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Toxicity of Thiocyanate to Fish, Plankton, Worm, and Aquatic Ecosystem

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Thioeyanates (Ammonium thiocyanate, Potassium thiocyanate, Sodium thiocyanate) are organic compounds and are used as non-selective contact herbicides for the control of weeds and poisonous plants (Thomson 1967). The effluents containing thiocyanate such as quinine factory effluents (Kaviraj and Saha 1997), or base metal mill effluents (Huiatt et al. 1983), and the washeries of thiocyanate containing herbicides are the potential sources of thiocyanate contamination of natural water bodies. However, thiocyanate (SCN-) is considered less toxic than cyanide and in many industries the effluents containing cyanides are treated to form thiocyanates to be disposed finally into the environment (Ingles and Scott 1987).

Although thiocyanate is often deliberately produced as a less toxic form of cyanide, several articles indicate sufficient alarm of this compound A number of deleterious effects of thiocyanate on rainbow trout have been documented (Heming et al. 1985; Parker and Doe 1982). The ability of thiocyanate to inhibit the transport of halides across the gill (Epstein et al 1973; Heming et al. 1985) of fish have also been reported. Thiocyanate containing effluents have been found to reduce the growth and reproduction (Kaviraj and Saha 1997) and appetite of fish (Eales and Shostak 1983). There are also reports that thiocyanate affects the enzyme system (Habig et al. 1975) and the neuromuscular functioning (Heming et al. 1985) of fish

Therefore, there is a need to investigate the thiocyanate toxicity to fish and aquatic ecosystem in detail. The aim of the present study was to evaluate acute toxicity of thiocyanate to animals of different trophic levels of aquatic ecosystem at different climatic conditions. To find out a safe level of the toxicant chronic toxicity of thiocyanate at sublethal doses was also carried out using fish as the test organisms.

MATERIALS AND METHODS

Test animals used in the bioassays were a cichlid fish, tilapia *(Oreochromis mossambicus),* a crustacean plankton *(Moina micruru)* and an oligochaet worm *(Branchiura sowerbyi).* Only adult tilapia of both sex (mean length 104 ± 7.71 mm; mean weight 14.25 ± 2.07g) were used for 96 hr acute toxicity bioassay and feeding test, while fingerlings (mean length 51.67 ± 4.25 mm; mean weight 1.36 ± 0.37g) were used for 90 d chronic toxicity bioassay. All the test organisms were acclimatized to the test condition for 96-192 hr before their use.

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For treatment of thiocyanate, analytical grade ammonium thiocyanate, NH_4SCN (purity 99%, E. Merck, A. G. Darmstad, made in Germany) was used. All doses were based on thiocyanate ion (SCN). Tap water (pH 7.02 ± 0.21, DO 6.5 ± 0.54 mg /L, alkalinity 180 ± 10.5 mg/L as $CaCO_3$ and hardness 105 ± 6.5 mg/L as $CaCO_3$) was used as diluent medium. Bioassay methods of APHA (1989) were followed strictly.

Acute toxicity bioassays were conducted to estimate 96 hr median lethal concentration (96 hr LC_{50} with 95% confidence limit) of thiocyanate to the test organisms and to evaluate the effects of thinocyanate on the respiratory rate of fish Separate bioassays were made in the months of December, March and September with mean water temperature of 20 ± 0.5°C, 25 ± 0.49°C and 28 ±0.51°C respectively. Respiratory rates of the fish under thiocyanate exposure was also tested under similar temperature conditions. The bioassays with worm and plankton were run in 500 mL beakers each containing 300 mL water, while the bioassays with fish were conducted in 15 L glass aquaria each holding 10 L of water. Each beaker contained 10 plankton or worms while each aquarium contained 4 fish. A set of five beakers or aquaria were exposed to one concentration of thiocyanate to make five replicates per concentration. Each set of tests was acconpanied by five replicates of control. Test animals were not fed during the acclimatization period and bioassays. Mortality of the test animals were recorded every 24 hr and dead animals were removed. The water of both experimental and control aquaria was siphoned every 24 hr and was replaced by fresh water. Desired quantities of thiocyanate were immediately added to this fresh diluent medium. Behaviour-al responses of the test animals were recorded every day. Total mortality of test organisms over 96 hr were used to calculate LC_{50} of thiocyanate (the concentration of toxicant at which 50% test animals died) and its 95% confidence limit by probit analysis (Finney 1971). Opercular movements of fish (number per minute) were recorded every 24 hr during the acute toxicity test.

Feeding rate of fish was evaluated in a separate 96 hr laboratory bioassay in 15 L glass aquaria with 10 L of water. Twelve adult fish (3 per aquarium) were exposed to each sublethal concentration of thiocyanate (0.0, 0.26, 0.57, 0.77 and 1.02 mg/L) in four replicates. Fish were given live earthworm pieces daily at 8 hr and were allowed to feed for 4 hr. Unconsumed food pieces were counted and removed to avoid any decomposition

Chronic toxicity bioassays were conducted in 60 L outdoor ear-hen vats for 90 d to assess the long term effect of thiocyanate on survival, growth and reproduction of fish. These bioassys were done during the months February to April when ambient water temperature ranged from 20 to 28°C. Vats were arranged in 5 different blocks each with 4 tanks as per Randomized Block Design (Gomez and Gomez 1984), thereby giving four replicates for each of the five treatments that were used for feeding test. Each vat was provided with a 3 cm thick soil sediment at the bottom and kept water filled for about one month before the start of the experiment. When sufficient plankton grew to serve as natural food for the fish, each tank was stocked with 15 fingerlings of tilapia followed by the treatment of thiocyanate. In addition to natural food, the stocked fish were fed a mixture of rice bran and mustared oil cake (1:1) 6 d a week. Initially, food ration was provided at the rate of 5% of the stocking weight. A 10% increase in the ration was provided every fortnight. pH, free CO_2, total alkalinity, hardness,

dissolved oxygen, primary productivity of water and phytoplankton and zooplankton abundances in the test medium were measured every 15 d during the bioassays (APHA 1989). Fish were sampled at the end of the experiment (90th d) and length, weight, visceral weight and gonad weight of fish were recorded. Final biomass was used to estimate the yield of fish in each treatment. Formulae used to estimate the condition factor (K), gastrosomatic index (GSI), maturity index (MI) and fecundity were adopted from LeCren (1951) and Bagenal(1978) and are given below:

K = [Body weight (g) / Body length $(mm)^3$] x 10^5; GSI = [Visceral weight (g) / Body weight (g)] x 100; MI = [Gonad weight (g) / Body weight (g)] x 100; Fecundity = Total number of ripening eggs/female.

All data except those of acute toxicity test were statistically analyzed by ANOVA followed by Duncan's Multiple Range Test (Gomez and Gomez 1984).

RESULTS AND DISCUSSION

Table 1. 96 hr LC_{50} (mg SCN/L) with 95% confidence limit in parentheses of thiocyanate to aquatic organisms at different temperature conditions

Test Organisms	Temperature conditions		
	$20^0C \pm 0.50$	$25^0C \pm 0.49$	$28^0C \pm 0.50$
O. mossambicus	10.20 (9.75 -10.67)	7.69 (7.19-8.23)	5.70 (5.12 – 6.34)
M. micrura	15.46 (13.23-18.08)	12.74(11.76- 13.80)	10.27 (9.29 – 11.34)
B. sowerbyi	217.79 (194.46-243.92)	186.45 (161.82-214.82)	166.88(136.03 - 204.72)

Table 1 represents the 96 hr LC_{50} values of thiocyanate to fish plankton and worms at different temperature conditions. Results indicate that water temperature plays a crucial role in determining the susceptibility of the aquatic organisms to thiocyanate. While the pH of water remaining the same, an increase in temperature from 20-28°C could drastically increase the susceptibility of the aquatic organisms to thiocyanate. Among the test organisms, fish were most susceptible and worms were least susceptible to thiocyanate, irrespective of water temperature (20-28°C).

Fish sharply reacted to higher doses of thiocyanate. Convulsion, gasping and flaring of the operculae were some of the immediate reactions. Finally, they lost their balance before they succumbed. Reactions of fish exposed to smaller doses of thiocyanate were less acute, but uneasiness of fish could be detected horn the increased opercular movements (Figure 1). Obviously, opercular movement increased with the temperature of water and dose of thiocyanate. Minimum concentrations of thiocyanate having a significant effect on the opercular movement of fish were 4 mg/L at 28°C, 6 mg/L at 25°C and 7 mg/L at 20°C.

A single factor ANOVA [$F_{(5,20)}$, 63.93, $P<0.01$] followed by DMRT showed that feeding rate of fish significantly varied among different doses of thiocyanate. A dose of 1.02 mg/L thiocyanate could reduce the feeding rate of fish to half of that

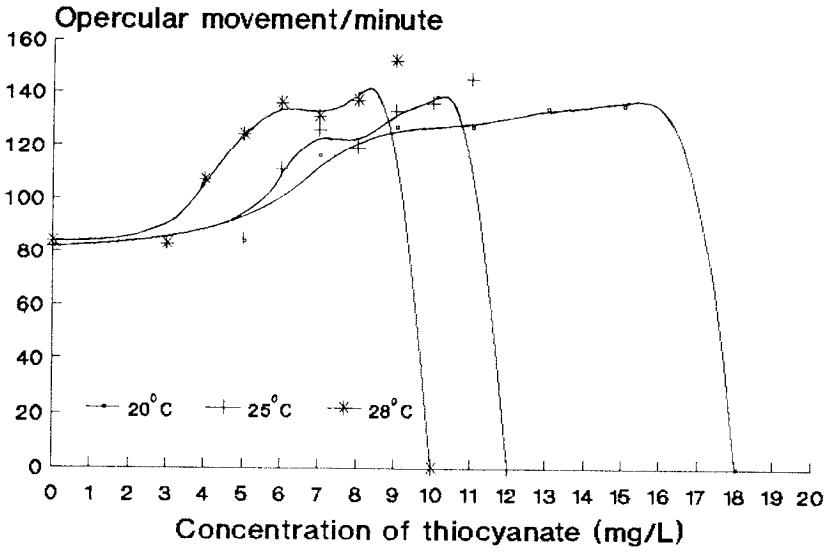

Figure 1. Effects of thiocyanate on the opercular movement of fish at different temperature conditions

control, while at 0.26 mg/L thiocyanate treatment feeding rate was comparable to that of control (Table 2).

No fish died during the outdoor experiment but the total yield of fish at the end of experiment significantly varied among the treatments.Table 2 documents the mean length, mean weight, condition factor (K), gastrosomatic index (GST), fecundity and maturity index (MI) of fish obtained from the experiment. 0.26 mg/L thiocyanate did not affect these parameters except mean length while a further increase in the dose significantly reduced all the parameters as compared with control. Changes in various limnological parameters during outdoor bioassay have been shown in Table 3. Single factor ANOVA carried with 90 d mean values showed a significant variation of free CO_2, dissolved oxygen (DO), primary productivity and plankton populations of water among various treatments ($p<0.05$) But no significant variation ($P>0.05$) was observed for the other limnological parameters (pH alkalinity, hardness). Dissolved oxygen, primary productivity and plankton populations were severely reduced at thiocyanate treatments fron 0.77 to 1.02 mg/L.

Although acute toxicity of thiocyanate to tialpia has not been reported earlier, reports of thiocyanate toxicity to many other fish are available for comparison, Threshold LC_{50} values of thiocyanate to rainbow trout *(Oncorhynchus mykiss)* have been found to range from 40-265 mg/L depending on the temperature, pH and hardness of water (Speyer and Raymond 1988). Heming et al. (1985) found 96 hr LC_{50} of thiocyanate to brook trout *(Salvelinus fontinails)* and rainbow trout *(Salmo gairdneri)* as more than 16.7 mg/L and 20.8 mg/L , respectively. These values are close to the LC_{50} values of thiocyanate to tilapia found in the present investigation Parkhurst et al. (1979) and Anderson (1946) reported thiocyanate toxicity to *Daphnia magna* as immobilization concentration at 11.3 mg/L The 96 hr LC_{50} values of *Moina micrura* observed in the present experiment are close to the concentration.

Table 2. Mean values (± SD) of feeding rate, mean length, mean weight, yield, condition factor (K), gastrosomatic index (GSI) fecundity and maturity index (MI) of fish exposed to sublethal doses of thiocyanate (0.26, 0.57, 0.77 and 1.02 mg/L) and control (0.00 mg/L).

Treatment (mg/L)	Food consumed (%)	Mean length (mm)	Mean weight (g)	Yield (g/M^2)	K	GSI	Fecundity	MI	
								Male	Female
0.00	100.00^{a}	63.50^{a}	4.65^{a}	251.03^{a}	1.83^{a}	8.25^{a}	254.25^{a}	1.00^{a}	8.00^{a}
		(2.21)	(0.12)	(8.91)	(0.21)	(0.36)	(5.85)	(0.07)	(0.60)
0.26	100.4^{a}	60.02^{b}	4.05^{a}	205.35^{b}	1.89^{a}	8.83^{b}	243.50^{a}	0.91^{ab}	7.85^{a}
		(2.26)	(0.22)	(16.81)	(0.29)	(0.24)	(19.94)	(0.10)	(1.02)
0.57	83.61^{b}	57.80^{bc}	2.62^{b}	96.10^{c}	1.36^{b}	8.80^{b}	196.75^{b}	0.83^{bc}	6.70^{b}
		(1.51)	(0.15)	(11.25)	(0.05)	(0.22)	(11.53)	(0.06)	(0.70)
0.77	79.30^{b}	56.56^{cd}	2.47^{b}	84.80^{c}	1.37^{b}	9.92^{c}	126.75^{c}	0.74^{cd}	6.09^{bc}
		(1.27)	(0.05)	(3.63)	(0.11)	(0.31)	(11.64)	(0.09)	(0.74)
1.02	50.61^{c}	54.13^{d}	2.16^{c}	61.13^{d}	1.37^{b}	11.37^{d}	89.50^{d}	0.66^{d}	5.52^{c}
		(1.28)	(0.12)	(9.41)	(0.09)	(0.38)	(9.33)	(0.16)	(0.53)

Values within columns indicated by the same superscript letter (a, b, c, d) are not significantly different at 5% level determined by Duncan's multiple range test.

Table 3. Mean values (± SD) of pH, free CO_2, alkalinity as $CaCO_3$, hardness as $CaCO_3$, DO, primary productivity, phytoplankton and zooplankton populations of thiocyanate treated water (0.26, 0.57, 0.77 and 1.02 mg/L) and control (0.00 mg/L).

Treatment (mg/L)	pH	Free CO_2 (mg/L)	Alkalinity as $CaCO_3$ (mg/L)	Hardness as $CaCO_3$ (mg/L)	DO (mg/L)	Primary productivity ($mgC/M^3/h$)	Phytoplankton (No/L)	Zooplankton (No/L)
0.00	7.65^a	2.48^a	141.75^a	111.75^a	7.27^a	146.25^a	5139^a	120^a
	(0.06)	(0.09)	(3.50)	(5.74)	(0.49)	(7.50)	(80.93)	(12.97)
0.26	7.65^a	2.50^a	139.75^a	110.25^a	7.04^a	145.00^a	5022^b	110^{ab}
	(0.01)	(0.08)	(3.40)	(8.46)	(0.36)	(5.47)	(69.35)	(9.42)
0.57	7.70^a	2.46^a	141.50^a	103.75^a	5.90^b	136.75^{ab}	4386^c	95^{bc}
	(0.08)	(0.13)	(5.07)	(6.60)	(0.18)	(6.70)	(32.46)	(7.94)
0.77	7.63^a	2.68^b	138.50^a	108.75^a	5.67^b	131.75^b	4403^c	92^{bc}
	(0.05)	(0.13)	(9.04)	(6.55)	(0.56)	(5.06)	(23.03)	(11.76)
1.02	7.70^a	2.70^b	141.75^a	109.00^a	5.97^b	127.75^b	4305^c	90^c
	(0.08)	(0.08)	(6.18)	(7.07)	(0.32)	(5.85)	(20.09)	(12.66)

Values within columns indicated by the same superscript letter (a, b, c) are not significantly different at 5% level determined by Duncan's multiple range test.

No report is available to compare the LC_{50} value of thiocyanate to oligochaet worms. Watson and Maly (1987) found that pH and temperature were the important modifying factors of thiocyanate *toxicity* to *Daphnia magna. They* reported that an increase in temperature from 8 to 16°C resulted a 5.5 fold increase in toxicity at pH 5 and a 10 fold increase at pH 6 and 7. Generally, temperature and toxicity are positively correlated for most chemicals (Mayer and Ellersieck 1986). In the present investigation, an increase of water temperature from 20 to 28°C, resulted an increase of toxicity of thiocyanate to fish, plankton and worm by 1.79, 1.50 and 1.30 fold, respectively at pH 7.02 ± 0.21. However, the present study shows that fish *(O. mossambicus)* is more sensitive than either the crustacean plankton *(M. micrura)* or oligochaet worm *(B. sowerbyi)* to the acute poisoning of thiocyanate, irrespective of water temperature (20-28°C). Thus, fish can be used as a good indicator organism to assess thiocyanate toxicity. The present investigation also indicates that thiocyanate, even in a low dose, interferes with the respiration of fish which is indicated from the increased opercular movement. There are reports that the thiocyanate ion (SCN^-) substitutes Cl^- in the gill and hampers the ionic balances, resulting in respiratory problems (Epstein et al. 1973). Heming et al. (1985) observed that acute toxicity of thiocyanate to trout was unpredictable, due to anomalous death of thiocyanate exposed fish. Healthy exposed fish suddenly started convulsions and quickly expired. These deaths were characterised by convulsions, gasping, loss of equilibrium and bouyancy, flaring of operculae, darkening of the skin epithelium and within minutes, cessation of ventilation and extreme rigor. These were characterized by Heming et al. (1985) as sudden death syndrome (SDS). Similar symptoms were also observed for tilapia in the present investigation

The SDS are not exhibited under low thiocyanate exposure (Heming et al. 1985). This is evident from the results of the feeding test and chronic bioassay made in the present investigation. However, the results indicated that even small doses of thiocyanate (except 0.26 mg/L) can produce profound effects on the feeding rate of fish. Eales and Shostak (1983) also found that sublethal doses of KSCN reduce the appetite of rainbow trout. Quinine factory effluents containing trace amounts of thiocyanate also reduced the feeding rate, growth and reproduction of tilapia (Kaviraj and Saha 1997). A loss in appetite appears to be the main reasons behind the reduction of growth of tilapia after chronic exposure to sublethal doses of thiocyanate. Apart from direct effect of the toxicants on the fish an alteration in the dissolved oxygen primary productivity, phytoplankton and zooplankton populations produce profound effects on the growth and reporduction of tilapia. Ability of even low sublethal concentrations of thiocyanate to alter primary productivity of water is a matter of great concern. Results of acute toxicity indicate that thiocyanate has the tendency to become more toxic if water temperature is increased. When water temperature naturally increased from 20 to 28°C during the months February to April in the present investigation, even very low concentrations of thiocyanate produced adverse effects on fish and aquatic ecosystem. Implications are that thiocyanate disposal, as such, is not safe and should be correlated with its lethal dose to aquatic organisms and temperature of the receiving water.

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