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Original Research Article

Studies of Ammonia Toxicity on Haematological parameters to Freshwater Fish *Cyprinus carpio* (Common carp)

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Keywords

Ammonia, *Cyprinus carpio,* RBC, WBC

A B S T R A C T

This study evaluates the ammonia toxicity in the blood of freshwater fish *Cyprinus carpio.* Table 2. and Figure 1. Represent the data on changes in the erythrocytes count of the blood of fish *Cyprinus carpio* to sublethal concentration of ammonia for 35 days. During the treatment, the erythrocyte count found to be decreased throughout the study period from 7 to 35 days showing percent decrease of -15.384, -30.00, -28.57, -26.531, -20.00 exposed to 7, 14, 21, 28 and 35 days respectively. During 7th day minimum percent decrease of -15.384 and a maximum percent decrease of -30.00 on 14th day was also observed in ammonia exposed fish. Table 3. and Figure 2. Reveal the changes in leucocytes count of the blood of fish *Cyprinus carpio* exposed to sublethal concentration of ammonia for 35 days. The WBC count was decreased throughout the study period showing a percent decrease of -25.257, -35.37, -45.85, -46.501, -51.041 from 7th to 35th day respectively.

Introduction

Ammonia is widespread in the environment due to agricultural runoff, industrial runoff and decomposition of biological waste. Ammonia is a major toxicant to fishes and other aquatic life. It enters natural water system from several sources including industrial waste, sewage effluents, coal gasification and liquefaction conversion process plants and agricultural discharges including feed lot runoff. It is a metabolic waste product to fishes. Ammonia is toxic to all vertebrates causing convulsions, coma and death, probably because elevated NH^{4+} displaces K and depolarizes neuron which leads to Ca^{+} and subsequent cell death in the central nervous system (Randall DJ, Tsui TK, 2002). The term ammonia refers to two chemical species which are in equilibrium in water, NH_3, ionized form and NH^{4+} unionised form.The toxicity of ammonia is primarily attributable to NH_3, as opposed to NH^{4+} and their equilibrium in water depending on pH and temperature (USEPA. 1999).

Ammonia is the major end product in the breakdown of feed in fish. Fish digest the protein in their feed and excrete ammonia through their gills and in their feacus.

Ammonia tends to block oxygen transfer from the gills to the blood and can cause both immediate and long term gill damage. Mucous producing membranes can be destroyed, reducing both the external slime coat and damaging the internal intestinal surfaces. Fish suffering from ammonia usually appear sluggish, often at the surface gasping for air (Robert *et al*., 1997). Total ammonia nitrogen (TAN) is composed of toxic (un-ionised) ammonia (NH3) and nontoxic (ionized) ammonia. Only a fraction of TAN exists as toxic (un-ionised) ammonia, and a balance exists between it and the nontoxic ionized ammonia. The final stage of the natural biological metabolic waste conversion is the result of the bacterial breakdown of ammonia, nitrite and nitrate. Ammonia toxicity to fish was investigated (Thurston, *et al*., 1986, Tomasso and Carmichael 1986), (Ogbonna and Chinomso.2010).

Reduction of RBC count in fish blood after cadmium in *Tilapia zilli* (Ruparelia *et al*., 1990), nickel in *Silurus glanis* (Sobecka, 2001), and in *Claries gariepinus* (Ololade and Ogini, 2009). In contrast, significant increase in RBC count was noticed in *Cyprinus carpio* (Drastichova *et al*., 2004) and *Tinca tinca* (Witeska *et al*., 2006). Similarly significant increase in RBC count was noted in *Prochilodus scorfa* exposed to copper (Cerqueira and Fernanders, 2002), in *Hoplias malabaricus* exposed to mercury (Oliveira Ribeiro *et al*., 2006), in *Clarias gariepinus* exposed to selenium (Abdel-Tawwab *et al*., 2007a) in *Tilapia zilli* exposed to aluminum exposure to *Rainbow trout Oncorhynchus mykiss* (Writters *et al*., 1990a). In this study toxicant ammonia exposed to Cyprinus carpio showed decreased result.

Variation in leucocyte count provides a more sensitive index of stress than the changes of erythrocyte abundance. A significant increase in WBC count was noted in fish exposed to various toxicants (Mandal and Lahiri, 1985; Sen *et al*., 1992; Witeska, 2004). Leucocyte was also noticed in *Colisa fasciatus* exposed to chromium (Srivastava *et al*., 1979), in *Scyliohinus canicula* exposed to zinc (Flos *et al*., 1987), in *Channa punctatus* exposed to copper (Singh *et al*., 2008). On the other hand, leucocytes count declined in Tilapia zillii exposed to cadmium (Ghazaly, 1992), in *Silurus glanis* exposed to nickel (Sobecka, 2001), in *Claries gariepinus* exposed to zinc (Ololade and Ogini, 2009) and in cadmium exposure in *Tilapia zilli* (Ghazaly, 1992). During the present study toxicant ammonia exposed to freshwater fish *Cyprinus carpio* results in decreased level.

Materials and Methods

To assess the haematological profile of the control and treated fish, RBC and WBC counts 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.

Erythrocyte count

Erythrocytes were counted by the method of Rusia and Sood (1992) using haemocytometer.

Calculation

$$\text{No. of erythrocytes (million/cu.mm of blood)} = \frac{\text{No. of erythrocyte X Dilution counted}}{\text{Area counted X Depth of fluid}}$$

Dilution - 200
Area counted - 5 X 0.04 = 0.2 square mm
Depth of fluid - 0.1 mm

Leucocyte count

Leucocytes were counted by the method of Rusia and sood (1992) using haemocytometer.

Calculation

$$\text{No. of leucocytes (1000/cu.mm of blood)} = \frac{\text{No. of leucocytes X Dilution counted}}{\text{Area counted X Depth of fluid}}$$

Dilution - 20
Area counted - 4 X 1 = 4 square.mm
Depth of fluid - 0.1 mm

Results and Discussion

Table 1 and Fig.1 Represent the changes in the erythrocyte count of blood of fish *Cyprinus carpio* exposed to sublethal concentration of ammonia exposed fish for 35 days. During the treatment, the erythrocyte count found to be decreased throughout the study period from 7 to 35 days showing a percent decrease of -15.384, -30.00, -28.57, -26.531, -20.00 exposed to 7, 14, 21, 28, and 35 days respectively. During 7^{th} day a minimum percent decrease of -15.384 and a maximum percent decrease of -30.00 on 14^{th} day was also observed in ammonia exposed fish.

Table 2 and Fig 2. Reveal the changes in leucocyte count of blood of fish *Cyprinus carpio* exposed to sublethal concentration of ammonia for 35 days. The WBC count was decreased throughout the study period showing a percent decrease of -25.257, -35.37, -45.85, -46.501, -51.041 from 7 to 35 days respectively. The minimum percent decrease on 7^{th} day is -25.257 and a maximum percent decrease on 35^{th} day is -51.041.

Ammonia is the main nitrogenous products excreted by teleost, and mainly excreted as the unionized form NH_3 (Smith, 1929b). The term ammonia refers to two chemical species which are in equilibrium in water (NH_3, Un-ionized form and NH^{4+}, ionized form). Tests for ammonia usually measure total ammonia nitrogen (NH_3 plus NH^{4+}). The toxicity to ammonia is primarily attributable to NH_3, as opposed to NH^{4+} and their equilibrium in water depending on pH and temperature (USEPA.1999). The toxicity of ammonia to fish and other aquatic organisms is primarily attributed to NH_3 and most biological membranes are permeable to NH_3, But relatively impermeable to NH^{4+} (Randall and Tsui, 2002).

Ammonia is a product of protein catabolism in animals and is one of the best known waste products with a direct and negative influence on fish and most fish do not produce urea due to the high energy cost as compared to excretion of ammonia via gills (Wood 1993). The toxicity of the total ammonia expressed as the sun of NH_3 and NH^{4+} depends on the waste pH. Ammonia toxicity becomes un-ionized ammonia and is more toxic than ammonium ion. The importance of unionized ammonia was first recognized when it was observed that increased pH cause total ammonia to appear to be much more toxic. The portion of the un-ionized form more toxic increases as the pH increases (Randall and Tsui 2002). Increased level of un-ionized level of ammonia (NH_3) in water cause an increases of its content in the blood of fish through diffusion and poisoning of the central nerves system (svobodova et al., 2007). However, Brinkman (2009) reported that chronic exposure in early life stages to ammonia concentrations as low of unionized ammonia nitrogen (UIAN) caused significant reductions in survival, growth and biomass.

Table.1 Changes in the red blood cell (RBC) count of Cyprinus carpio exposed to sublethal concentration of ammonia for 35 days

S.NO	EXPOSURE PERIOD	CONTROL	EXPERIMENT	CHANGE %	CALCULATED t VALUE
1	7	0.65 ± 0.45	0.55 ± 0.013	-15.384	0.91
2	14	0.60 ± 0.45	0.42 ± 0.71	-30.00	2.4
3	21	0.56 ± 0.71	0.40 ± 0.71	-28.57	2.57
4	28	0.49 ± 1.41	0.36 ± 0.45	-26.53	1.24
5	35	0.30 ± 0.71	0.24 ±1.013	-20.00	1.77

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

Table.2 Changes in the white blood cell (WBC) count of Cyprinus carpio exposed to sublethal concentration of ammonia for 35 days

S.NO	EXPOSURE PERIOD	CONTROL	EXPERIMENT	CHANGE %	CALCULATED t VALUE
1	7	14.61 ± 0.70	10.92 ± 0.43	-25.257	-1.18
2	14	13.09 ± 0.53	8.46 ± 0.50	-36.37	-0.90
3	21	12.91 ± 0.57	6.99 ± 0.34	-45.85	-0.59
4	28	10.86 ± 0.4	5.81 ± 0.38	-46.501	-0.57
5	35	10.09 ± 0.71	4.94 ± 0.39	-51.041	-0.39

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

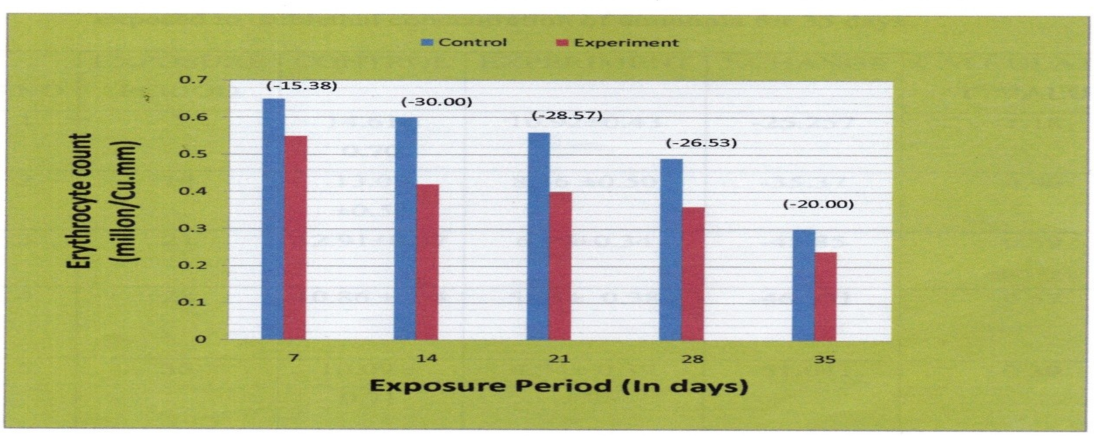

Fig. 1

Fig. 1 . Erythrocyte count of *Cyprinus carpio* exposed to sublethal concentration of ammonia for 35 days. Error bars indicate the standard error of the mean.

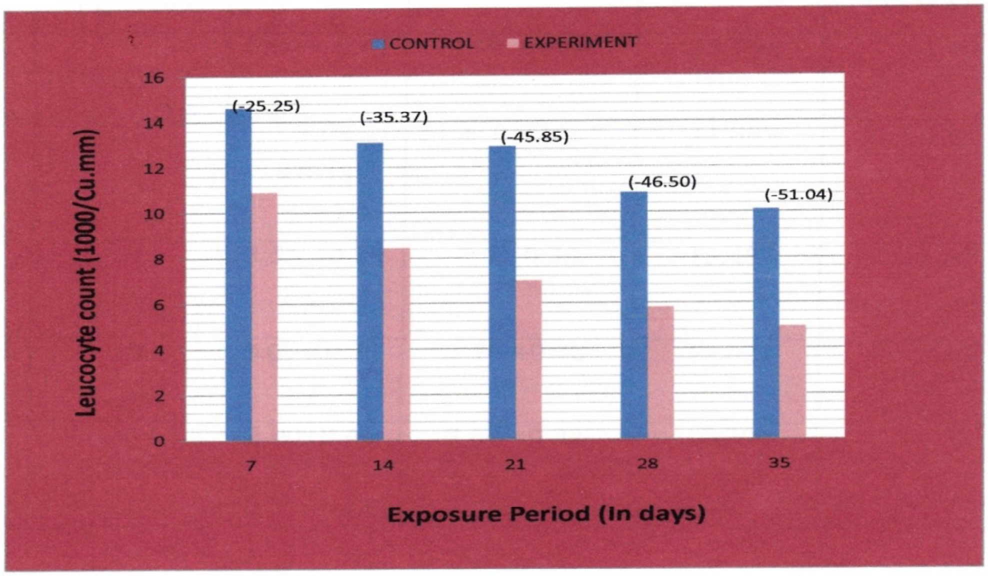

Fig. 2

Fig. 2. Leucocyte count of *Cyprinus carpio* exposed to sublethal concentration of ammonia for 35 days. Error bars indicate the standard error of the mean.

A significant decrease in erythrocyte count, and leucocyte count was observed in the blood of *Catfish* as a response to 24th exposure to increasing concentrations of nickel in water (Sobecka, 2001). He also suggested that the decrease in the number of circulating erythrocytes can be explained by a response of fish organisms to hormone increase as a result of stress caused by the presence of nickel. Exposure of *Colisa fasciatus* to sub lethal concentrations of lead produced hemolytic anemia due to lysis of erythrocytes with concomitant decrease in the Hb content, Ht value and the number of erythorcytes (Srivastava and Agarwal, 1979). It is well known that glycolsis concerned with the reduction of methemoglobin, thus maintaining the iron of the hemoglobin in the 2q ferrous form. In the present study during sublethal treatment decrease in erythrocytes count might be due to anemia, which acts as an indicator with subsequent result of inhibition of erythropoietin in the haemopoietic organisms.

McLeay and Gordon (1997) reported that the variation in leucocyte count provide more sensitive index of stress than do changes in erythrocyte abundance. (Sen *et al*., 1992). Increase in WBC count in fishes exposed to chronic and lethal doses indicates leucocytosis, Sen *et al*. (1992) reported that significant increase in WBC count in *Channa punctatus* exposed to sub lethal doses of zinc. The above authors reported that the increase in WBC count in *Oncorhynchus kisutch* exposed to bleached Kraft pulp mill effluent and suggested removal of cell debris. Changes in WBC in response to stress have reported in many fishes. While an increase in WBC has been reported by (Nussey *et al*., 2002), a reduction has been reported under stress (Svobodova *et al*., 2007). Almost similar to the initial nitrite exposure groups. A similar increase in the WBC in response to nitrite was also reported in *Mirgala* (Das 2004). However, in that similar TLC s with subsequent exposure after nitrite concentration to that of control and experiment, concentration treatment groups indicated that the toxic effect of nitrite on leucopoiesis did not persist (Nussey *et al*.,2002). Changes in leucocytes counts after exposure to pollutants may be associated to a decrease in nonspecific immunity of fish (Oliveira Riberiro *et al*., 2006). Although the response to environmental impacts varies with the type and severity of the stress, it often leads to a leucopenia, which is similar to the classic leucocytic response to stress in animals. In this study exposure of ammonia in fish blood of Cyprinus carpio results that significant decrease of WBC may be due to the manifestation of leucocytosis with heterophilia lymphopenia which are characteristics leucocytes responses in animals exhibiting stress.

References

Abdel – Tawwab, M., Mousa, M.A.A., Abbass, Ff., 2007a. Growth performance and physiological response of Affrican carfish, Clarias gariepin fed fed organic selenioum prior to the exporsure to environmental copper toxicity. Aquaculture, 272(1-4), 335-345.32.

Adhikari.s., sarkar, b., chatterjee, a., mahapatra c.t., ayyappan, s., 2004. Effects of cypermethrin and carbofuran on certain hematological paramenters and predication of theirrecovery in a freshwater teleost: Labeo rohita (Hamilton). Ecotoxicol. Environ. Saf., 58.220-226.

APHA, AWWA, WPCF, 185. In: Standard Methods for th Examination of water and Waste(16th Eds). American Public Health Asssociation Public Health Assciation, Washington, USA.

Avilez, i.m., altran, a.e., aquiar, l.h., Moraes, g., 2004. Hematological responses of the neotropical teleost Matrinxa (Brycon cephalus) to environmental itrite. Compp. Biochem. Physiol. C.135-139

Brinkman, S. F., 2009. Chronic toxicity of Ammonia to early life stage Rainbow trout. Trans. Am. Fish. Soc. 138: 433-440.

Cairns, J., 1984. Estimating hazard. Biosci., 3,7.

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.

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.

CCME. 1999. (Canadian Council of the Environment). Canaian Environmental quality Guidelines. Canadian Council of Ministers of the Environment. Winnipeg. Updated.

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.

Das, P. C., Ayyappan, S., Jena, J. K., Das, B. K., 2004. Acute toxicity of Ammonia and its sublethal effects on selscted hematological and enzymatic parameter of mrigala, Cirrhinus mrigala. (Hamilton). Aquacult. Res. 35, 134-143.

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.

Finney, D. J., 1978. Statistical method in biological assay (3rd Ed), Cambridge University Press. London, pp. 508.

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.

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.

Harding. J., Hoglund, L.B.m, 1983. On accuracy in estimating fish blood cvariables. Cop. Biochem Physiol. A. 75(1),35-40.

Hii, Y.S., Lee, M.Y., Tse, S.C. 2007. Acute toxicity of organochlorine insectide endosulfan and its effect on behavior and some hematologicalparameters of Asian swamp ell (Monopterus albus, Zniew).

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.

Kavitha, C., malavizhi, a., senthil kumaran, S., Ramesh, M., 2010. Toxicological effet of arsenate exposure on hematologil, biochemical and liver transaminases activity in an Indian major carp, Catls catls. Food chem.. toxicol., 48, 2848-2854.

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.

Kori-siakere, O., ake, jeg., idoge, E., 2005. Hemtologial characteristics of the African snakehead, Parachanna obscura. Afr.J.Biotechnol. 4(6),530.

Mandal, A., Lahiri, P., 1985. Haematological response to sumithion (Fenitrothion) in the blue rock pigeon, Columbia livia (Gmelin). Indian J.Exp. Biol., 23, 702-705.

McLeay, D.J. and M.R. Gordon. 1977. Leucocrit: a simple hematological Ntechnique for measuring acute stress in salmonids fish including stressful concentrations of pulpmill effluent. J. Fish. Res. Board Can. 34:2164 - 2175.

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.

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.

Nussey, g., van vuren, J.H.J., du Preez. H.H., 2002. The effect of copper and zzinc at neutral and acidic PH on the general hematology and osmoregulation of Oreochromis mossambicus. Afr.J.Aquat. Sci., 27,61-84.

Ogbonna, J. F., Chinomso, A., 2010. Determination of the concentration of Ammonia that could have lethal effect on fish APRN. J. Eng. Appl.Sci. 5, 1-5.

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.

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.

Ramesh, M., Sivakumari, K., Kanagaraj, M.K., Manavalaramanujam, R., 1994. Impat 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.

Randall. D.J.and T.K. Tsui. 2002. Ammonia toxicity in fish. Mar Pollut Bull. 45 (1-12):17-23.

Robert M.D., David M.C. and Martin W.B. 1997. Ammonia in fish ponds. Southern Regional Adquaculture Center (SRAC). Publication No.463.

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.

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.

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.

Singh. D., Nasth, K., Trivedi, S.P., Sharma, Y.K., 2008. Impact of copper on haematologivcal profile of freshwater fish, Channa punctatus. J.Environ. Biol., 29(2), 253-257.

Smith, H. W., 1929b. The excretion of Ammonia and urea by the gills of fish. J. Biol. Chem. 81, 727-742.

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.

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. Sprague, J., 1986. Measurement of pollutant toxicity to fish. I. Bioassay methods for acute toxicity. Water Res., 22, 793-821.

Spurny, P., 1998: Fin condition of fish kept in Aquacultural system. Acta. Uni. Agri. Silcul. Mend. Brun. Lxi. 138 pp. 80–7157–341.

Svobodova Z., Machhova J., Kroupova H., Smutna M., Groch L., 2007, Ammonia autointoxication of common carp, case studies. Aquacullt. Int. 15,227-286.

Tomasso, J.R., Carmicheal, 1986. Comparative toxicity of nitrite tofreshwater fishes. Aquat. Toxicol., 8,129-137.

Thurston, R.V., R.C. Russo, E.L. Meyn, R.K. Zajdel and C.E. Smith, 1986. Chronic toxicity of ammonia to fathead minnows. Trans. Am. Fish. Soci., 115: 196-207.

USPEA, 2001. Updae Of Ambient Water Quality Criteria For Cadmium. EPA-822-R-01.001, Office of water, US EnvironmentalProtection Agency., Washington, DC, pp.

USEPA Environmental Protection Agency, 1999. Update Of Ambient Water Quality Criteria For Ammonia. EPA 99-104, 822-R-014. 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.

Witeska, M., 2004. The effect of toxic chemicals of blood cell morphology in fish. Fresen. Environ. Bull., 13(12a), 1379-1384.

Witeska, M., Jezierska, B., Wolnickl, J., 2006. Respiratory and hematological response of tench. Tinca (L.) to a short-term cadmium exposure. Aquacult. Int, 14, 141-152.

Wood, C. M., Ammonia and urea metabolism and excretion. In: Ewans D. H., (Ed.) Physiology of fishes, CRC press Boca Raton, pp. 379-425.

Writters, H. E., Van puymbroeck, S., Van Den Sande. I., Vanderbordgt. O. L. J., 1990a. Haematological disturbances and osmatic shifts in Rainbow trout, Oncorhynchus mykiss (Walbaum) under acid and aluminium exposure. J. Comp. Physiol. B, 160, 563-571.

Wicks, B.J. and D.J. Randall, 2002. The effect of feeding and fasting on ammonia toxicity in juvenile rainbow trout *Oncorhynchus mykiss*. Aqua. Toxicol., 59: 71-82.