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MONITORING OF SUBLETHAL EFFECT OF CHROMIUM ON MELANOPHORES

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ABSTRACT

Some heavy metals are essential for normal physiological functioning of fish but become toxic when they accumulate in their body tissues and are not metabolized, dyes. Chromium is dangerous as it can accumulate in fish body as much as 4000 times greater than that of their surroundings. The present investigation deals with sublethal effects of chromium sulphate (10 ppm. 10% of 96 h LC_{50}) on the melanophores of *Catla catla* was investigated to understand its toxicity on Melanophores morphology. Observed toxic pathological alterations include statistically significant variations in the number, size and shape of the Melanophores. Due to the lysis of the Melanophores, the melanin contents were poured into the surrounding matrix of the connective tissue, between 20 and 30 days of exposure. The chromium induced morphological changes in the melanophores protected the delicate epidermis from the toxic medium. In addition to the physiological colour changes, there was slow morphological colour changes by the gradual regeneration of the chromatophores. The density of chromatophores was maximum after 45 days. These generated pigment cells were smaller in size and more in number when compared with the normal melanophores. The study show that higher concentrations of metals in fish can alter its physiological functioning that could lead to high mortality and ultimately loss of indigenous fish biota.

Figure : 00 References : 13 Table : 00

KEY WORDS : Heavy metals, Melanophores, Sub- lethal, Water pollution

Introduction

Every human society be rural, urban, industrial and most technologically advanced society dispose of certain kinds of by-products and waste products which when are injected into the biosphere in quantities so large that they effect the normal functioning of ecosystem and have an adverse effect on plant, animals and man are called pollutant. These pollutants may be natural pollutants or synthetic man made, anthropogenic or xenobiotic pollutants some of them are either biodegradable pollutants or non-biodegradable pollutants. Extensive urbanization and industrialization have increased the heavy metal concentrations in the aquatic environment. The occurrence of heavy metal contaminants, especially lead, chromium and cadmium in excess of the natural load has become a problem of increasing concern. The heavy metals lead and chromium usually enter water bodies either through industrial discharges or by the deterioration of galvanized pipes and during idol immersion[5]. Fish skin, being directly exposed to the ambient toxicants, is used extensively as a potent indicator of contaminated aquatic environment[11]. Therefore in the present study efforts have been made to investigate the toxic effects of sublethal concentration of chromium on the Melanophores of *Catla catla*.

Material and Method

Live specimens of *Catla catla* were collected locally and acclimated to the laboratory conditions

for a period of 30 days. During the acclimation period, fish were regularly fed. Water was renewed after every 24 hour (h) leaving no faecal matter, unconsumed food or dead fish if any. Prior to the commencement of the experiment the median. lethal concentration of chromium for 96 h (96 h LC_{50} value) was calculated following 24 h renewal bioassay system and trimmed Spearman Arber method[6] and it was found to be 100 ppm (95%confidence limit). For the present study, five groups of 10 fish each were exposed separately to 20 liters of 10 ppm (10%of 96 h LC_{50}) of chromium chloride solution prepared in tap water having dissolved oxygen 5.60 ppm, hardness23.2 mg/1; pH 7.5 and water temperature27±2°C. Feeding was allowed in the experimental as well as control groups for a period of 3 hours prior the renewal of the media throughout the period of the experiment. Parallel control groups were also kept along with the experimental groups. Three fish, each from the experimental as well as control groups were sacrificed at the expiry of 5, 10, 20, 30 and 45 days. Fragments of skin measuring about 5x8mm were excised from the back of the fish between dorsal fin and lateral line canal and were fixed in aqueous Bouins, fluid. Melanophores counting was done from alien blue (AB) pH 2.5/ periodicacid-schiff stained[9], permanent whole mount preparations of control as well as experimental tissues[3]. The density (number) of Melanophores was calculated following stan-136dard statistical procedures based on random sampling of five different sites from three control as well as experimental fish of each sacrificing interval following[3,4]. One-way analysis of variance (ANOVA) followed by Duncan's multiple range test was performed to determine whether Melanophores density was significantly affected by exposure periods.

Measurement methods Individual melanophores were measured with the help of ocular-meter and microscope. Melanophores size index was calculated. The observed values have been multiplied by unit of micrometer. Thereafter the mean was calculated and this value was divided by 100 to obtain a value in a digit with three decimal points. This was Mean Melanophores Size Index (MMSI). Statistical analysis of data was conducted.

Result and Discussion

Control Melanophores: In fish the Melanophores are mostly located in the sub-epidermal connective tissue of dermis just below the basement membrane. They appear to be uniformly distributed as asteroid cells having tentacle like processes of greatly variable length radiating from their central body. These cells (Melanophores) are gorged with pigment (melanin) granules and the distance between the adjacent melanophores seems to be more or less equal. There was no significant alteration in the density of melanophores. Whole mount preparation of dorsal skin epidermis of fish showing the normal distribution pattern of chromatophores and their stellate structure[2] (Alcian blue (AB) among the various control groups, the numerical average of all the control groups is taken into account. ***Experimental melanophores*** :Five days after transferring the fish into chromium solution the central body and tentacle like processes of Melanophores became less distinct as compared to control. However, the accumulation of melanin granules in the peripheral regions of melanophores gives a clear interlacing pattern as if they were thickly packed. Preparations of 10 ppm chromium chloride exposed dorsal skin epidermis of *Catla* showing toxic pathological alterations in the melanophores at different exposure periods. Interlacing of adjacent melanophores after 5 days of exposure, without any intercellular spaces. Consequently, ten days of exposure,the melanin granules concentrated at the periphery of the melanophores and drew back towards the astral arms as well as to the central body leading tothe disappearance of the interlacing patterns. As a result, the central body and the radiating arms became more discernible when compared withthe previous stage. Similarly, the intercellular spaces were also increased at this stage of exposure. Twenty days of exposure was marked by the disintegration of many of the melanophores and the subsequent release of melanin, the size of the melanophores decreased their density was highest after forty five days. The distribution pattern of chromatophores is the main factor that determines the ultimate pigmentation pattern of a species[2]. The embryo logically derived from the neural crest and they migrate to their final destinations to determine the pattern of distribution[1]. However, it appears that even after occupying their final destinations, the melanophores retain a high degree of plasticity. In the present study after 5days of exposure, eventhough there was no significant increase in the density of chromatophores they appear to be thickly packed and interlaced with each other

leaving no intercellular spaces. The reason for the interlacing pattern could partly be traced to the migration of the melanin granules towards the periphery of the asteroid structures in response to toxicity, as cations in the media are reported to cause dispersion of melanin[8]. However, the general outstretching of these cells, giving an impression of them being tightly packed, might be due to the high plasticity of the cells which they retain even after the postembryonic development. These observations are supported by the reports that the phenotypic expression of melanophores may vary according to environment[7,12,13]. The statistical analysis clearly shows that after 5 days of exposure no new chromatophores were produced. However, there appeared a marked difference in the melanization pattern when compared to the other stages of experimentation. A rapid change in the appearance of chromatophore without the production of any new cells may be considered as a physiological colourchange. On the contrary in all the subsequent stages the density of chromatophores in general shows a gradual increase with a maximum after 45 days of exposure. This gradual increase in the melanophore density was also accompanied by an observable darkening of the skin. While studying the role of epidermal melanocytes in the adaptive colour changes in amphibians have also reported increased melanophore mitosis and melanogenes is leading to morphological colourchange.;heavy metals like lead and chromiam interferes with the osmoregulation and osmotic balance. The variations in the osmotic and ionic factors play an important role in the melanization activity. In general, the increased melanization observed in the present study may be an attempt by the fish to protect itself from heavy metal toxicity. This assumption is again supported by the fact that melanin has the capacity to bind with aromatic compounds and cations[3,10]. It is worth noting that the regenerated cells after 45 days of exposure were smaller in size and had lesser number of branched radiating astral arms. However, their density was the highest among the various experimental groups, leading to the overall darkening of the epidermis. may be concluded that a gradual melanization activity by the fish is to protect itself from metal toxicity.

Conclusion

Present study shows that water pollution is due to toxic pollutants. Pollution due to toxic metal like chromium and lead may change hydro chemical parameters of water and cause damage to aquatic ecosystem *e.g.* increase in pH, temperature, acidity and BOD. Behavioural changes were also recorded in fins movements, swimming performance.. Fish skin is unique for its dynamic pattering speed of change due to aggregation and dispersion of chromatophores. Chromatophores are pigmentary organs that act simply as selective colour filter. Change in colour of aquatic organisms is a warning of toxicity. Change in functioning of chromatophores changes the colour of fishes. With this early observation we can detect presence of pollutants in water. The present study suggests that melanophores can be used successfully as bio marker of water pollutions mainly for heavy metals like Lead and chromium.

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