IOSR Journal of Engineering (IOSRJEN) **www.iosrjen.org**
ISSN (e): 2250-3021, ISSN (p): 2278-8719
Vol. 04, Issue 12 (December 2014), ||V2|| PP 45-48

Toxicity of Ammonia in Hemoglobin content to Freshwater Fish Cyprinus carpio (Common carp)

Y. Thangam, M. Perumayee, S. Jayaprakash, S. Umavathi, and S.K.Basheer
J.K.K.Nattraja College of Arts and Science, Kumarapalayam,
Namakkal (Dt), Tamilnadu, India.

Abstract: *The aim of the study was to determine the toxicity of ammonia in the blood of freshwater fish Cyprinus carpio. Changes in the haemoglobin content of fish Cyprinus carpio exposed to sublethal concentration of ammonia for 35 days were presented in Table 4 and figure. 3. During the above exposure period a biphasic trend was observed in the present study. The hemoglobin was decreased from 7th, 14th, 21st, 28th, and 35th days showing a percent decrease of -15.948, -11.809, -13.483, -12.621, -10.204. However, hemoglobin was declined showing a minimum percent decrease of -10.204 at the end of the treatment period (35th day) and a maximum percent decrease of -15.948 at the end of 7th day.*
Key words: *Ammonia, Cyprinus carpio, Hemoglobin.*

I. Introduction

Ammonia is unique among regulated pollutant because it is an endogenously produced toxicant that organisms have developed various strategies to excrete, which is in large part by passive diffusion of un-ionized ammonia concentration reduce or reverse diffusion gradient and cause the buildup of ammonia in gill tissues and blood (USEPA, 1999). In water, ammonia occurs two forms which together are called the total ammonia nitrogen or TAN chemically and these two forms are represented as NH^{4+} and NH_3 (Francis-Floyd and Watson, 2005). According to (Wood *et al*., 2011), a low concentration can positively effluence fish growth. Ammonia for vertebrate animals is highly toxic substrate even in low concentrations. It is formed in the organisms as the final product of protein and aminoacid. Due to its toxicity and especially its neurotoxin properties it cannot be accumulated in the organisms and must be eliminated in the brain and neurons. Among salts ammonia plays an important role in fish and fish, as a bio indicator organism, plays an increasingly important role in monitoring of water pollution because they respond with great sensitivity to changes in the aquatic environment (Chezhian and Senthamilselvan, 2012).

Ammonia is toxic to variety of aquatic organism including fish (Harris et al., 1998). Un-ionized form of ammonia is the most toxic form to aquatic organisms as it can readily diffuse through cell membranes and is highly soluble liquid. It can cause impairment to cerebral energy metabolism, damage to gill, liver, kidney, spleen and thyroid tissue in fish. Moreover, it is a common aquatic pollutant. Ammonia is the principal nitrogenous waste and involves the measurement of contaminant levels to characterize the hazards imposed on the aquatic environment. However, this field of study includes information on how contaminants can affect human and fishes in and around the aquatic environments. This study focused on the common carp Cyprinus carpio of monitoring the blood ammonia levels in fish blood in the period of metabolic attenuation, and handling of fish ability to withstand such changes under hematological changes (Busova and Stancelova, 2013).

Estimation of hemoglobin can be used as an index of anemia and fluid volume disturbance. Significant decrease in hemoglobin level was noted in Asian swamp eel Monopterus albuds, exposed to endosulfan (Hii et al., 2007, in European Catfish, Silurus gland L. exposed to cypermethrin (Adhikari et al., 2004). Ramesh et al. (1994) reported similar observation in Cyprinus carpio exposed to kitazin toxicity. Matkovics et al. (1987) observed a significant decrease in hemoglobin content of Cyprinus carpio when exposed to environmental ammonia showed a decrease content in hemoglobin (Avilez, et al., 2004). In crayfish decreased hemoglobin content due to nitrite exposure (Camargo and Alonso, 2006). Hemoglobin increased due to nitrite exposure in Oreochromis hybrid (Hrubec, et al., 2000), decrease in Pacu (Moraes, et al., 2006). The concentration of ammonia in the blood seems to decreased in Cyprinus carpio monitored ranged from 7 to 35 days µ mol/L do 141.4 µ mol/L (Spurny, 1998). Wick and Randall (2002) in their study demonstrated effect of concentration of ammonia in blood in Rainbow trout . Hence in the present study toxicant ammonia exposed in the freshwater fish Cyprinus carpio showed a decreased percent in the hematological parameters throughout the study period.

II. Materials and methods

To assess the haematological profile of the control and treated fish, hemoglobin, were measured in the whole blood of Cyprinus carpio. The changes in physico-chemical characteristics, such as temperature, pH, dissolved oxygen, alkalinity , hardness, salinity, calcium and magnesium of experimental water were recorded

throughout the experimental period. Fresh water fish Cyprinus carpio, weighing 5.0-6.0 gm and measuring 7-8 cm were collected from Tamilnadu Fisheries Development corporation, Aliyar fish farm, Aliyar, Tamilnadu, India. The median lethal concentration of ammonia was calculated by Probit analysis method (Finney,1978). The sublethal toxicity was conducted at $1/10^{th}$ of Lc50 of 24h value (1.05) ppm.

Estimation of haemoglobin

Hemoglobin content of the blood was estimated by Cyanmethemoglobin method (Drabkin, 1946) using Diagnostic Reagent kit supplied by Monozyme India Ltd.

Calculation

$$\text{hemoglobin concentration(gm/dl)} = \frac{\text{Abs. Of test}}{\text{Abs. Of standard}} \times 16.31$$

III. Result

Changes in the hemoglobin content of fish Cyprinus carpio exposed to sublethal concentration of ammonia for 35 days were presented in Table 4 and figure. 3. During the above exposure period a biphasic trend was observed in the present study. The hemoglobin was decreased from 7^{th}, 14^{th}, 21^{st}, 28^{th}, and 35^{th} days showing a percent decrease of -15.948, -11.809, -13.483, -12.621, -10.204 respectively. However, hemoglobin was declined showing a minimum percent decrease of -10.204 at the end of the treatment period (35^{th} day) and a maximum percent decrease of -15.948 at the end of 7^{th} day.

S.NO	EXPOSURE PERIOD	CONTROL	EXPERIMENT	CHANGE %	CALCULATED t VALUE
1	7	4.64 ± 0.41	3.90 ± 0.34	-15.948	-1.40
2	14	3.98 ± 0.38	5.1 ± 0.22	-11.809	-0.98
3	21	3.56 ± 0.25	3.0 ± 0.33	-13.483	-1.45
4	28	3.09 ± 0.35	2.70 ± 0.50	-12.621	-1.46
5	35	2.90 ± 0.41	2.64 ± 0.22	-10.204	-1.53

Table 4. Changes in the hemoglobin content of *Cyprinus carpio* exposed to sublethal concentration of ammonia for 35 days

Values are mean ± S.E. of five individual observations. (+) Denotes percent increase over control. (-) Denotes percent decrease over control.

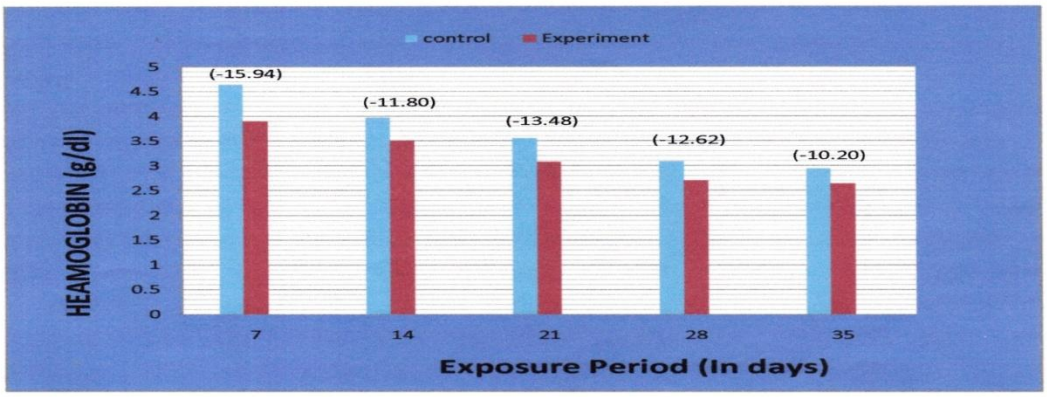

Fig. 3

Fig. 3. Hemoglobin content of *Cyprinus carpio* exposed to sublethal concentration of ammonia for 35 days. Error bars indicate the standard error of the mean.

IV. Discussion

The knowledge of the hematology is an important tool that can be used as an effective and sensitive index to monitor physiological and pathological changes in fishes (Kori-Siakpere *et al*., 2005). Previous studies on fish hematology have revealed that interpretation of blood parameters quite difficult, since variations in the blood are caused by internal and external factors. It is well known that blood sampling, laboratory techniques, seasonal variations, size, genetic properties, sex, population density, lack of food supply, environmental stress

and transportation could affect hematological data (Kori-Siakpere *et al*., 2005). When ammonia concentrations increase in water, ammonia excretion by fish decrease and the levels of ammonia in the blood tissue increase. High levels of ammonia increase oxygen consumption by tissues, damage gills, reduce ability of blood to transport oxygen. Increased level of ammonia in water increases its content in the blood of fish through diffusion and poisoning of central nervous system (Svobodova *et al*., 2007).In the present investigation reduction of hemoglobin may be due to inhibition of aerobic glycolysis could be the reason for the decrease of blood parameters in the stressed fish. Prolonged reduction in hemoglobin content is deleterious to transport any blood dyspepsia and degeneration and could be ascribed as pathological conditions in fish exposed to toxicants.

The concentration of ammonia in the blood of the common carp *Cyprinus carpio* was low in the monitored and the period ranged from 7 to 35 days µ mol/L do 141.4 µ mol/L. These values are in accordance with Spurny (1998), where in the winter season, the concentration of ammonia in the fish blood occur in values 50-100 µ mol/L. Fish in the winter season have subdued metabolism and do not ingest feed. Fish, especially stored in a pond, are kept after harvesting for approximately two months in storage ponds, where they also do not ingest feed. In this period they reduce weight and occur considerable hematologic change as well as significant hypoproteinemia (Svobodova *et al*., 2006). While the hypoproteinemia is also related in decreased concentration of blood ammonia. The situation is also demonstrated by several other authors. Wick and Randall (2002) in their study demonstrate the effects of starvation and feeding on the concentration of ammonia in the blood of rainbow trout. Fish that is fed reports in experimental conditions an increase in plasma ammonia concentration 30 minutes after feeding. The increased levels of ammonia according to the study return to the normal stage two hours after feeding, but after 8 hours since feeding is in the plasma noticeable the second increase in ammonia concentration 8 hours after feeding is caused by the higher physical activity during feeding in experimental conditions by a sudden change in lighting conditions, fright and stress response of fish to removal of the covers of the experimental tank with water.

Wick and Randall (2002) suggest that the pathway, in which is the ammonia utilized, is the formation of aminoacids of alanine, aspartate, glutamate and glutamine. All of these amino acids can thus serve as temporary storage of ammonia. In this way, the fish can be protected from the effects of ambient ammonia. These authors state that the endogenous concentration ammonia in the blood of rainbow trout varies with food intake. However, increasing the endogenous ammonia does not increase exogenous ammonia in water. The protective effect is observed in fish only for the first 24 hours. Ammonia formed in fish as a product of metabolism of proteins. Ammonia toxicant is a serious problem and can cause mass mortalities in fish. This study focused on the common carp Cyprinus carpio. It was focused on monitoring the ammonia levels in fish blood. The study results confirmed that the hematological parameters decreased may be due to the effect of sudden changes in water temperature and the ammonia in the blood of fish used to assess its physiological state and effect of hazardous substances, to determine the nonspecific resistance. On the contrary, there were no dramatically increased concentrations of ammonia in the blood of fish. Hence in the present study the literature on hemolglobin content on ammonia is almost negligible.

Reference

[1]. Abdel – Tawwab, M., Mousa, M.A.A., Abbass, Ff., 2007a. Growth Performance And Physiological Respons Of Affrican Carfish, Clarias Gariepin Fed Fed Organic Selenioum Prior To The Exporsure To Environmental Copper Toxicity. Aquaculture, 272(1-4), 335-345.32.

[2]. Adhikari.S., Sarkar, B., Chatterjee, A., Mahapatra, C .T., Ayyappan, S., 2004. Effects Of Cypermethrin And Carbofuran On Certain Hematological Paramenters And Predication Of Their Recovery In A Freshwater Teleost: Labeo Rohita (Hamilton). Ecotoxicol. Environ. Saf., 58.220-226.

[3]. APHA, AWWA, WPCF, 185. In: Standard Methods For The Examination Of Water And Waste(16th Eds). American Public Health Asssociation Public Health Assciation, Washington, USA.

[4]. Avilez, I. M., Altran, A. E., Aquiar, I. H., Moraes, G., 2004. Hematological Responses Of The Neotropical Teleost Matrinxa (Brycon Cephalus) To Environmental Nitrite. Compp. Biochem. Physiol. C.135-139.

[5]. Busova, M., Stancelova, K., 2013. Study Of The Adaptation Process In Common Carp (Cyprinus Carpio L.) After Harvesting. Jou. Micro. Biotech. Food Sci. 1(194-204).

[6]. Cairns, J., 1984. Estimating Hazard. Biosci., 3,7.

[7]. Camargo, J.A. Alonso. A., 2006. Ecological And Toxicological Effect Of Inorganic Nitrogen Pollution In Aquatic Ecosystems. A Global Assessment Environment Internations, 32.831-849.

[8]. Carvalho, C.S., Fernandes, M.N., 2006 Effect Of Temperature On Copper Toxicity And Hematological Responses In The Neotorpical Fish Prochilodus Scrofa At Low And High PH. Aquaculture, 251, 109-117.

[9]. CCME. 1999. (Canadian Council Of The Environment). Canadian Environmental Quality Guidelines. Canadian Council Of Ministers Of The Environment. Winnipeg. Updated.

[10]. Cerqueira, C. C. C. Fernandes. M. N., 2002. Gill Tissue Recovery After Copper Exposure And Blood Parameter Response In The Tropical Fish Prochilodus Scrofa. Ecotoxicol. Environ. Saf., 52,83-91.

[11]. Chezhian, A., Senthamilselvan, D ., 2012. Hormonal Responses In Freshwater Fish Cyprinus Carpio Var. Communis (Linnaeus) Exposed To Ammonia Toxicity. Inter. Jou. Of . Environ. Bio. Universal Research Publications 2 (3). 142-145.

[12]. Dratichova, J., Svobodova, Z., Luskova, Machova, J., 2004. Effect Of Cadmium Of Hematological Indices Off Common Carp Cyprius Carpio (L). Bull. Environ. Contam. Toxicol., 752,725-732.

[13]. Finney, D. J., 1978. Statistical Method In Biological Assay (3rd Ed), Cambridge University Press. London, Pp. 508.

[14]. Flos, R., Tort, L., Balasch, J., 1987. Effect Of Zine Sulphate On Haematological Parameter In The Dogfish Scyliorhinus Canicula And Influences Of MS222. Mar.Environ.Res., 21,289-298.
[15]. Francis-Floyd, R., Watson, C., Petty, D. And Poulder, D.B. (2005) Ammonia In Aquatic Systems1FA-16, Department Of Fisheries And Aquatic Sciences, Florida Cooperative Extension Service, Institute Of Food And Agricultural Sciences, University Of Florida.
[16]. Ghazaly, K.S., 1992. Hematological And Physiological Responses To Sub Lethal Ocncetrations Of Cadminum In A Freshwater Teleost, Tilapis Zillii. Water Air Soil Pollut., 64,551-559.
[17]. Harding. J., Hoglund, L.B.M, 1983. On Accuracy In Estimating Fish Blood Cvariables. Cop. Biochem Physiol. A. 75(1),35-40.
[18]. Harris, J.O., G. B. Maguire, S. Edwards And S. M. Hindrum. 1998. Effect Of
[19]. Ammonia On The Growth Rate And Oxygen Consumption Of Juvenile Greenlip Abalone,
[20]. Haliotis Laevigata Donovan. Aquaculture 160, 259-272.
[21]. Hii, Y.S., Lee, M.Y., Tse, S. C., 2007. Acute Toxicity Of Organochlorine Insectide Endosulfan And Its Effect On Behavior And Some Hematological Parameters Of Asian Swamp Ell (Monopterus Albus, Zniew).
[22]. Hrubec, T.C., Cardinale, J.L., Smith, S.A. 2000. Haematology And Plasma Chemistry Reference Intervals For Cultured Tilapis (Oreochromis Hybrid). Vet. Clin. Pathol., 29, 76-12.
[23]. Kavitha, C., Malavizhi, A., Senthil Kumaran, S., Ramesh, M., 2010. Toxicological Effet Of Arsenate Exposure On Hematological, Biochemical And Liver Transaminases Activity In An Indian Major Carp, Catla Catla. Food Chem.. Toxicol., 48, 2848-2854.
[24]. Kelson, N. J. R. M., Shaw, M.A., Minns, C.K., Mills, K.H.,1990. An Evaluation Of The Effect Of Atmospheric Acidic Deposition On Fish And The Fishery Resource Of Canada. Can.J.Fish Aquat. Sci., 47(3), 644-655.
[25]. Kori-Siakere, O., Ake, Jeg., Idoge, E., 2005. Hemtologial Characteristics Of The African Snakehead, Parachanna Obscura. Afr.J.Biotechnol. 4(6),530.
[26]. Matkovics, B., Witas, H., Gabrielak, T., Szabo, L., 1987. Paraquat As An Agent Affecting Antioxidant Enzymes Of Common Carp Erythrocytes. Comp. Biochem. Physiol. C., 87, 217-219.
[27]. Moraes, G., Avilez. I. M., Hori. T. S. F., 2006. Comparison Between Biochemical Reposnses Fo The Teleost Pacu And Its Hybrid Tambacu. Braz. J. Biol., 6(4). 1103-1108.
[28]. Nemcsok, J., Benedeczky, I., 1990. Effect Of Sublethal Concentration Of Phenol On Some Enzyme Activitys And Blood Sugar Level Of Carp, Cyprinus Carpio (L). Environ. Monit. Assess., 14,377-383.
[29]. Nussey, G., Van Vuren, J. H. J., Du Preez. H. H., 2002. The Effect Of Copper And Zinc At Neutral And Acidic Ph On The General Hematology And Osmoregulation Of Oreochromis Mossambicus. Afr. J. Aquat. Sci., 27, 61-84.
[30]. Ololade, I.A., Ogini, O., 2009. Behavioural And Hematological Effect Of Zinc On African Catfish, Clarias Gariepinus. Int.J.Fish. Aquacult., 1(2).022-027.
[31]. Oliveria Ribeiro, H., Santos, T.M., Ramalho-Santos, J., Pereira, M.L., 2006. Histopathological Effects Of Hexavalent Chromium In Mouse Kidney. Bull. Environ. Contam. Toxical., 76, 977-983. Parrish. P.R., 1985. Chronic Toxdicity Test IN: Fundamental Sof Aquatic Toxicology: Method And Applications. Rand, G.M., Petrocelli, S.R., (Eds.), Hemishphere Publishing Corporaion., Washington, Pp. 96-109.
[32]. Randall. D. J.And T. K. Tsui. 2002. Ammonia Toxicity In Fish. Mar Pollut Bull. 45 (1-12):17-23.
[33]. Ramesh, M., Sivakumari, K., Kanagaraj, M. K., Manavalaramanujam, R., 1994. Impact Of Acute Zinc Sulphate Toxicity And Salinity On The Carbohydrate Metabolism In Oreochromis Mossambicus During Different Exposure Periods. Indian J. Fish, 41(1),51-54.
[34]. Robert M. D., David, M. C. Martin, W. B., 1997. Ammonia In Fish Ponds. Southern Regional Aquaculture Center (SRAC). Publication No.463.
[35]. Ruparelia, S. G., Verma, Y., Saiyed, S. R., Rawal, U.M., 1990. Effect Of Cadmium On Blood Of Tilapia, Oreochromis Mossambicus(Peters), During Prolonged Exposure. Bull. Environ. Contam. Toxicol., 45, 305-3012.
[36]. Rusia, V., Sood S.K., 1992. Routine Hematological Tests. In: 'Medical Laboratory Technology' Kanai, L., Mucerjee (Ed.), Vol.I. Fifth Reprint. Tata Mcgraw Hill Publishing Company Limited, New Delhi, Pp. 252-258.
[37]. Sen, G., Behara, M. K., Patel, P., 1992. Effect Of Zinc On Hemato-Biochemical Parameters Of Channa Punctatus. J. Ecotoxicol. Environ. Monit., 2(2), 89-982.
[38]. Sprague, J., 1986. Measurement Of Pollutant Toxicity To Fish. I. Bioassay Methods For Acute Toxicity. Water Res., 22, 793-821.
[39]. Spurny, P., 1998: Fin Condition Of Fish Kept In Aquacultural System. Acta. Uni. Agri. Silcul. Mend. Brun. Lxi. 138 Pp. 80–7157–341.
[40]. Srivastava, A. K., Agarwal, S. J., 1979. Hematological Anomalies In A Freshwater Teleost, Colisa Fasciatus, On Acute Exposure To Cobalt. Acta. Pharmacol. Toxicol., 44, 197-199.
[41]. Singh. D., Nasth, K., Trivedi, S. P., Sharma, Y. K., 2008. Impact Of Copper On Haematological Profile Of Freshwater Fish, Channa Punctatus. J.Environ. Biol., 29(2), 253-257.
[42]. Sobecka, E., 2001. Changes In The Iron Leveling The Organs And Tissues Of Wells Catfish, Silurs Glanis L. Caused By Nickel, Acts. Ichthyol. Piscat., 31(2),127-143.
[43]. Svobodova, Z., Machhova, J., Kroupova, H., Smutna, M., Groch, L., 2007, Ammonia Autointoxication Of Common Carp, Case Studies. Aquacullt. Int. 15,227-286.
[44]. Svobodova, Z.,, Vykusova B., Modra, H., Jarkovsky. J., Smutna, M., 2006. Haematological And Biochemical Profile Of Harvest-Sizecarp During Harvest And Post-Harvest Storage.Aquacult Res 37:959–965
[45]. Tomasso, J. R., 1986. Comparative Toxicity Of Nitrite To Freshwater Fishes. Aquat. Toxicol., 8,129-137.
[46]. USEPA 1999. Update Of Ambient Water Quality Cuiteria For Ammonia. EPA-822-99-014, Office Of Water, Us Environmentol Proteetion Agency, USA.
[47]. USPEA, 2001. Updae Of Ambient Water Quality Criteria For Cadmium. EPA-822-R-01.001, Office Of Water, US Environmental Protection Agency., Washington, DC, Pp.
[48]. Wood, C. M., Tsui, T. K. N., Hung, C. Y. C., Nawata, C. M., Wilson, J. M., Wright, P. A., 2011. Ammonia Transport In Cultured Gill Epithelium Of Freshwater Rainbow Trout: Their Importance Of Rhesus Glycoproteins And The Presence Of An Apical $Na^+/NH4^+$Exchange. J. Exp. Bio. 212, 878-892.
[49]. Wicks, B. J., Randall, D. J., 2002. The Effect Of Feeding And Fasting On Ammonia Toxicity In Juvenile Rainbow Trout Oncorhynchus Mykiss. Aqua. Toxicol., 59: 71-82.