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Short Communication

Histochemical changes in the liver of freshwater fish, *Rasbora daniconius*, exposed to paper mill effluent

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Abstract: This investigation deals with the effect of paper mill effluent, on the histochemical components of the liver of *Rasbora daniconius*. The Paper mill effluent was collected directly from the 45 Km away from Aurangabad (MS) India. Histochemical studies on protein, lipid and glycogen contents of liver Rasbora daniconius showed a progressive decrease in staining intensity to Mercury bromophenol blue (Hg-BPB), Sudan black B and Bets's Carmine at 30 days exposure when treated at sublethal concentrations (1.9% and 0.95%) of paper mill effluent. The magnitudes of these changes were dose dependent. This study, therefore, concludes that paper mill effluent creates metabolic crisis and impairment in fish liver. The depletion in level of protein, lipid and glycogen points towards exhaustion of cell- energy to meet high demand of fish in stressful condition.

Key words: Paper mill effluent, liver, histochemistry, *Rasbora daniconius*.

التغيرات الهستوكيميائية في كبد أسماك المياه العذبة Rasbora daniconius عند تعرضها لمخلفات صرف مصنع الورق

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الملخص: تعنى تلك الدراسة بتأثير مخلفات صرف مصنع الورق على المحتوى الفسيوكيميائي لكبد اسماك *Rasbora daniconius*. تم تجميع مخلفات صرف مصنع الورق مباشرة من أورنجباد بالهند وبتعريض الأسماك لها لمدة 30 يوم أظهرت الدراسة الهستوكيميائية على البروتين والدهن والجليكوجين بالكبد، انخفاض متزايد في كثافة صبغات زئبق البروموفينول الأزرق واسود السودان (ب) والكرامين بيتا وعند تعريض الأسماك لتركيزات أقل من التركيزات القاتلة (1,9% ، 0,95 %) من مخلفات صرف مصنع الورق، فقد وجد أن التغيرات تعتمد على التركيز. أوضحت تلك الدراسة أن مخلفات صرف مصنع الورق تحدث مشكلة أيضية وتوقف عمل الكبد حيث أن الانخفاض في مستوى البروتين والدهن والجليكوجين يؤدي لنقص شديد في طاقة الخلية مما يجعلها لا تتحمل ظرف الإجهاد.

الكلمات المفتاحية: صرف مصنع الورق، كبد، هستوكيميائية، *Rasbora daniconius*.

Introduction

The rapid industrial growth throughout the world is common and in India, particularly due to alarming rise in human population caused tremendous environmental pollution. The aquatic environment is severely affected by different types of chemicals which are toxic to the aquatic organisms (Kopecka et al., 2006). The paper industry has been one of the major sources of aquatic pollution in India. Paper mill effluent consists variety of toxic components such as heavy metals, high Chemical Oxygen Demand (COD), Biological Oxygen Demand (BOD), soda, legnin, chlorine, resin acid, dioxin, furan, etc. which might be responsible for causing metabolic impartment in the aquatic

organisms which could even lead to their death.

Discharge of effluent into freshwater system deplete the dissolved oxygen content and, by interfering with respiratory metabolism cause heavy mortality (Quasim and Siddque, 1960; David and Ray, 1966; Venkataraman, 1966; Hingoroni et al., 1979). Pollution of aquatic by domestic and untreated or partially treated industrial effluent greatly contributes to massive kill of fish and other important aquatic biota (Kumari and RamKumar, 1977).

There are few reports available on the toxic effect of industrial effluent on protein, lipid and glycogen of fishes (Fujiay, 1961; Mcleay and Brown, 1974' 1979; Stonner and Living ston, 1978; Shaffi, 1980, 1981; Oikari and Nakari, 1985; Kumar and Gopal, 2001).

However, the information regarding assessment of the toxicity of paper mill effluent and its impact on freshwater fish, *Rasbora daniconius* belongs to the cyprinidae family, is very sensitive and available throughout the year in Godavari river (MS) India have been not received much attention. The fish, *Rasbora daniconius* (Ham.) locally knows as "Anjeri" is preferred as food by layman in the society and it is easy to maintain in the laboratory with minimum care.

Further, very little attention has been paid on a series of progressive histochemical changes in different tissues of the freshwater fishes such as function of exposure span, especially during chronic studies. Hence, in present investigation histochemical tests were used for localization of chemical products of cellular activities by the intensity of staining reactions. It is used for comparing the protein; glycogen and lipid contents present in the liver cells of the normal and treated fish, *Rasbora daniconius* at different concentrations of paper mill effluent for 30 days.

The results obtained from the present study reflect the ability of paper mill effluent to induce histochemical changes in *Rasbora daniconius.* These parameters could effectively be used as potential biomarkers of toxicity to the freshwater fish in the filed of environmental biomonitering.

Material and Methods

The fishes, *Rasbora daniconius* were collected from Godavari river at Kaigaon Toka, ((latitude 19^0 37.463 and longitude 75^0 01.409) 45 km away from Aurangabad (M.S.) India and brought to the laboratory. The fishes were maintained in glass aquaria and acclimatization for four weeks at laboratory conditions.

The Paper mill effluent was collected directly from the Kaigaon paper mill at releasing point 45 Km away from Aurangabad. The physicochemical variables of effluent and tap water were analyzed at every alternate day during the toxicity test period of 30 days according to APHA (2000) recorded in Table 1. The percentage concentration of test solution is obtained by using formula (FAO, 1984), which is given below.

$$\text{Volume percent} = \frac{\text{Volume of effluent}}{V_E + V_{DW}} \times 100$$

V_E = Vol. of Effluent, V_{DW} = Vol. of Dilution water.

The sublethal concentrations were selected on basis of the results of acute toxicity studies. The LC_{50} value for 96 hours was estimated at 9.5% concentration. The Sublethal concentrations for chronic test were selected at 1.9% (1/5) and 0.95% (1/10).

For the histochemical studies the 10 healthy fishes were exposed to paper mill effluent at two sublethal concentrations 1.9% (1/5) and 0.95% (1/10) for 30 days i.e. one month. Simultaneously a control tank was also maintained with 10 fishes. At the end of experimental period, surviving fishes were dissected. The livers were taken out and fixed in

caroyn's fluid for detection of glycogen and protein; in formal calcium for detection of lipid. Then subjected to routine microtechnique method and blocks were prepared in paraffin wax. Sections were cut at 6µ.

For histochemical detection of glycogen in these tissues, Best's carmine method (Best's, 1906) was employed. Where as for protein detection Mercuric bromophenol blue method (after Bonhag, 1955) was used and for lipid Sudan black-B method (Baker's 1944) and mounted in glycerin jelly. All the techniques followed as described by Pearse (1961).

Results

Physicochemical variables

The values of physicochemical parameters are recorded in Table 1. The values of physicochemical parameters of paper mill effluent fall within acceptable limits, except values of Chemical Oxygen Demand (COD), Biological Oxygen Demand (BOD) and hardness. The raised values of physicochemical parameters may imply an increased toxicity.

Protein

The liver of normal (control) *Rasbora daniconius* was characterized by high concentration of total protein. In the cellular cytoplasm, the Mercury bromophenol blue (Hg-BPB) reaction was either in the form of bluish granules of different size, or in a diffused state, perinuclear or peripheral in position and particularly concentrated adjacent to blood sinusoids.

In the nucleus, the chromatic granules and the nucleoplasm were stained less extent and gave a diffused faint blue colour (Figure 1 A). Total protein was found to exhibit a noticeable decrease in cytoplasm and nucleus of the liver cells of *Rasbora daniconius*, after exposure to 0.95% concentration of paper mill effluent for 30 days (Figure 1B). The liver of fish after exposure to 1.9% concentration of paper mill effluent showed completely devoid of protein and their remnants were mainly located at the peripheries of the cells which showed cytopalsmic vacuolation (Figure 1C).

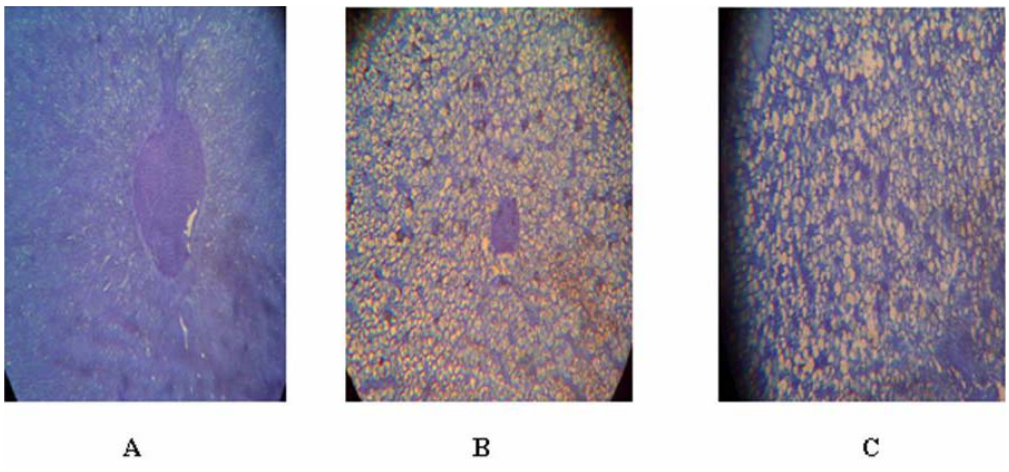

Figure 1 (A, B and C). Liver sections of *Rasbora daniconius* fixed in caroyn's fluid, stained with mercury bromophenol blue (Hg-BpB), 400x. A] Liver section of control fish. The protein appear at the marginal area of the hepatocytes, B] liver section of *Rasbora daniconius* exposed to 1.9% concentration of paper mill effluent for 30 days showing moderate decrease of protein in hepatocytes (400x). C] Liver section of *Rasbora daniconius* exposed to 0.95% concentration of paper mill effluent for 30 days showing apparent reduction in protein content (400x).

Table 1. Showing physico-chemical variables

Sr. No.	Variables	Tap water (Mean values)	Paper mill effluent (Mean values)
1	Temperature (^{o}C)	28±2 ^{0}C	29±2 ^{0}C
2	pH	7.2	6
3	Dissolved oxygen (DO)	6.9	0.92
4	Alkalinity	27	110
5	Total hardness	116	470
6	Biological Oxygen Demand (BOD)	10	285
7	Chemical Oxygen Demand (COD)	2.6	1190

*Except Temperature and pH, values expressed in mg/lit.

Lipid

The liver of normal (control) *Rasbora daniconius* was characterized by high concentration of lipid. Lipoid inclusion were uniformly distributed through out the cytoplasm, perinuclear and peripheral in position and particularly accumulated in the cytoplasm adjacent to sinusoid (Figure 2A). Other hand it was reduced in .95% sublethal concentration of paper mill effluent (Figure 2B). Such depletion is more pronounced in 1.9% concentration of paper mill effluent exposed fish (Figure 3B).

Glycogen

The liver of the normal fish, *Rasbora daniconius* revealed that glycogen was observed in the cytoplasm of the hepatocytes as indicated by large number of reddish coloured fine granules of different size (Figure 3A). On other hand after exposure to 0.95% concentration of paper mill effluent, glycogen content of the liver cells decreased. This diminution was quite evidenced in the amount and stainability (Figure 3B). This reduction of glycogen inclusion became more pronounced after exposure to 1.9% concentration of paper mill effluent. In this specimen glycogen content displayed weak intensity of reaction and became hardly detectable (Figure 3B).

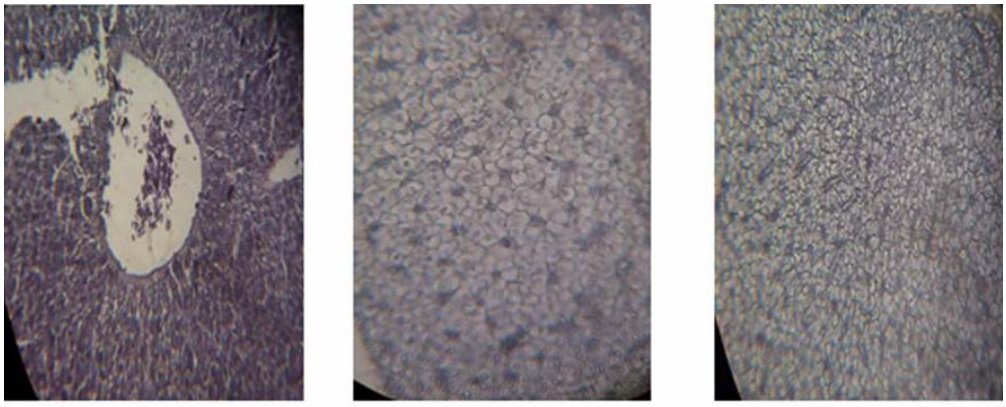

Figure 2 (A, B and C). Liver sections of *Rasbora daniconius* fixed in caroyn's fluid, stained with Sudan black-B, 400x. A] Liver section of control fish. The lipid inclusion distributed throughout the cytoplasm. B] Liver section of *Rasbora daniconius* exposed to 1.9% concentration of paper mill effluent for 30 days showing pronounced decrease of lipid in hepatocytes (400x). C] Liver section of *Rasbora daniconius* exposed to 0.95% concentration of paper mill effluent for 30 days showing obvious reduction in protein I protein content (400x).

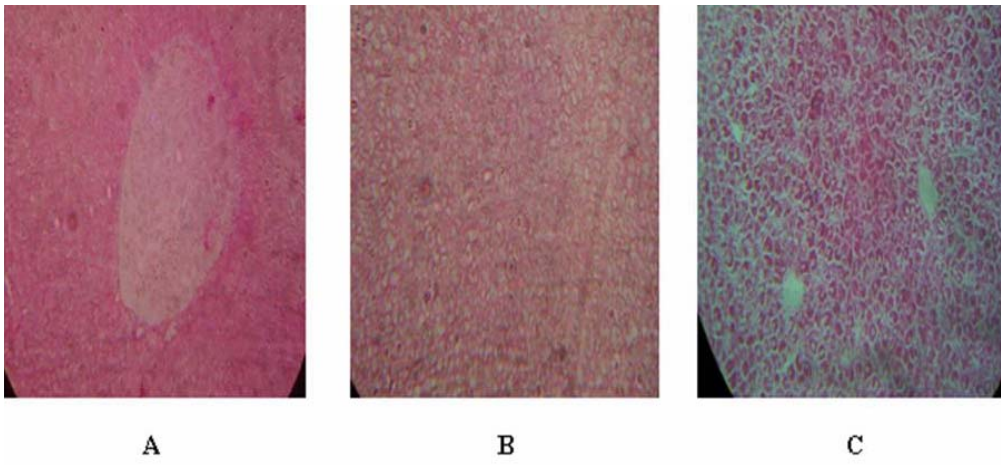

Figure 3 (A, B and C). liver sections of *Rasbora daniconius* fixed in formal calcium, stained with Bets's carmine, 400x. A] Liver section of control fish showing distribution of glycogen in the hepatocytes. B] Liver section of *Rasbora daniconius* to 1.9% concentration of paper mill effluent for 30 days showing marked decrease of glycogen in hepatocytes (400x). C] Liver section of *Rasbora daniconius* exposed to 0.95% concentration of paper mill effluent for 30 days showing reduction in protein I protein content (400x).

Discussion

Histochemistry has contributed a great deal not only to the understanding of biological phenomenon but also to clinical medicine. Histochemical techniques help to analyze not only the localization of protein, lipid and glycogen etc. but also molecular changes of at cellular level. The main advantage of histochemistry lies in the analysis of biological phenomena in the "particular cells". The altered state of cell constituent can be studied by using histochemical techniques.

The histochemical tests reveal the localization of chemical product of cellular activity. The intensity of staining can be used for comparing the protein, lipid and glycogen contents present in the liver cells of the normal fish with treated fish at different concenstartions.

Hepatic total protein content significantly decreased after treating freshwater fish, *Labeo rohita* with chlordane (Bansal et al.,1979). Singh and Srivastav (1981) mentioned that, the carbohydrates decreased as a result of exposure to sublethal concentration of a mixture of aldrin and formothion. Goel et al. (1988) illustrated a decrease in hepatic protein with the administration of n-hexane. Krapagaganapthy et al.,(1988) reported, the carbohydrate content of liver in *Colisa lalia* showed progressive decrease in staining intensity to PAS at all period expecting at 24 hrs of exposure, when treated at sublethal (0.04 mg/l) and median lethal 120 hrs (0.09 mg/l) concentrations of lindane. They also suggested that the carbohydrates in the liver are utilized to greater extent during the stressful condition. Jamal Al lail (2005) reported that, the marked reduction in glycogen content of the liver cell as compared to the control fish. They also stated that, these changes were time dependent.

Niak et al. (2004) reported the total protein, glycogen and lipid content underwent a significant depletion in the tissue of the tannery effluent treated fish, *Cyprinus carpio.* Khare and Singh (2004) observed histochemical changes in the gill of the fish, *Nandus nandus* exposed to sublethal concentration of endosulfan and carbaryl for one month. After long term exposure to both the pesticides,

there was reduction in the carbohydrate contents in all parts of the gills. Anandhi and Murthy (2006) studied the localization of lipid, glycogen and protein in the hepatic cells of *Glossogobius giuris* at different period of it reproductive cycle. They reported significant decrease in protein, glycogen and lipid in the liver of malathion treated fish.

The results showed that paper mill effluent induced reduction in protein, lipid and glycogen in liver. Similar type of decrease in the level of protein, lipid and glycogen were reported by Mcleay and Brown (1974), Yadav et al. (2007), Sakr and Jamal Al lail (2005) and D'cruz, and Miramda (2006). The gradual accumulation of toxicants present in industrial effluent has shown drastic reduction in the protein, lipid and glycogen contents progressively in the fish tissues (Rogar, 1980).

A sever dysfunction was noticed in the liver of the freshwater fish, *Rasbora daniconius* exposed to sublethal concentrations of paper mill effluent for 30 days. The depletion in the metabolites indicates that the whole metabolic pool of the fish gets disturbed or altered under toxic stress. Further, the change in the histochemical contents indicates their repaid utilization to provide excess energy in order to cope with stressful conditions.

According to present results on *Rasbora daniconius,* it is suggestive that decreased level of glycogen, protein and lipid in the tissues may be due to toxic effect of industrial effluent or may be due to toxicant imposed stress condition or may be due to impairment in the body and these effects were dose dependent. Besides this effluent has variable compositions whose values are far exceed the permissible limits thereby posing great danger to the aquatic biota. Therefore, it is needs that the authorities concerned to ensure that treated effluent discharge comply with acceptable standard to save our environment from destruction.

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