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Full Length Research Paper

Toxic stress and hematological effects of nickel on African catfish, *Clarias gariepinus,* fingerlings

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This laboratory study assessed the effects of nickel on the behavior and some blood parameters of African catfish, *Clarias gariepinus,* after a 96 h semi static method with a view to determining the safe concentration effect of the metal physiological functions of the fish. The mortality rate increased with increased concentrations of toxicants. The 96 h median lethal concentration (96 h LC_{50}) was 8.87mg Ni/l using the logarithmic method with dose-mortality regression line Y (% mortality) = 174.74 (log Concentration) – 97.711. All the blood parameters (erythrocyte, leucocytes, hematocrit and hemoglobin count) decreased with increasing concentration of toxicant and become significantly lower ($P < 0.05$) at higher concentration when compared with the control. The derived hematological indices of mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were equally lowered. It is believed that observed depression in hematocrit and hemoglobin values coupled with decreased and deformed erythrocytes are obvious signs of anemia. In conclusion, the changes observed indicate that hematological parameters can be used as an indicator of Ni related stress in fish on exposure to elevated Ni levels.

Key words: Nickel, *Clarias gariepinus*, hematology, stress, fish, 96 h acute toxicity.

INTRODUCTION

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 $NiSO_4$. $6H_2O$ and $NiCl_2.6H_2O$ 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. Stress reaction involves various physiological changes including alteration in blood composition and immune mechanisms (Svoboda 2001; Witeska, 2003). 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, 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 (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). The most common hematological variables measured during stress included red and white blood cells count, hemoglobin content, and hematocrit value and red blood cells indices. Fish hema-

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tological parameters are often determined as an index of their health status (Oshode et al., 2008).

Clarias sp. is a widely distributed fish which constitutes one of the major fisheries in Asia and Africa. Some records have shown that Clarias fishery contributes about 17% of over 6,000 tonnes of annual fish production from all fisheries sectors (Awachie and Ezenwaji, 1998). The common species found in Nigeria are *Clarias gariepinus*, *Clarias angullaris* and *Clarias buthupogon*. *Clarias* sp. is generally strong fish. The *C. gariepinus* is a prominent culture species because of its hardiness and fast growth rate. Several investigations have been carried out on various toxicants with *Clarias* sp. (Aguigwo, 1998; Maheswaran et al., 2008).

The toxic stress and hematological effects of various heavy metals such as Cu, Pb, Hg and Cd on the hematology of *Clarias* sp. have been reported. With these metals, various physiological and biochemical indices in fish has been investigated and consequently used in various scientific and ecological studies (Kori-Siakpere and Egor, 1999; Rogers et al., 2003; Kori-Siakpere et al., 2006; Maheswaran et al. 2008). 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, hemoglobin) may be increased (Vosyliene, 1996; Dethloff et al., 1999). However, not much work has been carried out on the effect of Ni on the toxic stress and hematology of *Clarias* sp. The present study was undertaken to evaluate some hematological effects resulting from the exposure of the freshwater fish, *C. gariepinus* to sublethal concentrations of Ni in water. The resulting data have implications for site-specific fish quality criteria for nickel and provide information on the levels of toxicants that are a threat to fish population recruitment.

MATERIALS AND METHODS

Healthy specimens of African catfish *C. gariepinus* were obtained from a fish farm in Ondo State, Nigeria. The choice of *C. gariepinus* was selected because of its ability to withstand stress and its high commercial value in Nigeria'). In the laboratory, fishes were kept in large plastic bowls of 120 L capacity containing 60 L of clean tap water. The fishes were 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. The size of the fish varied from 18.1 - 22.7 cm in standard length and 50.6 - 97.4 g 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. 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 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 were prepared after a range – finding test using a screening procedure. The concentrations prepared for the experiment were: 4, 6, 8, 10 and 12 mg/l based o literature guidance (Burba, 1999; Vinodhini and Narayanan, 2008).

Five sets of ten fishes each were subjected to serial dilutions of the stock solution of Ni (from 4 - 12 mg/l) in triplicates. Two sets of control (each consisting 10 fishes) which contains no toxicants were set up. The test was performed by the semistic (renewal) bioassay method in which the exposure medium was exchanged 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 were observed at 1 h intervals for the first 6 h after which they were observed at 2 h intervals. Dead fish were immediately removed from the experimental set-up. After the 96 h expiration of the experiment, blood was collected from the remaining fish to assess the effect of acute exposure to Ni sulphate on hematological parameters. During the experimental period, no death was recorded in the control set-up.

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 microhaematocrit method of Snieszko (1960) was used to determine the hematocrit (PVC). Hemoglobin (Hb) concentration was measured with Hb test kit using the cyanmethemoglobin method (Larsen and Snieszko, 1961). Red blood cell (RBC) and white blood cell (WBC) counts were counted under light microscope with an improved Neubauer haemocytometer (Mgbenka et al., 2003; Shah and Altindag, 2004, 2005). The derived hematological indices of mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated using standard formulae as described by Jain (1986): MCV was 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. Experimental data and those of control were statistically analyzed by means of analysis of variance (ANOVA). Standard deviation (SD) and Pearson correlation coefficient were calculated. Significance was set at P = 0.01 and 0.05. All analysis was performed using SPSS software (version 13.0).

RESULTS AND DISCUSSION

RESULTS

Figure 1 showed the percentage mortality for different exposure periods at different concentrations of nickel sulphate (4.0 - 12.0 mg/l). The 96 h LC_{50} value of $NiSO_4.6H_2O$ for the fish *C. garienpinus* was determined by the simple Logarithmic method (Litchfield and Wilcoxon, 1949). The calculated average LC_{50} is 8.87mg/l. The equation for the dose-mortality regression line was found to be Y (% mortality) = 174.74 (log Concentration) -97.711. Data on water physico-chemical parameters are presented in Table 1. The mean temperature, pH, alkalinity, hardness and dissolved oxygen of the water used were 27.4°C, 6.51, 193.3 mg l^{-1} (as HCO_3^-), 227.5 mg l^{-1} as $CaCO_3$ and 6.56 mgO_2 l^{-1} respectively.

The mean PCV, Hb, RBC, WBC, Platelet count, and derived erythrocyte indices (MCV, MCH and MCHC) of the fishes exposed to nickel sulphate are presented in

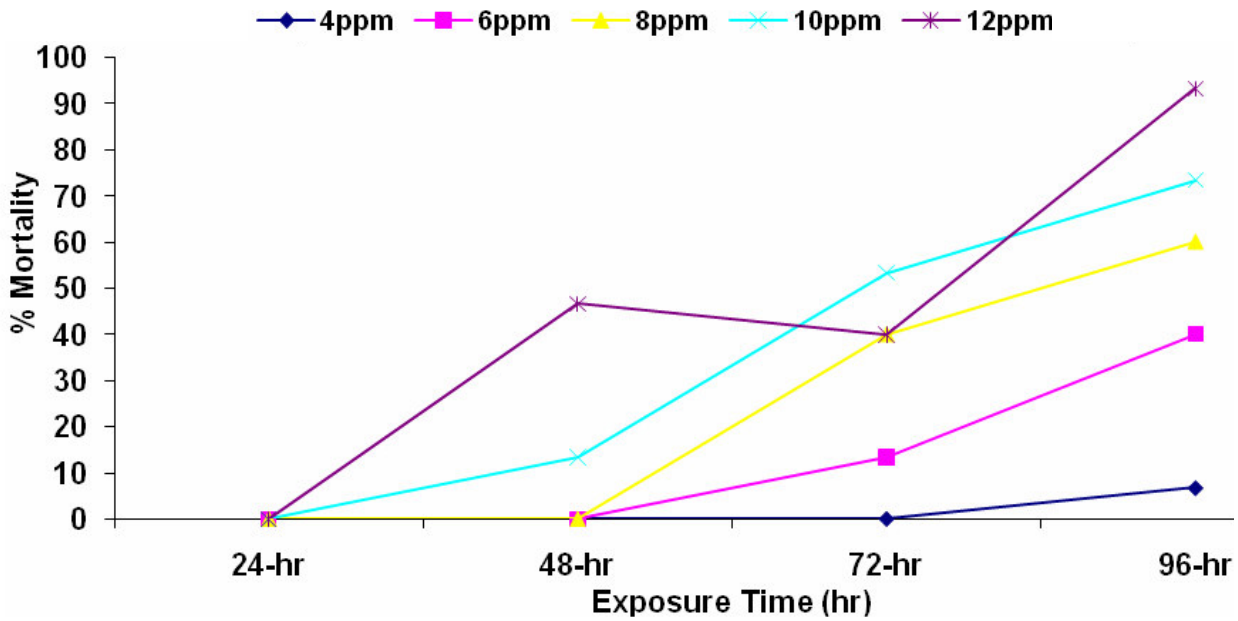

Figure 1. % Mortality of fish with durations of exposure to nickel sulphate at different concentrations.

Table 1. The physico-chemical (Mean ± SE) characteristics of the water used:

Parameters	4 pm	6 pm	8 pm	10 pm	12 pm	Control
Temperature (°C)	27.2 ± 0.4	27.2 ± 0.3	26.8 ± 0.7	26.8 ± 0.9	26.9 ± 1.2	27.4 ± 1.1
pH	6.5 ± 0.6	6.4 ± 0.9	6.4 ± 0.4	6.2 ± 0.8	6.0 ± 0.4	6.5 ± 0.2
Hardness (as $CaCO_3$)	233.4 ± 11.2	234.7 ± 9.7	236.1 ± 10.5	241.2 ± 11.6	241.9 ± 10.2	227.5 ± 3.1
Dissolved oxygen	6.5 ± 0.9	6.5 ± 0.5	5.6 ± 0.7	4.8 ± 0.5	4.3 ± 0.1	6.6 ± 1.2

Table 2. Blood parameters values of *Clarias gariepinus,* for various concentrations of nickel sulphate.

Blood parameters	4 (mg/l)	6 (mg/l)	8 (mg/l)	10 (mg/l)	12 (mg/l)	Control (mg/l)
Haematocrit(%V)	23.4±2.0^{a}	21.6±3.0^{a}	20.0±3.1^{a}	17.3±2.2^{a}	17.0±2.1^{a}	32.3±4.2
WBC(x 10^3mm^3)	1.7±0.5^{a}	1.4±0.9^{b}	1.0±0.5^{bc}	0.9±0.1^{bc}	0.9±0.1^{bc}	2.4±0.8
RBC(x 10^7/ µL)	13.6±2.4	12.9±2.5	12.4±2.8	10.9±2.1^{a}	9.8±1.9^{a}	14.2±1.7
Hb(x 10^2 g/dl)	74.2±3.2	72.1±2.2	66.2±6.1^{a}	53.1±3.1^{b}	48.2±3.3^{b}	93.3±4.2
MCV(x 10^{-7} Fl)	1.7±2.1	1.6±1.7^{a}	1.6±1.1^{a}	1.6±0.6^{a}	1.7±0.4^{a}	2.3±0.8
MCH (x 10^{-7} Pg)	5.4±1.4	5.6±0.8	5.3±1.2	4.9±0.6	4.9±1.0	6.6±0.3
MCHC (Pg)	316.2±14.2^{a}	333.3±11.2^{a}	330.0±9.2^{a}	306.4±16.3	282.4±19.4	287.9±14.5

The values are expressed as the mean ± S.E.
Means in the same horizontal column followed by different superscript are significantly different (α = 0.05) according to Duncan's New Multiple Range Test.

Table 2. Significant variations ($P < 0.05$) were observed between the various blood parameters with different concentrations of toxicants. The data shows that changes in hematological indices of fish may be due to Ni (as it may probably apply to any other metal) and are predetermined both by the concentrations of the metals in the water and time of exposure, and both these factors can cause reversible and irreversible changes in the homeostatic system of fish (Farkas et al., 2002; Javed, 2003). The erythrocyte, leucocytes, hematocrit and hemoglobin count of healthy control, under similar laboratory conditions, indicated a mean value of 14.2 ±

1.7 cells x 10^7 µl, 2.4 ± 0.8 x 10^3 mm^3, 32.3 ± 4.2% and 93 ± 4 x 10^2 g/dl respectively (Table 2).

DISCUSSION

Trend in mortality

A regular trend was generally observed in the mortality rate which increases with increased concentration. At the early stage (that is the first 24 h) of the toxicants introduction, all the fishes survived initial attack. This may be due to their protective adaptations and the hardy nature of *C. gariepinus*. During the second renewal (48 h exposure) the fish displayed physiological malfunctions such as erratic swimming, surfacing erosion of slime layer, skin lesion, nesting at the tank bottom and death. These were noticeable particularly among some fishes in the highest concentration (10.0 and 12.0 mg/l). These injuries are believed to weaken the organisms' resistance to toxins and consequently resulting to significant death of almost 50% within the highest concentration. With progressive exposure spanning 72 h, deaths become inevitable even at lower concentrations. This could be due to stress and cumulative impact of Ni-toxicity. Apart from the least concentration (4 mg/l), death, though at different rates, were recorded at every other concentration. Sub-lethal concentrations of toxicants in the aquatic environment will not necessarily result in outright mortality of aquatic organisms. However, the bioaccumulation of these pollutants over a period of time may constitute potential health hazards not only to the aquatic organisms like fish (as applied in this study) but also on higher trophic level especially man. It has been reported that toxicants and pollutants have significant effects, which can result in several physiological dysfunctions in fish (Omoregie et al., 1990).

The bioaccumulation process, which leads to mortality, 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 (NiO). 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.

Trend in some water physico-chemical properties

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 LC_{50} concentration of the fish. Skin damage, that is, body lesions associated with red spot disease as noticed especially after 72 h with fishes within 8 - 12 mg/l 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 tested medium decreased significantly especially at high levels of toxicants. 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 eggs or pre-swim up larvae were exposed even up to 0.134 mg/l.

Behavioral changes

Efforts were made to carefully observe the behaviour of the fishes during the 96 h study. Behavioral 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 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 per-formance 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 behavioral abnormality has been reported in various fish species on exposure to heavy metals (Little et al., 1993). Frequent surfacing with irregular opercula 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 res-ponse to lead treatment have been reported (Holcombe et al., 1976). In addition, Singh and Reddy (1990) in their study on *Heteropneustes fossilis*, has reported lethargy response and frequent surfacing along with gulping of air in exposure to just 0.25 mg/l copper. According to Little et al. (1993) behavioral measurements may be useful indicators of sub-lethal contamination due to concentrations even being lower than those that affect growth. These behavioral 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).

Variation in heamatological parameters

The hematological report shows that the mean PVC, WBC and Hb of fish in the control experiment were 32.3%, 2.4 x 10^3 mm^3 and 93.2 g/dl respectively. A progressive decrease in these parameters was observed in the experimental fish as the concentration increases as a response to the 96 h exposure to Ni in water. The decrease becomes very significant ($P < 0.05$) at higher concentrations (10 and 12 mg/l). 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; Witesta, 2003).

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) when they exposed fish to polluted environment under laboratory conditions. Thus, the significant reduction in these parameters is an indication of severe anemia caused by exposure of the experimental fish to Ni in the water. Flos et al. (1987) observed an increase in hematocrit levels in different fish species after zinc treatments. They attri-buted such an increase in hematocrit values to increase in the size of the erythrocytes as being demonstrated for chromium and zinc treated rainbow trout. Observed depression in hematocrit and hemoglobin values coupled with decreased and deformed erythrocytes are obvious signs of anemia (Maheswaran et al., 2008).

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 red blood cell 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 *O. niloticus* was observed (Annune et al., 1994b).

The decreased number of white blood cells (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 haematopoietic organs (Tomaszewski, 1997).

In the values obtained in the hematological indices, no significant change was recorded in the mean corpuscular volume (MCV) and mean corpuscular hemoglobin content (MCHC) but there was significant change in the mean corpuscular hemoglobin (MCH) especially at higher concentrations (that is, 10 and 12 mg/l). However, slight fluctuations were recorded in the MCV and MCHC when compared with the control. Cells 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 *Cyprinus carpio* after cadmium exposure (Koyama and Ozaki, 1984). 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, resulting in reduced oxygen carrying capacity of blood and eventually stimulating erythropoiesis (Hodson et al., 1978).

Conclusion

The present 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 behavior 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.

The changes in the hematological parameters indicate that they can be used as indicators of Ni related stress in fish on exposure to elevated levels in the water. Exposure of *C. gariepinus* to higher concentrations of Ni demonstrated a toxic poisoning. The report in the study may also infer that higher mortality is expected under a static bioassay method. This is almost the situation within most aquatic environment especially during drought when there's little or no flow of the river system. The study revealed the necessity to use other species of fish in order to evaluate their dose-response to Ni as toxicant. This would help in determining the sensitivities of individual species to Ni toxicity.

REFERENCES

Aguigwo JN (1998). Studies on acute toxicity of Cassava leaf extracts on African catfish *Clarias angullaris*. J. Aquat. Sci. 13: 29-32.

Allen P (1995). Accumulation profiles of lead and cadmium in the edible tissues of *Oreochromis aureus* during acute exposure. J. Fish Biol. 47: 559.

Annune PA, Ebele SO, Olademeji AA (1994a). Acute toxicity of cadmium to juveniles of *Clarias gariepinus* (Teugels) and *Oreochromis niloticus* (Trewawas). J. Environ. Sci. Health. A29: 1357-1365.

Annune PA, Lyaniwura TT, Ebele SO, Olademeji AA (1994b). Effects of sublethal concentrations of zinc on haematological parameters of water fishes. *Clarias gariepinus* (Burchell) and *Oreochromis niloticus* (Trewawas). J. Aquat. Sci. 9: 1-6.

Awachie JBE, Ezenwaji HMG (1998). The importance of *Clarias* species in the fishery development of Anambra river basin, Nigeria. CIFA Tech. Paper 8: 212-224.

Blaylock BG, Frank ML. (1979). A comparison of the toxicity of nickel to the developing eggs and larvae of carp (*Cyprinus carpio*). Bull. Environ. Contam.Toxicol. 21: 604-611.

Burba A (1999). The design of an experimental system of estimation

methods for effects of heavy metals and their mixtures on *Daphnia magna*. Acta. Zoologica Lituanica. Hydrobiologia 9 (2): 21- 29.
Dethloff GM, Schlenk D, Khan S, Bailey HC (1999). The effects of copper on blood and biochemical parameters of rainbow trout (*Oncorhynchus mykiss*). Arch. Environ. Contam. Toxicol. 36: 415-423.
Farkas A, Salanki J, Specziar A (2002). Relation between growth and the heavy metal concentration in organs of bream, *Abramis brama* L. populating lake Balaton. Arch. Environ. Contam. Toxicol. 43(2): 236-243.
Ghatak DB, Konar SK (1990). Acute toxicity of mixture of heavy metals cadmium, pesticide DDVP, detergent Parnol J and petroleum product n-heptane on fish, plankton and worm. Environ. Ecol. 8(4): 1239-1248.
Hodson PV, Blunt BR, Spray DJ (1978). Chronic toxicity of water borne lead and dietary lead to rainbow trout. (*Balmo garnderi*) in lake Ontario water. Water Res. 12: 869-878.
Holcombe GW, Benoit DA, Leonard EN, McKim JM (1976). Long term effects of lead exposure on three generations of brook trout, *Salvalinus fontinalis*. J. Fish. Res. Board Can. 33: 1731- 1741.
Jain NC (1986). Schalm`s Veterinary Heamatology.4th edition, Lea and Febiger, Philadelphia. pp. 1221.
Javed M (2003). Relationship among water, sediments and plankton for the uptake and accumulation of heavy metals in the river Ravi. IJP Sci. 2: 326-331.
Kori-Siakpere O, Ake JEG, Avworo UM (2006). Sub-lethal effects of some selected haematological parameters of Heteroclarias (A hybrid of *Heterobranchus bidorsalis* and *Clarias gariepinus*). Int. J. Zool. Res. 2: 77-83.
Kori-Siakpere O, Egor VE (1999). Haematological characteristics of African mudfish. *Clarias buthupogon*. (Pisces: Clariidae). Bull. Sci. Assoc. Niger. 21: 177-185.
Koyama J, Ozaki H (1984). Haematological changes of fish exposed to low concentrations of cadmium in the water. Bull. Japan Soc. Sci. Fish 50: 199-203.
Larsen HN, Snieszko SF (1961). Comparison of various methods of determination of haemoglobin in trout blood. Prog. Fish Cult. 23: 8-17.
Litchfield T, Wilcoxon FA (1949). A simplified method for evaluating dose –effect experiments. J. Pharmacol. Exptal. Theory. 96: 99-113.
Little EE, Fairchild JF, Delonay AJ (1993). Behavioral methods for assessing impacts of contaminants on early life stage fishes. Am. Fish. Soc. Symp. 14: 67-76.
Maheswaran R, Devapanl A, Muralidharan S, Velmurugan B, Ignaeimuthu S (2008). Haematological studies of fresh water fish, *Clarias batradrus* (L) exposed to mercuric chloride. IJIB. 2(1): 49-54.
Nebeker AV, Savonen C, Stevens DG (1985). Sensitivity of rainbow trout early life stages to nickel chloride. Environ. Toxicol. Chem. 4: 233- 239.
Omoregie E, Ufodike EBC, Keke IR (1990). Tissue chemistry of *Oreochromis nitoloticus* exposed to sub-lethal concentrations of gammalin 20 and actellic 25EC. J. Aquat. Sci. 5: 33-36.
Oshode OA, Bakare AA, Adeogun AO, Efuntoye MO, Sowunmi AA (2008). Ecotoxicological assessment using *Clarias gariepius* and microbial characterization of leachate from municipal solid waste landfill. Int. J. Environ. Res. 2(4): 391-400.
Rogers JT, Richards JG, Wood CM (2003). Ion regulatory disruption as the acute toxic mechanism for lead in the rainbow trout. Aquat. Toxicol. 64(2): 215-234.
Rose KA, Cowan JH, Houde ED, Coutant CC (1993). Individual based modeling of environmental quality effects on early life stages of fishes: A case study using striped bass. Am. Fish. Soc. Symp. 14: 125-145.
Schmitt CJ, Blazer VS, Dethloff GM, Tillitt DE, Gross TS, Bryant Jr. WL, DeWeese LR, Smith SB, Goede RW, Bartish TM, Kubiak TJ (1999). Biomonitoring of environmental status and trends (BEST) program: Field procedures for assessing the exposure of fish to environmental contaminants. Information and Technology Report USGS/BRD-1999-0007. U.S. Geological Survey, Biological Resources Division, Columbia 68p.
Singh HS, Reddy TV (1990). Effect of copper sulphate on heamatology, blood chemistry and hepato-somatic index of an Indian catfish, *Heteropneustes fossils* (Bloch), and its recovery. Ecotoxicol. Environ. Saf. 20: 30 - 35.
Snieszko SF (1960). Microhaematocrit as a tool in fishery research and management. U. S. Wildl. Serv. Sci. Rep. Fish. 314:15- 23
Svoboda M (2001). Stress in fishes (a review). Bull. VURH Vodnany. 4: 169- 191 [In Czech].
Tomaszewski JJ (1997). Diagnosyka laboratoryjna (laboratory diagnostics). PZWL, Warszawa. (in polish). 36(4):73 -76
U.S. Environmental Protection Agency (1986). Ambient water quality criteria for nickel. EPA 440/5-86-004. Technical report. Washington, DC. http://nepis.epa.gov/exe/zyPURL.cgi.
Vinodhini R, Narayanan M (2008). Bioaccumulation of heavy metals in organs of fresh water fish Cyprinus carpio (Common carp). Int. J. Environ. Sci. Tech. 5 (2): 179-182.
Vosyliene MZ (1996). The effect of long-term exposure to copper on physiological parameters of rainbow trout *Oncorhynchus mykiss*. 2. Studies of hematological parameters. Ekologija 1: 3-6.
Witeska M (2003). The effects of metals (Pb, Cu, Cd, and Zn) on hematological parameters and blood cell morphology of common carp. Rozprawa naukowa nr 72, Wydawnictwo Akademii Podlaskiej Siedlce [In Polish].