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Potential Application of Elemental Analysis of Fish Otoliths as Pollution Indicator

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The use of scales, otoliths, opercular bones, finrays and vertebrae which are commonly termed as hard parts for age and growth inferences, are in vogue since long (Tandon and Johal 1996). This is first report on the use of otoliths as pollution indicator. Elemental characteristics of the otolith have been studied by energy dispersive X-ray microanalysis (EDX). Two regions, median and marginal have been examined so as to reveal the overall change when the fish was subjected to various concentrations of malathion.

MATERIALS AND METHODS

The live specimens of *Channa punctatus* were brought from local fish markets of Chandigarh (76 °46' 30" E;30° 40' 0" N). These were acclimatized for 7 days and fed either with minced goat liver or mosquito fish *Gambusia affinis patruelis* (Baird and Giard). Toxicity tests were conducted in plastic tanks containing 10 L of dechlorinated water having following characteristics : pH 6.8; dissolved oxygen 8.91 mg/L and total hardness 84 mg/L. Stock solution of commercial grade malathion (EC 50%) was prepared in water and desired concentrations viz., 0.05, 0.10. 0.15, 0.20, 0.25 mg/L were used for further experimentation. Test water was replaced after every 24 hrs and the fish were not fed during the experimental period. A control group was maintained in toxicant free tap water. After 5, 15 and 30 days exposure, otoliths were removed. To collect the otoliths the cranium was opened dorsally, and the brain was removed carefully to expose the inner ears. Sagittae were gently pulled away, washed and dried. Dried otoliths were mounted on carbon stubs, sputter coated with gold (100 A") and studied by placing the scanner of Kevex Delta Class Analyzer on the area of interest of the otolith. This scanner was attached to JEOL, JSM-255 S scanning electron microscope. Peaks of gold were neglected.

To study the quantitative impact of the toxicant the values of the SD and SEM were used, however ANOVA and student 't' test (Snedecor and Cochran, 1967) were applied at 5 % level to test the significance, amongst various parameters and control.

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RESULTS AND DISCUSSION

Sagittae of normal *C. punctatus* consist of four major elements viz., calcium (SO%), aluminium (13%), iron (6.57%) and phosphorous (2.7%) (Table 1). Initially after 5d exposure, at median region the percentage composition of Al and P decreased while that of Ca and Fe increased with an increase in pesticidal concentration. In comparison to the values recorded at the median region, the marginal region exhibited an increase in the percentage composition of Al and Fe and a decrease in that of P and Ca. By increasing the exposure duration to 15d, the percentage composition of Al, P and Fe increased while that of Ca decreased at both median and marginal regions with increase in malathion concentration. After 30d exposure to malathion, an increased percentage composition of Al and P while a decreased percentage occurrence of Ca was observed in the median region of otoliths. Iron showed an upward trend with slight fluctuations. At the marginal region however, a decrease in the percentage compositions of Al and Ca were observed. P and Fe followed the opposite trend. To arrive at conclusion, values of SD and SEM were calculated. The use of these values is based on the fact that greater the value of SD or SEM , more is the variability in the occurrence of the specific mineral and later may be due to damage done by toxicant.

Based on the values of standard deviation, the following trends in the median and marginal zones of the otolith have been observed :

Exposure period	Median region	Marginal region
5d	Al>Ca>Fe>P	Ca>Al>Fe>P
15d	Ca>Al>Fe>P	Ca>Al>Fe>P
30d	Ca>Al=Fe>P	Ca>Al>Fe>P

When the entire data were subjected to analysis of variance (ANOVA), the F-values showed significant results at all levels i.e. within elements, within regions and within days. Likewise when elements vs. regions (F.DF.144 at 5% level = 18.75) and elements vs. days (F.DF.144 at 5% level = 9.75) were analyzed, the results were again significant, however when regions vs. days (F.DF.144 at 5% level = 0.019) were analyzed, insignificant differences were noted.
Analysis of multiple variance also showed significant results with respect to elements vs. regions vs. days (F.DF.144 at 5% level = 10.81).
Student ‘t’ test on the other hand showed highly significant results with respect to the four elements viz., Al vs. P (student ‘t’ test .DF. 70 at 5% level = 14.57) ; Al vs. Ca (student ‘t’ test .DF. 70 at 5% level = 73.02) ; Al vs. Fe (student ‘t’ test .DF. 70 at 5% level = 6.94) ; P vs. Ca (student ‘t’ test .DF. 70 at 5% level = 118.07) ; P vs. Fe (student ‘t’ test .DF. 70 at 5% level = 15.78) Ca vs. Fe (student ‘t’ test .DF. 70 at 5% level= 103.51).
Keeping in view the above observations, it can thus be concluded that all elements get affected by malathion irrespective of concentration and exposure period. However on the basis of values of SD and SEM, calcium can be considered as. a reliable pollution indicator in both the regions of otoliths, but marginal region is

Table 1. Percentage composition of major elements in median and marginal regions of otolith of *Channa punctatus* (Bloch).

Elements	Aluminium						Phosphorous						Calcium						Iron					
Regions	Median			Marginal			Median			Marginal			Median			Marginal			Median			Marginal		
Days	5	15	30	5	15	30	5	15	30	5	15	30	5	15	30	5	15	30	5	15	30	5	15	30
Control	11.90	8.65	8.54	14.35	10.86	11.84	2.96	1.08	1.45	3.04	1.30	1.74	80.81	84.00	83.32	78.77	82.35	81.49	4.34	6.26	6.69	3.83	5.49	4.93
Conc mg/L																								
0.05	10.96	8.42	8.65	16.35	10.96	11.12	2.61	1.40	1.65	2.91	1.07	1.43	81.55	82.32	82.81	76.22	82.23	80.87	4.88	7.86	6.90	4.53	5.74	6.58
0.10	10.05	9.37	8.63	19.90	10.81	8.24	2.06	1.63	1.50	2.10	1.39	1.72	80.95	80.24	81.42	72.58	81.84	83.02	6.94	8.76	8.45	5.42	5.97	7.02
0.15	10.31	11.41	10.50	20.51	16.36	8.08	2.00	2.87	1.94	2.40	1.94	3.14	80.90	77.63	81.43	71.62	73.88	80.13	6.79	8.09	6.13	5.47	7.82	8.65
0.20	9.20	11.64	10.79	19.74	15.20	7.40	1.95	2.64	2.47	2.53	1.62	1.32	82.05	76.53	78.80	72.42	76.28	79.87	6.81	9.19	7.94	5.31	6.90	8.41
0.25	7.82	11.90	11.31	21.67	17.61	7.65	1.58	2.66	2.87	1.35	2.24	4.88	84.22	74.51	76.33	70.27	73.98	77.25	6.78	10.93	9.50	6.71	7.16	10.22
Mean	10.04	10.23	9.74	18.75	13.63	9.05	2.13	2.05	1.98	2.39	1.59	2.87	81.75	79.20	80.68	73.65	78.43	80.44	6.09	8.51	7.60	5.21	6.51	7.63
S.D.	1.41	1.59	1.26	2.79	3.11	1.91	0.61	0.76	0.57	0.61	0.43	1.47	1.30	3.62	2.65	3.19	4.16	1.92	1.16	1.55	1.26	0.97	0.92	1.85
S.E.M.	0.63	0.71	0.56	1.25	1.39	0.86	0.45	0.34	0.26	0.27	0.19	0.66	0.58	1.62	1.18	1.43	1.86	0.86	0.52	0.69	0.56	0.43	0.41	0.83

more suitable than the median region. The present observations confirm the earliar observations on the scale of the same fish (Johal and Sawhney 1999). Saitoh(1993) conducted EDX studies and recorded high peaks of calcium in *Tilapia* as compared to low peaks of phosphorous and sulphur. As environmental conditions such as photoperiod (Jones 1986; Secor and Dean 1986); temperature(Campana 1984; Neilson and Geen 1985); feeding (Campana 1983); endogenous conditions (Geffen 1982); blood calcium (Mugiya *et.al.* 1981) and circadian rhythm (Inoue 1982) fluctuate cyclically, periodically, geographically and are species - specific, hence the occurrence of mineral profile in the otolith in fishes shows substantial variation. As all the fishes exposed to the present investigation belonged to same stock and were under similar conditions, hence the present studies have indicated that pesticides also affect the mineral profile of the fishes.

As already indicated in the beginning of this section that only little information is available about the chemical composition of otoliths, but the impact of the pesticides on the mineral profile especially of the median and marginal regions of the sagitta, has been described for the first time. It may be added here that the use of otolith as far as pollution monitoring is concerned should be encouraged especially in case of Silurid fishes because of absence of scales on the body. Hence, there is an urgent need to refine this technique and to develop some better computer software for this purpose.

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