programmes on environmental health and chemical safety (Ward and Parrish, 1982).

Fish have been the most popular choice as test organisms because they are presumably the best-understood organisms in the aquatic environment (Buikema et al. 1982) and also due to their importance to man as a protein source (Kime et al., 1996). As a result, aquatic pollution influences humans indirectly through the ingestion of fish due to bioaccumulation. It is therefore of great significance to evaluate pollution effects on fish for both environmental protection and socio-economic reasons. Juveniles and adult fish are often chosen due to their relative size, making them easier to use and maintain (Witeska et al., 1995). Several studies have shown that intoxication of fish with heavy metals may sometimes cause symptoms similar to the stress reaction (review- Witeska 2003). Usually, red blood cell (RBC) system of fish reacts to heavy metal intoxication with anemia but in some cases, particularly after short exposures, blood parameters (Haematocrit, RBC, mean corpuscular volume, Haemoglobin) may increase (Vosyliene 1996, Witeska 1998, Dethloff et al. 1999, Witeska and Jezierska 1994).

This work is therefore aimed at assessing the toxic stress of nickel on fish using a static bioassay technique (Reish and Oshida, 1987). The fish Clarias gariepinus is a hardy fish and highly valued in Nigeria. These tests then also provide information on the levels of toxicants that are a threat to fish population recruitment.

MATERIALS AND METHODS

MATERIALS AND METHODS

Healthy specimens of African catfish Clarias gariepinus were obtained from a fish farm in Ondo State, Nigeria. The choice of Clarias gariepinus was informed by its ability to withstand stress and its high commercial value in Nigeria. In the laboratory, fishes were kept in large plastic bowls of 120L capacity containing 60L of clean tap water and acclimatized for 6 weeks to the laboratory conditions, during which time they were provided with artificial feed (grower's mash) and ground shrimps obtained locally to avoid possible effects of starvation on any of the haematological parameters of the fish. The size of the fish varied from 18.1 – 22.7cm in standard length and 50.6 - 97.4g in weight. Fish of both sexes were used without discrimination. The fish were inspected for disease conditions and general fitness.

Water was changed every other day. The mean temperature, pH, alkalinity, hardness and dissolved oxygen of the water used were 27.4C, 6.51, 193.3 mg/L (as HCO_3^-), 227.5mg/L as $CaCO_3$ and 6.56 mgO_2/L respectively. Ten fingerlings were kept per bowl. There were five different treatment groups and each had three replicates. The fish were fed three times daily. Feeding was discontinued while aeration continued during the 96-hr test period.

Stock solution of the test metal compound, a chemically pure nickel tetraoxosulphate IV hexahydrate ($NiSO_4.6H_2O$) was prepared by dissolving 4.5g of Merck grade reagent equivalent to 1 g of nickel in 1000 ml distilled water at concentration of 1000 mg/L. From the stock solutions, different concentrations required were prepared after a range – finding test using a screening procedure. The 96-hr LC_{50} was found to be 8.87mg/L in C.gariepinus using the probit analysis method (Finney, 1971). The concentrations prepared for the experiment were: 4, 6,8,10 and 12 mg/L.

Five sets of ten fish each were subjected to serial dilutions of the stock solution of Ni (from 4mg/L – 12mg/L) in triplicates. Two sets of control (each consisting 10 fishes) which contains no toxicants were also set up. The test was performed by the semistic (renewal) bioassay method in which the exposure medium was exchanged every 24-hr to maintain toxicant strength and level of dissolved oxygen as well as minimizing the ammonia excretion levels during this experiment. Initially, the fish were observed at 1-hr intervals for the first 6-hr after which they were observed at 2-hr intervals. Dead fish were identified by an absolute lack of movement. They were removed as soon as this was noticed. No mortality was observed among control fish. The toxicity of the test chemical was determined using the logarithmic method of analysis (Litchfield and Wilcoxon, 1949).

RESULTS AND DISCUSSION

RESULTS

The results of the physico-chemical parameters (mean values) measured are given in Table 1. Table 2 represents

detail of mortality as recorded during the study. The estimation of the lethal concentration values (LC_{50}) was carried out using the Logarithmic method (Litchfield and Wilcoxon, 1949). The 96-hr LC_{50} values were determined from the graph (Fig.1) to be 8.87mg/L. Fig.1 depicts the percentage mortality for different exposure periods at different concentrations of nickel sulphate (4.0 ppm - 12.0 ppm). The LC_{50} value of $NiSO_4.6H_2O$ for the fish C. garienpinus was determined by the simple graphic method (Fig.2). The calculated average LC_{50} is 8.87mg/L. The equation for the dose-mortality regression line was found to be Y = 174.74X – 97.71. In the present study of C. garienpinus, it was observed that the behaviour and mortality rate was dependent on both duration of exposure and concentration of the toxicant. Even with the renewal bioassay method of study, using medium of equal physico-chemical characteristics and fishes of approximately similar sizes under the same experimental atmosphere, significant changes were observed behaviour of fishes.

Figure 1

Table 1: The physico-chemical characteristics of the water used

Parameters	Value
Temperature (^{0}C)	27.4 ± 1.1
pH	6.51 ± 0.23
Alkalinity (as HCO_3^-)	193.3 ± 2.7
Hardness (as $CaCO_3$)	227.5 ± 3.1
Dissolved oxygen	6.56 ± 1.23

Figure 2

Table 2: Variable Zinc Concentrations and Mortality (N = 20)

Concentration		Exposure	Duration	
(mg/L)	24-hr	48-hr	72-hr	96-hr
30	x	x	x	3
35	x	x	1	8
40	x	x	4	10
45	x	3	12	3
50	1	9	8	1
Control	x	x	x	x

x : No mortality

Figure 3

Figure 1: Trend in fish mortality with duration of exposure to zinc sulphate.

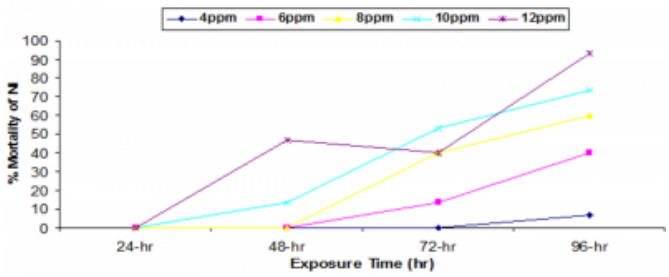
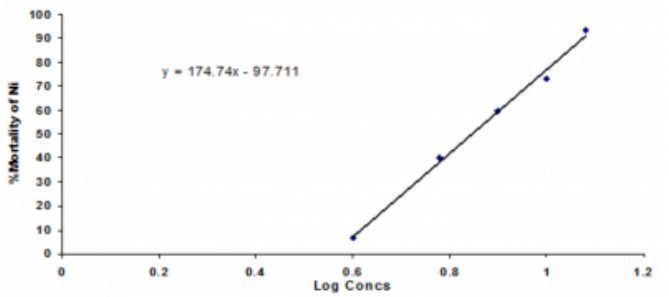


Figure 4

Figure 2: Logarithmic graph of mortality pattern



DISCUSSION

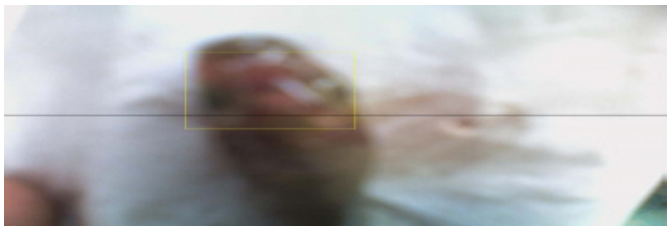
A regular trend was generally observed in the mortality rate which increases with increased concentration. At the early stage (i.e. the first 24hr) of the toxicants introduction, all the fishes survive initial attack. This may be due to their protective adaptations and the hardy nature of C. gariepinus. During the second renewal (48-hr exposure) some damages or injuries were noticeable particularly amongst some fishes in the highest concentration (10ppm and 12ppm). These injuries are believed to weaken the organisms' resistance to toxins and consequently resulting to significant death of 50% within the highest concentration. With progressive exposure spanny 72-hr, deaths becomes inevitable even at lower concentrations. This could be due to stress and cumulative impact of Ni-toxicity. Apart from least concentration (4ppm), death, though at different rates, were recorded at every other concentration.

Nickel toxicity to aquatic life depends on the species, pH, water hardness and other environmental factors (Blaylock and Frank, 1979). The water pH and hardness which increases with increased concentration of toxicants showed significant direct relationships with 96-hr LC_{50} concentration of the fish. Skin damage i.e. body lesions associated with red spot disease as noticed especially after 72-hr with fishes

within 8.0 – 12.0ppm is indicative of pH stress. It shows further that fish like C. gariepinus has limited tolerance to abnormal pH changes. The dissolved oxygen of the test medium decreased especially at high levels of toxicants. At higher 96-hr LC_{50} values of Ni, dissolved oxygen decreased significantly in the test medium. According to Nebeker et al., (1985), nickel has been shown as moderately toxic to fish and aquatic invertebrates when compared to other metals. Newly fertilized rainbow trout eggs, exposed to 0.035 mg/L nickel, produced smaller larvae than when eyed eggs or pre-swim up larvae were exposed even up to 0.134 mg/L

Figure 5

Figure 3 : A typical behavioural changes in the exposed fish. Curling of Spine and vertical movement of the fish due to loss of equilibrium is depicted. Due to complete loss of equilibrium, fish turned upside down and finally died as can be noticed in the picture.



Efforts were made to carefully observe the behaviour of the fishes during the 96-hr study. Behavioural functions are generally quite vulnerable to contaminant exposures, and fish often exhibited these responses first when exposed to pollutants (Little et al., 1993). Behavioral changes such as curling of spine and vertical movement (Fig.4.3) of the fish was observed. This may be due to loss of equilibrium at high intoxication which makes the fish to turn upside down and finally died. Thus, swimming performance is considered one of the measures which could serve as possible sensitive indicator of sub-lethal toxic exposure. Various methods have been used to quantify the effects of toxics on an organism's swimming performance (Rose et al., 1993). This kind of behavioural abnormality has been reported in various fish species on exposure to heavy metals (Little et al., 1993). Frequent surfacing with irregular opercular movement and loss of equilibrium in Tilapia mossambica has been reported when exposed to cadmium (Ghatak and Konar, 1990). Similarly, hyperactivity, erratic swimming, and loss of equilibrium in brook trout, Salvalinus fontinalis, in response to lead treatment have been reported (Holcombe et al., 1976). In addition, Singh and Reddy (1990) in their study on Heteropneustes fossilis, had reported lethargy response and frequent surfacing along with gulping of air in exposure to just 0.25ppm copper. According to Little et al. (1993) behavioural measurements may be useful indicators of sub-lethal contamination due to concentrations even being lower than those that affect growth. Behavioural changes usually occur much earlier than mortality.

Several factors have been attributed to behavioral changes/abnormalities in fish exposed to heavy metals like Ni (U.S. EPA,1986). These include nervous impairment due to blockage of nervous transmission between the nervous system and various effector sites, paralysis and depression of respiratory centre due to enzyme dysfunction, and alteration of energy pathway which results in energy depletion (Singh and Reddy,1990).

Bioaccumulation is not a valid criterion for judging the ecotoxicity of nickel substances because nickel is an essential element for many organisms and these organisms would suffer if they did not have the ability to accumulate and utilize nickel. Additionally, as a naturally occurring element, many organisms have mechanisms for detoxifying Ni through sequestration, thereby accumulating Ni in a non-toxic form. However, while the fish physiologically adapted to this environmental stressor, this trend does not always reflect a state of normality. The mortality recorded in the study is considered a consequence of stress induced on the immune system of fish. Thus, slow toxic progress and long continuance can result into chronic toxic response.

CONCLUSION

The presented results indicate that a short-term exposure to high levels of nickel induced stress reaction in fish. The gradual changes at lower concentration of toxicants in fish behaviour reflected a transient stress induced osmotic imbalance. However, deep changes observed showed that stress reduced the immune potential of fish. This reduced immunological status which persisted resulted in higher mortality especially at higher concentrations. Thus, it seems that even an incidental toxic stress may result in a considerable increase in susceptibility of fish to infections. Hence, good knowledge of fish response to various stressors will be of greater help in improving production of fish and in providing information on ways of effectively controlling and monitoring stress in aquaculture.

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