Pakistan J. Zool., vol. 46(4), pp. 915-920, 2014.

An Assessment of Nickel Sulphate Toxicity on Serum Electrolytes and Arterial Blood Gases in *Labeo rohita*

Abdul Latif[1] and Muhammad Ali[2]*
[1]*Institute of Pure and Applied Biology, Zoology Division, Bahauddin Zakariya University, Multan* 60800
[2]*Institute of Biotechnology. Bahauddin Zakariya University, Multan 60800*

Abstract.- In Pakistan, nickel is often detected as pervasive contaminant in aquatic environment, showing potential menace to both human health and environment. The objectives of our study were to determine the toxic impact of acute doses of heavy metal salt, nickel sulphate ($NiSO_4.7H_2O$) on serum electrolytes and arterial blood gases in freshwater cyprinid, *Labeo rohita*. The acute toxicity test revealed 96h LC_{50} values of 19.21 mg/L for $NiSO_4.7H_2O$ for *L. rohita*. In this study, two groups, one control and other experimental, each of 12 juveniles, were utilized. Based on LC_{50} values, experimental group was exposed to 19.21 mg/L of nickel sulphate for 96h. The assessment of nickel toxicity effects was based on comparison of analyzed concentrations of serum electrolytes and arterial blood gases of experimental group with control. In nickel sulphate treated groups, serum hyponatremia, hypokalemia, hypocalcemia, hypermagnecemia, hyperammonemia and hypoxia were observed. Based on momentous deviations in serum electrolyte levels and in arterial blood gases in experimental groups, it is concluded that $NiSO_4.7H_2O$ is an active toxicant for *L. rohita*.

Key words: *Labeo rohita*, serum electrolyte level, arterial blood gasses, nickel toxicity.

INTRODUCTION

Heavy metals such as copper, lead, cadmium and nickel are active ingredients of vast range of available pesticides (Mhadhbi *et al.,* 2012). In Pakistan, the massive use of synthetic pesticides, rotenone, biocides, herbicides, and fertilizers in agriculture, public health and forestry ultimately results in excess inflow of these heavy metals, mainly into aquatic ecosystem, causing serious threat and rapid deterioration of the aquatic environment. In turns, they cause adverse multiple effects in living organisms therein because of their characteristics toxicity, bioaccumulation, long persistence and biomagnifications via food chain (Malik *et al.,* 2010).

Nickel is a transition heavy metal placed in group VIII of the periodic table with oxidation states -1, 0, +2, +3, and +4. Among heavy metal toxicants in aquatic environment, it is of major concern. It is ubiquitous in its distribution in the environment (USDHHS, 1990). It plays numerous roles in the biology of animals including man and fish. For example, urease (an enzyme which assists in the hydrolysis of urea) contains nickel (Hodgson, 2004). Recently, several reports are available regarding the nickel toxicity on serum electrolytes and arterial blood gases in different animal species, particularly in fish (Pane *et al.,* 2003; Bersenyi *et al.,* 2004; Brix *et al.,* 2004; Doreswamy *et al.,* 2004; Hoang *et al.,* 2004; Gupta *et al.,* 2006). Water quality parameters play an important role in pond fish culture (Ali *et al.,* 2001). Admissible concentration of nickel in drinking water (as given by WHO) is 0.02 mg L. In Pakistan, after flood 2010, nickel concentration in ground water touched the range of 0 to 3.66 mg/L, while in surface water, it is found in the limit ranging 0 to 1.52 mg/L (Azizullah *et al.,* 2011), and in cultured farm it ranges from 5.3-8.1 µg/L (Farha *et al.,* 2013), thus acting as threat to the health of living organisms, particularly fish and human.

Plasma in freshwater fishes, particularly in cyprinids, is hyperosmotic. These fishes are active hyper-osmoregulators, as they have to face hyperhydration and ion losses by simple diffusion. Therefore they have to maintain osmotic and ionic homeostasis by means of uphill intake of ions across the intestinal and branchial epithelium at the expenditure of energy (Hoar and Randall, 2001). The prevalence of hyponatremia, hypokalemia and hypocalcemia in fish under stress is the reflection of the malfunctioning of gills and kidneys or it may be

* Corresponding author: abdullatif_126@yahoo.com
0030-9923/2014/0004-0915 $ 8.00/0

due to acidity in the water medium (Thrall, 2005). Hyponatremia results in hypervolemia, because damaged kidneys cannot maintain osmotic gradient due to water accumulation in plasma (Thrall, 2005).

To the best of our knowledge, very little attention is paid towards the serum electrolyte and arterial blood gases' levels in fish that could be used as a powerful biosensor tool in assessment of health of fish and environment in which they inhabit. Keeping in view the current status of our natural aquatic environments and the use of pesticides and their leaching impacts on these environments, the present study was conducted to investigate the effect of acute concentrations of nickel sulphate on serum electrolytes and arterial blood gases of most important commercially exploited fish, *L. rohita*.

MATERIALS AND METHODS

Procurement of fish

Juveniles (150) of *L. rohita* of both sexes were purchased from the local fish farm and stocked for one week in 1800 L capacity concrete water tank in the University for acclimatization under a natural photoperiod (March-May 2012). Water with a temperature of 25±1.0^{o}C, having pH values ranging from 6.7 to 7.0 was continuously aerated by the use of electric air pumps. Temperature, pH, oxygen and hardness were regularly monitored. The fish juveniles were allowed to feed on commercial pellets, having 35% protein, twice a day.

LC_{50} determination

For determination of 96h LC_{50} values, each group of 16 juveniles of *L. rohita* was exposed to one of the seven concentrations; 4.0, 8.0, 12.0, 16.0, 20.0, 28.0, 36.0 mg/L of nickel sulphate. (Technical grade Sigma Aldrich) in tanks of 200 L capacity at 25.0±1.0°C, pH, 6.5-6.8, total hardness, 65-75 mg/L (as $CaCO_3$) and alkalinity, 75-80 mg/L. The results obtained from the acute static toxicity experiments of nickel sulphate upon freshwater cyprinid, *L. rohita* were calculated by using Finney's Probit Analysis. The control mortality was corrected using Abbot (1925) formula, where necessary.

Experimental protocol

Based on 96h LC_{50} values protocol for acute toxicity test (Sprague, 1969), the trials in the experiments were conducted to investigate the effect(s) of 96h LC_{50} values of nickel sulphate on serum electrolytes of *L. rohita*. Two groups of juveniles of freshwater cyprinid, *L. rohita*, of both sexes, with body length of 9.324±1.547 cm and body weight of 11.461±1.251 g were randomly stocked in two separate tanks (12 fish per group) in two separate glass aquaria with a capacity of 200 L each. The fish juveniles of one group (Control) were kept untreated and are supplied with metal free tap water. Fish juveniles of second group (Experimental) were subjected to exposure to 19.21 mg/L of nickel sulphate for 96h. All experiments were carried out in triplicate in semi-static systems with renewal of water after every 12h interval. Temperature, pH and oxygen concentration of water were maintained throughout the experiments following Ali *et al.* (2006). All the experimental procedures and fish handling protocols were approved by the Ethical Committee of the Institute.

Sample collection and determination of blood serum electrolytes and arterial blood gases (ABG)

At the end of each experimental period, 1-2 ml of fresh blood sample was collected by making a caudal puncture with the help of fine needle, in non-heparinized glass vials, and allowing it to clot. Serum was separated by centrifugation at 10,000 rpm for 5-8 minutes in TG20-WS Tabletop High Speed Laboratory Centrifuge. Serum electrolytes such as Na^{+}, K^{+}, Ca^{2+}, Mg^{2+} and ABGs such as pO_2, pCO_2 were determined by using Hitachi 902 automatic analyzer (Japan), following Kengkoom *et al.* (2012). The test was performed as stated in service manual for model 902 automatic analyzer Hitachi, Ltd. (1997). All the tests were performed in triplicate.

Statistical analysis

All the data were expressed as mean and standard error of mean. The statistical package, Minitab-14 was used for the data analysis. The means were separated using one way ANOVA and the two means were considered significant at 5% ($P<0.05$), following the statistical protocol, introduced by Zar (1996).

RESULTS

96h LC_{50} value of nickel sulphate with 95% confidence for *L. rohita* was found to be 19.21 mg/L (Table I).

Table I.- The mortality rate of freshwater cyprinid (*Labeo rohita)* individuals in 96h at various nickel sulphate concentrations.

Nickle sukphate conc. (mg/L)	No. of fish	No. of dead fish	Mortality (%)
Control	16	0	0
4.0	16	0	0
8.0	16	2	12.5
12.0	16	3	18.75
16.0	16	8	50
20.0	16	10	62.5
28.0	16	12	75
36.0	16	16	100

Table II.- Serum electrolytes and arterial blood gases profile. All values are expressed as Mean ± SEM. One way ANOVA was used to compare the effect of Nickel on *L. rohita* with control fish.

Parameters	Control (n=12)	Ni treated (n=12)
Mg^{+2} (m.mol/L)	1.06±0.21	1.38±0.14***
Ca^{+2} (m.mol/L)	2.56±0.22	2.34±0.23*
Potassium (m.mol/L)	1.64±0.11	1.28±0.15***
Sodium (m.mol/l)	1.61±0.10	1.31±0.17***
pH	7.33±0.04	6.79±0.12***
Ammonia (m.mol/l)	707.82±2.76	859.8±74.6***
PO_2 (mm Hg)	58.44±1.07	44.47±1.38***
O_2 Sat %	96.33±1.15	88.23±4.57***
PCO_2 (mm Hg)	25.59±0.86	42.67±4.93***

*least significant (P<0.05); **significant (P<0.01); ***highly significant (P<0.001).

Table II shows effect of nickel, administered at a dose of 19.2 mg/L of nickel sulphate for 96 hours on the various serum electrolyte and ABGS. Mg^{++} ions, ammonia concentration and PCO_2 level increase 30%, 21.5% and 67%, respectively after nickel treatment. All other parameters Ca^{++}, K^+, Na^+, PO_2 and O_2 decreased 2.6%, 21.5%, 18.6%. 24% and 8.4%, respectively.

Reflexive cough and gill purge responses were also observed in fish exposed to Ni.

DISCUSSION

Any type of deviation in plasma divalent ions level can be used as indicator for the assessment of kidneys function. In the study, in toxicant exposed fish, serum hypermagnecemia is an indication of loss of renal function. It was informed by Little and Brewer (2001) that hypermagnecemia produces neuromuscular excitability and paralysis of voluntary muscles in vertebrates. From hypermagnecemic situation in exposed fish, it was inferred that selected heavy metal *i.e.* Ni^{+2} also acts as neurotoxicant, as changes in form, frequency, or posture of swimming movements of treated groups of fish was observed, with changes often occurring much earlier than mortality. The alterations in swimming behaviors observed in study were similar as demonstrated by Little and Brewer (2001) and Kwak *et al.* (2002) in pesticide exposed fish. Reflexive cough and gill purge responses were also observed, as probably stressed fish try to clear the opercular chamber of the toxicant or irritants, and could also increase rate and amplitude of the respiratory cycle. It is investigated that plasma Mg^{2+} levels in all freshwater fishes are lower than Ca^{2+}. It is an essential electrolyte in plasma and is found in bound state with plasma proteins (Bjornsson and Haux, 1985). It is interesting that Mg^{2+} is needed by erythrocytes to maintain their functional entity. That is why these erythrocytes have $\geq$10- fold higher Mg^{2+} than that in plasma. An erroneous plasma levels indicates haemolysis as it plays significant role in disturbing plasma values for Mg^{2+} (Houston, 1985).

To the best of our knowledge, very little attention is devoted towards the evaluation of plasma Ca^{+2} levels in fish and to use it as a powerful tool in assessment of health of fish and environment in which they inhabit. Ca^{+2} helps in clotting of blood, takes part in contraction of muscles, controls the excitability of nerves and also is involved in certain hormone activity (Hoar and Randall, 2001). In contrast to Mg^{+2}, the lower values of Ca^{+2} concentrations were observed in treated fish compared to control. It was suggested that the

experimentally stressed fish tried to control excitability of nerves and has lost integration of voluntary muscles, as stated by Hoar and Randall, (2001).

Normal serum blood potassium (K^+) level in cyprinids is about 3.00 m.mol/L (Hoar and Randall, 2001). It is investigated that plasma levels of K^+ in cyprinids remain unaffected by the factors affecting the gill electrolyte permeability. But the factors that cause acidosis such as strenuous exercise compels the muscle to release K^+ into plasma, thus resulting in an increase of the plasma levels of K^+ (Eddy, 1985). Eddy, (1985) stated that hypokalemia in fish might be associated with the alkalosis, cutaneous or intestinal losses of K^+ or due to metal toxicity. Thus hypokalemia in nickel treated fish is an indication of incidence of alkalosis cutaneous or intestinal loss of K^+.

The presence of sodium ion (Na^+) in the blood serum of an animal is called natremia. In cyprinids, the normal plasma natremia level in case of fish juveniles is 1.45 m.mol/L but in case of adult fish its level is 150 m.mol/L (Hoar and Randall, 2001). Hyponatremia results in hypervolemia, because damaged kidneys cannot maintain osmotic gradient due to water accumulation in plasma (Hoar and Randall, 2001). In our study, hyponatremia and hypokalemia was observed in Ni treated fish. The hyponatremia and hypochloremia in fish under stress is the reflection of the malfunctioning of gills and kidneys or it may be due to acidity in the water medium (Hoar and Randall, 2001; Thrall, 2005).

In our study, an increase in plasma levels of CO_2 and a decrease in O_2 were observed in fish serum after Ni treatment. The results of any abnormality in plasma levels of CO_2 and O_2 in fish are the signs of either damage of gills or hemoglobin abnormality (Hoar and Randall, 2001). Ammonia (NH_3) is the major end product of nitrogen metabolism in most freshwater fishes (Hoar and Randall, 2001). Review surveys suggest that more than 99% of NH_3 is present in ionized form, NH_4^+. NH_3 and NH_4^+ act as primary transport vehicle in plasma of most fish (Van Waarde, 1983). It is informed that gills are the pivotal organs for NH_3 excretion in freshwater fish, though skin plays a minor role in NH_3 excretion (Hoar and Randall, 2001). It is also reported that less than 15% NH_3 is excreted by fish kidneys (Hoar and Randall, 2001). Under normal aerobic situation, the primary site of NH_3 production is liver, but it is observed that in anoxic situation, the liver production is much more reduced and muscle proteolysis predominates and becomes the source of enhanced production of NH_3 (Van Waarde, 1983). High alkalinity or low acidity generally impairs its excretion by enhancing the NH_3/NH_4^+ ratio. Consequently, any factor that causes the increase of water pH becomes the source of plasma hyperammonemia leading to drastic physiological effects (Randall *et al.*, 1989). Plasma hyperammonemia may be an output response of exhaustive exercise (Walsh *et al.*, 1990). Plasma hyperammonemia is an indication for inflammation, mucinification and swelling in gills due to damage, consequently of an increased diffusion distance between water and blood. It is well documented that environmental toxic substances can cause damage to gills, resulting in plasma hyperammonemia (Hoar and Randall, 2001). Hypercapnia causes plasma hypoammonemia, which may be because of CO_2 mediated suppression of metabolic activities (Wright *et al*., 1988). In our study, hyperammonemia was observed after nickel treatment, indicating some kind of abnormality in physiological role of gills. $NiSO_4.7H_2O$ damage gills or perhaps the muscle proteolysis predominates over gill excretory role (Van Waarde, 1983).

From the present data, it is concluded that the nickel as an aquatic contaminant acts as hyperosmotic agent in *L. rohita*, strongly affecting on the serum electrolytes and arterial blood gases in fish and consumption of fish as diet from heavy metal polluted areas is directly threat to human blood serum characteristics. In humans, as nickel acts as carcinogenic and teratogenic (Dojlido and Best, 1993), thus consumption of affected fish with nickel could enhance the risks of cancer and abnormal development in human.

It has been observed that in waters with a low pH, the eminent levels of Ni metal can be dissolved, as is found in the acidic waters produced by pyrite oxidation. Liming (addition of calcium carbonate) is only the way to neutralize the effect of Ni in waters and soils and buffer them from rapid fluctuations in pH. Therefore the application of limestone to fish cultured ponds, and their surrounding watersheds is

recommended to shelter them from acidification, and to reinstate their important ecological, economic and natural values. Adding limestone to maintain a near-neutral pH (pH 7) keeps fish pond safe for aquatic life. It is also concluded that fish serum electrolytes and arterial blood gases can be used as biosensors of the health of the aquatic environment.

ACKNOWLEDGEMENT

The authors are grateful to Higher Education Commission of Pakistan for providing research grant for this study under Indigenous PhD Scholarship Scheme.

REFERENCES

ABBOTT, M.S., 1925. A method of computing effectiveness of an insecticide. *J. econ. En.*, **18:**265-267.

ALI, M., IQBAL, R., RANA, S. A., ATHAR, M. AND IQBAL, F., 2006. Effect of feed cycling on specific growth rate, body composition, condition factor and RNA/DNA ratio of *Labeo rohita*. *Afr. J. Biotechnol.*, **5:** 1551-1556.

AZIZULLAH, M., NASIR KHATTAKB, K., RICHTERA, R., HADERA, D.P., 2011. Water pollution in Pakistan and its impact on public health. *Environ. Int.*, **37:** 479-497.

BERSENYI, A., GY FEKETE, S., SZILAGYI, M., BERTA, E., ZOLDAG, L. AND GLAVITS, R., 2004. Effects of nickel supply on the fattening performance and several biochemical parameters of broiler chickens and rabbits. *Acta Vet. Hung.*, **52:** 185-197.

BJORNSSON, B.T. AND HAUX, C., 1985. Distribution of calcium, magnesium and inorganic phosphate in plasma of estradiol-17β treated rainbow trout. *J. Comp. Physiol.*, **155B:** 347-352.

BRIX, E., KEVIN JAMES KEITHLY, V., DAVID DEFOREST, K. AND LAUGHLIN, J.I.M., 2004. Acute and chronic toxicity of nickel to rainbow trout (*Oncorhynchus mykiss*). *Environ. Toxicol. Chem.*, **23:** 2221–2228.

DOJLIDO, J. AND BEST, G. A., 1993. *Chemistry of water and water pollution*. Ellis Horwood Limited, West Sussex.

DORESWAMY, K., SHRILATHA, B., RAJESHKUMAR, T. AND MURALIDHARA, T., 2004. Nickel-induced oxidative stress in testis of mice: Evidence of DNA damage and genotoxic effects. *J. Androl.*, **25:** 996-1003.

EDDY, E.B., 1985. Uptake and loss of potassium by rainbow trout (*Salmo gairdneri)* in freshwater and dilute sea water. *J. exp. Biol.*, **118:** 277-286.

FARHA, A., RAFIA, A., FARAH, J. AND BUSHRA, B., 2013. A comparative study of physico-chemical parameters of Keenjhar Lake, Thatta, Sindh, Pakistan. *Int. J. Adv. Res.* **1:** 482-488.

GUPTA, V.K., ARSHI RASTOGI, V.K. SAINI AND NEERAJ JAIN., 2006. Biosorption of copper (II) from aqueous solutions by Spirogyra species. *J. Colloid Interface Sci., Orland.*, **296:** 59-63.

HARRIS, W.S., 1997. n-3 fatty acids and serum lipoproteins: human studies. *Am. J. Clin. Nutr.* **65:** 1645S-1654S.

HOANG, T.C., TOMASSO, J.R. AND KLAINE, S.J., 2004. Influence of water quality and age on nickel toxicity to fathead minnows (*Pimephales promelas*). *Environ. Toxicol. Chem.*, **23:** 86-92.

HOAR, W.S. AND RANDALL, D.J., 2001. Ammonia toxicity, tolerance, and excretion. In: *Fish physiology*, vol. 20, *Nitrogen Excretion* (eds. Y.K. Ip, S.F. Chew and D.J. Randall), edited by W.S. Hoar, D.J. Randall, A.P.Farrell, Academic Press, San Diego, pp. 109-148.

HODGSON, E., 2004. *A textbook of modern toxicology*. 3rd edition. John Wiley and Sons, Inc, New Jersey, pp. 203-211.

HOUSTON, A.H., 1985. Erythrocyte magnesium in freshwater fishes. *Magnesium*, **4:** 106-128.

KENGKOOM, K., CHAIMONGKOLNUKUL, K., CHERDYU, S., INPUNKAEW, R. AND AMPAWONG, S., 2012. Acute and sub-chronic oral toxicity studies of the extracts from herbs in Phikud Navakot. *Afr. J. Biotechnol.*, **11:** 10903-10911.

KWAK, I.S., CHON, T.S., KANG, H.M., CHUNG, N.I., KIM, J.S., KOH, S.C., LEE, S.K. AND KIM, Y.S., 2002. Pattern recognition of the movement tracks of medaka (*Oryzias latipes*) in response to sub-lethal treatments of an insecticide by using artificial neural networks. *Environ. Pollut.*, **120**: 671-681.

LITTLE, E.E. AND BREWER, S.K., 2001. Neurobehavioral toxicity in fish. In: *Target organ toxicity in marine and freshwater teleosts new perspectives: Toxicology and the environment*. Vol. 2. Systems. (eds. D. Schlenk. and W. H. Benson. Taylor and Francis, London, pp 139-174.

MALIK, N., BISWAS, A.K., QURESHI, T.A., BORANA, K. AND VIRHA, R., 2010. Bioaccumulation of heavy metals in fish tissues of a freshwater lake of Bhopal. *Environ. Monit. Assess.*, **160**: 267-267.

MHADHBI, L., PALANCA, A., GHARRED, T. AND BOUMAIZA, M., 2012. Bioaccumulation of metals in tissues of *Solea vulgaris* from the outer Coast and Ria de Vigo, NE Atlantic(Spain). *Int. J. environ. Res.*, **6:** 19-24.

PANE, E. F., RICHARDS, J.G. AND WOOD, C.M., 2003. Acute waterborne nickel toxicity in the rainbow trout (*Oncorhynchus mykiss*) occurs by a respiratory rather than ionoregulatory mechanism. *Aquat. Toxicol.*, **63:** 65- 82.

RANDALL, D.J., WOOD, C.M., PERRY, S.F., BERGMAN, H., MALOIY, G.M., MOMMSEN, T.P. AND WRIGHT, P.A., 1989. Urea excretion as a strategy for

survival in a fish living in a very alkaline environment. *Nature*, **337:** 165–166.

SPRAGUE, J.B., 1969. Measurement of pollutant toxicity to fish. I. Bioassay methods for acute toxicity. *Water Res.*, **3**: 793-821.

THRALL, M.A., 2005. Veterinary hematology and clinical biochemistry. *Vet. Pathol*., **42:** 1.

USDHHS, US Department of Health and Human Services, 1990. *NIOSH Pocket Guide to Chemical Hazards*, Publication No. 90-117.

VAN WAARDE, A., 1983. Aerobic and anaerobic ammonia production by fish. *Comp, Biochem. Physiol.,* **74B:** 675-684.

WALSH, P.J., DANULAT, E. AND MOMMSEN, T.P., 1990. Variation in urea excretion in the gulf toadfish *Opsanus beta*. *Mar. Biol.*, **106:** 323-328.

WRIGHT, P.A., RANDALL, D.J. AND WOOD, C.M., 1988. The distribution of ammonia and H+ between tissue compartments in lemon sole (*Parophrys vetulus*) at rest, during hypercapnia and following exercise. *J. exp. Biol.*, **136:** 149-175.

ZAR, J.H., 1996. *Biostatistical analysis*. 3rd Edition. Englewood Cliffs, Prentice Hall, New Jersey.

(Received 30 April 2013, revised 28 March 2014)