Bull. Environ. Contam. Toxicol. (1992) 49:613–619

Environmental Contamination and Toxicology

Effect of Manganese and Iron at a Neutral and Acidic pH on the Hematology of the Banded Tilapia (*Tilapia sparrmanii*)

V. Wepener, J. H. J. Van Vuren, and H. H. Du Preez

Research Unit for Aquatic and Terrestrial Ecosystems, Department of Zoology, Rand Afrikaans University, PO Box 524, Johannesburg, 2000, South Africa

The pollution of natural water bodies is a common phenomenon in developing countries. Increases in population densities lead to increased mining and industrial activities in the area. With the establishment of gold and coal mines in South Africa, several industrial zones were created to support the mining industry. Many of these industries consist of heavy metal processing factories. Over the years pollution from the mines has led to acidification of the streams and lakes in the Transvaal (Schoonbee et al. 1985). It was also found that high concentrations of heavy metals occurred in the water, sediments, plants and fish tissue in the affected water systems (Bezuidenhout et al. 1990; De Wet et al. 1990). Of all the heavy metals, iron and manganese were found in the highest concentrations.

In order to determine the subtle, non-lethal effects induced by sublethal concentrations of heavy metals on the physiology of fish, it is necessary to monitor certain clinical parameters. The use of hematological methods as indicators of sublethal stress can supply valuable information concerning the physiological reactions of fish in a changing environment. The reason for this is the close association between the circulatory system of the fish and the external environment (Cassilas and Smith 1977). The objective of the present paper was to evaluate the effects of manganese and iron at a neutral and acidic pH on the hematology of Tilapia sparrmanii.

MATERIALS AND METHODS

Banded tilapia (T.sparrmanii) were obtained from the provincial hatchery at Lydenburg, South Africa. T. sparrmanii was chosen as a bio-indicator because of its wide distribution in the affected river systems. Different size fish (40-120g) were caught by seine-netting from the hatchery ponds. The fish were acclimatized for four weeks in well aerated aquarium tanks in the laboratory. During this time they were fed daily on commercial trout pellets (40% protein).

After the acclimation period the fish were transferred to the tanks of the experimental flow-through system. Two bioassays were conducted simultaniously in separate flow-through systems for manganese and iron

Send reprint requests to V. Wepener at the above address.

at concentrations of 4.43 $mg.L^{-1}$ manganese chloride and 1.57 $mg.L^{-1}$ ferric chloride respectively. The iron and manganese concentrations used in this toxicological study were derived by taking the mean iron and manganese concentrations in the river systems of the Witwatersrand as monitored by the Rand Water Board (1989). The bioassay consisted of eight 100 L glass tanks through which the water was circulated by means of a "Swimline" pump and filter. The water temperature was kept at 24 ± 0.5°C (average summer temperature on the Witwatersrand) by means of a heating element which was connected to a thermostat. The required pH was obtained by adding either 0.01 M H_2SO_4 or 0.1 M NaOH to the water of the tanks. The pH was monitored with a pH probe connected to a pump (Hanna Instruments DP/DR Control Pump System) which added the acidic or basic medium to the water depending on the required pH. Sublethal acute exposures were carried out at a pH of either 7.4 or 5 for 96 hours, while untreated fish were kept in the bioassay for two weeks without manipulating the pH before being analyzed to obtain control values for the hematological parameters investigated. A minimum of ten fish were exposed to both metals in the bioassay. The water quality of the well water during the bioassay was: pH 6.95, total hardness as $CaCO_3$- 61 $mg.L^{-1}$, calcium- 10.9 $mg.L^{-1}$, chloride- 6.1 $mg.L^{-1}$, sulfate- 2 $mg.L^{-1}$, nitrate- 1.8 $mg.L^{-1}$ and fluoride- 0.3 $mg.L^{-1}$.

The experimental fish were removed after 96-hrs and blood was immediately collected from the caudal aorta with a 1 mL heparinized (5000 U/mL) syringe. Blood parameters analyzed were red blood cell count (RBC), hemoglobin concentration (Hb), mean corpuscular volume (MCV), delta-aminolevulinic dehydratase activity (ALA-D), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH), hematocrit (Hct) and total white blood cell count (WBC). The hematocrit was determined according to the method described by Korzhuev (1964) and the RBC, WBC and MCV values were obtained by employing a "Sysmex CC-120 Microcell Counter". The Hb was determined by using the cyanmet-hemoglobin method (Blaxhall and Daisley 1973). The MCH was calculated in picograms/cell = Hb/RBC X 10 and the MCHC as the Hb in 100 mL blood/Hct X 100 (Dacie and Lewis 1963). The ALA-D activity was measured according to the method described by Hodson (1976). The porphobilinogen produced from the reaction between whole blood and amino levulinic acid was measured by adding Erlich's reagent and recording the optical density at 553 nm. Statistical analysis was performed using the unpaired Student's t-test (Zar 1984). The significance level was taken as $P < 0.05$.

RESULTS AND DISCUSSION

No mortalities of fish were found during exposures to manganese at either pH. During exposure to iron, nine mortalities were recorded at pH 5 and three at pH 7.4. After exposure to manganese at pH 5, significant decreases were found in the RBC ($P < 0.05$ Fig.1A), Hb ($P < 0.025$ Fig.1B), MCV ($P < 0.025$ Fig.1C), Hct ($P < 0.025$ Fig.1D) and WBC ($P < 0.01$ Fig.1H). The WBC (Fig.1H), RBC (Fig.1A), Hb (Fig.1B) and MCV (Fig.1C) of fish at pH 7.4 decreased significantly ($P < 0.005$). The MCHC (Fig.1E) of fish at pH 5 increased marginally, whereas there was a slight decrease in the case of pH 7.4. The MCH (Fig.1F) of T.sparrmaii at pH 5 decreased slightly and there was an insignificant increase ($P > 0.1$) at pH 7.4. At both

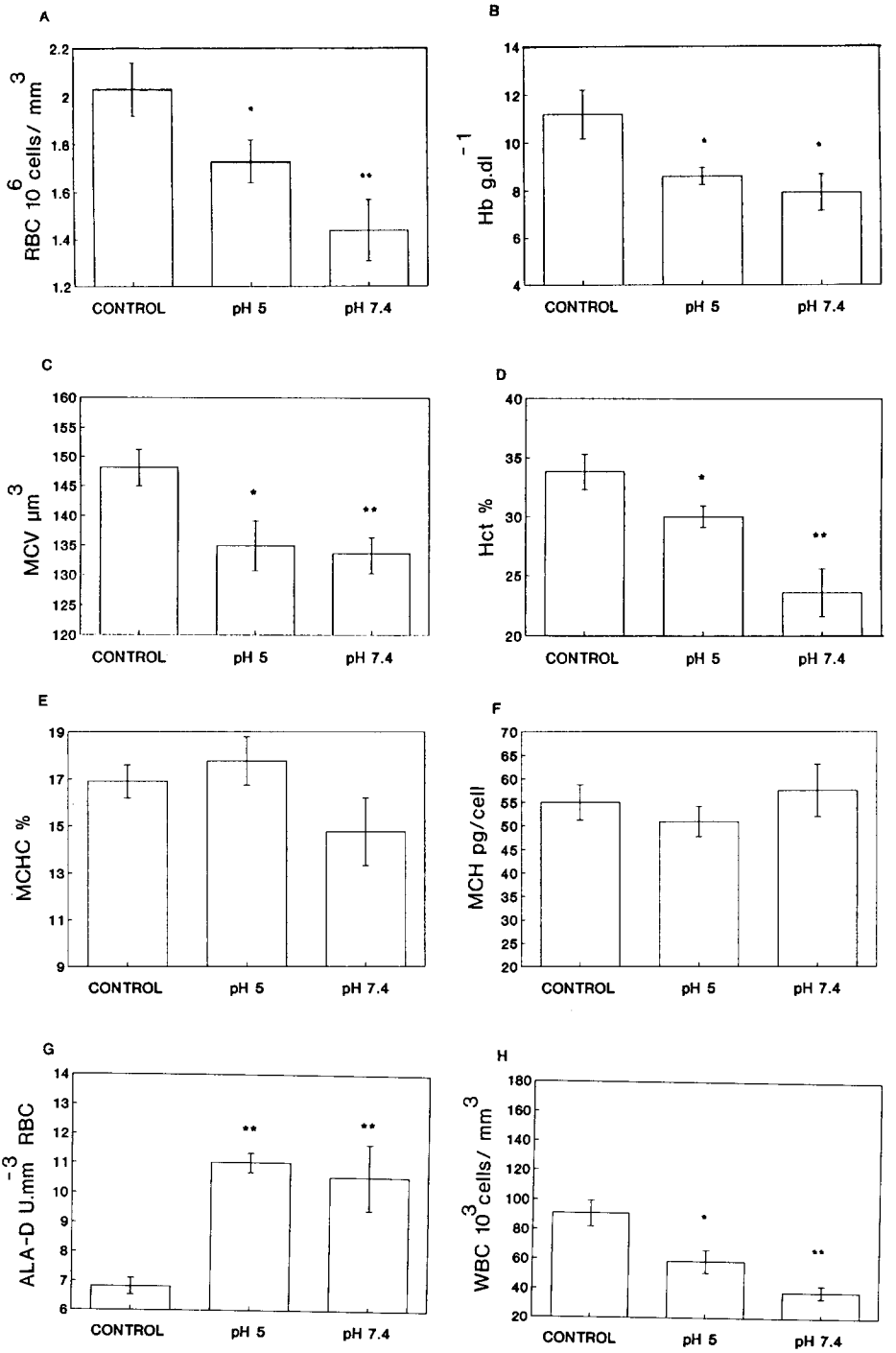

Figure 1.(A-H) Mean values ± standard error (n=10) of RBC (A), Hb (B), MCV (C), Hct (D), MCHC (E), MCH (F), ALA-D (G) and WBC (H) after 96-hour exposure to manganese. Asterisks indicate differences of $0.005 > P < 0.05$ and double asterisks indicate $P < 0.005$.

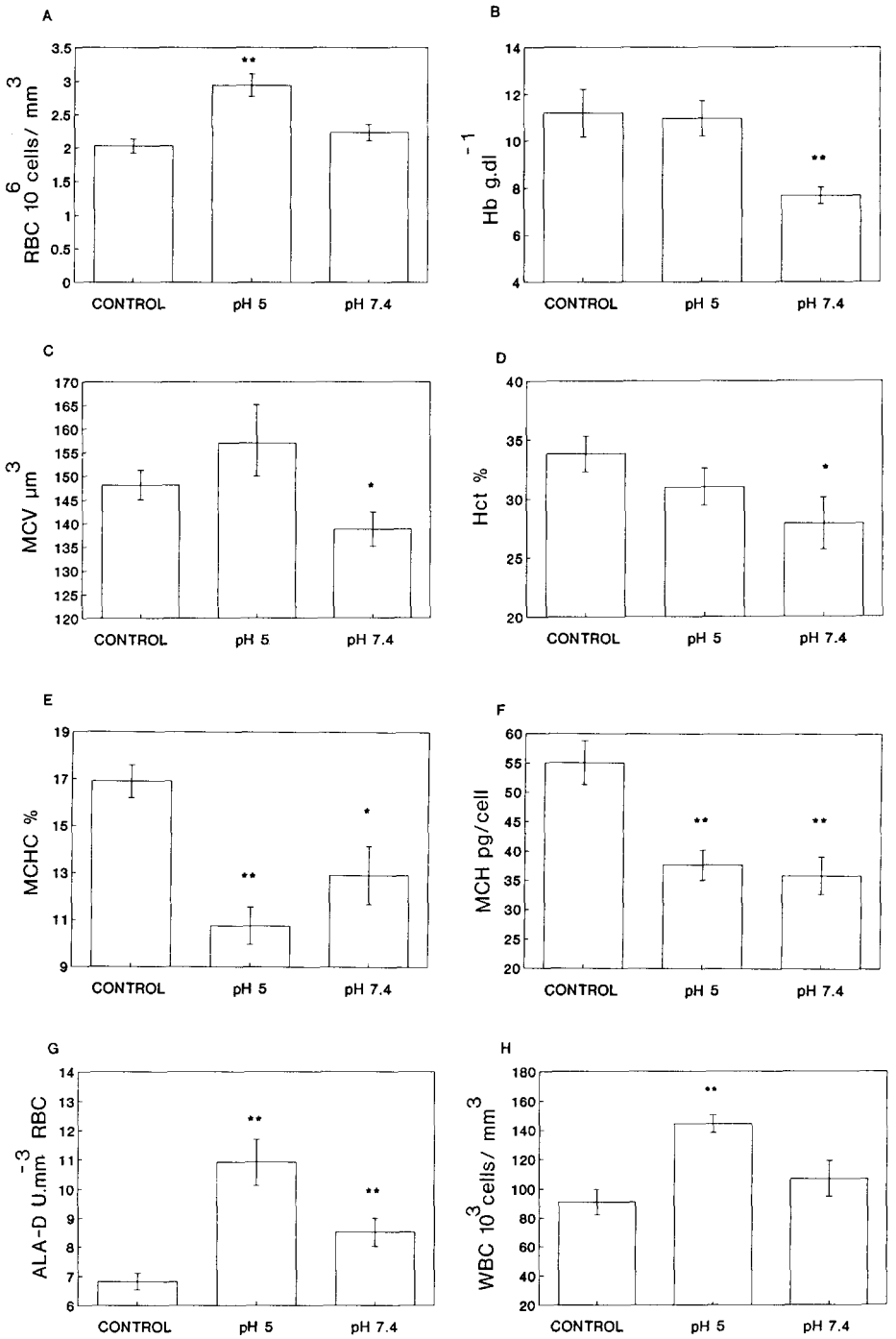

Figure 2.(A-H) Mean values ± standard error (n=10) of RBC (A), Hb (B), MCV (C), Hct (D), MCHC (E), MCH (F), ALA-D (G) and WBC (H) after 96-hour exposure to iron. Asterisks indicate differences of $0.005 > P < 0.05$ and double asterisks indicate $P < 0.005$.

pH values there was a significant increase ($P<0.005$) in the activity of ALA-D (Fig.1G).

After exposures to iron, T. sparrmanii showed an opposite reaction to manganese in that there were increases in the RBC (Fig.2A) and WBC (Fig.2H). Significant increases ($P<0.005$) only occurred at pH 5. At pH 7.4 significant decreases were observed in the Hb ($P<0.005$ Fig.2B), Hct ($P<0.025$ Fig.2D) and MCV ($P<0.05$ Fig.2C), while fish exposed to iron at pH 5 showed slight decreases in Hb ($P>0.1$ Fig.2B) and Hct ($P>0.25$ Fig.2D) whereas an increase was observed in MCV ($P>0.1$ Fig.2C) The MCHC (Fig. 2E) and MCH (Fig.2F) decreased significantly both at pH 5 ($P<0.005$) and pH 7.4 ($P<0.01$). Just as in exposure to manganese, fish exposed to iron at pH 5 and pH 7.4 showed significant increases ($P<0.005$) in ALA-D activity (Fig.2G) of red blood cells.

Manganese and iron are regarded as metals with a relatively low toxicity. It is evident from the available literature that very little work has been done on the effects of these metals on the physiology of fish. The significant decrease in the number of red blood cells and Hct of T.sparrmanii after exposure to manganese at pH 5 and 7.4 can be attributed to internal hemorrhaging that was observed. The bleeding is probably the result of necrosis of the intestinal mucosa and kidneys as observed by Agrawal and Srivastava (1980). The anemic conditions observed could also have resulted from damage to the hemapoietic tissue such as the kidney and spleen. Manganese-induced anemia could thus be seen as a secondary effect of manganese pollution. The significant decreases in the Hb concentrations observed also indicate the anemic conditions experienced by T. sparrmanii.

Decreased MCV is the result of the release of immature red blood cells from hemapoietic tissues. Immature cells are released to compensate for the loss of blood cells and increase the oxygen supply to relieve the hypoxic conditions which were experienced. The hypoxic conditions were caused by hyperplasia and necrosis of the secondary lamellae which led to elevated levels of blood lactic acid (Wepener 1990).

The enzyme ALA-D is a key enzyme in heme biosynthesis and an increase in ALA-D activity in the red blood cells would indicate a stimulation of heme synthesis in the spleen and kidney (Haux et al. 1985). The increase in ALA-D activity is not able to produce sufficient hemoglobin to compensate for the hypoxic conditions experienced. The slight increases and decreases observed in the MCHC and MCH values can not be attributed to cell shrinking or swelling but rather to a disproportional decrease in red blood cells and hemoglobin concentration.

The significant decreases in the WBC may be the result of increased secretion of corticosteroid hormones (Ellis 1981). The secretion of these hormones is a nonspecific response to any environmental stressor and is a fundamental mechanism in the increased susceptibility of fish to disease when exposed to a pollutant. The leucocytopenia (reduction in leucocytes) could further have been aggravated by the necrosis of the leucopoietic tissue.

It is evident that manganese caused greater stress at a neutral pH than an acidic pH. The reason for this is that the toxicity of manganese is not

dependant on the total manganese concentration but on the concentration of oxidized manganese which is available (Nix and Ingols 1981). The oxidation of manganese at low pH values is much slower than higher pH values (Bricker 1965).

Iron is one of the most common heavy metal pollutants found in aquatic ecosystems (Khalaf et al. 1985). In contrast to exposure to manganese, fish exposed to iron had an increase in RBC. The increased RBC is in reaction to hypoxic conditions caused by epithelial lifting of the gill lamellae (Wepener 1990). The Hb concentration of fish exposed to Mn at pH 7.4 decreased sharply due to the fact that there was an increase in the release of immature cells from the hemapoietic tissues. This is supported by the significant decrease in MCV (Fig. 1C). The increased MCV of fish exposed at pH 5 is the result of swelling due to beta adrenergic stimulation brought about by the hypoxic conditions experienced by the test organism (Butler et al. 1978). The decreased MCHC also points to the fact that cell swelling occurred at pH 5 while the decreased MCHC and MCH at pH 7.4 is the result of the increased number of immature red blood cells with a lower Hb concentration that were added to the circulation.

As with manganese, iron caused the stimulation of heme biosynthesis but the increase in ALA-D activity is not proportional to the increased RBC. This supports the hypothesis that there is a "safety factor" involved in the activity of ALA-D (Heath 1987). The increased WBC is the result of stimulation of the immune system to protect the organism against infections which may occur due to iron mediated damage of the gill tissue. From the results it is clear that iron in the form of aqueous cations caused the most stress to the test organism.

From the results of this study it is clear that any external stressor, even those which are considered non lethal, can have a detrimental effect on aquatic organisms. A change in the physical and chemical characteristics of the water could modify the toxicity of the heavy metals which already occur in the freshwater ecosystem.

REFERENCES

Agrawal SJ, Srivastava AK (1980) Haematological responses in a freshwater fish to exprimental manganese poisioning. Toxicology 17:97-100

Bezuidenhout LM, Schoonbee HJ, De Wet LPD (1990) Heavy metal content of the African sharptooth catfish, *Clarias gariepinus* (Burchell), from a Transvaal lake affected by mine and industrial effluents. Part 1. Zinc and copper. Water SA 16:125-129

Blaxhall PC, Daisley KW (1973) Routine haematological methods for use with fish blood. J Fish Biol 5:771-781

Bricker OP (1965) Some stability relationship in the system Mn-O_2-H_2O at 25^{o}C and one atmosphere total pressure. Am Mineral 50:1296-1354

Butler PJ, Taylor EW, Capra MF, Davidson W (1978) The effect of hypoxia on the level of circulating catecholamines in the dogfish *Scyliorhinus canicula*. J Comp Physiol 127:325-330

Cassilas E, Smith LS (1977) Effect of stress on blood coagulation and haematology in rainbow trout (*Salmo gairdneri*). J Fish Biol 10:481-491

Dacie JV, Lewis SM (1963) Practical haematology. J&A Churchill Ltd, London

De Wet LPD, Schoonbee HJ, Pretorius J, Bezuidenhout LM (1990) Bioaccumulation of selected heavy metals by the water fern, *Azolla filiculoides* Lam. in a wetland ecosystem affected by sewage, mine and industrial pollution. Water SA 16:281-286
Ellis AE (1981) Stress and the modulation of defence mechanisms in fish. In: Pickering AD (ed) Stress and Fish, Academic Press, London
Haux C, Sjöbeck ML, Larsson A (1985) Physiological stress responses in a wild fish population of perch (*Perca fluviatilis*) after capture and during subsequent recovery. Mar Environ Res 15:77-95
Heath AG (1987) Water pollution and fish physiology. CRC Press Inc, Boca Raton, Florida
Hodson PV (1976) δ-amino levulinic acid dehydratase activity of fish blood as an indicator of harmful exposure to lead. J Fish Res Board Can 33:268-271
Khalaf AN, Al-Jafery AR, Khalid BY, Elias SS, Ishaq MW (1985) The patterns of accumulation of some heavy metals in *Barbus grypus* (Hickel) from a polluted river. J Biol Sci Res 16:51-75
Korzhuev PA (1964) Methods of study in fish blood. In: Pavlovski EN (ed) Techniques for the Investigation of Fish Physiology, Israel program for Scientific Translations, Jerusalem, pp.2-10
Nix J, Ingols R (1981) Oxidized manganese from hypolimnetic water as a possible cause of trout mortality in hatcheries. Prog Fish-Cult 43:32-36
Rand Water Board (1989) Analyses of water streams in the catchment of the Vaal dam and Vaal River Barrage. Thirty eighth Report
Schoonbee HJ, Vermaak J, Swanepoel, JH (1985) Use of the Chinese grasscarp, *Ctenopharyngodon idella*, in the control of submerged water weed *Potamageton pectinatus* in an inland lake in the Transvaal, South Africa. In: Delfosse ES (ed) Proc VI Int Symp Biol Contr Weeds, Agric Can, Vancouver, pp. 557-565
Wepener V (1990) The effect of heavy metals at different pH values on the haematology and metabolic enzymes of *Tilapia sparmanii* (Cichlidae). M.Sc Thesis (in Afrikaans), Rand Afrikaans University, Johannesburg
Zar RH (1984) Biostatistical analysis. Prentice Hall, Englewood Cliffs, New York

Received March 11, 1992; accepted April 30, 1992.