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Cadmium induced vertebral deformities in an estuarine fish, *Ambassis commersoni* Cuvier

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Abstract. *Ambassis commersoni* was treated with 3 different concentrations (0·1, 0·25 and 0·5 ppm) of cadmium upto 120 days. Fishes treated in higher concentrations developed vertebral deformity and the deformation was prominent after 89th and 115th day. The levels of the vertebral minerals such as calcium, magnesium and phosphorus lowered in all exposure concentrations and minerals lost were directly proportional to the toxic concentrations. Nearly 2/3 of skeletal mineral composition was eliminated in the deformed fish. Vertebral fracture was attributed mainly to the large amount of vertebral components eliminated from the vertebrae. Vertebral cadmium accumulation was also linear in relation to the experimental concentrations.

Keywords. Cadmium; caudal curvature; vertebral deformities; calcium; phosphorus; magnesium; elimination.

1. Introduction

The toxic effects of cadmium (Cd) on fishes are known (Eisler 1971; Selvakumar 1981) and it has been identified as a causative factor for the outbreak of 'Itai-itai' disease in Japan. Pathological changes (Gardner and Yevich 1970) and depression of carbohydrate metabolism (Larsson 1975; Larsson and Haux 1982) and oxygen consumption (Selvakumar 1981) by Cd have also been reported. Exposure to Cd for longer durations are also known to induce vertebral deformities in fishes (Bengtsson *et al* 1975). Reports on skeletal deformities induced by chemical agents are very limited. Weis and Weis (1976) and Couch *et al* (1977, 1979) recorded pesticide-induced vertebral deformities in *Cyprinodon variegatus*. Linden (1976) noted that crude oil also caused vertebral malformation in *Clupea harengus membras* within a short period of exposure.

Bengtsson (1974) and Holcombe *et al* (1976) noted vertebral malformations in zinc-treated *Phoxinus phoxinus* and lead treated *Salvelinus fontinalis* after 4 months and 67 weeks of exposure respectively.

The present investigation was carried out on an estuarine fish *Ambassis commersoni* Cuvier by exposing them to 3 different sublethal Cd concentrations for 120 days. At the close of the experimental period, the Cd content and the mineral composition of the Cd treated fish vertebrae were also estimated.

2. Materials and methods

Specimens of *A. commersoni* were collected from the marine zone of the Vellar estuary. The length of the fishes used was $6{\cdot}3 \pm 0{\cdot}2$ cm and the weight $2{\cdot}5 \pm 0{\cdot}5$ g. The

fishes were kept in acclimatisation tanks for a week in the laboratory. The salinity of the test water ranged between 27 and 28‰. Fishes were fed regularly with chopped clam meat. Healthy fishes were selected and exposed to Cd concentrations of 0·1, 0·25 and 0·5 ppm (each set consisted of 12 specimens). These exposure concentrations were prepared on the basis of 96 h LC_{50} value (5·20 ppm). Cd solution was prepared from Analar $CdCl_2 \cdot H_2O$ salt. The experimental fishes were fed every day during all the 120 days.

After 120 days of experimental exposure period, the fishes were anaesthetized with MS 222 and the vertebral damages if any were located by X-ray photograph using a Siemen-160 (West Germany) X-ray equipment. The vertebral Cd concentration was estimated by the method of Smith and Windom (1972) using a Hilger-Watts Atmospek H 1550 AAS.

The vertebral column of the untreated and treated fishes were dried at 60°C for 24 h in a oven. The dried samples were ashed by heating them for 12 h at 500°C in a muffle furnace. The ashes were then dissolved in 2:1 of concentrated HNO_3 + concentrated $HClO_4$ and made upto 100 ml with double distilled water. The vertebral calcium (Ca) and magnesium (Mg) levels were estimated following the method of Katz and Narone (1964). Phosphorus (P) was estimated by the procedure of Strickland and Parsons (1972) and concentrations were noted in a Hitachi double beam UV spectrophotometer Model 220.

3. Observations

The concentration of Cd as well as those of Ca, Mg and P in the vertebrae of both untreated and treated fishes were estimated (figure 1).

Two fishes were observed with vertebral abnormalities out of 12 fishes exposed to 0·5 ppm Cd concentration. Caudal vertebral curvature (figure 2B) was clearly observed in one fish after 89 days of Cd exposure. After 115 days, another fish in the same experimental tank with abnormal swimming movement was noted. The damage (figure 2C) was located in the vertebral column through x-ray photograph in this fish with abnormal swimming movement.

The Cd content of the vertebrae in the control fishes revealed no traces of Cd. However in fishes exposed to Cd, its accumulation in vertebrae was seen. After 120 days of exposure the vertebral Cd concentrations were 8·32, 10·91 and 12·86 μg/g in respect of the 3 exposure concentrations, viz 0·1, 0·25 and 0·5 ppm (figure 1).

In the untreated fishes the vertebral mineral levels were found to be in the following order: (i) Ca 132·81 mg/g, (ii) P 52·97 mg/g and (iii) Mg 3·27 mg/g. After the experimental period the mineral levels were low in all Cd treated fishes and showed the following pattern: (i) Ca 120·72, 116·36 and 106·41 mg/g, (ii) P 46·59, 46·50 and 34·86 mg/g and (iii) Mg 3·08, 2·82 and 2·16 mg/g respectively in the 0·1, 0·25 and 0·5 ppm of Cd concentrations to which they were exposed. It is evident from the results that the loss of minerals from vertebrae was dose dependent (figure 1).

The Cd content (18·97 μg/g) in vertebrae of abnormal fish was higher than that in other treated fishes. Nearly 58% of Ca, 56% of P and 67% of Mg were lost from the vertebrae of the deformed fish. The skeletal mineral composition was found in the following order: (i) Ca 56·13 mg/g, (ii) P 23·49 mg/g and (iii) Mg 1·09 mg/g.

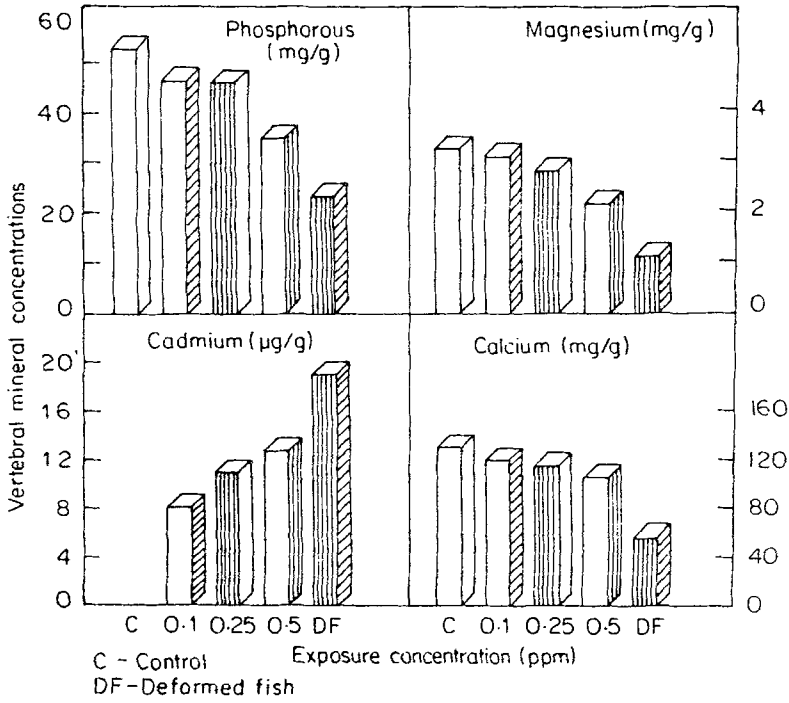

Figure 1. Concentrations of Cd, Ca, Mg and P in the vertebrae of both untreated and treated fishes.

3. Discussion

In the present investigation vertebral deformities were observed in treated fishes only after the 89th day (caudal curvature) and 115th day of exposures (with vertebral damage) at a Cd concentration of 0·5 ppm (figure 2). Eaton (1974), Bengtsson *et al* (1975) and Muramoto (1981a, b) also recorded similar vertebral anomalies in Cd treated *Lepomis macrochirus*, *Phoxinus phoxinus* and *Cyprinus carpio* L. respectively. From the results obtained in this study it is apparent that more than 2/3 of the vertebral Ca, Mg and P were found to be eliminated in deformed fishes. These were lowered to a considerable extent in all Cd treated normal fishes. The results of the present study corroborate the findings of Muramoto (1981a, b) on *Cyprinus carpio* L.

The caudal curvature and vertebral damages in *A. commersoni* might have been influenced by the loss of Ca, P and Mg from the vertebral column. The work of Matsunaga *et al* (1962) provides evidence to show the occurrence of as many as 72 fractures in 'Itai-itai' patients affected by Cd food poisoning, which led to large scale elimination of minerals from the bones. Koyama and Itazawa (1977a, b) and Itokawa *et al* (1978) also noted vertebral deformation, vertebral mineral elimination and skeletal lesions in Cd exposed *Cyprinus carpio* L. and rat. In the present study, abnormal swimming behaviour, altered movement and flexibility of body of fish might have resulted from excess elimination of skeletal mineral like Ca, P and Mg.

Larsson (1975) and Larsson *et al* (1981) have suggested that the vertebral demineralization in Cd-treated fishes might be mainly due to imbalance in Ca

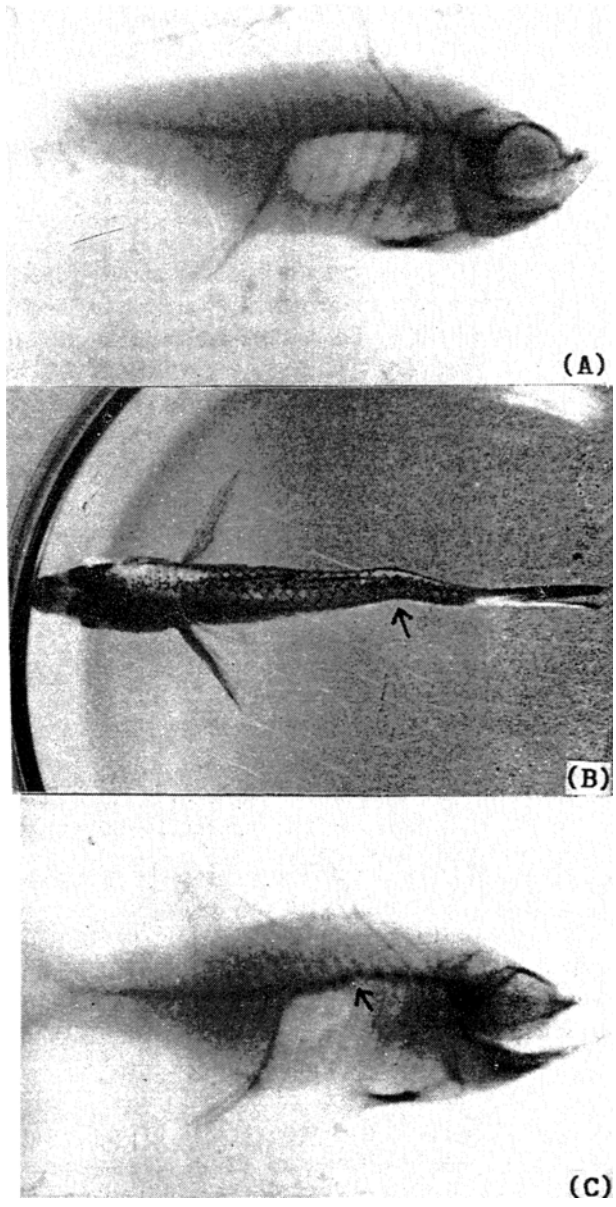

Figure 2. **A.** Normal fish. **B.** Vertebral anomalies with caudal curvature. **C.** Vertebral deformed fish.

metabolism controlled by endocrine glands. In the present study, during Cd exposure, the excess (body-accumulated) Cd ions might have affected the Ca and P metabolism of thyroid and parathyroid functions which in turn led to vertebral mineral elimination. This aspect needs further study.

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