

Fractionation of Metals in Water and Bed Sediments of a Chicken-fish System

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Received: July 7, 2017

Accepted: September 17, 2017

ABSTRACT

The study analyzed total and fractional concentrations of Cd, Cr, Cu, Mg, Pb and Zn in water and bed sediment samples collected monthly from fish ponds amended with chicken manure for one year. Four treatments in triplicate were used and the fish ponds were constructed directly under chicken sheds and all the ponds had a stocking density of 8 fingerlings/m² and a stocking ratio of 3 (Nile Tilapia):1(Catfish). Treatment 1 (T1) had a chicken density of 2000 birds/ha while Treatments 2 and 3 had chicken densities of 3000 and 4000 birds/ha respectively. Treatment 4 (T4) served as the control and had no chickens. Fish in T1, T2 and T3 fed on chicken manure and spilled feed that fell into the ponds while fish feed applied at 5% of fish body weight was given to fish in T4. Metal concentrations in chicken manure, water and sediments were determined monthly with Flame Atomic Absorption Spectrophotometry (FAAS) after wet acid digestion. The profile of mean total metal concentration in each treatment was Mn>Zn>Cu>Pb>Cr>Cd. Mean chromium concentrations in water for all the treatments exceeded the UNEP limit for freshwater fish culture while copper concentration exceeded the limits in T3 which received the highest chicken waste loading. Sequential extraction was carried out and Cd concentration was highest in the organic matter bound (OMB) fraction and lowest in the water soluble (WS) fraction. Cr concentration was highest in the oxide bound (OB) fraction in all the treatments while Cu was mainly associated with the OB and residual fractions. Pb on the other hand, was concentrated more in the OMB, OB and carbonatic fractions in all the treatments while Zn was predominant in the OB and carbonatic fractions with relatively insignificant amounts in the WS fraction.

KEYWORDS: total metals, pond, fractions, sequential extraction

INTRODUCTION

Trace metals easily get into animal feedstuff and are consequently discharged during excretion and defecation. Thus, sewage and animal manure serve as sources of trace metals in water bodies either when applied directly or via surface runoff when used as soil manure. A study analyzed some poultry feed samples for aflatoxins and trace metals and found high levels of Zn, Cu, Mn and Fe in the samples and concluded that levels of Pb in most of the samples exceeded the permissible limit of <1 mg kg⁻¹ in the United Kingdom[1]. Another study found that Pb and Cd concentrations were higher than European recommended levels in Bulgarian feedstuffs and feed given to pigs and poultry[2]. Poultry manure in Jiangsu province, China, have been reported to have Cu concentrations as high as 1726.3 mg kg⁻¹[3]. Chicken-fish farming (CFF) involves the combination of chicken and fish farming such that wastes from chicken becomes input for fish growth. Chicken manure undergoes microbial decomposition in the fish pond water releasing nutrients like nitrogen, phosphorus, calcium, magnesium, copper, zinc and iron [4]. Thus, there is the distinct possibility of trace metals accumulating in the chicken-fish system and being transferred to man when fish grown in such systems is consumed. Trace metals are partitioned between water, sediments, suspended solids and aquatic biota on entry into waterbodies and they tend to accumulate more in sediments than in water and aquatic organisms[5]. Fish ingests particulate matter in bottom and suspended sediments and this is a pathway for metal accumulation in fish. The ingestion of metal laden particulate matter affects the growth and reproduction of fish and consequently, the health of human consumers of fish [6]. Trace metals adsorbed on sediments are easily remobilized into the water column depending on factors like pH, concentration etc. This study assesses the total trace metal concentrations in water and sediments from fish ponds under 1-year integration with chicken. Metal partitioning in dissolved and particulate fractions of water was determined while sequential extraction was carried out to determine metal concentrations in soil fractions and these were compared to total metal concentrations.

MATERIALS AND METHODS

Experimental site and design. The Fish Farm of the National Institute for Freshwater Fisheries Research (NIFFR), New Bussa was the experimental site. Four treatments in triplicate were used for the experiment consisting of twelve earthen fish ponds (dimension; 5x4x1.5 m each). Nine chicken sheds (floor area; 2m x 1m each) were constructed over the ponds with wood and wire mesh was also used to cover the sides of each shed. The floors were constructed with iron rod mesh to enable faecal droppings and spilled feed fall directly into the ponds without hindrance. The sheds were at a height of 1m above the ponds. The treatments were; treatment 1 (T1) – pond integrated with four laying chickens,

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treatment 2 (T2) – pond integrated with six laying chickens, treatment 3 (T3) – pond integrated with eight laying chickens and treatment 4 (T4) – control without chicken. The layout of the experimental design is shown in Table 1.

Table 1. Layout of the Experimental Design

Treatments	Replicates	Number of Chickens	Chicken density/ha
T1	T1R1	Four chickens over the pond	2,000 Laying Chickens/ha
	T1R2	Four chickens over the pond	
	T1R3	Four chickens over the pond	
T2	T2R1	Six chickens over the pond	4,000 Laying Chickens/ha
	T2R2	Six chickens over the pond	
	T2R3	Six chickens over the pond	
T3	T3R1	Eight chickens over the pond	6,000 Laying Chickens/ha
	T3R2	Eight chickens over the pond	
	T3R3	Eight chickens over the pond	
T4	T4R1	Control – without integration	Nil
	T4R2	Control – without integration	
	T4R3	Control – without integration	

Oreochromis niloticus (Nile tilapia) and *Clarias gariepinus* (catfish) were stocked in each pond at a 3:1 ratio and a stocking density of 8 fingerlings/m². 20 week-old point-of-lay chickens (Nera Brown strain) were used for the experiment and their progress was monitored. Fish in T1-T3 fed on manure and spilled feed that fell into the pond while fish in T4 was given fish feed at 5 % body weight. The chickens were given commercial layers mash. Vitamin-mineral supplements and antibiotics were given to the chicken regularly for additional nourishment and for the prevention and cure of diseases. The chickens were also dewormed and vaccinated appropriately. The study lasted 12 months.

Determination of daily manure and spilled feed application rates

Thick waterproofs were used to cover the underneath of each chicken shed for a period of 24 hours weekly. Chicken droppings and spilled feed were collected on different waterproofs and weighed to give the 24 h manure and spilled feed loading rates. The average of these daily measurements in a month gave the daily manure and feed application rates per month for T1-T3.

Quality Assurance. Glassware, plastic containers, crucibles, pestle and mortar were washed with liquid soap, rinsed with distilled-deionised water and then soaked in 10% HNO₃ solution for 24 hrs. They were re-washed with distilled-deionised water and dried in a Gallenkamp drying oven at 80 °C for 5 h. Analar grade chemicals were used and triplicate digestion and analyses were done. The Flame Atomic Absorption Spectrophotometer (FAAS) was calibrated with multi-element standard Solution (MESS) and the calibration standards were analyzed after 10 sample runs to ensure that the instrument remained calibrated.

Sampling of ponds. Collection of water samples was done with acid washed polyethylene bottles from 5 points on each pond (depth, 10cm). The samples were mixed to give a composite sample followed by acidification with concentrated HNO₃ at 1.5 cm³ L⁻¹ [7] and refrigerated pending analysis. Sediment samples were collected with acid washed polyethylene corer at a depth of 0 – 5 cm. Extraneous materials were removed from sediment samples. The samples for trace metal analyses were oven dried at 110 °C. Dry sediment samples from each treatment were ground into fine powder with acid washed plastic mortar and pestle and were passed through 0.2 mm sieve. They were kept in corked dry acid washed polyethylene bottles at ambient temperature, prior to analyses.

Metal fractionation in samples. Fractionation of water samples was done as follows[8,9]:

Fraction I (Dissolved): 1000 cm³ of water sample was collected and 500 cm³ was filtered through 0.45µm pore diameter cellulose membrane filter. The filtrate was stored at 4 °C until it was analyzed for dissolved metals.

Fraction II (Total): The remaining 500 cm³ of water sample from fraction I was used for total metal analysis. Fractions I and II were acidified with conc. HNO₃ and refrigerated pending analysis.

Fraction III (Particulate): The particulate matter retained on the 0.45 µm filter paper was oven-dried at 45±5 °C to constant weight and stored at 4 °C before being digested and analyzed for particulate metals.

Sediment samples were subjected to a six step sequential extraction (SE) using a modification of the method by [10] which divided them into six fractions; water soluble (WS), exchangeable, Fe and Mn oxide-hydroxide bound or reducible (OB), organic matter bound or oxidisable (OMB), carbonate bound or acid soluble (carbonatic) and residual or silicate bound fraction (residual) fractions. Each extraction step was followed by centrifugation of the resulting sample for 15 min at 4500 rpm [11]. Supernatants were collected with pipette and passed through Whatman no. 42 filter paper. The residues were then washed with distilled-deionized water, vigorously shaken and centrifuged for 15 min prior to the next extraction. Excessive solubilization of solid materials was avoided by keeping rinse water volume at a minimum. Metal (Cd, Cr, Cu, Mn, Pb and Zn) concentrations were determined by analyzing the extracts collected with FAAS.

Digestion of samples for total metal determination. Unfiltered water samples, filtrates obtained by filtration and particulate matter retained on the 0.45 µm membrane filters were all digested with 20 cm³ of concentrated HNO₃ at 100

°C for 6 h to almost dryness in a Teflon beaker. The metals were recovered with 20 cm³ of 1 % HNO₃ and the solution was centrifuged and decanted. The supernatant was then transferred into a 50 cm³ volumetric flask, cooled and made up to mark with distilled-deionised. Portions of this solution were analyzed for metal contents. Sediment samples for total metal determination were digested according to [12]. 2 g of sediment sample was placed into a beaker followed by the addition of 9 cm³ of concentrated HNO₃ and 3 cm³ of concentrated HCl respectively. The beaker was placed in a microwave oven and digested at 700 W for 5 min in order to reach 180 °C. It was then digested at 500 W for 10 min to maintain a temperature of 180 °C. The sample was allowed to cool followed by centrifugation at 3500 rpm for 5 min. The extract was removed with pipette and filtered with Whatman no. 42 filter paper into a 25 cm³ volumetric flask and made up to mark with distilled/deionised water prior to analysis [8].

RESULTS AND DISCUSSION

The mean daily manure application rates (DMAR) into each treatment for all the months during the experiment are shown in Figure. DMAR depends on the feed consumption by chickens because higher feed consumption translates to

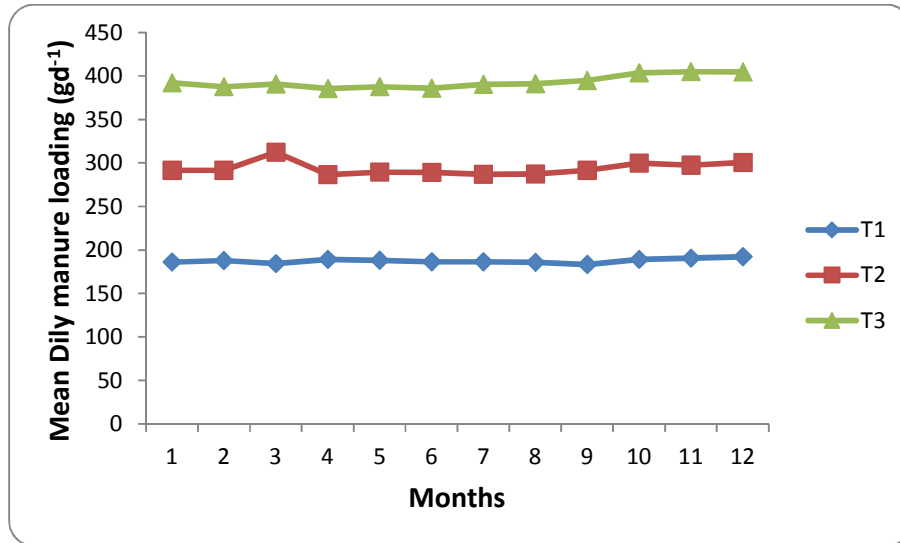


Figure 1. Mean daily manure loading rates for the 12 months

higher manure production. DMAR was highest (192.064 ± 1.840 g day⁻¹) in the month of September and lowest (183.266 ± 9.270 g day⁻¹) in June for T1. For T2, it was highest in September (300.449 ± 5.428 g day⁻¹) and lowest in January (286.544 ± 2.760 g day⁻¹). For T3, it was highest in August (404.838 ± 10.594 g day⁻¹) and the lowest in January (385.518 ± 6.754 g day⁻¹). DMAR was significantly higher ($P < 0.05$) in T3 compared to T1 and T2 but was similar ($P > 0.05$) across the months within each treatment. DMAR values in T3 (integrated with eight chickens) were effectively double the values obtained in T1 (integrated with eight chickens) across the months.

The daily feed application rates (DFAR) are shown in Table 2. DFAR for the integrated treatments depends on chicken activity. The higher the activity of the chickens, the higher their ability to spill feed and this translates to higher DFAR.

Table 2. Mean Daily Feed Loading Rates (g day⁻¹)

Month	Integrated Treatments (spilled chicken feed)			Control (fish feed)
	T1	T2	T3	T4 (5% of body weight)
1	11.630 ±1.824	17.850 ±0.636	24.265 ±1.506	91.680
2	11.505 ±1.084	16.870 ±1.504	24.165 ±1.791	260.470
3	11.738 ±0.961	15.984 ±1.126	23.374 ±1.387	522.920
4	11.970 ±0.692	17.045 ±0.915	24.715 ±1.087	646.800
5	10.385 ±1.457	16.530 ±0.768	24.698 ±1.207	854.250
6	10.528 ±1.306	16.805 ±0.462	24.563 ±0.731	1248.600
7	12.354 ±0.918	16.852 ±0.357	24.522 ±1.256	1518.900
8	13.485 ±0.902	16.343 ±0.641	24.373 ±1.504	1856.524
9	13.583 ±0.359	17.123 ±0.864	22.130 ±1.139	2106.870
10	14.074 ±0.399	18.628 ±0.794	25.326 ±0.956	2350.653
11	13.930 ±1.099	19.266 ±1.182	27.663 ±1.070	2692.724
12	14.009 ±0.495	18.650 ±0.575	26.506 ±1.269	3001.220

In T3, DFAR values differed significantly ($P < 0.05$) from those of T1 and T2 and this is due to the higher chicken density in T3. For T4, DFAR was the fish feed quantity determined as 5% of fish body weights obtained from sampling and weighing of fish.

Figure 2 shows mean metal concentrations in fish and chicken feed.

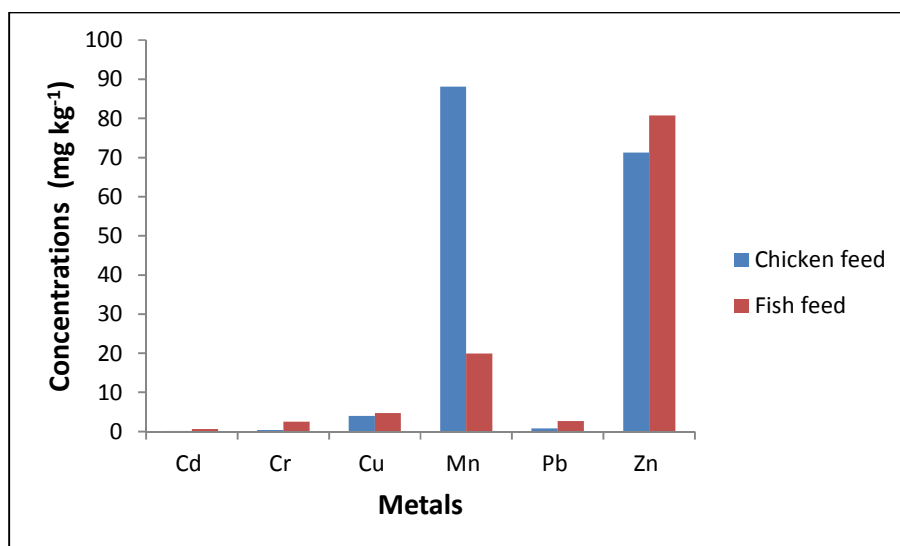


Figure 2. Mean Metal concentrations in chicken feed and fish feed samples

Range of values for metal concentrations in chicken feed were; 0.137 – 0.150 for Cd, 0.321 – 0.552 for Cr, 3.110 – 5.223 for Cu, 0.623 – 0.917 for Pb, 75.280 – 95.227 for Mn and 65.285 – 77.270 mg kg⁻¹ for Zn. Mean Mn and Zn concentrations were similar ($P>0.05$) but significantly different ($P<0.05$) from mean Cd, Cr, Cu and Pb concentrations. The descending order for metal concentrations in chicken feed was Mn>Zn>Cu>Pb>Cr>Cd across the months. Cd in fish feed ranged from 0.435 to 0.744 mg kg⁻¹ while Cr ranged from 1.688 to 3.114 mg kg⁻¹. Cu had a range of 3.641 – 5.662, Pb 1.743 – 3.300, Mn 18.310 – 21.465 and Zn, 76.905 – 88.713 mg kg⁻¹. Zn concentrations were significantly higher ($P<0.05$) than the concentrations of other metals in fish feed. The general profile for metal contents in fish feed across the months was Zn>Mn>Cu>Pb>Cr>Cd

Mean metal concentrations in chicken manure samples in the three integrated treatment are shown in table 3.

Table 3. Mean metal concentrations in Chicken manure samples (mgL⁻¹)

Treatments	Cd	Cr	Cu	Mn	Pb	Zn
T1	0.258 ±0.032	0.675 ±0.093	15.941 ±1.628	131.632 ±3.554	1.931 ±0.269	95.310 ±4.268
T2	0.249 ±0.031	0.709 ±0.087	15.435 ±1.645	131.019 ±4.203	1.889 ±0.185	97.770 ±2.757
T3	0.253 ±0.027	0.670 ±0.095	15.516 ±1.621	130.436 ±2.413	1.978 ±0.220	96.461 ±3.031

There was wide variation in ranges for Cd, Cr, Cu and Pb concentrations in T1 with values of 0.200 – 0.318, 0.541– 0.865 and 12.075 – 17.480 mg kg⁻¹ respectively. Ranges for Pb, Mn and Zn concentrations were 1.610 – 2.311, 125.800 – 135.480 and 88.160 – 100.274 mg kg⁻¹ respectively.

In T2, range of values for metal concentrations were: Cd 0.210 – 0.311 mg kg⁻¹; Cr 0.600 – 0.850 mg kg⁻¹; Cu 12.054 – 17.370 mg kg⁻¹; Pb 1.631 – 2.272 mg kg⁻¹; Mn 120.104 – 135.232 mg kg⁻¹ and Zn 92.730 – 101.230 mg kg⁻¹. Metal concentration ranges in T3 were: Cd 0.216 – 0.305 mg kg⁻¹, Cr 0.536 – 0.871 mg kg⁻¹, Cu 12.101 – 17.970 mg kg⁻¹, Pb 1.640 – 2.326 mg kg⁻¹, Mn 125.300 – 134.531 mg kg⁻¹ and Zn 91.580 – 100.418 mg kg⁻¹. Mean Mn concentration were significantly higher ($P<0.05$) than the mean concentrations of other metals in all the treatments while mean Cd concentrations were significantly lower ($P<0.05$). The descending order for metal concentrations in fresh chicken manure samples in the treatments was Mn>Zn>Cu>Pb>Cr>Cd across the months.

Table 4 shows the mean total, particulate and dissolved metal concentrations in water samples under the four treatments.

Table 4. Mean total, particulate and dissolved metal concentrations in water samples (mgL⁻¹)

Treatments	Parameter	Metal concentrations					
		Cd	Cr	Cu	Mn	Pb	Zn
T1	Total	0.039 ±0.018	0.065 ±0.037	0.378 ±0.180	1.996 ±1.569	0.071 ±0.051	0.428 ±0.278
	Particulate	0.016 ±0.008	0.026 ±0.015	0.245 ±0.112	1.259 ±0.970	0.043 ±0.031	0.278 ±0.175
	Dissolved	0.024 ±0.011	0.039 ±0.022	0.133 ±0.069	0.737 ±0.604	0.030 ±0.020	0.150 ±0.104
T2	Total	0.051 ±0.025	0.074 ±0.043	0.553 ±0.279	2.962 ±2.375	0.085 ±0.061	0.613 ±0.407
	Particulate	0.019 ±0.008	0.029 ±0.017	0.343 ±0.179	1.819 ±1.463	0.053 ±0.038	0.355 ±0.233
	Dissolved	0.033 ±0.016	0.045 ±0.026	0.210 ±0.102	1.144 ±0.921	0.032 ±0.023	0.258 ±0.174
T3	Total	0.069 ±0.037	0.095 ±0.053	0.860 ±0.484	4.231 ±3.563	0.132 ±0.103	0.882 ±0.587
	Particulate	0.031 ±0.017	0.037 ±0.020	0.602 ±0.361	2.631 ±2.290	0.086 ±0.066	0.506 ±0.352
	Dissolved	0.038 ±0.019	0.058 ±0.033	0.250 ±0.138	1.598 ±1.279	0.046 ±0.037	0.376 ±0.236
T4	Total	0.042 ±0.020	0.055 ±0.033	0.168 ±0.093	0.472 ±0.379	0.066 ±0.050	0.335 ±0.224
	Particulate	0.020 ±0.009	0.026 ±0.014	0.105 ±0.058	0.298 ±0.245	0.043 ±0.032	0.190 ±0.125
	Dissolved	0.023 ±0.011	0.029 ±0.018	0.064 ±0.035	0.174 ±0.136	0.023 ±0.018	0.145 ±0.099

Total Cd concentrations in T1 and T4 were similar ($P>0.05$) but were significantly lower ($P<0.05$) than the value for T3. Cd concentration and manure loading were positively correlated (T1 $r = 0.340$; T2 $r = 0.125$; T3 $r = 0.717$, $P<0.01$). There's significant positive correlation ($P<0.01$) between feed loading rate and Cd content of water in T1 ($r = 0.708$) and T4 ($r = 0.932$). Correlation coefficients for T2 and T3 were 0.484 and 0.493 respectively. Particulate and dissolved Cr were significantly different ($P<0.05$) in T1, T2 and T3 but were similar ($P>0.05$) in T4. Cr concentration and manure loading were positively correlated (T1 $r = 0.406$; T2 $r = 0.150$; T3 $r = 0.732$, $P<0.01$). Feed loading was positively correlated with Cr concentrations in water (T1 $r = 0.767$ significant at $P<0.01$; T2 $r = 0.590$, significant at $P<0.05$; T3 $r = 0.508$; T4 $r = 0.979$, $P<0.01$). Total Cu was significantly affected ($P<0.05$) by the treatments with T3 having the highest values and T4 the lowest. Cu concentration and manure loading were positively correlated (T1 $r = 0.445$; T2 $r = 0.091$; T3 $r = 0.671$, $P<0.05$). There's also significant positive correlation ($P<0.01$) between feed loading rate and Cu content of water in T1 ($r = 0.722$) and T4 ($r = 0.961$). Correlation coefficients for T2 and T3 were 0.471 and 0.417, respectively. Pb concentration and manure loading were positively correlated (T1 $r = 0.441$; T2 $r = 0.155$; T3 $r = 0.835$, $P<0.01$). Feed loading rate and Pb content of water in T1 had significant positive correlation ($r = 0.800$ at $P<0.01$), T2 ($r = 0.649$ at $P<0.05$) and T4 ($r = 0.996$ at $P<0.01$). Correlation coefficient for T3 was 0.563. Manure loading significantly affected Mn concentration ($P<0.05$) and the final value in T3, which received higher manure loading, was much higher than that of T4 which received only fish feed. Mn concentration and manure loading were positively correlated (T1 $r = 0.512$; T2 $r = 0.149$; T3 $r = 0.843$, $P<0.01$). There was significant positive correlation between feed loading rates and Mn concentrations in all the treatments, T1 ($r = 0.799$) and T1 ($r = 0.991$) at $P<0.01$; T2 ($r = 0.667$) and T3 ($r = 0.589$) at $P<0.05$. Total Zn concentration was significantly higher ($P<0.05$) in T3 compared to other treatments. Zn concentration and manure loading were positively correlated (T1 $r = 0.451$; T2 $r = 0.161$; T3 $r = 0.797$, $P<0.01$). There was significant positive correlation between feed loading rate and Zn content of water in T1 ($r = 0.811$ at $P<0.01$), T2 ($r = 0.610$ at $P<0.05$) and T4 ($r = 0.995$ at $P<0.01$). Correlation coefficient for T3 was 0.512.

Mn concentrations were highest in all the treatments compared to other metals followed by Zn while Cd had the lowest concentrations overall. There was a general increase in metal concentrations with increasing chicken waste loads and total Cd and Cr concentrations in water increased with increasing manure and spilled feed loading. In all the treatments, higher Cd and Cr concentrations were recorded in the dissolved fraction than in the particulate fraction. These results are at variance with the results obtained by [13] who found higher Cd, Cr, Pb and Zn concentrations in the particulate fraction of water samples. An experiment in sewage fed fish ponds of Eastern Kolkata, India to determine trace metals in water, sediment and fish samples, concluded that trace metals existed mostly in particulate phase (60–80%) and had the profile $\text{Fe} > \text{Al} > \text{Mn} > \text{Zn} > \text{Pb} > \text{Cu}$ [5]. However, Cu, Pb, Mn and Zn concentrations were higher in the particulate fraction than in the dissolved fraction of water in this study. Initial total Cr was lower than the UNEP limit of 0.05 mg L^{-1} in freshwater [14]. Mean Cr concentrations in the treatments exceeded this limit even in the control treatment which received only compounded fish feed. The UNEP limit of 1.0 mg L^{-1} for Cu was not exceeded in T3 which received the highest inputs of chicken wastes while the total Pb and Zn limits of 0.1 and 0.5 mg L^{-1} , respectively, were exceeded in all the treatments.

Table 5 shows the results of from total metal analysis in the sediment samples. Manure application substantially increased total metal concentrations in the sediment and mean total Mn concentrations were considerably higher than those of the other metals. This was probably due to the fact that initial Mn concentrations in the sediments were considerably higher than those of the other metals. In addition, inputs into the system increased the level of Mn with the integrated treatments having higher Mn levels due to the higher Mn content in chicken manure. The profile of mean metal concentration in each treatment was $\text{Mn} > \text{Zn} > \text{Cu} > \text{Pb} > \text{Cr} > \text{Cd}$. This profile is not very different from the results of [5], who concluded that total metal concentrations in sediments for the East Kolkata sewage-fed ponds had the profile: $\text{Al} > \text{Fe} > \text{Mn} > \text{Zn} > \text{Cu} > \text{Ni} > \text{Cr} > \text{Pb} > \text{Co}$. An investigation of the nutrient status and load of pollution for Cu, Zn, Fe, Mn, Pb and Cd in the sediment of municipal sewage fed Jagannath canal, India concluded that trace metal concentrations had the profile: $\text{Fe} > \text{Mn} > \text{Zn} > \text{Cu} > \text{Pb} > \text{Cd}$ [15]. This profile is similar to the profile of metal concentrations in sediments of this study. The USEPA threshold effects limits for metals in sediments are: Cd 0.676, Cu 18.7, Pb 30.2 and Zn 124 mg kg^{-1} [16]. Mean concentrations of these metals in the sediment samples were below the limits.

Table 5. Mean total metal concentrations in sediments (mg kg^{-1} , dry wt.)

Treatments	Metal concentrations					
	Cd	Cr	Cu	Mn	Pb	Zn
T1	0.341 ± 0.128	2.168 ± 0.504	11.397 ± 4.374	166.819 ± 24.574	4.047 ± 1.317	61.725 ± 14.689
T2	0.402 ± 0.188	2.329 ± 0.736	12.630 ± 5.427	176.696 ± 29.021	4.589 ± 1.732	57.126 ± 20.269
T3	0.514 ± 0.258	2.499 ± 0.856	14.014 ± 6.291	197.364 ± 41.662	5.127 ± 2.224	66.159 ± 20.261
T4	0.324 ± 0.113	2.222 ± 0.716	7.692 ± 0.316	141.864 ± 13.207	3.7941 ± 0.142	50.949 ± 10.368

Total trace metal concentration is a poor indicator of metal bioavailability but existing permissible limits of trace metals are based on total concentrations. The USEPA limits (thresholds effects levels) for sediments is Cd 0.676, Cu 18.7, Pb 30.2 and Zn 124 mg kg^{-1} . Metal concentrations in the sediments of this study did not exceed any of these limits except final Cd and Cu concentrations in the sediments of T2 and T3. Total metal concentrations in wastewater-fed fishpond sediment have been reported as Zn $502.8 \pm 91.0 \text{ mg kg}^{-1}$, Mn $369.0 \pm 14.15 \text{ mg kg}^{-1}$ and Pb $210.4 \pm 30.97 \text{ mg kg}^{-1}$ [17]. These values were higher than the values obtained in this study. Results of total metal concentrations in the sediment

samples of this study indicates that total metals in the sediments were much higher than total metal concentrations in the water samples across the treatments.

Table 6 shows mean metal concentrations in the sediment fractions. In all the treatments, significantly higher ($P<0.05$) concentrations of Cd was observed in the OMB fraction than in other fractions. Significantly higher ($P<0.05$) concentrations of Cr was also observed in the OB fraction compared to other fractions. In all the treatments, Cu concentrations in OB fraction was similar ($P>0.05$) to those in the residual fraction but was significantly higher ($P<0.05$) than concentrations in other fractions. Pb was more concentrated ($P<0.05$) with the OMB fractions of all the treatments while Mn was more concentrated ($P<0.05$) in the carbonatic fraction for T1-T4. Significantly higher ($P<0.05$) concentrations of Zn was observed in the OB fraction in all the treatments. Sequential extraction provides information on the distribution, mobility and bioavailability of metals and is also important in determining whether metals are of anthropogenic or lithogenic origin[18]. Metals associated with the exchangeable fraction, carbonatic, reducible and organic fractions are regarded as having higher mobility and higher remobilization potential from the sediment to the water column and are mostly from anthropogenic sources[19]. They provide information on the nature and magnitude of the pollution that has impacted a particular environment. Metals associated with the residual (inert) fraction may be considered as products natural sources or lithogenic sources. Cd is known to be a highly mobile metal and it was associated more with the OMB fraction in this study than in other fractions. Only about 10% of all Cd was associated with the inert (residual) fraction in the treatments. An experiment to determine metal contents in three coastal ecosystems, reported that about 70% of total Cd was associated with the exchangeable, OB and OMB phases[20].

Table 6. Mean Metal Concentrations (Mean \pm SD) in Sediment Fractions (mg kg⁻¹)

Treatments	Sediment Fractions	Metal concentrations					
		Cd	Cr	Cu	Mn	Pb	Zn
T1	WS	0.022 \pm 0.013	0.186 \pm 0.039	0.374 \pm 0.153	3.547 \pm 0.737	0.078 \pm 0.040	0.628 \pm 0.158
	Exchangeable	0.028 \pm 0.014	0.167 \pm 0.046	0.496 \pm 0.237	18.724 \pm 3.676	0.100 \pm 0.042	3.635 \pm 1.235
	OB	0.069 \pm 0.035	0.807 \pm 0.229	4.072 \pm 2.156	49.186 \pm 9.626	1.046 \pm 0.415	24.403 \pm 6.871
	OMB	0.141 \pm 0.058	0.413 \pm 0.098	1.342 \pm 0.552	10.799 \pm 1.906	1.381 \pm 0.553	8.452 \pm 2.924
	Carbonatic	0.049 \pm 0.024	0.306 \pm 0.106	0.687 \pm 0.352	66.940 \pm 12.087	0.742 \pm 0.319	13.816 \pm 4.213
	Residual	0.034 \pm 0.014	0.254 \pm 0.083	4.050 \pm 1.844	16.055 \pm 3.301	0.608 \pm 0.243	10.167 \pm 3.159
T2	WS	0.027 \pm 0.019	0.227 \pm 0.086	0.358 \pm 0.158	4.149 \pm 1.066	0.084 \pm 0.045	0.589 \pm 0.242
	Exchangeable	0.033 \pm 0.023	0.280 \pm 0.132	0.553 \pm 0.272	18.882 \pm 3.878	0.105 \pm 0.053	3.563 \pm 1.762
	OB	0.099 \pm 0.065	0.789 \pm 0.268	4.690 \pm 2.827	53.271 \pm 11.674	1.209 \pm 0.585	21.735 \pm 9.119
	OMB	0.150 \pm 0.068	0.419 \pm 0.137	1.530 \pm 0.821	10.869 \pm 2.289	1.537 \pm 0.729	7.665 \pm 3.347
	Carbonatic	0.057 \pm 0.039	0.298 \pm 0.141	0.523 \pm 0.252	69.338 \pm 13.479	0.827 \pm 0.411	13.593 \pm 6.068
	Residual	0.033 \pm 0.016	0.273 \pm 0.130	4.542 \pm 2.478	17.562 \pm 3.926	0.682 \pm 0.323	9.780 \pm 4.586
T3	WS	0.034 \pm 0.025	0.184 \pm 0.087	0.414 \pm 0.224	4.737 \pm 1.766	0.112 \pm 0.080	0.713 \pm 0.251
	Exchangeable	0.049 \pm 0.030	0.206 \pm 0.098	0.574 \pm 0.307	21.215 \pm 5.357	0.131 \pm 0.089	4.333 \pm 1.651
	OB	0.106 \pm 0.063	0.807 \pm 0.270	5.498 \pm 3.244	58.235 \pm 15.169	1.428 \pm 0.802	25.149 \pm 9.521
	OMB	0.194 \pm 0.115	0.508 \pm 0.243	1.753 \pm 0.984	12.651 \pm 4.003	1.738 \pm 0.964	9.198 \pm 3.309
	Carbonatic	0.071 \pm 0.046	0.408 \pm 0.207	0.596 \pm 0.294	75.321 \pm 19.247	0.887 \pm 0.493	15.099 \pm 6.002
	Residual	0.037 \pm 0.023	0.298 \pm 0.164	4.837 \pm 2.898	19.195 \pm 5.153	0.785 \pm 0.444	10.976 \pm 4.513
T4	WS	0.026 \pm 0.012	0.163 \pm 0.054	0.349 \pm 0.169	3.332 \pm 0.849	0.067 \pm 0.026	0.632 \pm 0.205
	Exchangeable	0.031 \pm 0.018	0.185 \pm 0.071	0.428 \pm 0.185	15.527 \pm 1.917	0.087 \pm 0.023	3.394 \pm 0.865
	OB	0.065 \pm 0.027	0.811 \pm 0.305	2.939 \pm 1.230	42.664 \pm 4.577	0.997 \pm 0.343	19.728 \pm 4.648
	OMB	0.126 \pm 0.043	0.409 \pm 0.187	1.164 \pm 0.407	9.812 \pm 1.580	1.215 \pm 0.408	6.669 \pm 1.878
	Carbonatic	0.047 \pm 0.023	0.311 \pm 0.151	0.487 \pm 0.258	56.149 \pm 5.399	0.664 \pm 0.228	12.074 \pm 2.754
	Residual	0.043 \pm 0.015	0.272 \pm 0.134	2.410 \pm 0.988	14.022 \pm 1.838	0.567 \pm 0.199	8.680 \pm 2.196

A study of metal speciation in bottom sediments of Goreckie Lake, Poland found that 43% Cd was associated with the OMB fraction, 28 % was associated with OB fraction, 18% was associated with carbonatic fraction while 5.9 % was associated with the residual fraction [20]. The profile for Cd concentrations in the different fractions was OMB>OB>carbonatic>residual>exchangeable>WS under T1 and T4 while the profile in T2 and T3 was OMB>OB>carbonatic>exchangeable>residual>WS. This shows that the least concentrations occurred in WS fraction. In this study, the profile for final Cr concentration in the fractions was OB>OMB>Carbonatic>Residual>WS>Exchangeable for T1 and OB>OMB>Carbonatic>Residual>Exchangeable>WS in T2, T3 and T4. This shows that the highest Cr concentration was in the OB fraction. Cr in the sediments of Goreckie Lake was contained mostly in the residual fraction (56%) and in OMB fraction (38%), while in smaller amounts in the OB fraction (7.2%) [21]. Cu in this experiment was mainly associated with the OB and residual fractions. The profile for final Cu concentration in T1, T2 and T4 was OB>Residual>OMB>Carbonatic>Exchangeable>WS while the profile in T3 was OB>Residual>OMB>exchangeable>Carbonatic>WS. Pb was concentrated more in the OMB, OB and carbonatic fractions of sediments in all the treatments. The profile for final Pb concentration in all the treatments was OMB>OB>carbonatic>Residual>exchangeable>WS. The highest concentrations of Mn (about 40%) was in the carbonatic fraction in the different treatments. Indeed, the profile for final Mn concentration in all the treatments was carbonatic>OB>exchangeable>Residual>OMB>WS. The profile for final Zn concentrations in all the treatments was OB>carbonatic>Residual>OMB>exchangeable>WS. Zn was concentrated in the OB and carbonatic fractions with relatively insignificant amounts in the WS fraction.

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