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Impact of nickel-chrome electroplating effluent on the protein and cholesterol contents of blood plasma of *Channa punctatus* (Bl.) during different phases of the reproductive cycle

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Abstract: *Sublethal toxicity of nickel-chrome electroplating effluent on blood plasma protein and cholesterol was investigated in the fish Channa punctatus(Bl). The fish was most sensitive to stress during the spawning phase followed by preparatory and prespawning phase of the reproductive cycle. An increase in the concentration of biomolecules corresponding to a decline in GSI clearly indicates that the metabolism of the fish is affected and the biomolecules are not taken up by the body under the stress of the effluent.*

Key words: *Blood plasma, Protein, Cholesterol, Spawning phases, Channa punctatus.*

Introduction

A fair amount of the heavy metals released in industrial effluents find their way into the aquatic bodies. Metals like nickel, chromium, zinc, mercury and manganese tend to accumulate in the organisms living therein even when present at levels considered safe for survival. This ultimately affects the biomoleucles, growth and reproductive ability of the organisms. Protein and cholesterol, the two important components of maturing oocytes are transported to the gonads via blood. These two biomolecules being very sensitive to stress bring about marked changes in the composition of blood plasma under stress of pollutants.

The short term effects of nickel and chromium on macromolecular variations in fishes have been reported by a number of workers such as Jha and Jha (1995) in *Anabas testudineus,* Sornarej *et al.* (1995) in *C. punctatus*, Kondal *et al*. (1990) in *Heteropneustes fossilis* and Nanda *et al.* (2000) in *H. fossilis.* But no report is available on the effect of nickel chrome electroplating effluent on the blood plasma protein and cholesterol contents during various phases of the reproductive cycle in fishes. Therefore, the present study was undertaken to investigate the effects of nickel chrome electroplating effluent on the blood plasma protein and cholesterol levels of *C. punctatus* during preparatory, prespawning and spawning phases of the reproductive cycle. In addition the gonado somatic index (GSI) was estimated to have a clear picture of the effect of the changed concentration of plasma protein and cholesterol on the reproductive potential of fish. *C. punctatus* was selected for the present study because it is found abundantly in the waters of Punjab and is an esteemed food fish.

Materials and Methods

The test fish *C. punctatus* were collected from the ponds situated in the vicinity of Ludhiana city. Fish were acclimated to the laboratory conditions for 15 days by keeping them in glass aquaria (95 x 45 x 45 cm) of 150l capacity. Length and weight of the experimental fish ranged from 11.54 – 18.28 cm and 16.24 – 61.72 g respectively. The fish were fed *adlibitum* on pig liver during acclimation and experimental period except for 48 hrs preceding sacrifice. The effluent was collected from the Hero Cycles Pvt. Ltd. Ludhiana.

The experiments were conducted in triplicate in glass aquaria (95 x 45 x 45 cm). The dechlorinated tap water having pH 7.3 $\pm$ 0.2, temperature 22 $\pm$ 2ºC, dissolved oxygen 5.5 $\pm$ 0.5 mg/l and hardness 272 $\pm$ 2 mg/l as $CaCO_3$ was used as diluent as well as control. Safe and sublethal concentrations of the effluent were calculated by following the bioassay studies of Kaur and Kaur (1996). Ten specimens were exposed to each 0% (control), 3% (T_1) and 15% (T_2) concentrations of the effluent for 30 and 60 days during preparatory, prespawning and spawning phases of the reproductive cycle. Five fish were sacrificed at the zero (initial for that particular phase) 30 and 60 days of exposure during each phase. Blood was taken from the heart of fish and pooled separately for each replicate in EDTA rinsed tubes kept in ice. Blood plasma was pipetted out after centrifuging the blood for 10 minutes at 3,000 rpm and stored at –20$\pm$2º. Bloodplasma protein and cholesterol contents were measured following the methods of Lowry *et al.* (1951) and Zlatkis *et al*. (1953), respectively. The gonadosomaticindex (GSI) was calculated with the help of the following formula:

$$\text{GSI} = \frac{\text{Weight of gonads (g)}}{\text{Weight of fish (g) - Weight of gonads (g)}} \times 100$$

The help of Punjab Pollution Control Board, Ludhiana was taken for analysis of the physico-chemcial parameters of the effluent (Table 1).

Results

The protein and cholesterol contents of the blood plasma of fish after giving 30 and 60 days exposure to 0%, 3% and 15% concentration of the effluent during the preparatory

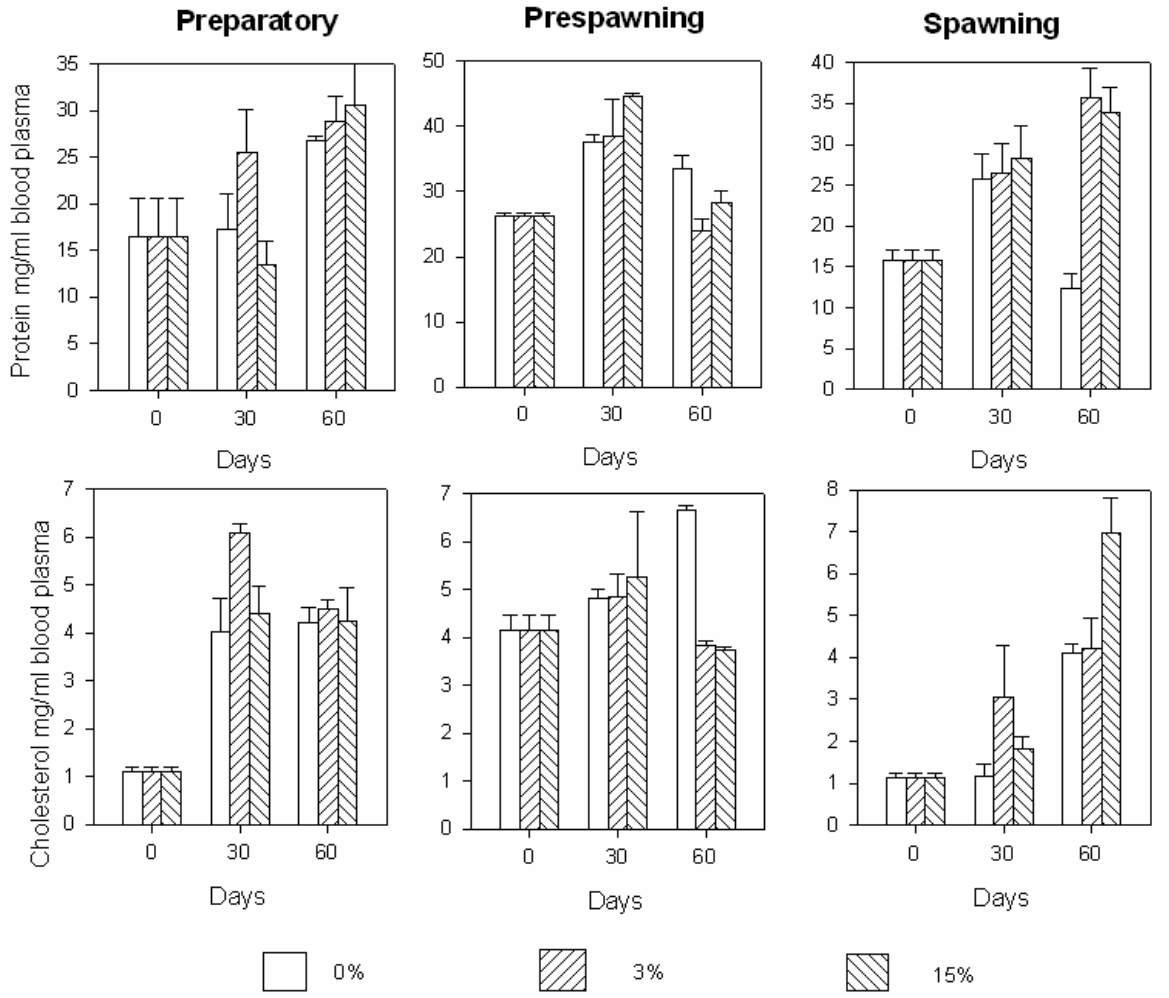

Fig. 1: Variations in the blood plasma protein and cholesterol contents (mg ml^{-1}) of female *Channa punctatus* on exposure to nickel-chrome electroplating effluent during different phases of the reproductive cycle.

Table – 1: Physico-chemical analysis of untreated nickel-chrome electroplating effluent.

Parameter	Value
pH	3.0
Color	Light yellow
Total suspended solids	78
Total dissolved solids	1624
a) Fixed solids	1412
b) Volatile solids	212
BOD	00
COD	22.4
Chloride as Cl	288
Sulphate as SO_4	680
Ammonical nitrogen	2
Chromium total	34
Chromium hexavalent	22
Nickel	40
Cyanides	1.0

Values except pH and color are given in mg L^{-1}

prespawning and spawning phases are illustrated in Fig. 1 and the percentage variations over control are documented in Table 2.

Protein: During the normal course of preparatory phase i.e. 15th Jan to 15th April, the protein content initially increased slowly till 30 days and then after 60 days it increased rapidly and came to be 26.75 mg/ml. Exposure to T_1 induced an increase of 48.31% over control after 30 days, which gradually decreased and came to be only 8.00% more over control after 60 days of exposure. T_2 initially rather decreased the protein content which was 22.12% less as compared to control but after 60 days it rapidly increased the content to the level of 30.56 mg/ml which was 14.24% more over control.

During the course of normal prepspawning period (15th April – 15th July) protein content increased rapidly upto 30 days and then there was some decline upto 60 days. Similar pattern was observed with both the concentrations of the effluent but the increase after 30 days (+2.53% and + 19.08%, respectively in T_1 and T_2) as well as decline after 60 days

Table – 2: Percent change over control in the GSI and contents of blood plasma protein and cholesterol of female *Channa punctatus* on exposure to nickel- chrome electroplating effluent during different phases of the reproductive cycle.

Parameter	Treatment	Preparatory		Prespawning		Spawning	
		30φ	60	30	60	30	60
GSI	T_1#	-39.39*	-71.08	-26.90	-17.83	-41.81	-30.57
	T_2	-29.29	-84.33	-83.05	-81.20	-85.93	-53.49
Protein	T_1	+48.31	+8.00	+2.53	-28.49	+3.23	+190.35
	T_2	-22.12	+14.24	+19.08	-15.68	+10.20	+174.85
Cholesterol	T_1	+51.37	+6.65	+0.60	-42.56	+159.83	+2.68
	T_2	+9.72	+0.95	+9.13	-43.91	+55.55	+65.79

* +ve is increase over control and –ve is decrease over control in the contents.
\# T_1 and T_2 are 3% and 15% concentrations of the effluent.
φ 30 and 60 are the days of exposure.

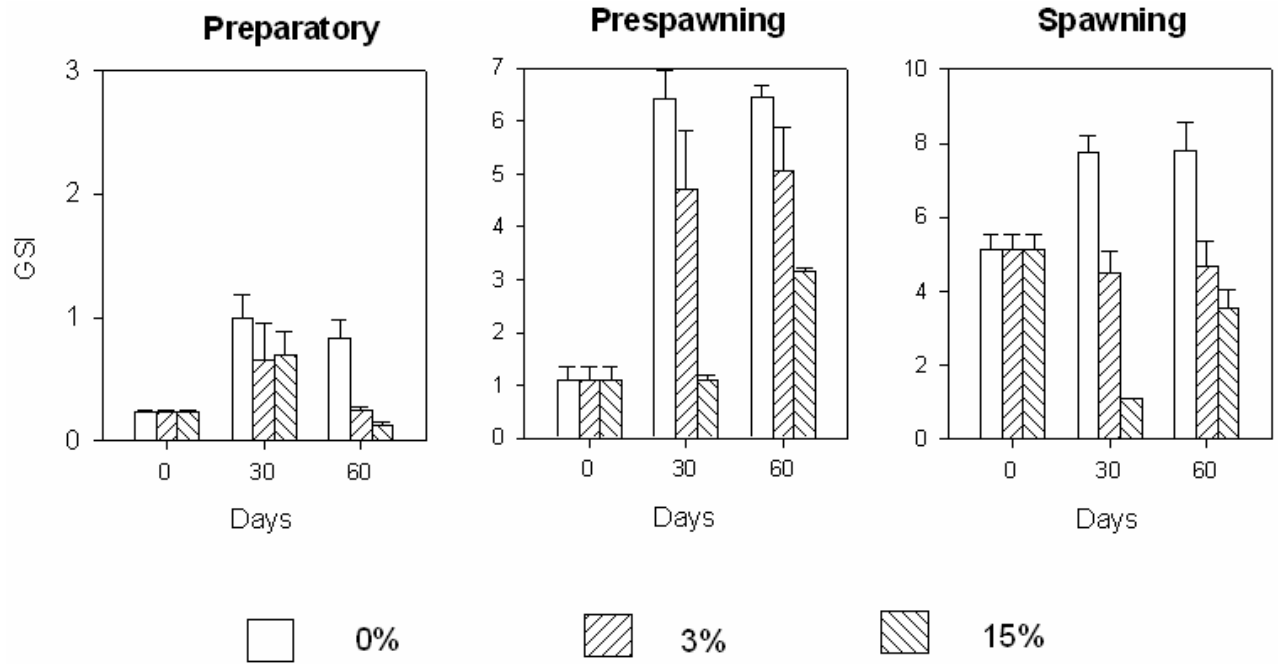

Fig. 2: GSI of *Channa punctatus* on exposure to nickel-chrome electroplating effluent during different phases of the reproductive cycle.

(-28.49% and –15.68% in T_1 and T_2, respectively) was more as compared to control.

In the spawning phase (15th July – 15th Oct.) protein content increased rapidly in control till 30 days but then there was a decline and it came to be 12.33 mg/ml. In both the treatments, however, protein content increased after both the durations and maximum content 35.80 mg was observed in T_1 after 60 days of exposure.

Cholesterol: During preparatory phase in control fish there was a rapid increase in cholesterol from initial 1.10 mg to 4.01 mg (30 days of exposure) and to 4.21 mg (60 days of exposure). Both the concentrations of the effluent on the other hand induced an increase after 30 days followed by a decline after 60 days of exposure. Increase was more in T_1 (6.07 mg) after 30 days but decline was more in T_2 (4.25 mg) after 60 days. The cholesterol content of effluent exposed fish was, however, more than control after both the durations of exposure.

During the normal course of prespawning phase, there was an increase in the cholesterol content from initial after both the durations of exposure. In both T_1 and T_2 an increase from initial after 30 days of exposure was followed by a decline after 60 days of exposure and it came to be minimum in T_2 (3.73 mg) after 60 days.

In the spawning phase cholesterol continued to increase from an initial 1.15 mg in control as well as the two concentrations of the effluent after both the durations of exposure. Both T_1 and T_2 induced more increase in the contents as compared to control after both the durations. Maximum, increase 6.98 mg was brought by T_2 after 60 days of exposure.

GSI: The variation in GSI of the fish on exposure to 0%, 3% and 15% concentrations of the effluent are given in Fig. 2 and Table 2. The GSI, showed a decrease in preparatory phase with both the treatments compared to control after both the time intervals.

The decrease was more after 60 days and maximum in T_2 (84.33% less than control) and came to be even less than initial, during the preparatory phase.

In prespawning phase GSI increased after both the durations in 0% as well as T_1 but the increase was delayed in T_1 as it increased slowly after 30 days (26.90% less than control) and continued to increase upto 60 days but was 17.83% less than control. T_2 on the other hand, brought a decline in GSI after 30 days of exposure (-83.05%) over control and then it slightly increased but it was 81.20% less than control. During spawning phase GSI showed an increase after both the durations of exposure in control. In both the treatments a rapid decline in GSI till 30 days of exposure (-41.81% and -85.93% in T_1 and T_2 respectively) was followed by a small increase after 60 days of exposure but this was much less than control (-30.57% in T_1 and -53.49% in T_2).

Discussion

Protein: Plasma protein content increased over control in the effluent exposed fish during all the phases of the reproductive cycle in the present study. Increase in the serum protein content has also been reported by Garg *et al.* (1989) in *H. fossilis* exposed to manganese for 48hr and Kondal *et al.* (1990) in *H. fossilis* exposed to vegetable oil factory effluent. This increase can be attributed to synthesis of metal binding proteins as heavy metals are known to form metallothioniens for their excretion from the body. This gets support from the findings of Pierson (1983) and Ariyoshi *et al.* (1990), who reported synthesis of metallothionein like proteins for detoxification of heavy metals in rainbow trout exposed to zinc and in red carp exposed to heavy metals, pesticides and surfactants.

These proteins being different from normal proteins are not taken up by the organs of the body and tend to accumulate in the body. The decrease over control in the GSI of the effluent exposed fish at the times of increase in plasma protein content supports this hypothesis of synthesis of stress proteins. Sharma and Davis (1980), however, attributed the increase in the soluble protein concentration to interference in the function of coenzyme A in methyl mercury exposed *C. carpio*, as the mercurials on one hand decrease de novo synthesis and on the other hand inhibit protein degradation leading to an elevation in its concentration. This may be another reason for the observed increase in plasma protein content during the present study.

The decrease observed in the protein content due to T_2 after 30 days of exposure in preparatory phase and after 60 days of exposure in prespawning phase gets corroboration from the findings of Rao *et al.* (1983) and Nanda *et al.* (2000), respectively in *Sarotherodon mossambicus* exposed to benthiocarb and *H.fossilis* exposed to nickel. Van Vuren (1986) has even observed changing patterns as well as a decline in fraction numbers of blood protein in *Labeo umbratus* exposed to toxicants. This decrease might be due to the intensive proteolysis as a result of activity of lysosomal enzymes leading to depletion of both tissue and circulating proteins as has been suggested by Dhanapakiam and Ramasamy (2001) in *C. carpio* exposed to copper and zinc mixtures. This inference gets further supported by the observations of Singh *et al.* (1993) who attributed the decline in protein content to an alteration in the nucleic acids in the *H. fossilis* exposed to sublethal concentrations of aldrin.

Cholesterol: Plasma cholesterol increased over control by both T_1 and T_2 in all the three phases except for a decrease over control in the prespawing phase after 60 days of exposure. Hypercholesterolemia observed during the present study gets corroborated by the works of Dhawan and Saxena (1988), Kondal *et al.* (1990) and Tulasi *et al.* (1992) in *H. fossilis* exposed to dyeing factory and vegetable oil factory effluent and *Anabas testudenius* exposed for 30 days to lead nitrate respectively. Wasserman *et al.* (1970), however, suggested involvement of thyroid hormones in cholesterol metabolism. They further related hypercholesterolemia in people exposed to organochlorines to enhanced breakdown due to hyperthyroidism under stress. Hilmy *et al.* (1980), on the other hand related hypercholesterolemia under the stress of mercuric chloride in killi fish to inhibition of cholesterol excretion through bile duct.

The decline in GSI at the times of increase in cholesterol during the present study clearly indicates that the accumulated cholesterol is not being used for gametogenesis by the fish. Ogawa *et al.* (2001) reported that an irregular rise and fall in the levels of plasma, cortisol, estradiol and ketosterone lead to adverse effects on gametogenesis in *C.carpio* due to acid stress.

Hypocholesterolemia observed, on the other hand, during prespawning phase gets support from the works of Tewari *et al.* (1987) and Ruprelia *et al.* (1989) who reported that lead exposure brought a decline in de novo synthesis of cholesterol in *Barbus conchonius* and to mobilization of cholesterol for conversion into bile acids in *Oreochromis mossmambicus*, respectively. Jyothi and Narayan (2001), however, attributed the decline in serum cholesterol and inturn a disturbed steroidogenesis to liver disfunction due to carbaryl stress in *Clarias batrachus*. On the other hand, Gill and Pant (1988) reported that the decrease in serum cholesterol was due to the cadmium stress affecting absorption of dietary cholesterol in fish. In the present study also hypocholesterolimia is accompanied by fall in GSI of the fish, which clearly shows that gametogenesis is affected because the cholesterol the precursor to all biologically important steroids is present in insufficient amounts.

The present study clearly indicates that both the sublethal (3% and 15%) concentrations of the effluent upset metabolism and inturn greatly affect the reproductive potential of the fish as there was a regular decline in GSI during all the three

phases of the reproductive cycle even when there was an increase in the concentration of the two biomolecules in the blood plasma of the fish. The stress leads to accumulation of biomoleculels in the blood but retards the onset of maturation of oocytes (low GSI during all the phases). It was observed that fish was most sensitive to the stress of the effluent during spawning phase followed by preparatory and prespawning phase of the reproductive cycle.

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