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Influence of Industrial Pollutants on Thyroid and Interrenal Functions in the Fresh Water, Air-breathing Teleost, *Channa punctatus* (Bloch)

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Abstract

The fresh water air breathing teleost *Channa punctatus* was exposed to mercuric chloride (0.15 ppm) and cadmium chloride (35.5 ppm) for 1, 2, 7, 15 and 30 days to examine the short as well as long term effects of sublethal doses of these industrial pollutants on the hypothalamo-pituitary-thyroid axes. The study demonstrates how pollutants influence the release of thyroxine mediated peroxidase system of iodination to teleosts. Inhibition of head kidney peroxidase enzymes is usually associated with a decrease in iodide peroxidase activities and blood thyroxine along with tri-iodothyronine level. Alterations in the head kidney acid phosphatase activities indicate changing profile in the lysosomal membrane characteristics caused by the pollutants. The stabilization of lysosomal membrane may be explained by a relative scale reduction of head kidney lysosomal protease activities, which is essential for thyroxine and tri-iodothyronine release from the follicular cells of the head kidney. Guaiacol or non-iodide peroxidase is also hyperactive in the head kidney of *Channa punctatus*. An increase in guaiacol peroxidase activities has been noticed at certain periods of exposure to each of the pollutants. The changing histopathological aspect in the head kidney induced by the pollutants also reveals dispersions of the interrenal, chromaffin tissues and necrosis of the haemopoetic elements. The secretion of adreno-cortical cells is mainly concerned with the organism reaction to stress. The cellular damage in the interrenal tissues due to exposure to industrial pollutants probably causes the lower production of cortisol with an overall decrease in the capacity of the fish to fight against stress. Analysis of available data suggests that

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mercury and cadmium cause the depletion of energy resources and disturb the metabolic pathway as indicated by the adverse effects on haematological parameter, enzyme system of head kidney and histopathological lesions in the head kidney.

Introduction

The exposure of animals to xenobiotics produces a variety of responses and it cannot be denied that the survival of the organism depends upon the efficiency of the endocrine function. The thyroid and interrenal tissues are considered as the major endocrine systems for the maintenance of the general health of the animals. It is known that the thyroid gland of fish is normally diffused and occurs as follicular patches in the sub and para-pharyngeal regions. It appears in the head kidney too. The interrenal, chromaffin tissue in the head kidney is homologous to the mammalian adrenal cortex and medulla, respectively (Chavin & Kovacevic 1961; Hanke & Jones 1966; Yaron 1970; Hooli & Nadkarni 1975; 1976). Iodide peroxidase and lysosomal enzyme activities are important to thyroxinogenesis. Iodide peroxidase oxidizes inorganic iodide to make active iodine prior to incorporation into tyrosine, and lysosomal protease cleaves T_4 and T_3 from thyroglobulin for the release of these hormones in the blood. Thus disturbances in the activities of lysosomal protease and cytosolic iodide peroxidase may affect not only the rate of thyroxine production but also its release.

It has been documented that the head kidney peroxidase in *C. punctatus* (De and Bhattacharya 1976) is iodide peroxidase (IPOD), and some industrial pollutants including phenol, ammonia cause significant inhibition to the head kidney peroxidase within a short period either in vivo (Mukherjee & Bhattacharya 1975) or in vitro (De and Bhattacharya 1976). Besides IPOD, guaiacol or non-iodide peroxidase (GPOD) is also proactive in the head kidney of *C. punctatus* and *A. testudineus* and possibly plays a significant role in the detoxification of pollutants (Mukherjee & Bhattacharya 1975; Chatterjee & Bhattarcharya 1985).

Thyroid hormone and cortisol have an important physiological role in regulating metabolism; cortisol influences the physiological fitness of a fish through its effects on reproduction, growth and immune functions (Pickering 1993). Chronic exposure of yellow perch to sub-lethal levels of heavy metals (cadmium, zinc and copper) impairs growth and alters the activities of metabolic enzymes (Lavesque et al. 2002). Copper normally

reduces growth rate in rockfish *Sebastes schlegeli* and there is an inverse relationship between the growth rate and the copper concentration as reported by Kim & Kang (2004). There are some evidences that Hg may adversely affect the functional integrity of the interrenal and thyroid axis of fish. The qualitative relationship between exposure to mercury and cadmium and perturbations of the thyroid and interrenal function in fish has not been investigated.

Industrial effluents contain a variety of pollutants such as heavy metals, pesticides, chemical fertilizers, detergents, organic, inorganic salts, oil etc. which generate serious problems to the aquatic organism, especially the fish species. The changing biochemical constituents of tissues and enzyme activities are important in determining the nature and extent of toxicant effects on organisms. The objective of the present study is to clarify the role of the industrial pollutants in thyroid and interrenal functions in the fresh water teleost, *C. punctatus* (Bloch).

Materials and Methods

The fresh water air breathing teleost, *C. punctatus* was exposed to mercuric chloride (0.15 ppm) as well as cadmium chloride (35.5 ppm) for 1, 2, 7, 15 and 30 days to determine the short and long term effects of sublethal doses of these industrial pollutants on thyroid and interrenal function, enzyme system of head kidney. The fishes were collected locally and acclimatized to laboratory conditions for 15 days. During the experimental period all the fishes were fed once daily at 1800 hrs with commercial fish feed containing 8% protein, 3% lipid and 20% carbohydrate. The average length and weight of fish were supposed to be 16 ± 1 cm and 35 ± 2 g, respectively. Fishes were kept in batches of 10 in a glass aquaria measuring 60 × 30 × 30 cm. Each aquarium contained 30 liters of diluent water. The range for water pH, dissolved oxygen content, temperature and hardness (as $CaCo_3$) were 6.5 to 7, 6 to 8 ppm, 26 to 30^oC and 160 ppm, respectively.

The process of changing water took place daily during the exposure period and the desired level of pollutant poured in a fresh manner. The head kidney lysosomal protease, lysosomal acid phosphatase, guaiacol peroxidase, Iodide peroxidase, blood tri-iodo thyronine (T_3) and thyroxine (T_4) were assayed on days 1, 2,7,15 and 30 respectively. In contrast, fishes under control were sampled during the exposure period on days 1, 2, 7, 15

and 30, and the data under controlled situations for each time period represent the average value in the respective days mentioned earlier. The small pieces of head kidney were collected from fishes under control as well as under experiment on the 1st, 2nd, 7th, 15th and 30th days of exposure, and fixed in Bouin's fluid for routine haematoxylin and eosin study and Zenker's fluid for Mallory's triple staining study. The tissues were fixed in Orth's fixative (Lillie 1954) to identify the chromaffin cells in the head kidney.

Estimation of peroxidase

Head kidney was instantly dissected out and the 0.5% homogenate prepared with 0.05 M phosphate buffer of pH 6.5 was subjected to centrifugation at 10,000X g for 10 minutes. The supernatant was analyzed for peroxidase activity using a spectrophotometer (ECIL) with guaiacol as the hydrogen donor (Bergmeyer et al. 1974) at 470 nm instead of 436nm since maximum absorbance was obtained at this wavelength. Enzyme activity is expressed as $\Delta OD \cdot min^{-1} \cdot mg\ protein^{-1}$. The method of Alexander (1962) was followed to determine whether the head kidney peroxidase contains iodide. The results are expressed in terms of $\Delta OD \cdot min^{-1} \cdot mg\ protein^{-1}$. Results are depicted as percentage of change in enzyme activity against the control.

Lysosomal enzymes

The 5% head kidney homogenate in 0.44 M sucrose was centrifuged at 2000 g for 5 minutes at 4°C. The supernatant was then centrifuged at 13000 g for 10 minutes to isolate the lysosomal particles (Fukuzawa et al. 1971). The particles were washed thoroughly with 0.44 M sucrose and suspended in 0.44 M sucrose solution containing 0.177 M KCI and assayed for lysosomal acid phosphatase (Nakagawa et al. 1980) and lysosomal protease (Spies 1957). Enzyme protein was estimated following the method of Lowry et al. (1951), using bovine serum albumin as standard.

Radio-immuno assay of blood thyroxine and tri-iodothyronine

Blood drawn from the caudal vein by means of a heparinized syringe was assayed for thyroxine (T_4) and tri-iodothyronine (T3) following a radio-immuno assay technique (Chopra 1972). All reagents required for this assay were procured from the Bhaba Atomic Research Center, Trombay, India. Data were tested statistically following Student's *t* test (Snedecor & Cochran 1971).

Results

The exposure of *C. punctatus* to mercuric chloride (Fig. 1) resulted in significant inhibition of iodide peroxidase activity in all the days of treatment except in the 1st day when a 30% increase was noticed compared to the control value. Lysosomal protease (Fig. 2) activity however, remained inhibited for fish treated with mercuric chloride and it was accompanied by an increase in the lysosomal acid phosphatase (Fig. 3) activity. Blood thyroxine and tri-iodo thyronine contents were reduced throughout the experiment period (Figs. 4 and 5). In the case of guaiacol peroxidase activity, inhibition was maximal (62%) on the 1st day of exposure compared to the control value, while on the 15th day only 30% inhibition was recorded. Interestingly enough activation of guaiacol peroxidase activity occurred at 18% on the 30th day of exposure (Fig. 6).

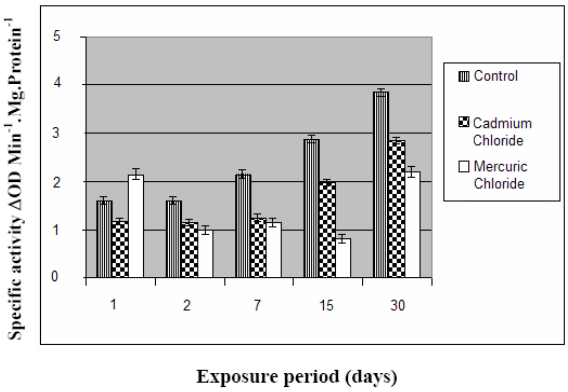

Figure 1. Iodide peroxidase activity ±S.E. in head kidney of C. punctatus exposed chronically to cadmium chloride and mercuric chloride

Iodide peroxidase activity (Fig. 1) in *C. punctatus* decreased consistently throughout the experimental duration owing to cadmium chloride exposure. The iodide peroxidase activity, blood thyroxine and tri-iodo thyronine titre (Figs. 4 and 5) also decreased dramatically during the first two days of the exposure pe

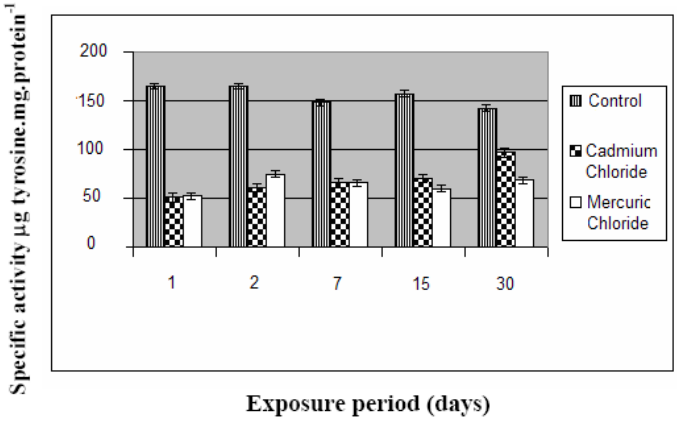

Figure 2. Lysosomal protease activity ±S.E. in head kidney of *C. punctatus* exposed chronically to cadmium chloride and mercuric chloride

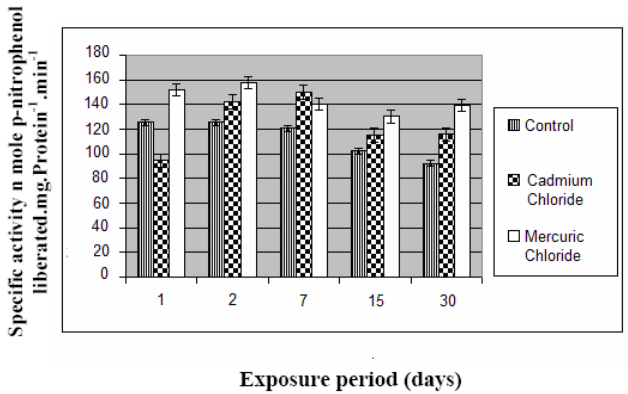

Figure 3. Lysosomal acid phosphatase activity ±S.E. in head kidney of *C. punctatus* exposed chronically to cadmium chloride and mercuric chloride

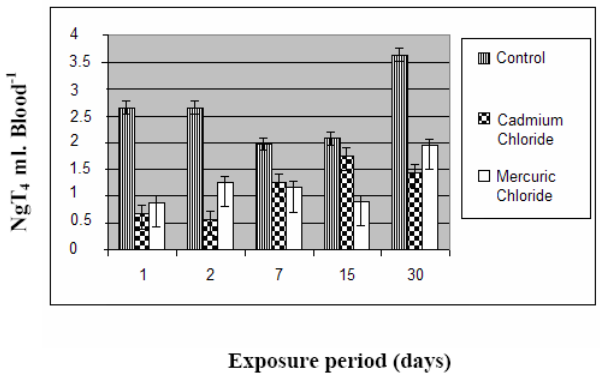

Figure 4. Thyroxine level ±S.E. in blood of *C. punctatus* exposed chronically to cadmium chloride and mercuric chloride

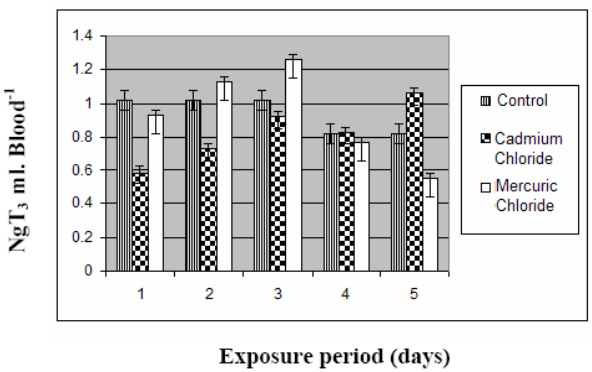

Figure 5. Tri-iodo thyronine level ±S.E. in blood of *C. punctatus* exposed chronically to cadmium chloride and mercuric chloride

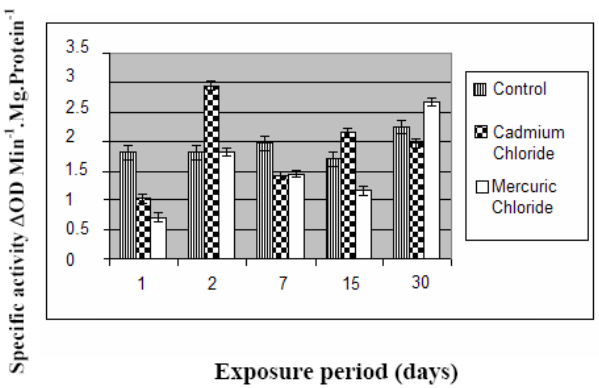

Figure 6. Guaiacol peroxidase activity ±S.E. in head kidney of *C. punctatus* exposed chronically to cadmium chloride and mercuric chloride

riod. They rose on the 15th day and subsequently declined significantly on the 30th day. Lysosomal protease activity was remarkably inhibited by cadmium chloride throughout the experimental period. Maximum inhibition occurred on the 1st day (Fig. 2). While lysosomal acid phosphatase demonstrated an increase in its activity on the 2nd day (Fig. 3), guaiacol peroxidase activity was found to be inhibited by 45% after the 1st day of the exposure period. Therefore, a significant increase (68%) was recorded on the 2nd day followed by a decline, which reached the control level within 30 days of the treatment (Fig. 6).

Histopathology of the head kidney exposed to mercuric chloride and cadmium chloride

The normal head kidney has a concentration of haemopoietic tissue with chromaffin and interrenal tissue distributed along the posterior cardinal veins and tributaries. Interrenal tissues are arranged in the form of small lobules. Each cell of the interrenal tissue is columnar having a rounded nucleus towards its base. Chromaffin cells are found to lie within the interrenal tissue to form an adrenal complex (Fig. 7). A few thyroid follicles are also found in the head kidney, which are confirmed by positive PAS reaction. Each thyroid follicle consists of a homogeneous colloid encircled by cuboidal epithelial cells.

Due to 30 days chronic exposure to mercuric chloride and cadmium chloride, interrenal tissues were found to lose their regular arrangement resulting in gradual degeneration. Chromaffin tissues remained in dispersed conditions. The haemopoietic cells appeared to shrink considerably and were fewer in number compared to those in control. This degeneration was persistent up to the 15th day in the case of cadmium chloride but on the 30th day histopathology of the head kidney was found to gain towards normalization of the histological picture. But in the case of mercuric chloride, degeneration continued until the end of the experiment period (Figs. 8 to 12).

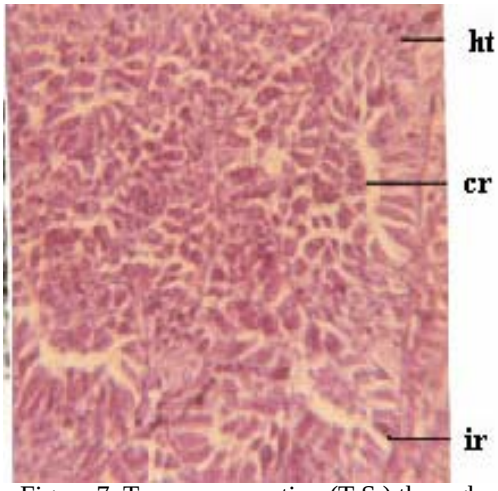

Figure 7. Transverse section (T.S.) through the head kidney of the control fish X500

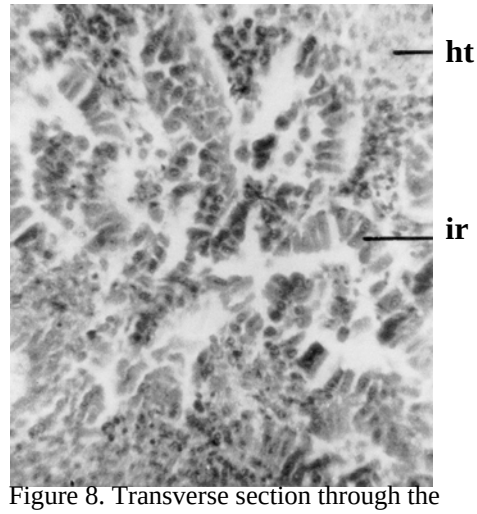

Figure 8. Transverse section through the head kidney of fish exposed to cadmium chloride 15 days X500

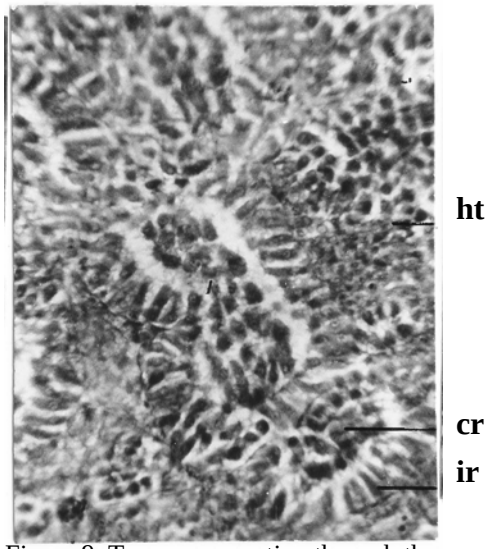

Figure 9. Transverse section through the head kidney of fish exposed to cadmium chloride for 30 days X 500

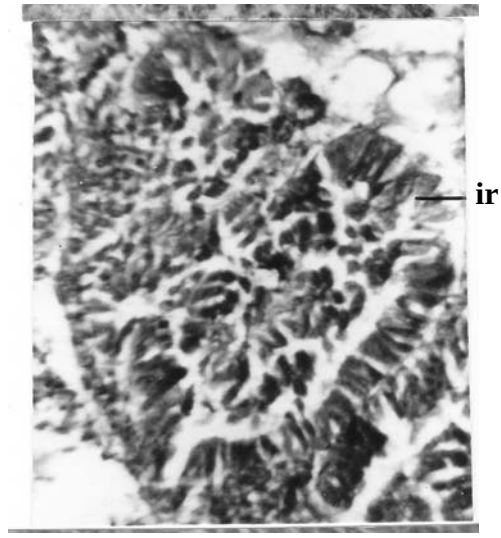

Figure 10. Transverse section through the head kidney of fish exposed to mercuric chloride for 1 day X 500

Legend :

cr: chromaffin tissue; **ir:** inter-renal tissue; **ht**: haemopoietic tissue; **dit**: degenerating inter-renal tissue

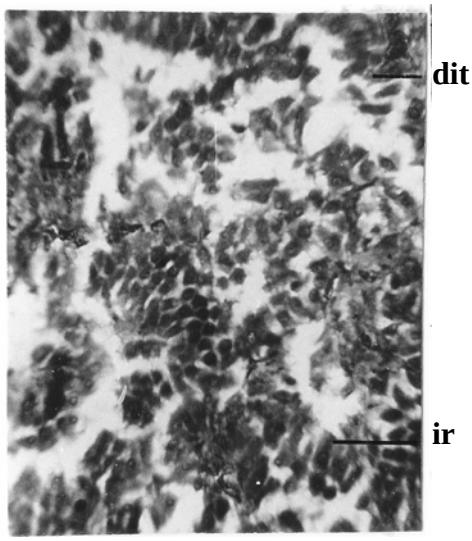

Figure 11. Transverse section through the head kidney of fish exposed to mercuric chloride 15 days X 500

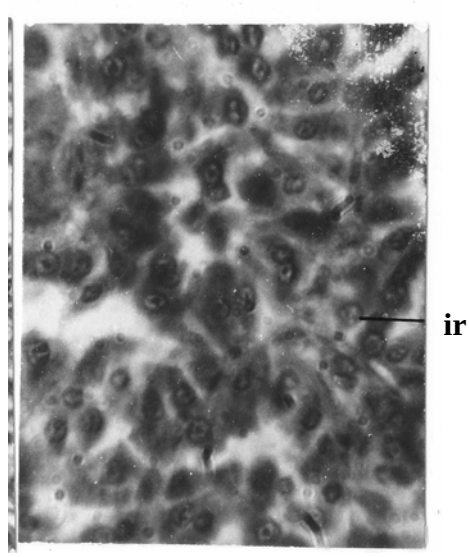

Figure 12. Transverse section through the head kidney of fish exposed mercuric chloride for 30 days X 1400

Legend :
cr: chromaffin tissue; **ir:** inter-renal tissue; **ht**: haemopoietic tissue; **dit**: degenerating inter-renal tissue

Discussion

In fishes thyroid hormones contribute to the control of growth and development, metabolism and osmoregulation, often in association with growth hormone and cortisol. One of the most spectacular actions of T_4 is the stimulation of metamorphosis. Thyroid hormones also appear to be involved in triggering the migratory behavior and part of the adaptive osmoregulatory changes in salmonids as they move in sea water (Prunet et al. 1989). Although thyroxine (T_4) is the major circulatory thyroid hormone in fish, there is strong evidence that T_3 (tri-iodo thyronine) is the physiologically relevant hormone, monodeiodination of T_4 probably occurs in target tissues (Hazon & Balment 1997).

The head kidney peroxidase from *C. punctatus* is found to form I^-_3 suggesting that this peroxidase is of physiological importance (De & Bhattacharya 1976). Chavin & Bouwman (1965) demonstrated that thyroid

hormone synthesis does take place in the kidney of some fish and this is catalysed by iodide peroxidase (Kumar et al. 1973). In *Channa punctatus* iodide peroxidase activity is in general depressed by the treatment of mercuric chloride and cadmium chloride. The profile of blood T_3 and T_4 concomitantly traced during the exposure of fish to the toxicants, in most cases demonstrated a significant decline as noted in *Anabas testudineus* exposed to phenol, ammonia, mercury and cadmium (Chatterjee & Bhattacharya 1985). Experiment with mouse has also revealed that acute and sub-acute treatment with $HgCl_2$ reduce T_4 fraction significantly (Nishida et al. 1986).

Environmental toxicants such as organochlorines and carbon disulphide are known to depress thyroid functions in fish (Grant & Schoettger 1972; Cavalleri 1972). Thyroid hypofunction due to environmental toxicants may be mediated through inhibition of iodide peroxidase as this enzyme from fish kidney source is readily inactivated by toxicants or inhibition of lysosomal protease as evidenced by the present study. Mensi et al. (1982) reported that nitrate intoxication in rainbow trout causes lysosomal damage. In the present investigation it is noted that cadmium chloride have a stabilizing effect on the membrane as evidenced by decrease release of lysosomal acid phosphatase while mercuric chloride produced a labilizing effect with an increase in the acid phosphatase activity. The low blood T_3 and T_4 litre in toxicant treatment was accompanied by 15 to 85% inhibition of lysosomal protease activity. It is surmised that due to affinity of these toxic compounds to bind to sulphydryl groups the lysosomal membrane is stabilized. The stabilization may account for a lowered protease activity, which is essential for T_3 and T_4 release from follicular cells of the head kidney (Gorbman et al. 1983). Plasma T_4 decreased significantly in *Anguall anguilla* (L) owing to exposure to chromium and copper reported by Teles et al. (2005). Lead nitrate impairs thyroid function involving the hypothalamo hypophysiothyroid axis in catfish, *Clarias batrachus* (L) reported by Katti & Sathyanesan (1987). Lead induced thyroid dysfunction and lipid peroxidation in the fish *Clarias batrachus* reported by Chawrasia et al. (1996).

It has been established that guaiacol peroxidase is one of those enzymes that responds rapidly to the action of various external agents including chemicals (Matkovics et al. 1977). In *Channa punctatus* an increase in guaiacol peroxidase activity is noted at certain periods of exposure to each of the toxicants. This suggests that in *C. punctatus* elevated guaiacol peroxidase may have a role in the detoxification of industrial pollutants.

Cortisol is the major corticosteroid released by the inter-renal gland into the blood of teleost fishes. The major functions of cortisol relate to energy metabolism, ion regulation and in stress response. In teleost fishes cortisol activates key enzymes for intermediary metabolism, ion regulation and in stress response. In teleost fishes cortisol activates key enzymes for intermediary metabolism in the liver (Vijayan et al. 1991). Cortisol may also interact with hormonal systems and has been reported to inhibit prolactin release (Borski et al. 1991) and modify thyroid hormone levels in coho salmon during smoltification (Redding et al. 1984).

During the present investigation significant degenerative changes in the head kidney were found to occur in *Channa punctatus* owing to cadmium chloride and mercuric chloride. Bleau et al. (1996) showed that in rainbow trout sub-lethal concentration of inorganic and organic mercury stimulated the pituitary inter-renal and pituitary thyroid axes. There is some evidence that plasma cortisol and plasma T_4 follow a similar pattern in response to contaminants as to other treatments (Vijayan & Moon 1992; Hontela et al. 1995) and an activation of the inter-renal gland by T_4 has been documented in fish (Young & Lin 1988). According to the report of Lacroix & Hontela (2006), the deleterious effect of (Cd^{++}) on cortisol steroidogenesis may be enhanced when the endocrine stress response is triggered. Thophon et al. (2003) reported that in fish *Lates calcarifer,* gill lamaellae and kidney were the primary target organs for the acute toxic effect of cadmium.

The cellular damage in the inter-renal tissue due to exposure to mercury and cadmium points to a lowered production of cortisol with an overall decrease in the capacity of the fish to fight stress. Analysis of the available data suggests that the pollutants cause depletion of energy resources and disturb the metabolic pathway as indicated by the adverse effects on haematological parameter, enzyme system and histopathological lesions in the head kidney.

Conclusions

The immune system is a key point for the toxic effects of industrial pollutants both in humans and animals. In human it has been observed that occupational and environmental exposure to mercury may cause clinical and sub clinical effects (Salmo et al. 2002). Consumption of fishes containing pollutants has been identified as a health risk. Weekly plasma mercury

level was correlated to average fish eating frequencies in the corresponding week of blood collections. Fish intake within 24 hours influenced plasma mercury levels significantly in Japanese men reported by Karita & Susuki (2002). Exposure to fish with Hg during pregnancy and lactation were studied in 100 women and new borns from Porto Velho, Amazon, by Marques et al. (2007). According to their report the development delay of exclusively breastfed infants is a component of the health inequalities that accompanies socioeconomic disadvantages. So the mercury levels in the blood of fish-eaters and non-fish-eaters are perhaps well distinguishable signifying that the health damage is the aftermath of fish intake for the group of fish eaters. As a result the loss of human capital because of the side effects of biomagnifications (and other species also) is not only highlighted but also intensified negatively the growth rate of gross domestic product (GDP) of a nation, as labour is one of the means of production. The valuation of health hazards and the opportunity cost, due to ailment, of human capital are relevant, in addition to the cost of health protection.

The methods of Revealed Preference and Stated Preference (Cropper & Oates 2001) could be applied to the economic valuation of resource loss (health damage) ,subject to availability of data. The future is that the group of fish-eaters must think twice before they make sure whether fish-food is good for nutrition and health protection. The valuation of resource loss, due to the impact of water pollution upon fish would supplement the understanding of the estimated social cost borne by the common people. The amount of social cost is perhaps greater than the water pollution abatement cost. It could have been better to maintain water quality rather than bear the unaffordable social cost of large-scale water pollution.

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