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Full Length Research Paper

Haematological indices of *Channa punctatus* as an indicator of heavy metal pollution in waste water aquaculture pond, Panethi, India

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Investigations on the concentration of heavy metals (Cu, Ni, Fe, Co, Mn, Cr and Zn) in water and their effects on haematological parameters were observed in *Channa punctatus.* The results reveal that heavy metals available in water were in the order Fe > Mn > Zn > Co > Ni > Cu = Cr. In the present study, the haematological data of *C. punctatus* revealed that significant (P<0.05 and <0.01) decrease was observed in red blood cell count (RBC) count and hemoglobin (Hb) content respectively, but white blood cell (WBC) count showed significant (P<0.01) increase when compared to the control. Increase and decrease in haematological indices means that fish was exposed to effluents containing heavy metals and was under stress.

Key words: Heavy metals, haematology, *Channa punctatus*, RBC, WBC.

INTRODUCTION

Human activity has continuously disturbed the natural environment, particularly the aquatic ecosystems. The use of heavy metals in industries has lead to the wide spread environmental contamination. Consequently the waste water from industries and also the sewage water from domestic sources containing heavy metals find their way into the nearby water bodies. The aquatic pollution due to heavy metals is of major concern, due to their persistence and accumulative nature. Aquatic animals live in very intimate contact with their environment thus, absorb heavy metals from the surrounding contaminated water which ultimately affect their health. Among these animal species, fishes are the inhabitants that cannot escape from the detrimental effects of these pollutants (Olaifa et al., 2004) and are therefore very susceptible to physical and chemical changes which may be reflected in their blood components (Wilson and Taylor, 1993). The metal once absorbed is transported by the blood to either a storage point, such as bone or to the liver for transportation. If transported by the liver, it may be stored there, excreted in bile, or passed back into the blood for possible excretion by kidney or gills or stored in extra hepatic tissues such as fat. This is how heavy metal gets accumulated in different tissues of the fish via blood. Blood parameters are therefore considered as patho-physiological indicators of the whole body and therefore are important in diagnosing the structural and functional status of fish exposed to toxicants (Adhikari et al., 2004). In fish, exposure to chemical pollutants can induce either increase or decrease in haematological levels. A number of haematological indices such as haematocrit (Hct), haemoglobin (Hb), red blood cells (RBCs) and so on, are used to assess the functional status of the oxygen carrying capacity of the bloodstream and have been used as an indicator of metal pollution in the aquatic environment (Shah and Altindag, 2004a). Furthermore, it should be noted that haematological indices are of different sensitivity to various environmental factors and chemicals (Vosyliene, 1999b). Previous haematological

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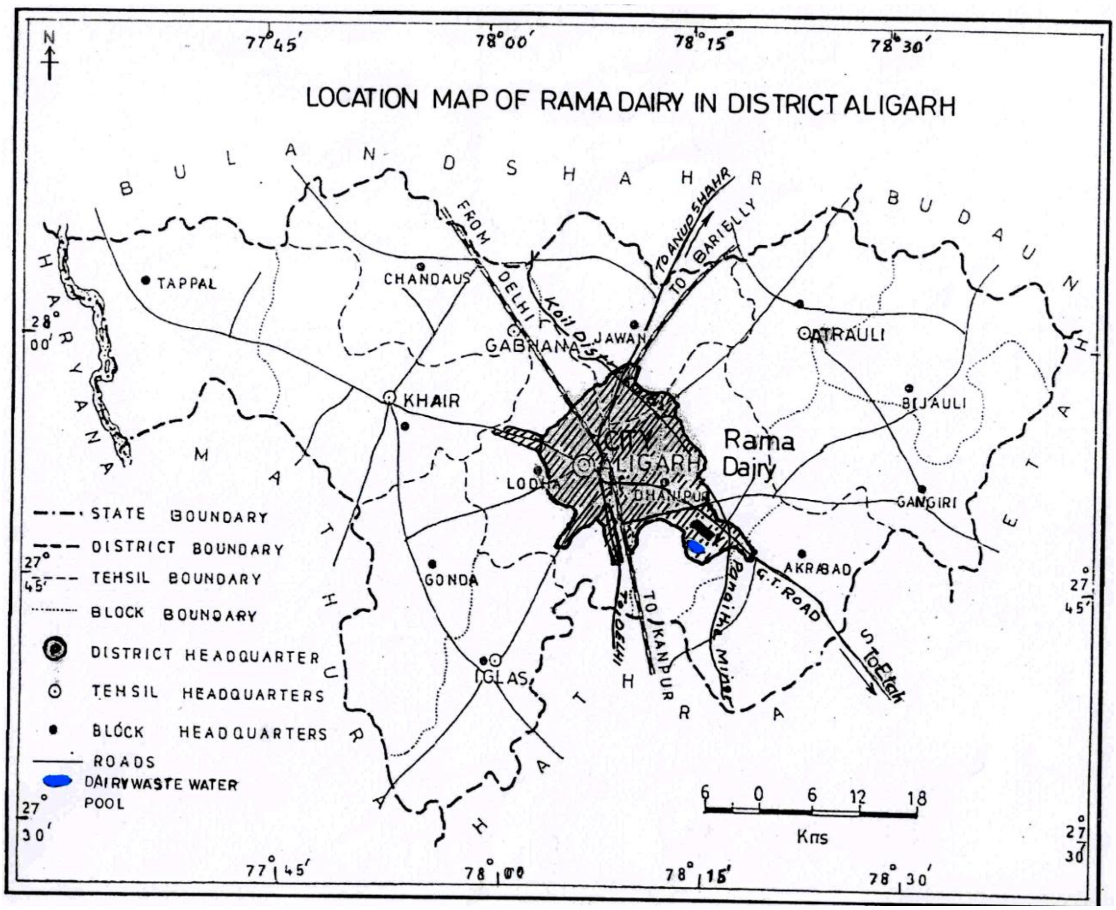

Figure 1. Map showing the location of waste water aquaculture pond near Rama Dairy at Panethi, Aligarh.

study of pollutants brought knowledge that erythrocytes are the major and reliable indicators of various sources of stress. Blood tissue truly reflects physical and chemical changes occurring due to heavy metal accumulation in body of fish. Haematological studies are useful in assessing the health of fish subjected to changing environmental conditions.

Hematological abnormalities have also been studied in various toxicant exposed fishes: *Labeo rohita* to synthetic detergents (Chellan et al., 1999), *Channa punctatus* to Pb and Cd (Hymavathi and Rao, 2000; Karuppasamy et al., 2005), *C. punctatus* to Cu (Singh et al., 2008), *Mastacembelus armatus* to thermal power plant effluents (Mehjbeen Javed and Nazura Usmani, 2012) and *Clarias gariepinus* to metal finishing company effluents (Adakole, 2012).

This report presents our findings on the effect of various types of pollutants from sewage and from treated and untreated effluents discharged from dairy processing factory, Rama Dairy into the aquaculture pond. Therefore, considering the importance of hematological parameters as indicators of fish health, the present work dealing with the toxic stress and synergistic effect of various heavy metals on fish reared in the pond was investigated.

MATERIALS AND METHODS

Description of study area

The study pond is located at Panethi (Latitude 27.88969; Longitude 78.07594) in Aligarh district, India. This waste water pond is situated at a distance of about 1 Km from Rama Dairy (Figure 1) and few cold stores are also present nearby. This dairy supplied the milk and other processed products in and around the Aligarh region. The waste water from this factory reached the study pond via some small streams. This pond also received the domestic waste water of communities living in the area. Fishes thriving in this pond fulfill the need of local peoples living around.

Collection and analysis of water sample from waste water aquaculture pond

Water was collected in pre-cleaned and acidified glass bottles. The bottles were immediately brought to the laboratory and acidified with concentrated HNO_3 acid to pH less than 2.0. Water samples were then analyzed for the presence of heavy metals (Cu, Ni, Fe, Co, Mn, Cr and Zn) according to the standard techniques for

Table 1. Physicochemical parameters of waste water aquaculture pond water.

Parameter	Water
Temperature	30.0°C
pH	7
Dissolved oxygen (D.O.)	5.8 mgL^{-1}
Total solids (TS)	1700 mgL^{-1}
Total Dissolved solids (TDS)	1500 mgL^{-1}
Total Suspended solids (TSS)	200 mgL^{-1}

Values are Mean (n = 3).

Table 2. Heavy metal concentrations (mgL^{-1}) in waste water aquaculture pond water.

Heavy metal	Water
Cu	0.07± 0.01
Zn	0.45±0.03
Ni	0.08± 0.02
Fe	8.08± 2.88
Co	0.24± 0.02
Mn	2.32± 0.10
Cr	0.07± 0.02

Values are Mean ± S.D (n= 3).

treatment of waste water APHA (2005).

Fishing to collect fish samples

Fishing to collect the representative samples was performed with the help of professional local fisherman. Cast nets with a strong rope attached to the apex and a number of weights of iron or lead attached along the margin were used for fishing. The total fish catches were harvested and samples of live *C. punctatus* were transferred to water buckets and then to the laboratory. The sampled fish were then immediately washed with distilled water for removing salts and sterilization. Blood was collected by heart puncture of the fish by heparinised syringe in vials containing ethylenediaminetetraacetic acid (EDTA) as an anticoagulant, which was used to estimate the haematological parameters.

The sampled fish were then immediately killed by using the concussive blow to the head of each selected fish, and placed on ice to carry out the morphometric measurements of each of these fish. Fishes had mean length of 21.32 cm and mean weight of 58.00 g (stock = 5, n= 6). The healthy controls of similar length and weight were procured from nearby fish farm which was unpolluted.

Haematological analysis of *Channa punctatus*

Total count of RBC

The RBC counts were made by Neubauer haemocytometer (Shah and Altindag, 2004a). Blood was diluted 1:200 with Hayem's solution (Mishra et al., 1977). Erythrocytes were counted in the loaded haemocytometer chamber and total numbers were reported as10^6 mm^{-3} (Wintrobe 1967). Counting was done in the five smaller squares that is, in the 1st, 5th, 13th, 21st and 25th. The RBC's on the lower and right sides of a square were added in the total, while those on the upper and left sides were rejected.

Total count of WBC

WBC counts were made by Neubauer haemocytometer (Shah and Altindag, 2005). Blood was diluted 1:20 with Turk's diluting fluid and placed in haemocytometer. Four large (1 sq mm) corner squares of the haemocytometer were counted under the microscope. The cells touching the boundary lines were not counted. The total number of WBC was calculated in mm^3 x 10^3 (Wintrobe 1967).

Estimation of haemoglobin

Haemoglobin (Hb) was determined with haemoglobin test kit (DIAGNOVA, Ranbaxy, India) using the cyanmethaemoglobin method.

Statistical analysis

Experiments were conducted in triplicates. All haematological values are given as means ± standard error of mean (S.E.M). Values of RBC, WBC and Hb of fish blood were compared statistically with control by using student's t test (2- tailed) with the help of SPSS 17. The level of significance was established at $P < 0.05$ and $P<0.01$.

RESULTS

Physicochemical parameters of waste water aquaculture pond water are given in Table 1. Table 2 shows the mean concentration of heavy metals (mg L^{-1}) in water. The heavy metal content in pond water were in the order of Fe (8.08) > Mn (2.32) > Zn (0.45) > Ni (0.08) > Cu (0.07) = Cr (0.07) > Co (0.04). The heavy metal content in pond

Table 3. Total count of RBC's, WBC's, and Haemoglobin in the control and exposed *Channa punctatus* collected from waste water aquaculture pond.

Variable	Control (mean±SEM)	*Channa punctatus* (mean±SEM)
Number of RBC (10^6 mm^{-3})	5.37± 0.034*	2.15±0.329*
Number of WBC (10^3 mm^{-3})	4.56 ± 0.183**	8.23±1.655**
Haemoglobin (g/dL)	13.06 ± 0.265**	7.75±0.713**

Values are given as Mean **±** SEM (n = 6). **Significantly different at (P<0.01); *Significantly different at (P<0.05).

Table 4. Summary of water quality guidelines and standards by International Organization or country.

Parameter	WHO (guidelines)	E U (standards)	Canada (guidelines)	Australia (guidelines)	N Z (guidelines)	Japan (standards)	USA (standards)	Present Study
Copper	2	2	2	1	2	1	1.3	0.07
Nickel	0.02	0.02	0.02	0.02	0.02			0.08
Iron		0.2	0.2	0.3	0.01	0.3	0.3	8.08
Cobalt								0.04
Manganese	0.5	0.05	0.05	0.5	0.5	0.05	0.05	2.32
Chromium	0.05	0.05	0.05		0.05	0.05	0.1	0.07
Zinc	3			3		1	5	0.45

Values are in mgL^{-1}. Adapted for Water Quality for Ecosystem and Human Health, 2006 (prepared and published by the United Nations Environment Programme and Global Environment Monitoring System (GEMS)/ Water Programme). Blank cells indicate that no citable information was available.

water was compared with the quality guidelines and standards of United Nations Environment Programme Global Environment Monitoring System (UNEPGEMS 2006) (Table 4). The values for the heavy metals such as Fe, Ni and Mn are higher than the limits suggested by UNEPGEMS. Whereas the concentration of heavy metals Cu, Cr and Zn are within the limits proposed. Table 3 presents the data on haematological profiles of the fish *C. punctatus*.

Total RBC count

The erythrocyte count of healthy controls showed a mean value of 5.37 x 10^6 mm^{-3}. The fishes that were collected from waste water aquaculture pond water showed mean value of RBC as 2.15 x10^6 mm^{-3} (Table 3). The values mentioned above showed a significant (P<0.05) decrease when compared to the control.

Total WBC count

The results of the total count of white blood cells revealed that the blood of the control fish showed a mean value of 4.56 mm^3 x 10^3. The fishes collected from waste water aquaculture pond showed mean value of WBC as 8.23 mm^3 x 10^3 (Table 3). The values mentioned above showed a significant increase (p<0.01) when compared to the control.

Estimation of haemoglobin

The control fishes showed mean value of 13.6 g/dL for haemoglobin. The fishes collected from waste water pond showed mean value of haemoglobin as 7.75 g/dL (Table 3). The values for treatments showed a significant (p<0.01) decrease when compared to the control.

DISCUSSION

Water parameters are one of the major factors responsible for individual variation in fish hematology. Since hematological parameters are necessary for clinical diagnosis of a disease and pathological conditions in human, these criteria should receive enough attention in assessing the health of the fish with regard to aquatic pollution and has been accepted by many workers such as McCarthy et al. (1973) and Christensen et al. (1978). While evaluating the total effect of zinc and lead on the hematological indices of carp, a synergetic effect of these metals was found on the erythrocyte count, concentration of hemoglobin, and the leucocytes (Vosylienë, 1999b). Similar changes are reported in this study on the polluted waste water pond fish *C. punctatus*.

This study on hematological changes in fish serves as an effective tool in the diagnosis of the extent of environmental pollution and also the abiotic fish diseases. Hypoxia, anemia, and hyperthermia are related stresses causing an osmotic imbalance and decreased capacity of the RBC to carry sufficient oxygen unless otherwise compensated by erythropoiesis or suitable physiological adjustments. Decreased availability of oxygen generally causes increased synthesis of hemoglobin, release of blood cells from storage sites, and enhanced erythropoiesis.

Adakole (2012) showed that when *C. gariepinus* was exposed to metal finishing company effluents, RBC was initially increased and decreased after chronic exposure. In the present study, lowering of total RBC count coupled with low Hb content may be due to destructive action of pollutants on erythrocytes which was after chronic exposure and as a result of which the viability of the cells may be affected as was also reported by Karuppasamy (2000). Multiple form of hemoglobin allows fish to adjust more efficiently to physiological stress such as varying water temperature and oxygen concentration (Hochachka and Somero, 1973). Hemolysis occurs in response to toxicity that leads to alteration in the selective permeability of the membrane (Das et al., 1987). Chandanshive et al. (2012) also reported decrease in RBC of fish *Labeo rohita* after exposure to mixture of heavy metals. All these reports are in agreement with the present study of reduction in total RBC count and Hb content of fish from polluted sewage fed pond due to the inhibition of aerobic glycosis curtailing of iron and hemoglobin via the lowered energy status in fish. Joshi et al. (2002) and Banerjee and Banerjee (1988) have suggested that heavy metal exposure decreases the total RBC count, and Hb content due to impaired intestinal absorption of iron.

Increase in total WBC count in the present study was a result of direct stimulation for its defense from diseases due to the presence of heavy metals. Progressive increased levels of total WBC count have also been reported in *C. punctatus* exposed to lead (Hymavathi and Rao, 2000), *Clarias batrachus* exposed to mercuric chloride (Joshi et al. 2002) and *Clarias gariepinus* to metal finishing company effluents (Adakole J.A 2012). Leukocytosis is directly proportional to severity of stress condition in maturing fish and is a result of direct stimulation of immunological defense due to the presence of heavy metals in waste water pond. This is in correlation with the report by Saravanan and Harikrishnan (1999) in freshwater fish, *Sarotherodon mossambicus*, when exposed to sublethal concentration of copper and endosulfan. This may be attributed to alteration in blood parameters and direct effects of various pollutants. These observations are also in good agreement with those of Karuppasamy et al. (2005) and Hardikar and Gokhale (2000).

Hence, the present investigation results confirm that stress due to heavy metals present in the waste water pond, does create hematological disturbances, erythrocyte destruction (hemolysis), and leukocytosis in fish population, affecting the immune system and making the fish vulnerable to diseases. Therefore, the fish is provided as a bioindicator of deteriorating water quality and due care should be taken to monitor the environment of waste water aquaculture pond.

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