J.K.A.U.: Sci., vol. 2, pp. 55-62 (1410 A.H. / 1990 A.D.)

Biochemical and Behavioural Responses of Common Carp *Cyprinus carpio* to Sulphuric Acid

H.F. AL-KAHEM, ALI S. AL-AKEL, Z. AHMAD,
M.J.K. SHAMSI AND M.A. CHAUDHARY
Department of Zoology, College of Science,
King Saud University, Riyadh, Kingdom of Saudi Arabia.

ABSTRACT. Common carp, *Cyprinus carpio*, was exposed to different concentration of sulphuric acid (H_2SO_4). The calculated LC_{50} value was 25 µl/l. The treated fish showed remarkarble behavioural changes. A marked decline in haemoglobin and glycogen content was observed. The physiological/behavioural responses of the fish were directly related to the acid concentrations.

Introduction

The effect of acid deposition in aquatic species has been observed. The fish species are of prime interest due to their economic and ecological significance[1]. In many countries, there is a decrease in the size of populations of freshwater fish associated with increasing acidity in rivers, lakes, watersheds and reservoirs. Mining operations, especially for coal, run off from peat waters, intensive mineral fertilization of cultivated lands, industrial waste water and acid rains are the main sources of acidification[2-8].

The elimination of fishes from habitats polluted by acid precipitation is usually gradual and generally related to reproductive failure[9] or mortality of eggs and fry[10-11], although occasional kills of adult fish do occur[12].

Recent researches have shown that physiological changes reflected in such parameters as depletion of certain plasma ions[1,13-16], reduced ionoregulatory ability[17] and anoxia[5] appear to be important factors contributing to the fish death in acidified water.

Histopathological changes in liver, epithelial lining, muscle layers and connective tissue of the gut in carp exposed to pH 5.0-7.0 have been documented[18]. In the present study, authors have studied the behavioural and biochemical changes in the fish exposed to different concentrations of sulphuric acid. LC_{50} of this to carp, *Cyprinus carpio* was also investigated.

Material and Methods

Specimens of carp, *Cyprinus carpio* (Length 14-15 cm, weight 62-68 gm), obtained from fish farm (Dirāb), were kept in aquaria of 70 gallon capacity and acclimatized to laboratory conditions (water temperature, 22.0 ± 0.5°C, dissolved oxygen, 7.8 ± 0.4 mg/l, hardness of water as $CaCo_3$ 132.0 ± 3.5 mg/l, and pH, 7.5 ± 0.4) for 15 days. During the period of acclimation, the fishes were fed a commercial fish food to satiety. As per the prevalent practice in toxicological investigation, the fishes were deprived of food during the bio-essay.

Five different concentrations (15, 20, 25, 30 and 40 µl/l with pH values 3.9, 3.7, 3.5, 3.3 and 3.0, respectively) were prepared by adding required quantity of sulphuric acid into 30 liters of stored tap water. Ten fishes were transferred in each test solution and exposed for 96 hours. The experiment was run in two replicates. Equal number of fish (ten) representing control set were also released in a separate container having the same volume of water but without sulphuric acid. Dead fishes were removed immediately after death and their number recorded. LC_{50} and regression equation :

Y (Probit Kill) $= a + b\,x$ (Log concentration were calculated by the method of Finney[19].

In another set of experiment, fishes were exposed to four different concentrations (10, 15, 20 and 25 µl/l) for 48 hours. Six fishes from each solution were taken and sacrified. Blood samples were collected in heparinized vials by excising the caudal peduncle to allow the blood from dorsal aorta to flow out. Same number of fishes were also sacrificed from the control group. Haemoglobin was estimated according to the method of Blaxhal and Daisley[20]. Intensity of colour was read on Backman spectrophotometer at 540 mµ. Haemoglobin was quantified with the help of a calibration curve prepared by using commercially available cyanmethaemoglobin.

Muscle samples were taken from the trunk region below the dorsal fin. Extraction of glycogen from muscle, liver and kidney was done by the method of Ashman and Seed[21] and estimated by the method of Montogomery[22]. The behavioural observations being based on direct examination and were made at 12 hours intervals for 15 minutes. Student "t" test was employed to test the significance of the results obtained.

Results

Data related to mortality of carp exposed to sulphuric acid are represented graphically by Fig. 1 and 2. The LC_{50} of sulphuric acid to *Cyprinus carpio* was found to be 25

µl/l and this corresponds to pH 3.5. The regression equation describing the dosage-mortality relationship was :

Y (Probit Kill) = 4.513 + 6.78 x (Log concentration).

Some of the behavioural changes exhibited by acid exposed fish include: faster rate of opercular beats, coughing and yawning, jerky movement with occasional convulsion and profuse secretion of mucus. Loss of equilibrium was also noted.

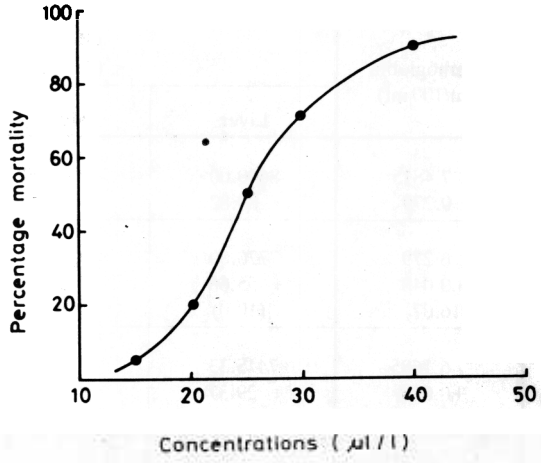


FIG. 1. Percentage of mortality of *C. carpio* in 96 hours at different concentrations of sulphuric acid

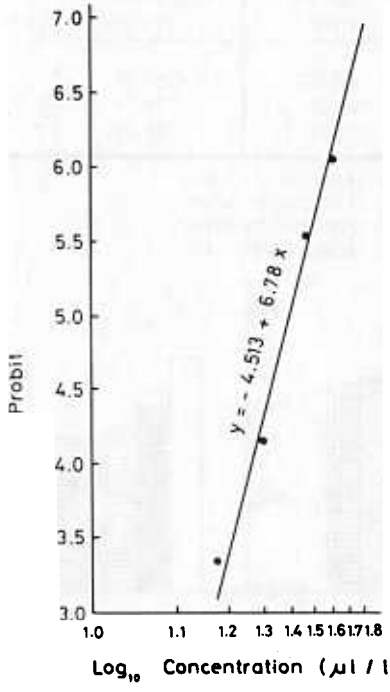


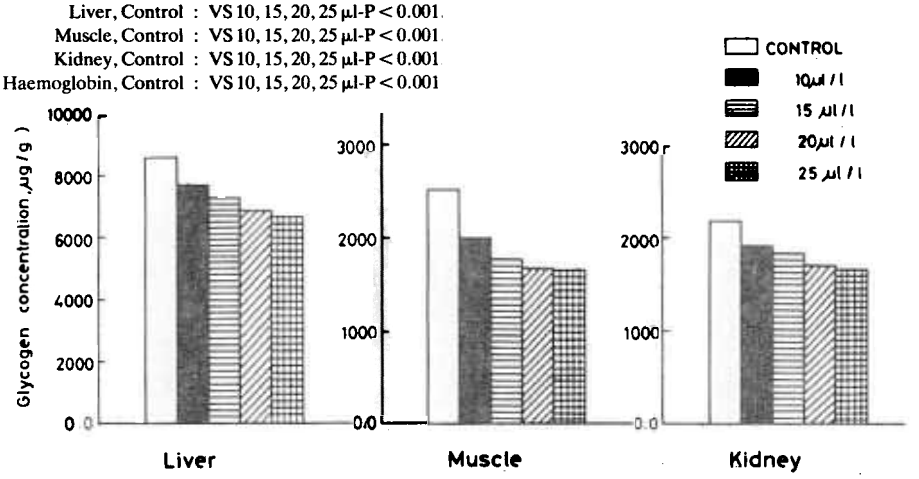
FIG. 2. Relationship between Probit Kill (y) and log concentration (x).

Data pertaining to the changes in the concentration of haemoglobin and glycogen in acid exposed fish are given in Table 1. The data reveal a significant ($P < 0.001$) decline in haemoglobin concentration of the acid exposed fish. A significant ($P < 0.001$) fall in the glycogen level of muscle, kidney and liver of acid treated fish was registered. The magnitude of depletion was directly related to the acid concentration.

TABLE 1. Haemoglobin and tissue glycogen contents of *Cyprinus carpio* exposed to sulphuric acid. Values are mean ± standard deviation. Values given in parentheses indicate the percentage changes from the respective control.

Concentrations (µl/l)	Haemoglobin (gm/100 ml)	Glycogen (µmg/gm)		
		Liver	Muscle	Kidney
Control	7.4815 +0.279	8640.00 + 84.82	2528.00 + 24.15	2189.33 + 31.42
10	6.279 +0.048 (16.07)	7776.00 + 55.66 (10.0)	2010.00 + 21.88 (20.49)	1914.66 + 48.97 (12.54)
15	6.1685 +0.066 (17.55)	7445.33 + 29.37 (13.83)	1773.33 + 25.41 (29.85)	1848.00 + 28.16 (15.59)
20	6.123 +0.072 (18.16)	6949.33 + 47.35 (19.57)	1682.00 + 27.30 (33.46)	1733.33 + 22.20 (20.83)
25	6.0321 +0.128 (19.37)	6789.33 + 78.71 (21.42)	1664.00 + 42.15 (34.18)	1682.00 + 21.80 (23.17)

Liver, Control : VS 10, 15, 20, 25 µl-$P < 0.001$
Muscle, Control : VS 10, 15, 20, 25 µl-$P < 0.001$
Kidney, Control : VS 10, 15, 20, 25 µl-$P < 0.001$
Haemoglobin, Control : VS 10, 15, 20, 25 µl-$P < 0.001$



3. Changes in glycogen content of liver, muscle and kidney of *C. carpio* exposed to different concentrations of sulphuric acid.

Discussion

Exposure of *Cyprinus carpio* to different concentrations of sulphuric acid brought about a general hyperexcitability. It is clear from the behavioural pattern that acid causes severe irritation and fish struggled in an attempt to get relieved from stressful environment. The fish exposed to low pH value secreted mucus profusely to protect the gills and body from the irritating effect of toxicant. The deposition of mucus on the gill filaments may reduce the interfilamentous space, thus probably causes obstructions in the passage of water. The mucus deposition may also reduce diffusion of gases causing reduced supply of oxygen[23,24]. Elevated muscular activity of acid treated fish reported above also demands more supply of oxygen. The increased demand for oxygen and reduced diffusion due to mucus deposition on gill filaments may be compensated by increasing the frequency of yawning, coughing and opercular beats. The former two are associated with the clearing of gill surfaces from mucus[25,26], while the opercular beats are mainly related to increase the passage of water through gills. Thus, all the above measures enable the acid exposed fish to get more oxygen. The foresaid hypothesis has also been supported[27].

Data pertaining to haemoglobin and glycogen embodied in Table 1 reveal a sharp decline in the level of these molecules in exposed fish. Depletion in the level of haemoglobin could be attributed to the probable disruption in the pathway of haemoglobin synthesis, possibly by checking the haemoglobin synthesizing ability of fish. Secondly, to the inhibition in the secretion of haemopoietin from haemopoietic organs, is checking the production of erythrocytes. Further works are required to know the real mechanism involved in the depletion of haemoglobin.

The stress exerted by acid enhanced the breakdown of muscle, liver and kidney glycogen reserve. Increased glycolysis should be expected in exercising fish as a means of stepping up the glucose supply to the mechanical tissue. The available evidences leave no doubt that glycogen is the stored form of energy which can be easily mobilized and glucose is the most readily utilizable source of energy[28]. The percentage depletion of glycogen level was more in muscle compared to kidney and liver, Table 1. This indicates that muscle glycogen is utilized first by the acid exposed fish to meet the energy requirements and save its reserves in the liver to perform other vital functions of life. The findings[29-31] lend a considerable support to the present investigation. They reported a significant decline of glycogen content in the fish exposed to metals and attributed it to increased muscular activity of fish brought about by toxicants.

References

[1] **Powell, J.F.** and **McKeown,** The effect of acid exposure on the ion regulation and sea water adaptation of coho salmon *(Onchorhynchus kisutch)* parrs and smolts, *Comp. Biochem. Physiol.* **83C:** 45-52 (1986).

[2] **Cogbell, C.V.** and **Likens, G.E.,** Acid precipitation in north eastern United States, *Water Resource Res.* **10:** 1133-1137 (1974).

[3] **Oden, S.,** The acidity problem, an outline of concepts, *Water, Air and Soil Pollution* **6:** 137-166 (1976).

[4] **Dillon, P.J., Jeffries, D.S., Snyder, W., Reid, R., Yan, N.D., Evans, D., Mass, J.** and **Schneider, W.A.**, Acidic precipitation in south-central Ontario: Recent observations, *J. Fish. Res. Bd. Can.* **35:** 809-815 (1978).

[5] **Ultsch, G.R.**, Oxygen consumption as a function of pH in three species of freshwater fishes, *Copeia.* **2:** 272-279 (1978).

[6] **Ultsch, G.R.** and **Gros, G.**, Mucus as a diffusion barrier to oxygen: possible role in O_2 uptake at low pH in carp *(Cyprinus carpio)* gills, *Comp. Biochem. Physiol.* **62A:** 685-689 (1979).

[7] **Haines, T.A.**, Acidic precipitation and its consequences for aquatic ecosystem: a review., *Trans. Amer. Fish. Soc.* **110:** 669-707 (1981).

[8] **Fugelli, K.** and **Vislie, T.**, Physiological response to acid water in brown trout (*Salmo trutta* L.): Cell volume regulation in heart ventricle tissue, *J. Exp. Biol.* **101:** 71-82 (1982).

[9] **Beamish, R.**, Acidification of lakes in Canada by acid precipitation and the resulting effects on fishes, pp. 479-498, *in:* **Dochinger, L.** and **Seliga, T. (eds.)** *Proceedings of the First International Symposium on Acid Precipitation and the Forest Ecosystem USDA, Forest Service General Technical Report* **NE-23**, pp. 479-498 (1976).

[10] **Johansson, N.** and **Kihlstron, J.**, Pikes (*Esox lucius* L.) shown to be affected by low pH values during first week after hatching, *Environ. Res.* **9:** 12-17 (1975).

[11] **Leivestad, H., Hendry, G., Muniz, I.** and **Snekvik, E.**, Effect of acid precipitation on freshwater organism: *in:* **Braekke, F. (ed.)** Impact of acid precipitation on forest and freshwater ecosystems in Norway, *Research Report* **6176,** *SNSF. Project MSK, 1432 Aas-NLN, Norway,* 87-111 (1976).

[12] **Leivestad, H.** and **Muniz, I.**, Fish kill at low pH in a Norwegian river, *Nature* **259:** 391-392 (1976).

[13] **Packer, R.K.** and **Dunson, W.A.**, Effect of low environmental pH on blood pH and sodium balance of brook trout, *J. Exp. Zool.* **174:** 65-72 (1970).

[14] **Packer, R.K.** and **Dunson, W.A.**, Anoxia and sodium loss associated with the death of brook trout at low pH, *Comp. Biochem. Physiol.* **41A:** 17-26 (1972).

[15] **Lyons, J.**, Effect of lethal acidity on plasma sodium concentrations in yellow perch *(Perca flavescens)* from a naturally acidic and a naturally alkaline lake, *Comp. Biochem. Physiol.* **73A:** 437-440 (1982).

[16] **McKeown, B.A., Green, G.H., Walson, T.A., Powell, J.F.** and **Parker, D.B.**, The effect of pH on plasma electrolytes, carbonic anhydrase and ATPase activities in rainbow trout *(Salmo gairdneri)* and large scale suckers *(Catostomus macrocheilus), Comp. Biochem. Physiol.* **80A:** 507-514 (1985).

[17] **Saunders, R.L., Henderson, E.B., Harmon, P.R., Johnston, C.E.** and **Eales, J.G.**, Effects of low environmental pH on smolting of Atlantic salmon *(Salmo salar), Can. J. Fish. Aquat. Sci.* **40:** 1203-1211 (1983).

[18] **Shrivastava, R.S.** and **Dwivedi, P.**, Studies on the effect of acidic medium on the fry of *Cyprinus carpio, Acta. Hydrochem. Hydrob.* **7:** 421-430 (1979).

[19] **Finney, D.J.**, Probit analysis, *in:* **Goulden, C.H. (ed.)**, *Methods of statistical analysis,* John Wiley, New York (1952).

[20] **Blaxhal, P.C.** and **Daisley, K.W.**, Routine haematological methods for use with fish blood, *J. Fish Biol.* **5:** 771-781 (1973).

[21] **Ashman, P.U.** and **Seed, J.R.**, Biochemical studies in the vole, *Microtous montamus.* I. The daily variation of hepatic glucose-6-phosphatase and liver glycogen, *Comp. Biochem. Physiol.* **45:** 365-378 (1973).

[22] **Montogomery, R.C.**, Determination of glycogen, *Arch. Biochem. Biophys.* **67:** 378-387 (1957).

[23] **Plonka, A.C.** and **Neff, W.H.**, Mucopolysaccharide histochemistry of gill epithelial secretion in brook trout *(Salvelinus fontinalis)* exposed to acid pH, *Proc. Pa. Acad. Sci.* **43:** 53-55 (1969).

[24] **Dively, J.L., Mudge, J.E., Neff, W.H.** and **Anthony, A.**, Blood PO_2, PCO_2 and pH changes in brook trout *(Salvelinus fontinalis)* exposed to sublethal levels of acidity, *Comp. Biochem. Physiol.* **57A:** 347-351 (1977).

[25] **Cairns, M.A., Garton, R.R.** and **Tubb, R.A.**, Use of fish ventillation frequency to estimate chronically safe toxicant concentrations, *Trans. Amer. Fish. Soc.* **111:** 70-77 (1982).

[26] **Henery, M.G.** and **Atchison, G.J.**, Behavioural effects of methyl parathion on social groups of bluegill *(Lepomis macrochirus), Environ. Toxicol. Chem.* **3:** 399-408 (1984).

[27] **Mustafa, S.** and **Murad, A.**, Survival, behavioural response and hamotological profile of Catfish, *Heteropneustes fossilis,* exposed to DDT, *Jap. J. Ichthyol.* **31:** 55-65 (1984).

[28] **Love, R.M.,** *The Chemical Biology of Fishes,* vol. 2, Academic Press, London, 943 p. (1980).
[29] **Shafi, S.A.,** Zinc intoxication in some freshwater fish. I. Variation in tissue energy reserve, *Annals. Limnol.* **16:** 91-97s (1980).
[30] **Shamsi, M.J.K.** and **Al-Akel, A.S.,** Effect of mercuric chloride on the glycogen content in the various tissue of freshwater fish, *Oreochromis niloticus, Proc. Saud. Biol. Soc.* **9:** 271-276 (1986).
[31] **Al-Akel, A.S., Shamsi, M.J.K., Al-Kahem, H.F., Chaudhary, M.A.** and **Ahmad, Z.,** Effect of cadmium on the cichlid fish, *Oreochromis niloticus.* Behavioural and physiological responses, *J. Univer. Kuwait (Sci.)* **15:** 341-345 (1988).

التأثير البيوكيمائي والسلوكي لحمض الكبريتيك على سمك الشَّبُّوط

حمود القحم ، علي بن سليمان العقل ، زبير أحمد ، محمد جاويد و محمد أصغر شودري
قسم علم الحيوان - كلية العلوم - جامعة الملك سعود
الرياض - المملكة العربية السعودية

لقد تم غمر سمك الشبوط *(Cyprinus carpio)* في تركيزات مختلفة من حمض الكبريتيك ونتيجة لهذا الغمر لوحظت تغيرات سلوكية ونقص في الهيموجلوبين والجليكوجين .

وتبين من الدراسة ارتباط الاستجابات السلوكية والفسيولوجية مباشرة مع تركيزات الحمض . وكذلك حسبت الجرعة الحرجة (Lc_{50} = 25µl/l) لهذا الحمض .