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Full Length Research Paper

Impact of nickel (Ni) on hematological parameters and behavioral changes in *Cyprinus carpio* (common carp)

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The effect of nickel on hematological parameters and behaviour in *Cyprinus carpio* after a 96 h exposure to nickel test was investigated. *Cyprinus carpio* fingerlings were obtained from local fish hatchery. Morphometric characteristics of experimental fish were recorded. Fish of both sexes were stocked without discrimination. The fish were exposed to different concentrations (0, 6, 9, 12, 15 and 18 mg/l) of nickel sulphate using standard screening procedure. The mortality rate of the experimental fish was increased with increase in concentration of nickel. The 96 h median lethal concentration (96 h LC50) was 12.44 mg Ni/L using the logarithmic method with dose-mortality regression line y = 188.224x – 86.52. The dissolved oxygen concentration decreased with increase in the level of Ni. All the blood parameters (erythrocyte, leucocytes, hematocrit and hemoglobin count) decreased with increasing dose of nickel and become significantly lower (*$P < 0.05$*) at higher concentration when compared with the control. Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were also lowered with concentration of toxicant when compared with the control. The results of the present study showed that a short-term exposures to high levels of nickel induced stress reactions in *C. carpio*. Some adaptive changes were observed; preparing the organism to an increased energy expense, whereas other changes showed a considerable immunosuppressive effect of stress. It was concluded that changes observed indicate that hematological parameters can be used as an indicator of Ni stress in *C.* carpio.

Key words: Cyprinus carpio, nickel, bioaccumulation, mortality, haematology, behaviour

INTRODUCTION

Toxic effects may include both lethality and sublethal effects such as changes in behaviour, development, reproduction, pathology biochemistry and behaviour (Rand and Petrocelli, 1985). Heavy metals from natural and anthropogenic sources are continuously released into aquatic ecosystem (Oymak et al., 2009). Due to their toxicity, long persistence, bioaccumulative and non-biodegradable properties in the food chain, heavy metals constitute a core group of aquatic pollutants (Uysal et al., 2008; Moorthikumar and Muthulingm, 2010). Different kinds of organisms may be used to determine the mechanisms of action of pollutants on specific physiological function (Gul et al., 2004; Kandemir et al., 2010). According to Eisler and Gardener (1973) heavy metals are being passed on into aqueous environments through industrial processes, sewage disposal, soil leaching and rainfall. They further reported that concentrations of these heavy metals are sublethal or lethal to aquatic organisms when the duration of exposure to these metals are prolonged. The effect of heavy metals on aquatic organisms is currently attracting widespread attention, particularly in studies related to pollution. With an early use of metals, there was little concern about environmental contamination (Ololade and Oginni, 2010). However, salts of the metals began to find their way into commercial and industrial applications and then it became evident that metallic salts possess certain biocide properties. Though, many metals play a vital role

Abbreviations: **MCV**, Mean corpuscular volume; **MCH,** mean corpuscular hemoglobin; **MCHC**, mean corpuscular hemoglobin concentration; **RBC,** red blood cell; **WBC,** white blood cell; **Hb,** hemoglobin; **Hct,** haematocrit; **EDTA,** ethylenediaminetetraacetate; **PVC,** polyvinyl chloride; **LSD,** least significant differences.

in the physiological processes of plants, animals and humans, yet excess concentration of metals is harmful (Ololade and Oginni, 2010).

Pollution infers deleterious effects and is usually assessed relative to biological system (Bhilave et al., 2008). Nickel is introduced into the hydrosphere by removal from the atmosphere by surface run-off by discharge of industrial and municipal waste and also following natural erosion of soils and rocks (Babukutty and Chacko, 1995). Heavy metal contamination may have devastating effects on the ecological balance of the recipient environment and a diversity of aquatic organisms (Farombi et al., 2007). Among animal species, fishes are the inhabitants that cannot escape from the detrimental effects of these pollutants (Olaifa et al., 2004; Clarkson, 1998; Dickman and Leung, 1998). Fish are widely used to evaluate the health of aquatic ecosystems because pollutants build up in the food chain and are responsible for adverse effects and death in the aquatic systems (Farkas et al., 2002; Yousuf and El-Shahawi, 1999). The various human activities acting on the natural environment result in the release of different chemicals, including heavy metals such as cadmium and nickel. When in excess, the metals adversely affect the habitats and organisms they support (Ewa and Mikomaj, 2005). Chemical changes disturb the equilibrium (homeostasis) of ecosystems and thus prevent their normal functioning. In polluted water bodies, concentrations of compounds containing both essential metals (nickel) and those playing no part in an organism's functioning (cadmium) may increase to toxic levels (Jezierska and Witeska, 2001). Transport of metals in fish occurs through the blood where the ions are usually bound to proteins.

The metals are brought into contact with the organs and tissues of the fish and consequently accumulated to a different extent in different organs and tissues of the fish. Most heavy metals released into the environment find their way into the aquatic environment as a result of direct input, atmospheric deposition and erosion due to rainwater, therefore aquatic animals may be exposed to elevated levels of heavy metals due to their wide use for anthropogenic purposes (Kalay and Canli, 2000). Heavy metals are non-biodegradable and once they enter the environment, bioconcentration occurs in the fish tissue for aquatic environment, by metabolic and biosorption processes (Wicklund-Glynn, 1991). Biological effects of disturbed chemical equilibrium in the habitat appear early, before individual organisms show symptoms and biochemical changes. Environmental pollution and the resultant changes are related to constant matter flow and exchange. The fish are intimately associated with water and constitute an important food item in human diet. Nickel is a natural element in the earth's makeup. This must be a factor in assessing its ability to harm the environment. Although, trace metals like Ni are essential for normal physiological process, aquatic ecotoxicity testing has shown that NiSO4. 6H2O and NiCl2.6H2O fall into the "harmful" classification where their abnormally high concentrations can become toxic and disturb the homeostasis of an animal (Farkas et al., 2002; Javed, 2003). 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 factors disturbing homeostasis (Witeska, 2003). Svoboda (2001) reported that stress reaction involves various physiological changes including alteration in blood composition and immune mechanisms. It has also been linked as one major factor of disease outbreaks, low productivity and mortality in aquaculture. 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, O2 concentration and pollution).

Changes in environmental quality can therefore be a major cause 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 (Oshode et al., 2008). Haematological variables remain veritable tools in deter mining the sub-lethal concentration of pollutants such as heavy metals in fish (Witeska, 2003). Oshode et al. (2008) reported observation of haematological parameters allows the most rapid detection of changes in fish. Disrupted haematological patterns appear very quickly and precede changes in fish behavior and visible lesions. The rapidity of toxic effects, exerted by heavy metals, is related to the blood's transport function, with the blood distributing the metals to all the body parts. Evaluation of toxic effects of the metals is eased by results of the basic haematological assays Red blood cell (RBC), White blood cell (WBC), Hemoglobin (Hb) haematocrit (Hct), leukocyte and erythrocyte appearance). Several investigations have been carried out on various toxicants with *Clarias* sp. (Aguigwo, 1998; Maheswaran et al., 2008; Al-Akel et al., 2010). Usually, RBC system of fish reacts to heavy metal intoxication with anemia but sometimes, particularly after short exposures, blood parameters (Hct, RBC, mean corpuscular volume, Hb) may be increased (Vosyliene, 1996; Dethloff et al., 1999; Sing et al., 2010). Exiting literature shows that not much work has been carried out on the effect of Ni on the toxic stress and hematology of common carp. The present study was planned to evaluate some hematological effects and behavioural changes resulting from the exposure of the freshwater fish, *Cyprinus carpio* to sublethal concentrations of Ni in water.

MATERIALS AND METHODS

Experimental diets

Healthy specimens of *Cyprinus carpio* were obtained from local fish

Table 1. The Physico-chmical (Mean ± SE) characteristics of the freshwater used.

Parameter	10 am	1 pm	4 pm	7pm	10 pm	Control
Temperature (°C)	38.4 ± 0.9	38.8 ± 0.6	37.4 ± 0.7	37.1 ± 0.9	36.8 ± 0.5	38.58 ± 0.8
pH	7.1 ± 0.5	6.8 ± 0.7	6.6 ± 0.5	6.3 ± 0.8	6.1 ± 0.6	6.6± 0.9
Hardness (as CaCO3) (mg / L)	544.5 ± 6.8	549.2± 7.6	557.8 ± 5.5	560.2 ± 7.2	564.0 ± 5.2	526.2 ± 7.1
Alkalinity (mg / L)	423.7 ± 4.2	429.2 ± 8.3	434.9 ± 6.6	441.2 ± 8.9	447.9 ± 7.7	430.7 ± 8.2
Dissolved oxygen (mg / L)	7.6 ± 1.0	6.7 ± 0.9	5.7 ± 1.1	4.4 ± 0.6	3.9 ± 0.8	5.9± 1.2
Total ammonia (mg/ L)	9.1± 1.4	10.6 ± 2.2	11.8 ± 1.5	12.4 ± 1.8	12.5 ± 2.4	1016± 1.6

hatchery and their initial morphometric characteristics were recorded. *C. carpio* were selected because of its ability to withstand stress and its high commercial value in the Kingdom. The fingerlings were stocked in 50-L glass aquaria containing deep tube-well water stored in an overhead tank. The fish was acclimatized to this condition for 1 week before using in any trial during which time they were provided with artificial feed (35% crude protein) obtained locally. Fish of both sexes were stocked without discrimination. The fish was inspected for disease conditions and general fitness. Water was changed every other day. Altogether, 18 aquaria were arranged according to randomized block design with three replicates. Each aquarium was stocked with ten fishes. Five different treatment groups with three replicates were used. The fish was fed three times daily.

Feeding was ended while aeration continued during the 96 h test period. Toxicant stock solution of the tested metal, a chemically pure nickel tetraoxosulphate IV hexahydrate ($NiSO_4.6H_2O$) was prepared by dissolving 4.5 g 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 will be prepared after a range – finding test using a screening procedure. The concentrations prepared for the experiment was: 0, 6, 9, 12, 15 and 18 mg/l based on literature guidance (Burba, 1999; Vinodhini and Narayanan, 2009). Six sets of ten fishes each were subjected to serial dilutions of the stock solution of Ni (from 6 to 18 mg/l) in triplicates. Two sets of control (each consisting 10 fishes) which contains no toxicants were set up. The test was performed by following semistic (renewal) bioassay method in which the exposure medium was exchanged after every 24 h to maintain toxicant strength and level of dissolved oxygen as well as minimizing the ammonia excretion levels during this experiment. Initially, the fish was observed at 1 h intervals for the first 6 h after which they were observed at 3 h intervals. Dead fish from different treatments were immediately removed from the experimental set up. Blood was collected from the remaining fish to assess the effect of acute exposure to Ni sulphate on hematological parameters after 96 h.

Hematological studies

Blood samples were collected from both the control and experimental fishes that survived the 96 h toxicant exposure period. The blood samples were taken by puncturing posterior caudal vein using ethylenediaminetetraacetate (EDTA) as anticoagulant (Schmitt et al., 1999). Blood, 2.0 ml, was decanted in heparinzed bottles for determination of blood parameters. The micro-haematocrit method of Snieszko (1960) was used to determine the Hct Polyvinyl chloride (PVC). Hb concentration was measured with Hb test kit using the cyanmethemoglobin method (Larsen and Snieszko, 1961). RBC and WBC counts were counted under light microscope with an improved Neubauer haemocytometer (Mgbenka and Oluah, 2003; Shah and Altindg, 2004, 2005). The derived hema-tological indices of MCV, MCH and MCHC were calculated using standard formulae as described by Jain (1986) MCV will be calculated in femtoliters = PCV/RBC x 10; MCH was calculated in picograms = Hb/RBC x 10; and MCHC = (Hb in 100 mg blood / Hct) x 100.

Statistical analysis

Treatment effects were compared by the least significant difference method using MstatC software of Michigan State University, MI, USA. Significance of difference has been presented as probability (P) values. Treatments were compared, Least Significant differences (LSD) to determine significant variation among the dietary levels (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Mortality trends and physico-chemical parameters

The physico-chemical parameters (mean values) were measured during experimental period (Table 1). Mortality of experimental fish was recorded during the study period under different treatments (Figure 1). The estimation of the lethal concentration values (LC50) was carried out using the Logarithmic method (Litchfield and Wilcoxon, 1949). Figure 1 shows the percentage mortality for different exposure periods at different concentrations of nickel sulphate (6.0 to 18.0 ppm). The LC50 value of $NiSO_4.6H_2O$ for the fish *C. carpio* was determined by the simple graphic method. LC50 values after 96 h were determined from the graph to be 12.44 mg/L. The equation for the dose-mortality regression line was found to be Y = 188.224x – 86.52. The mortality rate was generally increased with increased concentration of Ni. At the early stage (that is, the first 24 h) of the toxicants introduction, all the fish survive initial attack. This may be due to their protective adaptations and the hardy nature of *C. carpio* in particular. During the second renewal (48 h exposure), some damages or injuries were noticeable particularly amongst some fishes in the highest concentration (12 and 18 ppm). These injuries probably weaken the organisms' resistance to toxins and consequently resulting to significant death of 50% within the highest concentration. With progressive exposure after 72 to 96 h, deaths become inevitable even at lower concentrations. This could be due to stress and cumulative impact of Ni-toxicity. Apart from least concentration (6 ppm), death, though at different rates,

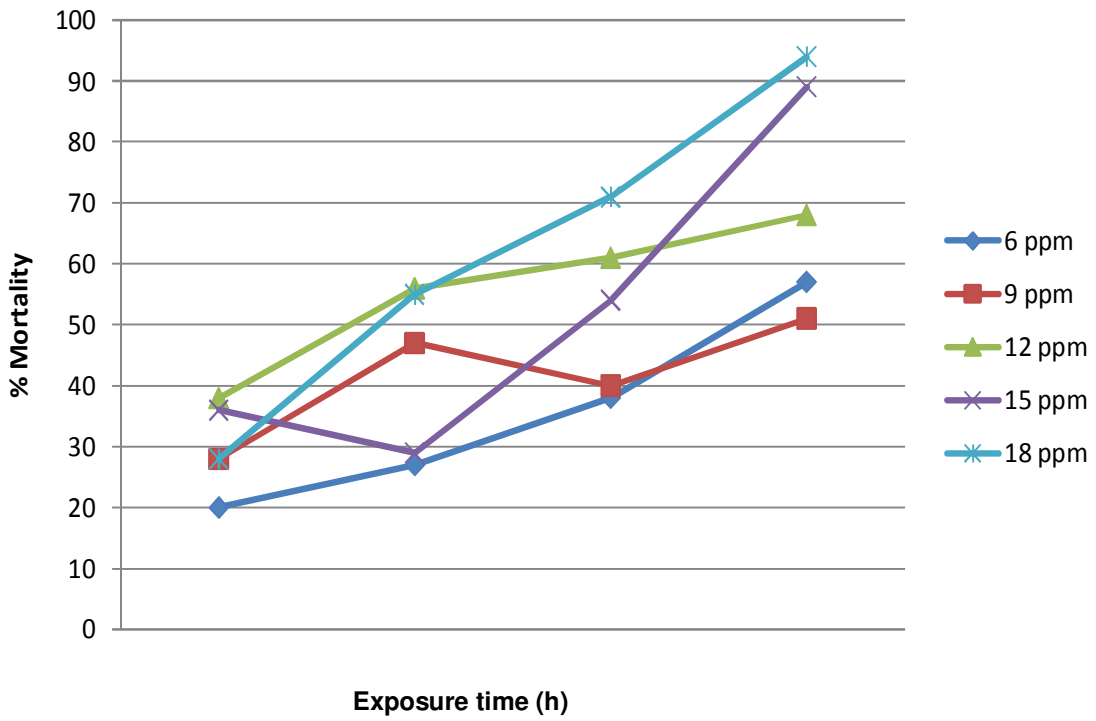

Figure 1. Trend in fish mortality with duration of exposure to nickel sulphate.

were recorded at other concentrations. 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 h LC50 concentration of the fish. Skin damage that is, body lesions associated with red spot disease as noticed especially after 96 h with fishes within 9.0 to 18.0 ppm indicates pH stress. It shows further that in *C. carpio* has limited tolerance to abnormal pH changes. The dissolved oxygen of the test medium decreased especially within the level of toxicants. At 96 h LC50 values of Ni, the 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.

Hematological parameters

Haematological parameters of fish are highly variable between and within species and seasons (Luskova, 1997), with the values of individual indicators differing relative to temperature, season, sex, food, and type of culture (Sopi*ƒ*ska, 1985; Thomas et al., 1999). Blood parameters may also show within-population differences (Allen, 1993; Thomas et al., 1999), which explain wide variations within the control during the experiment. Values of the haematological parameters recorded in the control close to those typical of the healthy carp (Svobodova et al., 1994, 1997; Singh et al., 2010). The hematological report showed that the mean PVC, WBC and Hb of *C. carpio* in the control were recorded as 35.6%, 2.7 x 10^7 mm^3 and 88.6 g/dl, respectively (Table 2). In the present study, a gradual decrease in these parameters was observed in the experimental fish as the concentration increases with span of time of exposure to Ni in water under the various treatments. The decrease was very significant ($P < 0.05$) at higher concentrations of Ni (12 and 18 mg/l). Similar findings were reported in *Clarias garipenus* by Ololade and Oginni (2010). The reduction in WBC count of the treatment groups may be due to the release of epinephrine during stress which is capable of causing the contraction of spleen and a decrease of leucocytes count, which can result in the weakening of the immune system (Svoboda, 2001; Witeska, 2003).

The exposure resulted in the erythrocyte system dysfunction, as evidenced by haemolytic anaemia, observed on the onset of the experiment and related to, that is, rapid erythrocyte disintegration. The observed depiction in the hemoglobin and hematocrit values in the fish could be attributed to the lysing of erythrocytes. Similar reductions have been reported by Musa and Omoregie (1999) and Al-Akel et al. (2010) when they exposed fish to polluted environment under laboratory conditions. Thus, the significant reduction in these parameters might be an indication of severe anemia caused by exposure of the experimental fish to Ni in the water. Erythrocyte

Table 2. Blood parameters values of *Cyprinus carpio,* for various concentrations of nickel sulphate.

Blood parameter	6 mg / L	9 mg / L	12 mg / L	15 mg / L	18 mg / L	Control
Haematocrit (% V)	28.4 ± 1.9[a]	25.5± 1.2[a]	22.8 ± 1.4[b]	20.3 ± 2.3[b]	18.2 ±1.5[c]	34.7 ± 2.5
WBC (x 10^3 mm^3)	2.2 ± 0.4[a]	1.8 ± 0.6[a]	1.3 ± 0.3[c]	0.9 ± 0.2[c]	0.6 ± 0.1[d]	2.7± 0.8
RBC (x 10^{-7}µL)	15.7 ± 2.5[a]	13.6± 1.8[a]	11.1 ± 2.7[b]	8.8 ± 1.9[c]	6.4 ± 2.7[d]	15.1 ± 2.2
Hb (x 10^2 g /L)	79.5 ± 4.7[a]	73.8± 5.7[a]	60.5 ± 5.2[b]	50.2 ± 3.7[c]	46.8 ± 417[d]	88.6 ± 4.6
MCV (x 10^{-7}Fl)	2.2 ± 0.5[a]	1.8 ± 0.2[a]	1.4 ± 0.1[b]	0.9 ± 0.2[c]	0.6 ± 0.1[d]	1.9 ± 0.4
MCH (x 10^{-7}pg)	6.6 ± 1.1[a]	5.2 ± 0.8[b]	4.1 ± 1.7[c]	3.4 ± 0.9[d]	2.9 ± 0.6[d]	5.4± 172
MCH (pg)	421.4 ± 7.8[a]	395.7± 10.4[a]	333.7 ± 8.5[b]	260.6 ± 943[c]	188.8 ±6.7[d]	294.5 ± 12.6

Values are expressed as the mean ± S.E. Means in the same horizontal column followed by different superscript are significantly different (≤= 0.05) according to Duncan's new multiple range test.

swelling is related to intracellular osmotic disorders and stress. Erythrocyte haemolysis is associated with blood serum acidification and intracellular alkalisation (Nikinmaa and Huestis, 1984). Flos et al. (1987) observed an increase in Hct levels in different fish species after zinc treatments. They attributed such an increase in Hct values to increase in the size of the erythrocytes as being demonstrated for chromium and zinc treated rainbow trout. Francis-Floyd (1992) sug-gested that microcytic anaemia usually occurs concurrently with haemolytic anaemia in fish. Observed depression in Hct and Hb values coupled with decreased and deformed erythro-cytes are obvious signs of anemia. Our findings were substantiated by Maheswaran et al. (2008). They further discussed that decrease or increase in certain blood parameters can be associated with the nature of species and the toxicants in different studies. Annune et al. (1994a) reported a significant increase in RBC count of *C. gariepinus* when subjected to Zn treatment. They attributed the RBC elevation to blood cell reserve combined with cell shrinkage as a result of osmotic alterations of blood by the action of the metal.

In another study, a non-significant decrease in red cells for Oreochromis *niloticus* was observed (Annune et al., 1994b; Singh et al., 2010). Our results are supported by previous research work that various heavy metals such Ni and toxins enter the aquatic system exerted a specific toxic effect on fish blood and tissues (Mousa and Khattab, 2003; Vosyliene and Kazlauskiene, 2004).The decreased number of WBC (leucopoenia) may be the result of bioconcentration of the tested metal in the kidney and liver. Other authors have associated the cause to hindering of granulopoiesis or lymphpoiesis, induced by primary or secondary changes in haema-topoietic organs (Tomaszewski, 1997; Al-Akel et al., 2010). In the present study, the values obtained for the hematological indices, no significant change was recorded in the MCV and MCHC. It was observed that there was significant change in the MCH especially at higher concentrations (that is, 12 and 18 mg/l). However, slight fluctuations were recorded in the MCV and MCHC when compared with the control. Ololade and Oginni (2010) reported that cell released from the spleen, which is an erythropoietic organ would have the lower MCV values when compared with the control. A similar observation was made for *C. carpio* after cadmium exposure (Koyama and Ozaki, 1984; Al-Akel et al., 2010; Singh et al., 2010). The significant change ($P < 0.05$) in the MCH of the experimental fish when compared with the control may be due to the reduction in cellular blood iron. These results were upheld by the findings of Hodson et al. (1978).

Behavioural changes

Efforts were made to observe carefully the behaviour of the fish during the 96 h exposure of Ni in the present 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; Ololade and Oginni, 2010). Behavioral changes such as curling of spine and vertical movement of the fish was observed during the experimental period. This may be due to loss of equilibrium at high intoxication which makes the fish to turn upside down and finally died. The swimming performance is considered one measure which could serve as possible sensitive indicator of sub-lethal toxic exposure (Kandemir et al., 2010). 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 swim-ming, and loss of equilibrium in brook trout, Salvalinus fontinalis, in response to lead treatment have been reported (Holcombe et al., 1976). 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 0.25 ppm of copper.

According to Little et al. (1993) and Ololade and Oginni (2010) behavioural measurements may be useful

indicators of sub- lethal contamination due to concentrations even being lowers than those that effect 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 among the nervous system and various effecter 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 use nickel (Ololade and Oginni, 2010). Additionally, as a naturally occurring element, many organisms have mechanisms for detoxifying Ni through sequestration, thereby accumulating Ni in a non-toxic form. We are of the opinion the fish physiologically adapted to this environmental stressor, this trend does not always reflect a state of normality. The mortality recorded in the study it might be a consequence of stress induced by Ni on the immune system of *C. carpio*. Probably slow toxic progress and long continuance exposure of toxic metal like Ni can result into chronic toxic response.

Conclusion

The changes in the hematological parameters indicate that they can be used as indicators of Ni related stress in fish on exposure to higher levels in the water. Exposure of *C. carpio* to higher concentrations of Ni demonstrated a toxic poisoning. It is concluded that higher mortality is expected under a static bioassay method. The findings of the present study revealed the necessity to use other species of fish to evaluate their dose-response to Ni as toxicant. This would help in determining the sensitivities of individual species to Ni toxicity. 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. The persisted reduced immunological resulted in higher mortality especially at higher concentrations. Information ascertained the fish response to stressors like Ni 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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