Histopathological Changes In Lymphoid Organs Of Fish After Exposure To Water Polluted With Heavy Metals

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Abstract
Pollution of water with heavy metals may suppress the immune system of fish leading to increased susceptibility to diseases, decreased production and mortality. Polluted water from a brook of Ludhiana city called Buddha Nallah was used to investigate the effect of heavy metals on fish lymphoid organs. In the present study, the water sample showed abnormally high concentrations (in mg/L) of all the 8 heavy metals investigated - Iron (35.86), Zinc (2.75), Manganese (0.166), Nickel (0.090), Chromium (0.065), Copper (0.045), Lead (0.044) and Cadmium (0.025). Common Carp fish was reared in a water tank containing polluted water from the Nallah. Exposure of fish to the polluted water (3%) for 2 weeks led to sudden mortality among 60% of the test fish. The dead fish showed generalized congestion and cyanosis on the outer surface of the body and congestion and hemorrhage in the intestines. Histopathology of lymphoid tissues of the dead fish revealed congestion, haemorrhage, lymphocytic infiltration and degenerative changes in kidney, spleen, gut and liver.

It is evident from the study that heavy metals cause significant pathological changes in fish lymphoid organs and mortality of fish.

INTRODUCTION
Pollution of water with harmful chemicals, heavy metals, trace elements etc. may suppress the immune system of fish leading to increased susceptibility to diseases, decreased production and mortality. In Ludhiana city of Punjab state of India, the growth of industries has led to an increase in water pollution. Agrochemicals and industrial effluents pollute the available water resources. The city has a large number of industries for manufacturing bicycle parts, Nickel – Chrome plating, dyeing, woolen hosiery etc. These industries discharge their liquid waste directly, or through sewage, into a brook called Buddha Nallah running through densely populated areas of Ludhiana (population 4.5 million). The inflow of waste waters into the nallah has been recorded at 32.4 million gallons per day. The pollutants include 0.5 kg per capita per day of refuse.

This brook used to be a good source of fisheries several years ago and 56 fish species had been recorded in it in 1944. However, only 18 species were found in it during 1970. Later, in 1984, only 4 air – breathing species of fish could be recorded. Now the brook is without any fish due to the heavy pollution. The pollution has led to increase in temperature, pH, hardness, B. O. D. and total solids in this brook. Now only septic conditions prevail in it (Kaur, 1997).

Since the brook ultimately joins river Satluj, a major source of drinking water and fisheries in Punjab, at about 25 kms from Ludhiana city, the river Satluj too is now getting polluted. It would, therefore, be useful from economic and public health points of view to assess the effect of this pollution on the immune system of fish which makes it vulnerable to diseases.

MATERIALS AND METHODS
EXPERIMENTAL FISH AND POLLUTED WATER:
The present studies were conducted on Common Carp fish (Cyprinus carpio carpio), 20 cm long and weighing 250 gm, available at the Punjab Agricultural University (P.A.U.) Fish Farm, Ludhiana. Polluted water was obtained from the brook called Buddha Nallah at a village near Ludhiana city.

EXPOSURE OF FISH TO POLLUTED WATER
Fishes were divided into 3 groups of 8 fish each and kept in plastic tanks of size 160 x 110 x 110 cm. Aerators and ample daylight were provided in the tanks and the water temperature was maintained at 32°C. The fishes were acclimatized under lab conditions for one week and then exposed to polluted water. One group of fish was reared in water with 3% concentration while the other group was exposed to 1.5% concentration of polluted water. The third
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group was kept in normal unpolluted water as untreated control under the same conditions. The fish were fed once in a day and the water in the aquaria was changed every day. The same concentration of polluted water was added every day in the aquaria having the test fish.

ANALYSIS OF HEAVY METAL CONTENTS OF POLLUTED WATER
A sample of the polluted water was analyzed for its contents of heavy metals by the Central Testing Laboratory of the Department of Soils, PAU, Ludhiana.

PATHOLOGICAL STUDY
Postmortem examination was conducted on the dead fish and lymphoid organs (kidney, spleen, gut and liver) were collected. Sections of tissues were stained with H&E stain and examined microscopically.

RESULTS
ANALYSIS OF WATER
Analysis of the polluted water (pH 6.81) revealed heavy contamination with Iron, Zinc, Manganese, Nickel, Chromium, Copper, Lead and Cadmium (Table 1).

<table>
<thead>
<tr>
<th>S. no.</th>
<th>Element</th>
<th>Contents in the polluted water (mg/L)</th>
<th>Maximum permissible limit for water (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Iron</td>
<td>35.86</td>
<td>1.00</td>
</tr>
<tr>
<td>2</td>
<td>Zinc</td>
<td>2.75</td>
<td>15.00</td>
</tr>
<tr>
<td>3</td>
<td>Manganese</td>
<td>0.166</td>
<td>0.50</td>
</tr>
<tr>
<td>4</td>
<td>Nickel</td>
<td>0.090</td>
<td>0.10</td>
</tr>
<tr>
<td>5</td>
<td>Chromium</td>
<td>0.065</td>
<td>0.50</td>
</tr>
<tr>
<td>6</td>
<td>Copper</td>
<td>0.045</td>
<td>1.50</td>
</tr>
<tr>
<td>7</td>
<td>Lead</td>
<td>0.044</td>
<td>0.10</td>
</tr>
<tr>
<td>8</td>
<td>Cadmium</td>
<td>0.025</td>
<td>0.01</td>
</tr>
</tbody>
</table>

PATHOLOGICAL FINDINGS
Exposure of fish to a low concentration (3%) of polluted water from Buddha Nallah for a short period (2 weeks) led to sudden mortality among majority (60%) of the test fish. No mortality was observed in the control fish while the fish exposed to 1.5% of polluted water showed 37.5% mortality.

GROSS LESIONS
Generalized congestion and cyanosis on the outer surface of the body was observed in the dead fish (Fig. 1). Congestion and hemorrhages on the intestines were also seen.
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Figure 2
Figure 1: Dead fish with generalized congestion and cyanosis on the body.

HISTOPATHOLOGICAL LESIONS
Histopathological examination of lymphoid organs of dead fish revealed congestion and haemorrhages in gut, spleen, kidney and liver. Engorged blood vessels and extravasated erythrocytes could be seen in tissue sections of these organs. Moderate lymphocytic infiltration was observed in gut and kidney. Aggregates of round cells with large blue staining nuclei were clearly visible. Liver and kidney also showed degenerative changes in the parenchyma. Cellular architecture of tissues was lost and homogeneous pink staining areas of the tissue devoid of blue staining nuclei were seen in liver and kidney (Figs. 2 to 12).

Figure 3
Figure 2: Congestion, hemorrhage and lymphocytic infiltration in kidney (20x). Engorged blood vessels, extravasated nucleated erythrocytes and round lymphocytes with dark basophilic nuclei are visible.

Figure 4
Figure 3: Degenerative changes and hemorrhages in kidney (20x) with loss of cellular architecture and absence of nuclei from cells in homogeneous pink mass and extravasated nucleated erythrocytes.

Figure 5
Figure 4: Congestion and lymphocytic infiltration in gut (20x). Engorged blood vessels and round lymphocytes with dark basophilic nuclei are visible.
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**Figure 6**
Figure 5: Hemorrhages in gut (20x). Extravasated nucleated erythrocytes are visible.

**Figure 7**
Figure 6: Degenerative changes in gut (20x). Note the loss of cellular architecture and absence of nuclei from cells in homogeneous pink mass.

**Figure 8**
Figure 7: Hemorrhages in spleen (20x). Dark pigment derived from hemoglobin and nucleated erythrocytes are visible.

**Figure 9**
Figure 8: Lymphocytic infiltration in spleen (20x) showing dark basophilic nuclei.
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Figure 10
Figure 9: Degenerative changes in spleen (20x). Note the loss of cellular architecture and absence of nuclei from cells in homogeneous pink mass.

Figure 11
Figure 10: Congestion in liver (20x). Engorged blood vessels are visible.

Figure 12
Figure 11: Lymphocytic infiltration in liver (20x). Round lymphocytes with dark basophilic nuclei are visible.

Figure 13
Figure 12: Degenerative changes in liver (20x). Note the loss of cellular architecture and absence of nuclei from cells in homogeneous pink mass.

DISCUSSION

In the present study, the fish died after exposure to polluted water showed changes in lymphoid organs suggestive of heavy metal toxicity. The surviving fish from the other groups did not show such changes.

Stress can have a depressive effect on the immune responses in fish. It has been reported (Weeks et al., 1986) that fish from contaminated river from heavily industrialized urban area had 30 – 40% decrease in macrophage chemotactic activity. Chronic exposure of fish to sewage sludge decreased leukocyte bactericidal activity and B cell numbers (Secombes et al., 1992). Good correlations have been found between concentrations of chemicals in sediment and tissues and prevalence of disease (Malins et al., 1988). There is
increased occurrence of neoplasms in fish exposed to contaminants (Morra, 1993). The density of melanomacrophage centers of spleen may decrease in fish from contaminated waters (Wester et al., 1994).

In the present study, abnormally high levels of eight heavy metals (Iron, zinc, manganese, nickel, chromium, copper, lead and cadmium) were detected in the polluted water of Buddha Nallah. Exposure of fish to 3% of this polluted water for 14 days led to the death of exposed fish. The cause of mortality may be the toxicity of heavy metals in fish.

It has been speculated (O'Neill, 1981) that metals may disturb the metabolism, ionic balance and cell division of immunocompetent cells. Among the metals, Manganese has a relatively low toxicity to fish (Hetrick et al., 1982). It is an essential trace element for various functions. It stimulates NK cell activity in Carp both, in vitro as well as in vivo (Jones, 1964). It has been shown to have a strong enhancing effect on phagocytosis by Carp macrophages in an in vitro test (Ghanmi et al., 1990).

Chronic exposure of trout and carp to nickel, zinc, copper or chromium has been reported to suppress to a variable extent the primary humoral response to bacteriophage. Copper was found to cause immunosuppression of antibody producing cells in rainbow trout when tested in vitro (Khangarot et al., 1991) and in air breathing catfish in vivo in a dose dependant manner along with depressed phagocytic activity of spleen and kidney macrophages, and suppression of T cell activity as indicated by prolongation of allograft rejection time (Anderson et al., 1989). Defense against internal infections can be compromised by prolonged exposure to Copper (Malins et al., 1988).

Different concentrations of copper or zinc have been reported to cause dose – dependant suppression of kidney lymphocyte numbers and natural cytotoxic cells (Merchant & Packer, 1983). Copper caused a marked decrease in macrophage activity both in vitro and in vivo, but zinc caused a modest increase in macrophage activity under the same conditions. In a study, exposure to Copper reportedly caused a strong inhibition of the phagocytic response. However, Cadmium caused an initial stimulation followed by a variable decrease (Roubal, 1988).

Cadmium causes both, immunosuppression and immunostimulation in mammals depending on a variety of factors. T cell activities are usually suppressed whereas the effects on B cells are more varied. A concentration of 10 - 12 g/ml is about half the LC50 of cadmium. This concentration has been reported to cause inhibition of serum antibody titres in one species of fish but a six fold stimulation in another (Newman & MacLean, 1974). A concentration of 0.7 or 3.6 g Cd/L has been reported to cause suppression of T lymphocyte function but enhancement of antibody response to bacterial challenge (Nielsen et al., 2001). Cadmium can have a marked effect on differential leukocyte counts in fish. A dose-dependant three-fold increase in neutrophils and decrease in lymphocytes has been reported (Morra, 1993). Lesions in hematopoietic areas of lymphoid organs following exposure to cadmium have been reported (Plumb & Arechon, 1990). Elevated cortisol may be a primary mechanism for immune system suppression in fish exposed to a variety of pollutants. Most fish exhibit an elevated plasma cortisol level in response to nearly any stressor. However, cadmium alone among the metals fails to induce this hormonal change.

Exposure of fish to Lead for up to 183 days was reported to produce a reduction in spleen size but an increase in leukocyte number and an especially large increase in the number of thrombocytes (Robohm, 1986). In fish exposed to Lead for at least 60 days, lymphoid tissue in head kidney was greatly reduced. Similar changes were reported in case of fish chronically exposed to Zinc (Rougier et al., 1994).

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