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# Elemental Lepidological and Toxicological Studies in *Channa punctatus* (Bloch) upon Exposure to an Organochlorine Pesticide, Endosulfan

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The chemical composition of the scales of bony fishes has been described by various workers (Takahashi and Yokoyama 1954; Burley and Solomon 1957; Van Oosten 1957; Snyder 1958; Bai and Kalyani 1960; Cowgill et al. 1968; Stapleton 1968; Alwadhi 1993; Kaur 1993a,b; Tandon and Johal 1993; Johal and Dua 1994). Element deposition in different parts of the scale depends upon the chemistry of water in which the fish lives. Alwadhi (1993), Kaur (1993 a,b), Tandon and Johal (1993) pointed out that in carps, different regions of the scale have different chemical composition. Johal and Dua (1994) reported that the circuli of the marginal zone of *Channa punctatus* (Bloch) undergo disorganization and loose their identity upon exposure to sublethal concentrations of Endosulfan. It is obvious that this disorganization is due to the altered chemical composition in response to the toxic effect of Endosulfan.

Keeping this in view, chemical composition of different parts of the scale of *Channa punctatus* (Bloch), when kept in captivity and on exposure to different concentrations of Endosulfan (an organochlorine pesticide) for 5 and 15 d, has been investigated.

## MATERIALS AND METHODS

Live specimens of *Channa punctatus* (Bloch) were procured from a local pond, transported to the laboratory and acclimatized for 7d at room temperature (22± 3°C, S.D.= 1.81). The average size of the fish was 14.54 cm (S.D.= 2.68). Technical grade of Endosulfan was provided by Hoechst under the trade name Thiodan 35 EC. This formulation had 35% W/W technical Endosulfan, whereas 65% W/W was comprised by the solvents and the emulsifiers. Toxicity studies were conducted in PVC containers of the capacity of 100 L. Aliquots of stock solution (1 gm/L) were added to bring the Endosulfan concentration to 0.0022 mg/L and 0.0035 mg/L. A parallel control group was maintained in the toxicant free tap water (pH 6.8; dissolved oxygen 8.91 mg/L and total hardness 86 mg/L). To each experimental tank 15 fish

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were released. Fresh solutions were supplied daily so as to maintain the required concentration of the pesticide.

After 5 and 15 d of exposure, scales were removed from all the three groups (control and two pesticide concentrations) from below the dorsal fin and above the lateral line. Scales were cleaned with triple distilled water and dust particles were removed by placing the scales in a sonicator for 10 min; scales were air dried and mounted on carbon stubs, keeping dorsal surface upwards. The elemental composition of the different regions of the scales was determined by energy dispersive X-ray microanalysis (EDX). This was done by placing the scanner of "Kevex Delta Class Analyser" attached to JEOL JSM 255 S Scanning Electron Microscope. The material was then subjected to an accelerating voltage of 25KV and was analysed by using an operating system DEC RT-11. This system had a software package "Quantax" for acquisition and processing of the X-ray data from the sample. The spectrum was then processed (i.e., removal of background noise and identifying the peaks) and was analysed using standardless analysis to calculate the quantitative results. This was done by extracting net peak intensities.

This system was calibrated, using Al, Cu sample to an accuracy of 0.003 Kev. Final results of the quantitative analysis were normalized to 100%. The X-ray detector has a Be (Beryllium) window which transmits X-rays from elements with atomic number 11 and greater. X-rays from elements lighter than Na are absorbed by the Be window and are therefore undetected.

## RESULTS AND DISCUSSION

The changes in the elemental profile in the control group at 0 d, 5 d and 15 d and the fish that were exposed to 0.0022 mg/L and 0.0035 mg/L were observed. To interpret the comparison between control and the exposed groups, the values of control at 5 d and 15 d were used, whereas for elemental assay the values at 0 d were considered as the control values.

Various elements present in the scales of *Channa punctatus* (Bloch) are P, Ca, Al, Fe, S, Si, and Cl. The percentage composition of various elements in three different parts, viz., focus, middle and margin is given in Table 1. Amongst elements, Al has maximum concentration followed by Fe, Cl, P, Ca, S, and Si respectively. In the focus, Fe has the maximum concentration whereas in the middle and marginal part it is Al. A sufficient amount of Ca is present in the margin.

There were four categories of patterns in which the elemental changes occurred in response to pesticide concentrations. They were : (I) decreased percentage composition with increase in Endosulfan concentration (negative correlation) (II) increased percentage composition with increase in Endosulfan concentration (positive correlation) (III) increase in percentage composition at 0.0022 mg/L but decrease in percentage composition at 0.0035 mg/L, as compared to the controls at 5 d and 15 d (IV) decrease in percentage

composition at 0.0022 mg/L but increase at 0.0035 mg/L of Endosulfan as compared to the controls at 5 d and 15 d. These results are given in Table 2. S and Cl belong to category I and showed this trend at 5 d exposure in the focus and marginal parts. In the second category P, Al and Si are included and show these changes in the focus and middle parts. Majority of the elements belong to categories III and IV. Fe exhibited the characteristics of III whereas Ca represented IV category. Thus it can be concluded that Fe and Ca showed antagonistic trends in their relative deposition on exposure to Endosulfan concentrations.

To study the variability in elemental concentrations the S.D. values were employed. The results are given in Table 3. It is quite apparent from these data that Ca and Fe showed maximum variability whereas S, Si and Cl exhibited minimum variation in their percentage composition. P and Al along with Fe showed moderate levels of variation. These investigations indicate that Ca reveals maximum variation followed by Fe in their deposition in response to Endosulfan exposure.

Different elements, e.g., Al, Ca, P, Si, F, Mg, Li, Na, Cu, and Ba have been described in the scales of bony fishes. Rare occurrence of Cr in the posterior part of the scale of *Catla catla* (Ham.) from Gobindsagar reservoir in India has been reported by Kaur (1993a) and that of F in the scale of *Alosa* (Van Oosten 1957). Twenty elements namely Ca, Cl, Cr, Co, Cu, Fe, I, Pb, Mg, Mn, Ni, K, Rb, Se, Si, Na, Sr, S, Ti, and Zn have been reported by Cowgill et al. (1968) in the scale of *Latimeria chalumnae* Smith. In the scales of freshwater bony fishes the elements like Al, Ca, Cl, Fe and P are of common occurrence (Van Oosten 1957; Alwadhi 1993; Kaur 1993a,b; Tandon and Johal 1993). However the occurrence of other elements in minor quantities, i.e., F (Van Oosten 1957), Li, Cu, Na (Snyder 1958), Ba (Stapleton 1968) and Cr (Kaur 1993a) depend upon their occurrence in the surrounding waters. This observation is further substantiated by the occurrence of 20 elements in *Latimeria chalumnae* (Cowgill et al. 1968) collected from sea bed.

On the basis of present investigations, it is thus concluded that Ca deposition gets adversely affected on exposure to Endosulfan. Out of the three different regions on the scale, the middle and the marginal areas are the regions of choice because of their reliable responses. This was further substantiated by the findings of Johal and Dua (1994), who reported the disorganization of circuli in the marginal part upon exposure to Endosulfan.

As the overall growth of the fish directly depends upon Ca metabolism, ultimately the altered deposition of Ca in the hard part exhibits an overall deterioration in the general health of the fish.

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Table 1. Elemental composition (expressed in percentages) in different regions of the scale of *Channa punctatus* (Bloch) upon exposure to sublethal concentrations of Endosulfan

| Treatment, time | | P | Ca | Al | Fe | S | Si | Cl |
|---|---|---|---|---|---|---|---|---|
| | F | 8.43 | 2.36 | 29.18 | 38.55 | 9.43 | 5.71 | 6.26 |
| | Mi | 8.94 | 9.46 | 30.65 | 20.33 | 9.38 | 3.20 | 18.05 |
| 0 d | Ma | 17.56 | 20.30 | 22.55 | 17.05 | 6.03 | 1.81 | 14.70 |
| | Mean | 11.64 | 10.71 | 27.46 | 25.31 | 8.28 | 3.57 | 13.00 |
| | SD | 5.13 | 9.03 | 4.31 | 11.58 | 1.95 | 1.98 | 6.07 |
| | F | 7.13 | 3.26 | 38.42 | 31.27 | 7.81 | 4.60 | 7.51 |
| Control | Mi | 2.93 | 0.52 | 43.03 | 45.21 | 2.86 | 3.02 | 2.43 |
| 5 d | Ma | 23.31 | 31.82 | 22.56 | 12.16 | 4.36 | 1.06 | 4.72 |
| | Mean | 11.12 | 11.86 | 34.67 | 29.54 | 5.01 | 2.89 | 4.86 |
| | SD | 10.76 | 17.33 | 10.74 | 16.59 | 2.54 | 1.77 | 2.54 |
| | F | 8.82 | 0.61 | 12.88 | 67.98 | 3.97 | 1.26 | 4.49 |
| 0.0022 mg/ L | Mi | 2.86 | 0.06 | 16.62 | 78.97 | 0.59 | 0.24 | 0.66 |
| 5 d | Ma | 11.56 | 10.56 | 40.80 | 27.10 | 2.47 | 3.24 | 4.50 |
| | Mean | 7.55 | 3.74 | 23.43 | 58.02 | 2.34 | 1.58 | 3.22 |
| | SD | 4.39 | 5.91 | 15.16 | 27.33 | 1.69 | 1.52 | 2.21 |
| | F | 21.15 | 20.42 | 31.01 | 20.43 | 2.89 | 1.93 | 2.17 |
| 0.0035 mg/L | Mi | 23.31 | 30.20 | 26.30 | 6.80 | 1.60 | 9.85 | 1.93 |
| 5 d | Ma | 22.57 | 46.14 | 17.60 | 7.22 | 1.07 | 0.38 | 2.08 |
| | Mean | 24.34 | 32.25 | 24.97 | 11.48 | 1.83 | 4.05 | 2.06 |
| | SD | 1.09 | 12.98 | 6.80 | 7.75 | 0.93 | 5.08 | 0.12 |
| | F | 26.34 | 29.52 | 26.20 | 11.74 | 2.68 | 1.38 | 2.14 |
| Control | Mi | 26.13 | 31.32 | 30.42 | 7.54 | 1.75 | 1.25 | 1.59 |
| 15 d | Ma | 25.26 | 38.51 | 21.56 | 11.32 | 1.28 | 0.42 | 1.64 |
| | Mean | 25.91 | 33.12 | 26.06 | 10.20 | 1.90 | 1.02 | 1.79 |
| | SD | 0.57 | 4.76 | 4.43 | 2.31 | 0.71 | 0.52 | 0.30 |
| | F | 4.23 | 0.23 | 30.18 | 57.80 | 2.74 | 2.49 | 2.23 |
| 0.0022 mg/L | Mi | 6.17 | 2.04 | 40.48 | 27.44 | 8.37 | 5.29 | 10.21 |
| 15 d | Ma | 7.33 | 4.66 | 49.19 | 19.18 | 4.56 | 5.34 | 9.34 |
| | Mean | 5.91 | 2.31 | 39.95 | 34.88 | 5.22 | 4.37 | 7.26 |
| | SD | 1.56 | 2.22 | 9.52 | 20.34 | 2.87 | 1.63 | 9.38 |
| | F | 23.18 | 21.43 | 35.42 | 12.89 | 2.61 | 2.73 | 1.73 |
| 0.0035 mg/L | Mi | 28.18 | 42.14 | 20.74 | 5.78 | 1.15 | 0.34 | 1.37 |
| 15 d | Ma | 27.23 | 38.93 | 21.45 | .67 | 1.36 | 2.97 | 1.38 |
| | Mean | 26.20 | 34.17 | 25.87 | 8.44 | 1.51 | 2.01 | 1.49 |
| | SD | 2.65 | 11.15 | 8.28 | 3.87 | 0.45 | 1.45 | 0.20 |

F = Focus; Mi = Middle; Ma = Margin;
SD = Standard Deviation.

Table 2. Categorisation of different elements depending upon their pattern of change in the scale of *Channa punctatus* (Bloch) upon exposure to sublethal concentrations of Endosulfan

| | Overall | | Focus | | Middle | | Margin | |
|---|---|---|---|---|---|---|---|---|
| | 5 d | 15 d | 5 d | 15 d | 5 d | 15 d | 5 d | 15 d |
| I | S,Cl | - | S,Cl | - | - | - | Cl | - |
| I | - | - | P | Al,Si | P | - | - | - |
| III | Fe | Al,Fe,S,Si,Cl | Fe | Fe,S,Cl | Fe | Al,Fe,S,SCl | Al,Fe,Si | Al,Fe,S,Si,Cl |
| IV | P,Ca,Al,Si | P,Ca | Ca,Si,Al | P,Ca | Ca,Al,S,Si,Cl | P,Ca | P,Ca,S | Ca,P |

I = Decrease in element percentage composition with increase in Endosulfan concentration
II = Increase in element percentage composition with increase in Endosulfan concentration
III = Increase in element percentage composition at 0.0022 mg/L but decrease on exposure to 0.0035 mg/L of Endosulfan
IV = Decrease in element percentage composition at 0.0022 mg/L but increase on exposure to 0.0035 mg/L of Endosulfan

Table 3. Degree of variation of elemental composition (on the basis of S.D. values) in the scale of *Channa punctatus* (Bloch) upon exposure to sublethal concentrations of Endosulfan

| | Overall | | Focus | | Middle | | Margin | |
|---|---|---|---|---|---|---|---|---|
| | 5d | 15d | 5d | 15d | 5d | 15d | 5d | 15d |
| Maximum | Fe | Ca | Al | Fe | Ca | Ca | Ca | Ca |
| Moderate | Ca,P,Al | P,Al,Fe | Ca,P,Al | P,Al,Ca | P,Fe,Al | P,Fe,Al | P,Al,Fe | P,Al,F |
| Minimum | Si,Cl,S | Si,Cl,S | Si,Cl,S | Si,Cl,S | Cl,S,Si | Cl,S,Si | S,Cl,Si | S,Cl,Si |

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