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Toxic Effects of Sewage Sludges on Freshwater Edible Fish *Cirrhina mrigala*

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Municipal sewage sludges have been advocated by several investigators (Tacon and Ferns 1976; Yip and Wong 1977; Ghosh et al. 1985) as a suitable dietary supplement in aquaculture because of the protein contained in it. Other researchers (Wong et al. 1982; Gaigher and Toerien 1985; O'Grady and Spillet 1987) discourage their usage for a variety of reasons, the most common being the presence of heavy metals and pesticides that accumulate in various organs, thereby hampering growth in fish. Settled sewage, if palatable however, would prove to be an excellent low-cost nutrient in intensive aquaculture farms. Sludges may be administered in the aqueous or dehydrated condition, and in either case would be suitable for even fry and fingerlings, if consumed soon after application because of the small particle size.

The absence of reports on the effect of sewage sludges from the wastewater treatment plant on animals prompted this investigation, though heavy metal analysis revealed the presence of appreciable quantities of Zn, Cu. Pb and moderate amounts of Ni, Cr, Cd in aqueous activated sludge (Coutinho 1989). In this study the effect of activated sludge (AcS), raw sludge (KS) in acute and chronic bioassays and hydrated digested sludge (DS) and dehydrated (sun-dried) digested sludge (KS) in chronic feeding experiment on survival, behavior and whole body acetylcholinesterase (AchE) activity of Cirrhina mrigala (Carp), a freshwater edible fish, were investigated.

MATERIALS AND METHODS

One hundred Cirrhina mrigala fingerlings measuring 4-5 cm were obtained from Central Institute of Fisheries, Government of India, and were maintained in aerated glass aquaria measuring 2ft X 1ft X 1ft in triplicate for each acute feeding trial with 5% of the body weight neat activated sludge and raw sludge, and for chronic studies with 2% body weight AcS, KS, DS and KS, supplemented with 2% body weight dried prawn fish meal. Control sets were maintained simultaneously, the fish being fed

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on appropriate quantities (2% body weight) of fish meal. Sludges were obtained from a local municipal sewage treatment plant.

During the acute investigation, mortality, behavioral changes, and whole body acetylcholinesterase (AchE) activity were studied and the enzyme activity and mortality at the termination of the 21-d chronic dietary substitution study. Ten fish were killed from each aquarium by cold nacrosis on 5, 7, 9, 10 days of treatment and on 21st day of the long term study. Individual whole body homogenates (10%) were passed through the glass wool to remove bone, skin, scale and fat debris. The filtrate was passed through Whatman 42 at 4°C; The filtrate thus obtained was used for estimating AchE activity by Hestrin (1949) method with slight modification as follows: The assay medium contained 1 mL each of 0.1M NaCI, 0.2M $MgC1_2$ and 0.1M Phosphate buffer pH 7.0 0.5 ml each of 0.01% gelatin, 50 X 10 M acetylchol ine and glass distilled water. To this assay mixture 0.5 mL of whole body homogenate was added and the tubes were incubated at 37°C for 30 min. The blank was prepared by adding 0.5 mL of distilled water instead of 50 X 10 acetylcholine. At the end of 30 min, 2 mL of 2M alkaline hydroxylamine was added to each tube. After 1 min, 2 mL of HCI and 1 mL of $FeCL_3$ (0.27M in 0,1N HCI) were added. The tubes were shaken and optical density was read at 540 nm. One unit of acetylcholinesterase is defined as µmol of acetylcholine converted per 30 min under assay conditions. For the analysis of heavy metals, an extract from the dehydrated sludge was prepared according to the method described in standard methods for the examination of water and waste water (Bupp 1989) with slight modification as follows: 1 g of dehydrated activated sludge was transferred to a round bottomed flask, 50 mL of glass distilled water and 5 mL of nitric acid was added to it. This mixture was allowed to evaporate to dryness in a water bath maintained at 90°C. Subsequently the round bottom flask was removed from the water bath and glass distilled water was added to resuspend the acidified sludge. The extract was then filtered through Whatman No. 1 filter paper to obtain a clear filtrate. The filter paper was washed ten times with small quantities of glass distilled water to remove all traces of metals. The volume of clear filtrate was made upto 100 mL with glass distilled water. An acid blank was prepared by using 5 mL of fuming nitric acid and the above procedure followed. The acid extract was analysed for heavy metals such as zinc, copper, lead, nickel, cadmium and chromium on automic absorption/flame emission spectrophotometer AA-630-12. The QA/QC procedure used for heavy metal analysis by atomic absorption spectroscopy is as follows: During the analysis, a blank was analysed between each sample analysis to verify baseline stability. The baseline was rezeroed whenever necessary. Since only five analysis were carried out for the sludge sample, a known amount of copper and nickel were added to the blank and analysed for recovery. The amount of metal was almost

equal to the amount found in the samples analysed. Recovery was found to be 90 to 92%. To further assure ourselves and to confirm the test was in control, a standard sample was analysed for each sample analysis.

Statistical analysis of the results was done by using Newman-Keuls test (Hassard 1991).

RESULTS AND DISCUSSION

At the onset of the experiment the fishes were very active; behavioral changes indicating the initiation of physiological deterioration were manifest only after the fourth day. From Table 1 it is evident that mortality was significantly increased among activated sludge-fed fish (AcS) than those maintained on raw sludge (KS).

Mortality in all experimental groups of fish was a slow process extending over a period of 3 to 4 hr and associated with characteristic behavioral changes in all the affected fish. initially the fish were agitated, surfacing and holding their heads perpendicular to the surface of the water, spiralling for 50-90 sec at the surface of the water, after which they swam obliquely, diving to the bottom of the aquaria where they remained for 1 to 3 min. Later they lay on their sides, the posture being numb and jerky when prodded, indicating paralysis.

Although, the AchE activity (Table 1) initially showed a statistically significant increase as compared to the control, later a decreasing trend was notable. While elevated levels of upto 44.71% and 49.15% with respect to the control in hyperactive AcS and KS-fed fish were observed on day 5, the AchE levels plummetted to very low values with upto 54.54% and 14.78% inhibition in comparison to the control at the termination of the acute study in the two respective groups of fish. This is consistent with the behavioral changes mentioned earlier.

Chronic feeding studies with different sewage sludges showed maximum survival (96%) with stabilised dehydrated digested sludge while its native form when used as a dietary additive caused maximum mortality (28%) (Table 2). In sharp contrast to this, while a significant and consistent inhibition of AchE was recorded in all groups of sludge-fed fish of the long term study, greatest inhibition of AchE was observed in KS-fed fish and least in hydrated digested sludge-fed individuals. The former group of animals were more lethargic.

The results of analysis of heavy metals in activated sludge are given in Table 3. It is evident from this analysis that the sludge was contaminated with heavy metals and it would increase the body burden of fish which may lead to their death (Foulkes 1990 ; Niimi and Kissoon 1994). The other cause

Table 1. Mortality and acetylcholinesterase activity µmol of acetylcholine converted/g wet weight of tissues/min ± S.D. of whole body homogenate of C. mrigala under control and treated conditions during acute bioassay. Mean of 10 individual samples.

Days	Ache activity of control fish	AcS			RS		
		Mortality %	AchE activity	% change	Mortality %	AchE activity	% change
5	226.43 ±11.41	5	327.67 ±14.77*	+ 44.71	2	337.72 ±15.33*	+ 49.15
7	229.28 ±15.64	8	320.33 ±19.38**	+ 39.71	5	324.56 ±21.74**	+ 41.56
9	222.54 ±10.90	10	139.34 ±17.67**	- 37.67	9	239.70 ±16.33*	+ 7.71
10	236.12 ±12.40	13	107.34 ±20.34**	- 54.54	12	201.32 ±15.12*	- 14.78

Significance is given at * $P < 0.01$, ** $P \leq 0.05$.

AcS = Activated Sludge ; RS = Raw Sludge

Table 2. Mortality and AchE activity µmol of acetylcholine converted/g of wet weight of tissue/min ± S.D. of C. mrigala under control and treated condition at the termination of chronic bioassay on the 21st day

Groups of Fish	Mortality	AchE activity	% inhibition
Control	0%	236.12±12.40	-
AcS-Fed Fish	10%	37.07± 0.88*	84.30
RS-Fed Fish	14%	42.15± 1.34*	82.15
DS-Fed Fish	28%	55.16± 2.84*	76.64
KS-Fed Fish	4%	12.00± 0.74*	94.88

Significance is given at * $P \leq 0.05$. % inhibition compared with control

AcS = Activated Sludge ; RS = Raw Sludge ; DS = hydrated digested sludge ;
KS = Dehydrated digested sludge.

could be due to the presence of pesticide residues and other organic contaminants, which were not analysed in this study.

Table 3. Results of heavy metals analysed in activated sludge (5 analysis) ±SD

Metals	Values in mg/g (Dry weight sludge)
Zinc	1.820±0.001
Copper	0.053±0.0002
Lead	0.017±0.0001
Nickel	0.273±0.0002
Chromium	0.006±0.0003
Cadmium	0.005±0.0008

Several investigators have reported changes in AchE activity as an ideal tool to examine neurophysiological changes in response to toxicants (Coppage and Braidech 1976; Nemcsok et al. 1985a). An enigmatic increase in AchE activity in both sets of sludge-fed fish on the 5th and 7th days of the acute study indicate neuromuscular imbalances possibly due to the penetration of the blood brain barrier by xenobiotics. It may result in elevated de novo synthesis of the enzyme as suggested by Szabo et al. (1992), who recorded similar elevation in the levels of AchE (130%) following 2 wk of chronic treatment of Cyprinus carpio with sublethal concentrations of herbicide paraquat and fungicide coppersulfate independently.

A decrease in AchE activity during the latter phase of the acute bioassay and at the termination of chronic exposure, on the other hand, infer a plausible irreversible damage to the central nervous system and the neuromuscular junction in the fish fingerlings. Vijayalakshmi (1980) reported depressed levels of AchE in brain, gill, liver and muscle of Etroplus maculatus following exposure to Sumithion. Nemcsok et al. (1985b) also observed reduced levels of AchE in carp with exposure to increasing concentration of copper (10-50 ppm) and attributed this discrepancy to accumulation of the ions in vital organs. In a study conducted by Radhakrishniah (1988) on Labeo rohita treated with sublethal quantities of Cu as a xenoblotic, the metal was found to accumulate rapidly in the brain under acute conditions causing muscular dystrophy and uncoordinated movements, both features similar to those observed in the present study on Cirrhina mrigala, sustained on sewage sludge supplemented diets.

Moreover, high rate of mortality observed in activated sludge treated group compared with raw sludge treated group could be explained on the basis of activated sludge being a concentrated suspension of aerobic microorganisms. These microorganisms are responsible for decomposing sewage with consumption of appreciable oxygen, thereby causing a depletion in dissolved oxygen in the aerated aquaria and high mortality of fish under study. On the other hand, raw sludge was nitrogen-rich, low microbe medium that would undergo decay if allowed to remain unconsumed in the aquaria. The high mortality and neuromuscular aberration brought about by the synergistic action of xenobiotics in the sludges fed to juvenile C. mrigala prompt further investigation and caution in their usage as a nutritive substitute in pisciculture.

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REFERENCES

Bupp SM (1989) Metals by flame atomic absorption spectrometry. In: Clesceri LS, Greenberg AE, Trussel RR (ed) Standard methods for the examination of water and waste water, 17th ed. Am Pub Hlth Assoc, Washington DC p 3-13

Coppage DL and Braidech TE (1976) River pollution by anticholinesterase agents. Water Res 10: 19-24

Coutinho CJ (1989) Study of feasibility of secondary sewage effluent in the pisciculture of freshwater edible fish. Cyprinus carpio and Sarotherodon mossambicus. Ph.D thesis, Bombay University

Foulkes EC (1990) The concept of critical levels of toxic heavy metals in target tissues. Crit Rev Toxicol 20:327-340

Caigher IJ and Toerien DF (1985) Cage culture of Mozambique Tilapia (Oreochromis mossambicus) without artificial feeding in maturation ponds of the Phuthaditjhaba sewage system: Water S. A. 11:19-24.

Ghosh TK, Rao AVJ, Krishnamoorthi KP and Joshi P (1985) Settled sewage for pisciculture. Asian Environ, 7:31-35

Hassard TH (1991) Understanding Biostatistics. Mosby Year Book, St Louis, USA

Hestrin S (1949) Estimation of acetylcholinesterase by hydroxylamine hydrochloride and Ferric chloride method. J Biol Chem 180: 249-251

Nemcsok J, Orban L, Dobler L, Szepfalussy J (1985a) Acetylcholinesterase activity measurement as a tool for demonstrating the possible cause of fish decay. Acta Biol Szeged 31:9-12

Nemcsok J, Orban L, Vig E, Aszatols B, Boross L (1985b) Effect on Cu^{+2} on some biochemical parameters of fishes Symp Biol Hung 29:413-426

Niimi AJ, Kissoon GP (1994) Evaluation of the critical body burden concept based on inorganic and organic mercury toxicity to Rainbow Trout (Oncorhyncus mykiss) Arch Environ

Contam Toxicol 26:169-178
O'Grady K, Spillet P (1987) Pond and tank feeding trilas with carp (Cyprinus carpio) : Gross nutrition, conversion efficiency and cost effective diets. Aquaculture Fish Manage 18:73-93
Radhakrishniah K (1988) Accumulation of copper in the organs of freshwater fish Labeo rohita on exposure to lethal and sublethal concentrations of copper J Environ Biol 9:319-326
Szabo A, Nemcsok J, Aszatols B, Rakonczay 2, Kasa P, Hien LH (1992) The effect of pesticides on carp (Cyprinus carpio), Acetylcholinesterase and its biochemical characterization. Ecotoxicol Environ Safe 23:39-45
Tacon AGJ, Fern PN (1976) Use of activated sludge from domestic sewage in trout diets. Nutr Rep Inter 13:549-562
Vijayalakshmi S (1980) In vivo effects of Sumithion on tissue respiration and enzyme activity in the fish. Etroplus maculatus Experientia 36:1280-1281
Wong MH, Chang YH, Lau WM (1982) Toxic effects of animal manure and sewage sludge as supplementary feed for the common carp. Toxicol Let 12:65-73
Yip SW and Wong MH (1977) The effects of sewage sludge on the growth rate of carp. Environ Pollut 14:127-132