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Influence of Poultry Litter on the Toxicity of Cadmium to Aquatic Organisms

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Increased deposition of cadmium in impounded waters through atmospheric fallout and runoff water (Borg et al. 1989) is a growing concern for aquaculture. In India, pisciculture practices are threatened by frequent low to moderate deposition of Cd in ponds (Das et al. 1994). Although several studies have been conducted on Cd toxicity to freshwater organisms (Alabaster and Lloyd 1980; Kaviraj and Das 1994), little is known about the interaction of Cd with other chemicals present in the receiving water system. There is evidence that Cd , in the presence of other chemicals, may produce synergistic, additive or antagonistic effects on aquatic organisms (Finlayson and Verrue 1982). Aquatic ecosystems, heavily enriched by nitrogen and phosphorus, have reduced the stress imposed by Cd (Hendrix et al. 1981). In contrast, chemicals such as $KMnO_4$ and $CoCl_2$ used in aquaculture increase Cd toxicity to fish and plankton (Das and Kaviraj 1994). Poultry litter is frequently used in pisciculture ponds to enrich nutrients (Jhingran 1983). However, interaction of poultry litter with Cd is not known.

MATERIALS AND METHODS

Test organisms used in this investigation were fry of common carp Cyprinus carpio (mean total length 3.34 ± 0.23 cm and wet weight 0.33 ± 0.03 g), calanoid copepods Diaptomus forbesi (average size 0.58 ± 0.08 mm) and the worm Branchiura sowerbyi (mean length 2.0 ± 0.7 cm and wet weight 2.05 ± 1.20 mg). All organisms were collected from local unpolluted natural freshwater reservoirs and they were acclimatized to laboratory conditions for 48- 192 hr before being used in bioassays. Poultry litter was collected from four different poultry farms, one of which was located near the study site and three others at three suburban towns each about 40-50 km away from the study site. Fresh poultry litter contained 75-80 % moisture and average values (± SD) of nitrogen, phophorus (P_2O_5) and potassium (K_2O) among the sources were, respectively , 1.67 ± 0.1 % , 1.78 ± 0.24 % and 0.84 ± 0.03 %. The values of pH, conductivity, nitrate nitrogen and BOD loadings contributed by 250 mg/L poultry litter from each source to the test mixture showed 0.3-1.0 % variation among the sources. To test the variability of the effects of poultry litter four different bioassays with Cd were made for each test organism. Only 250 mg/L of poultry litter were used for such tests. To test the dose-dependent effects of poultry litter, bioassays were made with different doses of poultry

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llitter (250, 125, 65 and 30 mg/L) from a single source. Bioassays with carp fry were conducted in 20-L glass aquaria each containing 15-L of unchlorinated tap water (pH = 7.2 ± 0.02, temperature = 25 °C, alkalinity = 165 ± 12 ppm as $CaCO_3$, hardness = 185 ± 15 ppm as $CaCO_3$). Bioassays with the copepods and worms were performed in 250-mL glass beakers each containing 200 mL of the same water. The 96 hr LC50 values and 95 % confidence limits were derived by probit analysis (Finney 1971) and the values were compared according to the procedure outlined by APHA (1976).

Separate experiments were performed to study the growth of nitrifying bacteria, changes in physico-chemical parameters of water and bioaccumulation of Cd during exposure to Cd alone (2.5 mg/L), poultry litter (250 mg/L), or the combination of the two. Water samples were collected at 24 and 96 hr, plated and were incubated for 4 d in Wingradsky's medium containing a suspension of magnesium carbonate. Color intensity was read after the addition of sulphanilic acid and α-naphthylamine reagents (Salle 1967). Conductivity, pH, NO_3-nitrogen and BOD loadings were estimated in the 96 hr samples of water by the procedures of APHA (1976). Separate bioassays, with similar treatment as above, were conducted to estimate the accumulation of Cd in the test animals. After 96 hr of exposure, test animals were removed and digested in conc. HNO_3, $ClHO_4$ and H_2SO_4 (Churnoff 1975). Cadmium concentrations in the test organisms were then estimated by atomic absorption spectrophotometry using the flame method. The detection limit for Cd determination was around 0.001 mg/L. The accuracy of determination was checked regularly by subjecting standard sample (bovine liver) to the overal analytical procedure. All analyses of the standard materials yielded estimates within ± 10 % of stated concentrations.

RESULTS AND DISCUSSIONS

Poultry litter up to a dose of 250 mg/L , irrespective of sources, was found non-toxic to all organisms. Table 1 summarizes Cd LC50 (with 95% confidence limits) for the test animals. Poultry litter at 250 mg/L increased the susceptibility of copepods and fry to Cd, but the susceptibility of worms to Cd was reduced below 250 mg/L. Bioassays with various sources of poultry litter did not produce any significant variation in Cd LC50 values, but the effects of poultry litter were dose-dependant (Table 2).

A combination of 250 mg/L poultry litter and 2.5 mg Cd/L increased nitrate N and BOD loadings of water (Table 3). Such combination also increased the nitrifying bacterial population of water in 96 hr (Table 4). The bioaccumulation of Cd in response to 2.5 mg Cd/L in copepods, fry and worms was respectively 3.53 ±0.06, 13.67 ± 0.27 and 45.99 ± 0.75 (µg/g). Poultry litter significantly increased Cd accumulation in worms (99.16 ±2.81 µg/g) and carp fry (26.61±2.93 µg/g), but did not affect that accumulated by copepods (2.47 ± 0.05 µg/g).

Toxicity of Cd to aquatic organisms varies with the species tested, water quality, Cd salts used and other physical parameters (Kaviraj and Das 1990). The 96-hr Cd LC50 to common carp fry *Cyprinus carpio*, worm *Branchiura sowerbyi* and copepods *Diantomus forbesi* reported earlier by Das and Kaviraj (1994) are similar to the data reported here. Poultry litter increased average toxicity of Cd to copepods and fry, but reduced average Cd toxicity to worms. Treatment of poultry litter increased pH, NO_3-N, BOD and

bacterial loadings of the water. Cadmium toxicity to most organisms is reduced at higher
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Table 1.96-hr LC50 values of Cd (mg/L) to copepods, common carp fry and worms, with 95 % confidence limits in parentheses.

Treatment	LC50		
	Copepods	Worms	Carp Fry
Cadmium	5.70 (4.96-6.54)	58.02 (55.78- 60.34)	220.77 (198.89-245.50)
Cd + 30 mg/L poultry litter	4.90 (4.26-5.65)	48.55 (45.71-51.56)	226.03 (211.24-242.37)
Cd + 65 mg/l poultry litter	4.20 (3.92-4.52)	68.73 (61.86-76.36)	234.16 (220.90-249.97)
Cd + 125 mg/L poultry litter	3.45 (2.80-4.24)	77.56 (67.67-88.90)	123.13 (109.78-138.10)
Cd + 250 mg/L poultry litter (source-1)	2.86 (2.25-3.65)	85.76 (76.57-96.12)	72.68 (66.67-79.67)
Cd + 250 mg/L poultry litter (source-2)	3.046 (2.25-4.14)	85.53 (73.06-95.50)	72.518 (67.96-77.43)
Cd + 250 mg/L poultry litter (source-3)	3.093 (2.34-4.11)	88.78 (86.37-91.26)	74.157 (69.89-78.68)
Cd + 250 mg/L poultry litter (source-4)	2.996 (2.33-3.86)	86.418 (71.59-104.31)	70.169 (65.58-75.19)

Table 2. Significance of difference in Cd LC50 between single Cd treatment and combined Cd + poultry litter treatment.

Test organisms	Doses of poultry litter (mg/L)			
	30	65	125	250
Copepods	N	N	D	D
Fry	N	N	D	D
Worms	N	I	I	I

I = Significant increase
D = Significant decrease
N = Significant no change

Table 3. Changes in water quality parameters due to Cd - poultry litter interaction (± SD values within parentheses).

Treatments		Parameters			
Cd (mg/L)	Poultry litter (mg/L)	pH	Conductivity (mMho x 10^{-2})	Nitrate (ppm)	BOD (ppm)
0	0	7.57 (0.036)	0.56 (0.008)	0.012 (0.001)	0 0
2.5	0	7.95 (0.047)	0.46 (0.004)	0.322 (0.002)	0.021 (0.002)
0	250	9.09 (0.13)	0.70 (0.01)	2.00 (0.43)	0.92 (0.078)
2.5	250	8.95 (0.09)	0.69 (0.03)	2.88 (0.52)	1.025 (0.147)

Table 4. Intensity of nitrifying bacterial growth in the test medium.

Test medium (Treatments)	Intensity 24 hr	Intensity 96 hr
Control	–	–
Cd (2.5 mg/L)	–	+
Poultry litter (250 mg/L)	–	++
Cd (2.5 mg/L) +Poultry litter (250 mg/L)	–	+++

pH (Forstner and Wittman 1983) because at higher pH more Cd precipitates out into the sediment (Borg and Andersson 1984) leading to reduction in the availability of free Cd ion responsible for toxicity. Cadmium stress is also reported to be counteracted by higher nutrient levels (Hendrix et al. 1981). Inspite of higher pH and nutrients (NO_3-N) of water, Cd toxicity to fry and copepods, in the present study, was increased by poultry litter. Higher nitrate levels may become toxic to fish, but the concentration of NO_3-N observed in the present study occurs naturally in water with no apparent toxicity (Goldman and Horne 1983). However, there is a possibility of formation of $CdNO_3$ in water. Although no information is available whether $CdNO_3$ is more toxic than $CdCl_2$ we tested the toxicity of Cd to fry using $CdNO_3$ salt assuming that $CdNO_3$ would be formed as a result of the interaction between $CdCl_2$ and poultry litter. Only 1-2 % variation in the LC50 value of Cd was observed, however, between the salts of $CdNO_3$ and $CdCl_2$. Common carp fry accumulated more Cd during treatment with poultry litter. This could have accounted for the increased Cd toxicity to common carp fry with the

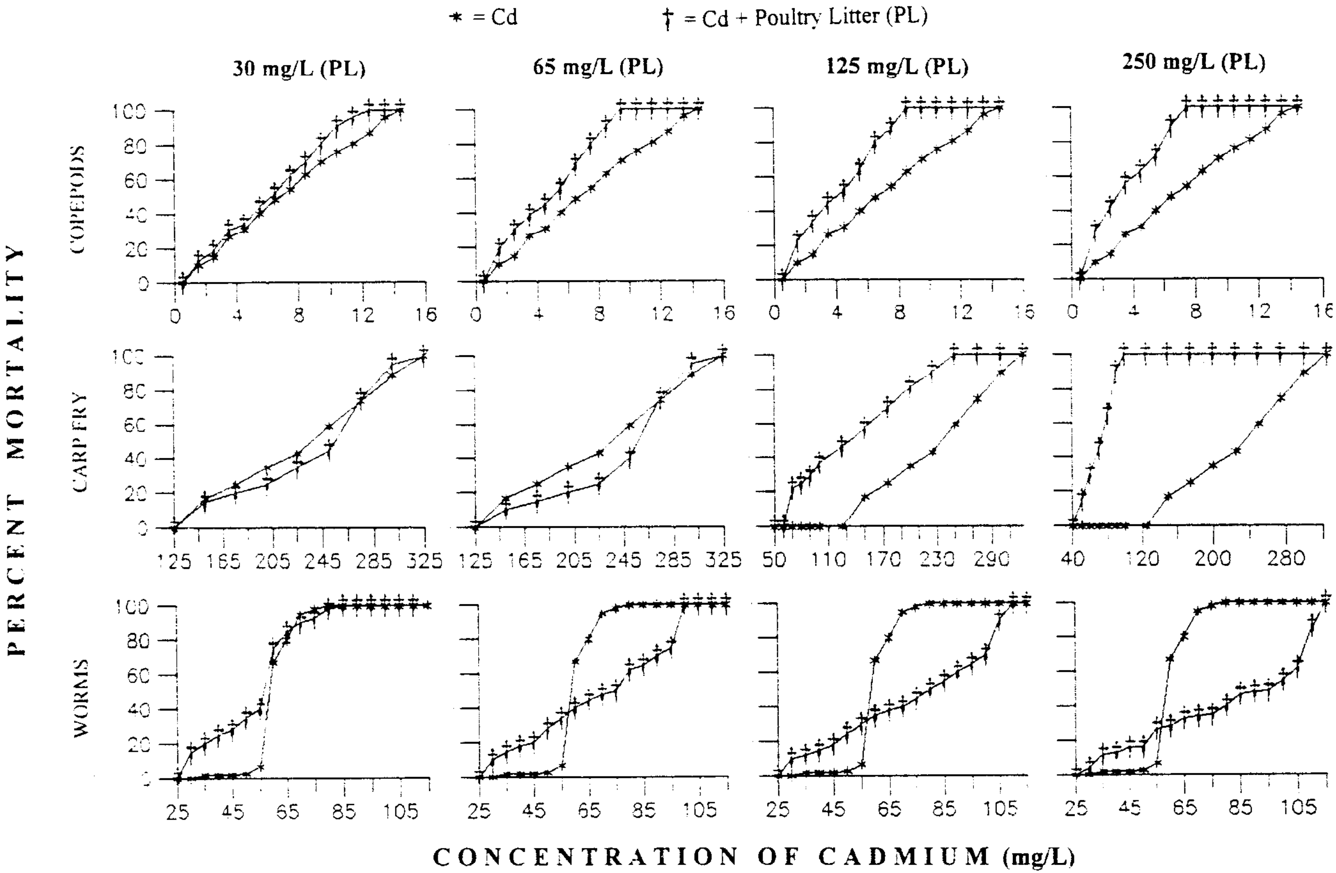

Figure 1. Cadmium toxicity curve for copepods. fry and worms under various exposure treatments with poultry litter.

addition of poultry litter. Accumulation was also higher in worms, but Cd toxicity to worms was reduced under higher poultry litter doses.

Poultry litter treatment did not affect the accumulation rate of Cd in copepods, yet toxicity of Cd to copepods increased with poultry litter treatments. Poultry litter, being an organic manure, forms complexes with metals like Cd (Forstner and Wittman 1983). Higher pH of water assisted in the sedimentation of such complexes. Worms being bottom feeders accumulated maximum concentrations of Cd. Common carp also consume particulate matter from the bottom and, thus, may have ingested a high amount of Cd. In contrast, the copepods obviously could not ingest particulate matter at a rate comparable to fry. Moreover, the copepods were restricted to the water column and could avoid metals that sedimented to the bottom. However, the copepods were most sensitive to Cd and probably altered physico-chemical and bacteriological conditions of the water under the combined treatment of Cd and poultry litter further increased their sensitivity to Cd, although the rate of Cd accumulation remained unchanged. It is, however, difficult to explain the reduction of Cd toxicity to worms under the combined treatment. It appears that a comparison of mean mortality data (LC50) for interpretation of interaction between Cd and poultry litter is not useful for all organisms. Figure 1 shows that the response of the three test organisms to the combined dose of Cd and poultry liiter was different.Worms, in contrast to carp fry and copepods, were more susceptible to the combined dose at the lower range of Cd treatments, but 65 mg/L or more poultry litter reduced the susceptibility of worms in the upper range of Cd treatment (55 mg Cd/L or more). Such a difference in sensitivity can be revealed only from the complete toxicity curve and not from the LC50 value. The limitations of LC50 data have been emphasized earlier (Sprague 1969). The present results also indicate that conventional method for evaluating LC50 provides misleading values on the susceptibility of organisms to the interaction of Cd and poultry litter. However, it is clearly revealed from the present investigation that poultry litter, notwithstanding its nutritive effects on water, significantly alters the acute toxicity of Cd to aquatic organisms and one must be careful of using this manure in Cd-polluted water.

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