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Accumulation of lead, cadmium, manganese, copper and zinc by sludge worms; *Tubifex tubifex* in sewage sludge

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ABSTRACT: *Tubifex tubifex* has been shown to survive in organic polluted environments, however, not much has been done on its inorganic pollution tolerance. Samples of *T. tubifex* and their respective sewage sludge were taken from Soche wastewater treatment plant in Blantyre City, Malawi during July 2007 to November 2008. The total number of sludge samples taken was fifty one which was made into seventeen composite samples. A total of seventeen *T. Tubifex* samples were also collected. The samples were analyzed for copper, lead, manganese, zinc and cadmium using standard methods from American Public Health Association and Association of Official Analytical Chemists. The concentrations of metals in sewage sludge and *T. tubifex* were on dry weight basis and the metals determined were acid extractable. In general, heavy metals concentration was lower in *T. tubifex* than in sewage sludge. The range of heavy metals concentrations were (in sludge and (*T. tubifex)*): zinc 275.3 - 361.5 mg/kg (45.0 - 82.2 mg/kg), manganese 293.7 - 230.1 mg/kg (1.21 - 3.69 mg/kg), copper 86.5 - 120.1 mg/kg (1.6 - 4.7 mg/kg), lead 11.2 - 22.4 mg/kg (Below detection limit – 0.95) and cadmium 1.12 - 2.31 mg/kg (1.08 - 2.18 mg/kg). The results showed significant differences between the concentrations of manganese, copper, lead and zinc in sewage sludge and *T. tubifex* ($p < 0.05$). However there was no significant difference between the concentrations of cadmium in sewage sludge and *T. tubifex* ($p > 0.05$). *T. tubifex* did not show the ability to accumulate heavy metals (attributed to its high defecation and metabolic rate) except for cadmium hence cannot be used as a bioindicator for heavy metal pollution in sludge.

Keywords: *Biological indicator; Heavy metals; Pollution; T. tubifex; Wastewater treatment plant*

INTRODUCTION

Environmental degradation is a widely recognized global challenge. Some of the problems now affecting the world are acid rain, global warming, hazardous wastes, over population, ozone depletion, smog and water pollution. In Malawi, the major environmental problems are ranked in the order (from more to least important); soil erosion, deforestation, water resources degradation and depletion threat to fish resources, threat to biodiversity, human habitat degradation, high population growth, air pollution and climate change (GoM, 1994).

Environmental pollution is one of the major causes of environmental degradation worldwide. One of the major causes of environmental pollution is the use of sewage sludge in agriculture (Nouri, 1980; Nouri *et al.*, 2008). Sewage sludge is the residue from the treatment of domestic and industrial wastewater.

It contains useful organic matter and nutrients for plants. High cost of inorganic fertilizers and high inflation rates in most developing countries have resulted in waste materials like sewage sludge providing an alternative cheap input of nutrients in agriculture (Cooke, 1982). Sewage sludge does not only provide a cheap source of fertilizer, but it also solves the problem of waste disposal and protects the resource base and their reuse. The continual use of sewage sludge can result in accumulation of heavy metals and other toxic materials in soil and crops in particular leafy vegetables (Abdel-Ghani *et al.*, 2007). This can be detrimental to the environment, the production resource base and to

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the consumers of the produce (Alloway and Ayres, 1997; Campos *et al*., 2009).

Organisms acquire toxic substances from the environment along with nutrients and water. Some of the poisons are metabolized and excreted, but others accumulate in specific tissues. This capacity is widely recognized as offering one way of monitoring the distribution of toxins in the environment. One of the reasons these toxins are so harmful is that they become more concentrated in successive trophic levels of a food web, a process called biological magnification (Woodwell, 1972; Grimanis *et al*,. 1978; Adams *et al*, 1992; Campbell, 1996; Manly, 1996).

Monitoring the distribution of toxins in the environment is important because it provides data required for planning, helps in the determination of the health and condition of a particular environment, provides a means to record environmental changes and trends over time and finally it helps in focusing conservation efforts by relevant authorities towards decision-making (Roth *et al*., 1997). The use of living organisms to monitor the distribution of toxins in the environment is known as biomonitoring while the organisms are called bioindicators.

Although, there are a number of organisms that can be used as indicators, invertebrates and periphyton are easy to use (Stauber and Florence, 1987; Kaitala, 1988; Kale, 1998). A number of studies have been done on accumulation of heavy metals by organisms which include Kara (2005) and Kaonga *et al*. (2008). Examples of invertebrates commonly used as indicators are earthworms, midges and stoneflies. For example, Ireland (1983) and Bamgbose *et al*. (2000) reported that earthworms could accumulate, in their tissues, heavy metals in contaminated environment. For the terrestrial environment, earthworms are the standard soil toxicity test organism and are ideally suited for assessing the bioavailability of metals, as a result of their ecological importance in most temperate and tropical soils (Spurgeon *et al*., 2003; Lanno *et al*., 2004). Closely related to earthworms are *Tubifex tubifex*.

T. tubifex, also called sludge worm, sewage worm, or lime snake; is a species of tubificid segmented worm that inhabits the sediments of lakes and rivers on several continents. *T. tubifex* probably includes several species, but distinguishing between them is difficult because reproductive organs, commonly used in species identification, are reabsorbed after mating and also external characteristics of the worm vary with changes in salinity. These worms ingest sediments and gain nutrition by selectively digesting bacteria and absorbing molecules through the body wall. The worms can survive with little oxygen through the use of their hemoglobin rich tail-ends, which they wave in order to absorb oxygen. They can also survive in heavily polluted areas with organic matter that almost no other species can endure. *T. tubifex* can also survive drought and food shortage by forming a protective cyst and lowering its metabolic rate (Ward, 1997; Suthar and Sing, 2008).

Studies done in Blantyre City, Malawi have already shown that sludge has high heavy metal levels. For example, Kadewa *et al*. (2001) found levels of copper, cadmium and chromium in soils fertilized by sewage sludge from Soche wastewater treatment plant to be higher than the range for critical concentration for sludge amended soils. However no studies have been done on the accumulation of heavy metals by *T. tubifex* hence the need for this study which was carried out in Blantyre City, Malawi from July 2007 to November 2008.

MATERIALS AND METHODS

Study area

The project was conducted in the city of Blantyre, Malawi from July 2007 to November 2008. Blantyre city is the largest urban settlement in Malawi covering an area of 228 km^2. It is the industrial and commercial center of Malawi situated in the Shire Highlands at an altitude of approximately 1150m above sea level. It lies at 15° 47'S, 35° 0'E. The samples were taken at Soche waste water treatment plant. Soche wastewater treatment plant was constructed in 1958 and at that time it had only one trickling filter. After 20 years, the plant's capacity to treat wastewater was doubled with the addition of another trickling filter tank. It now serves a population of 24,000 (BCA, 1995). It has to be noted however that during the sampling period only one trickling filter was working.

Sewage sludge sampling

Sewage sludge eesamples were collected from the filtering tank. A total of fifty one sewage sludge samples were collected. Three random grab samples were collected from the filtering tank during each sampling session and were mixed in a bucket to make one composite sample. The total number of composite samples was therefore seventeen.

T. tubifex sampling

T. tubifex were collected in the same location as sewage sludge samples above. A total of seventeen *T. Tubifex* samples were collected for analysis. The *T. tubifex* samples were collected in 400 mL plastic bottles into which a few holes were poked on the lid.

Instrumentation

Atomic absorption spectrophotometer (AAS): GBC 933A AAS model was used for the determination of heavy metals. A glass electrode Sargent-Welch digital pH meter, model Pax S-29998 with pH reading to 0.01 in the range 0 to 14 was used to measure pH.

Sewage sludge preparation and heavy metal determination

Sewage sludge was dried in a shallow tray left in a well ventilated room until it could be easily ground and sieved. The dry samples were then passed through a 2 mm sieve and a representative sample (100 g) was retained after quartering and coning (Anderson and Ingram, 1993). Sewage sludge samples were digested using concentrated nitric acid, HNO_3 and concentrated hydrochloric acid, HCl. A dried sample (4 g) of sewage sludge was transferred to a round bottom flask and moistened with 3 ml distilled water. For each gram of sample, 7.5 mL of 6M HCL and 2.5 mL of concentrated nitric acid were added and the flask was left to stand at room temperature over night. Thereafter, the mixture was boiled gently under reflux for 2 h, cooled and then filtered using a filter paper washed in HNO_3. The solution was diluted in a 100 mL volumetric flask with HNO_3 (2 mL) to volume and the absorbance of the diluted solution was measured using AAS (MAFF, 1987; AOAC, 1990; Alloway, 1995).

T.tubifex preparation and heavy metal determination

The *T. tubifex* were cleaned with distilled water, placed in petri dishes and refrigerated at 10^{o}C for 24 h in order to purge the sludge in the gut. Thereafter they were removed and rinsed slightly with distilled water and then frozen pending analysis. In preparing for analysis, after thawing, 3 g of the *T. tubifex* sample (done in triplicates) was weighed and digested with 2 mL concentrated nitric acid and heated to dryness on a hotplate. The digest was redissolved in 1 mL concentrated nitric acid (AR) and filtered after which it was made up to 50 mL with distilled water in a volumetric flask (Bamgbose *et al*. 2000). The sample was then run on AAS.

Preparation of standard stock solution

The standard stock solutions of all the metals were prepared as in American Public Health Association (APHA, 1985).

Determination of moisture content in T. tubifex

About 2.5 g of the sample was weighed accurately and placed in a pre-weighed crucible. The sample was then placed in an oven and dried for 24 h at 105 ºC. The weights of the sample were recorded before and after the drying and the change in weight were calculated as percentage moisture (AOAC, 1990; Kaeser and Sharpe, 2006). Moisture content determination was done on the same day of sampling.

Determination of sewage sludge pH

The sample was dissolved in water to form a solution (50:50) and a pH meter (saturated with potassium chloride, KCl) was used to measure pH (AOAC, 1990).

Data analysis

Data was analyzed using statistical package for social scientists (SPSS) windows program, version 11.5 (Independent sample t- test) and microsoft excel windows program (Pearson correlations and ranges). Independent sample t-test was chosen because it was assumed that the samples were different from each other. Pearson correlations were used because it was assumed that the levels of heavy metals in sewage sludge were linearly related to those found in *T. tubifex*.

RESULTS AND DISCUSSION

Moisture content of T. tubifex

The moisture content of *T. tubifex* ranged from 70 – 79 % with an average of 74 % (Table 1). This is not significantly different from studies that were done on earthworms in which it was found that they contained moisture content of 75 % (Beyer, 1996).

pH of sewage sludge

The range of pH in sludge was 7.3 to 8.9 (Fig. 1). This range is not conducive to the availability of cations. Penney (2004) and Iretskaya and Chien (1999) declare that the pH is the most important parameter that governs the adsorption of inorganic ions. One reason is that a large part of the particle charge is variable, and therefore electrostatic attraction is different depending on the pH value. Hence anions are adsorbed more strongly at low pH (when the oxides contain many positively charged groups) whereas cations are more strongly sorbed at high pH (because humic substances and oxides become more negatively charged). However the pH range was conducive for the survival of *T. tubifex which* can tolerate pollution more than earthworms. Earthworms prefer a pH range of 6.5 to 7.5.

Zinc levels in sewage sludge and T. tubifex

The range of zinc concentration in sludge was 275.3 – 361.5 mg/kg while for *T. tubifex* it was 45.0 – 82.2 mg/kg (Table 2). The levels of zinc in sewage sludge were significantly higher than those in *T. tubifex* ($p < 0.05$) (Table 2). Sewage sludge and *T. tubifex* zinc levels were not strongly correlated ($r = 0.016$). The mean concentration of zinc in sewage sludge was lower than that found by Kadewa *et al.* (2001) (374.4 mg/kg as compared to 308.4 mg/kg) who analyzed heavy metals in sludge from the same sampling area (the research was about sludge utilization in agriculture whereby he used the sludge in vegetables as a fertilizer and determined heavy metal content). This is attributed to the fact that only one trickling filter was working during the time this study was conducted as such their could be a dilution effect on the waste water treatment facility as it is handling the load for two components. The levels of zinc in sludge were lower than the maximum permissible levels from other countries (Table 1) which was also the case with the study done by Kadewa *et al.* (2001). The presence of zinc in sewage sludge is due to corrosion of galvanized iron in the domestic plumbing systems (Koch and Rotard, 2001). The presence of Queen Elizabeth Central hospital which also releases part of its waste water into the municipal sewer line, might also be contributing to zinc levels in sewage sludge. Tolosana and Ehrlich (2000) found that effluent from medical institutions in South Africa had higher levels of zinc. This is compounded by the use of shampoos to combat dandruff which can contain up to 0.5 % Zn as an active ingredient (Leeper, 1978). In Blantyre, there are a lot of hair dressing saloons whose zinc contribution to wastewater may be significant.

Manganese levels in sewage sludge and T. tubifex

The range of manganese concentration in sludge was 293.7 - 230.1mg/kg while for *T. tubifex* it was 1.21 – 3.69 mg/kg (Table 2). The results showed significantly higher ($p < 0.05$) concentrations of manganese in sewage sludge than in *T. tubifex* (Table 2). Sewage sludge and *T. tubifex* manganese levels were not strongly correlated and showed an inverse relationship i.e. as the levels were increasing in sludge there was a decreasing trend in *T. tubifex* ($r = -0.012$). The mean levels of manganese in sludge were lower than that found by Kadewa *et al.* (2001) (323.3 mg/kg as compared to 254.3 mg/kg) which is also attributed to dilution. The maximum permissible levels of manganese in sewage sludge for other countries were not available. Manganese is used in industrial processes and in various consumer products. The sources of manganese in sewage sludge are effluents from alloy, steel and iron production (Jaques, 1987 and Moore, 1991).

Copper levels in sewage sludge and T. tubifex

The range of copper concentration in sludge was 86.5 – 120.1mg/kg while for *T. tubifex* it was 1.6 – 4.7 mg/kg (Table 2). The results showed significantly higher ($p < 0.05$) levels of copper in sewage sludge than *T. tubifex*. Sewage sludge and *T. tubifex* copper levels were not strongly correlated ($r = 0.092$). The mean copper sludge levels were lower than those found by Kadewa *et al.* (2001) (122.0 mg/kg as compared to 101.97 mg/kg) which is also attributed to dilution. The levels were also below the maximum acceptable limits from other countries (Table 2). The presence of copper in sludge may be due to corrosion of copper plumbing systems (Koch and Rotard, 2001). The presence of Queen Elizabeth Central Hospital might also be contributing to copper levels in sludge. Tolosana and Ehrlich (2000) found that the effluent from medical institutions in South Africa contained high levels of copper.

Lead levels in sewage sludge and T. tubifex

The range of lead concentration in sludge was 11.2 – 22.4 mg/kg while for *T. tubifex* it was from below detection limit – 0.95 mg/kg (Table 2). The results showed significantly higher ($p < 0.05$) levels of lead in sewage sludge than in *T. tubifex*. Sewage sludge and *T. tubifex* lead levels were not strongly correlated ($r = -0.082$) and also showed an inverse relationship i.e. as the levels of lead were increasing in sludge it was not the same case for *T. tubifex*. The mean lead levels in sludge were slightly lower than those found by Kadewa *et al.* (2001) (61.1 mg/kg as compared to 16.353 mg/kg) which is attributed to dilution and also the banning of leaded petrol in Malawi. The lead values in sludge were below the maximum permissible limits from other countries (Table 2). Sewage sludge is not the only source of lead into soils. For example, Grandjean, (1992) reported that lead in soil results from various municipal and industrial wastes, automobile emissions, decomposition of paints in aged homes and sewage waste.

Table 1:Moisture content in *T.tubifex*

Sample No.	Moisture content (%)
1	74
2	73
3	72
4	79
5	73
6	79
7	75
8	70
9	75
10	73
11	77
12	74
13	76
14	77
15	71
16	70
17	73

Cadmium levels in sewage sludge and T. tubifex

The range of cadmium concentration in sludge was 1.12 – 2.31 mg/kg while for *T. tubifex* it was 1.08 – 2.18 mg/kg (Table 2). The results showed no significant differences ($p < 0.05$) for cadmium in sewage sludge as compared to *T. tubifex*. Correlations showed an inverse relationship of cadmium levels in sewage sludge and *T. tubifex* ($r = -0.174$) i.e. as the levels were increasing in sludge it was not the case in *T. tubifex*. These results were not very different from Kadewa (2001). Kadewa reported a mean of 1. 42 mg/kg of cadmium for Soche sewage sludge as compared to 1.644 mg/kg for this study. Cadmium in sewage sludge may have come from fertilizer producing industries and the use of cadmium batteries. The cadmium levels in sewage sludge were low as compared to the maximum acceptable limits from other countries (Table 2). In earthworms studies, cadmium tends to be more mobile in soils and therefore more available to earthworms than other heavy metals (Ma, 2004). The apparent ability to accumulate high concentrations of cadmium in earthworms is related to the induction of cadmium-binding proteins with the

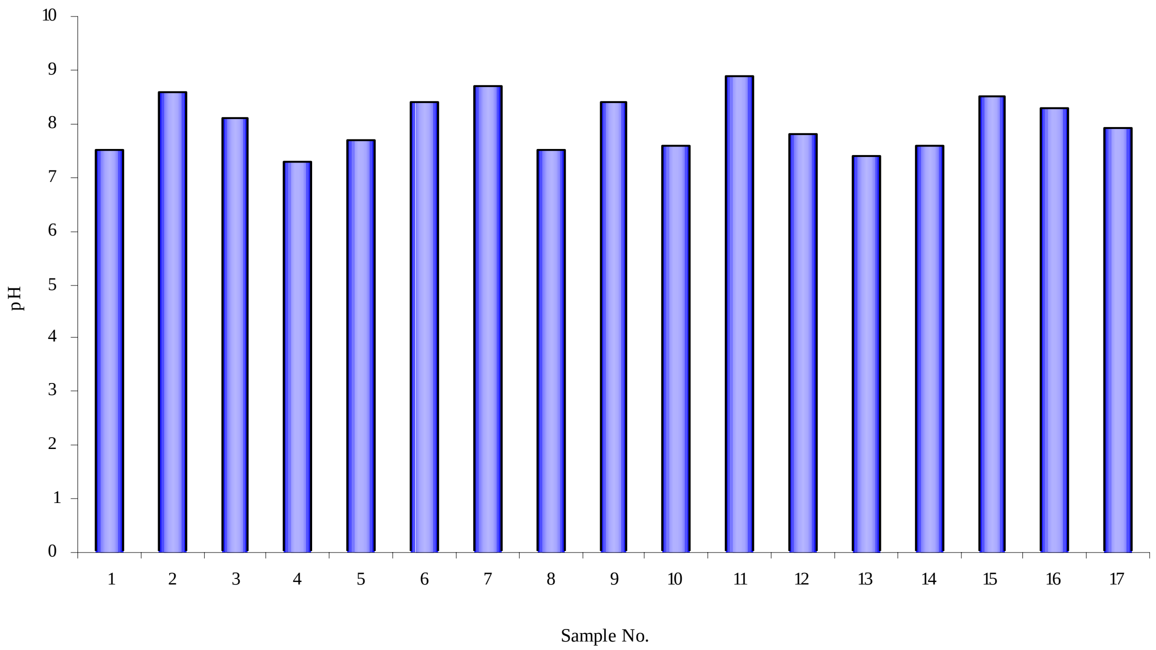

Fig. 1: Sludge pH levels

Table 2: Heavy metals in sludge and *T. tubifex*

Sludge					*T. tubifex*				
Zn	Mn	Cu	Pb	Cd	Zn	Mn	Cu	Pb	Cd
316.2 ± 0.123	257.4 ± 5.652	112.7 ± 14.911	0.56 ± 0.033	1.51 ± 0.566	51.9 ± 2.108	2.83 ± 0.057	3.3 ± 1.201	0.56 ± 0.033	1.67 ± 1.233
293.5 ± 0.152	244.8 ± 13.157	99.2 ± 9.744	19.3 ± 2.506	1.83 ± 0.751	49.3 ± 0.002	3.07 ± 1.622	2.9 ± 0.803	ND	
361.5 ± 1.321	297.5 ± 11.371	115.3 ± 9.744	22.4 ± 2.619	1.25 ± 0.023	62.2 ± 1.324	2.88 ± 1.081	1.8 ± 0.711	0.11 ± 0.018	1.88 ± 0.561
302.7 ± 2.343	252.3 ± 4.796	120.1 ± 16.416	11.2 ± 1.204	1.29 ± 0.211	50.6 ± 3.626	2.61 ± 0.600	3.7 ± 1.523	0.37 ± 0.014	1.35 ± 0.501
324.6 ± 5.146	246.3 ± 15.511	98.2 ± 16.314	14.8 ± 1.718	1.53 ± 0.301	55.6 ± 0.127	2.53 ± 0.042	2.3 ± 0.916	0.95 ± 0.116	1.60 ± 0.042
301.1 ± 0.146	230.1 ± 5.016	86.5 ± 7.114	13.6 ± 1.092	2.05 ± 0.396	45.0 ± 0.143	4.18 ± 0.147	4.1 ± 0.042	0.33 ± 0.045	1.33 ± 0.043
285.3 ± 5.649	241.9 ± 9.128	90.7 ± 6.118	16.5 ± 2.331	1.33 ± 0.991	71.6 ± 4.900	1.21 ± 0.023	1.9 ± 0.571	ND	
303.0 ± 6.005	234.0 ± 7.112	116.5 ± 5.311	13.5 ± 1.008	1.12 ± 0.451	62.1 ± 9.120	3.16 ± 0.151	2.8 ± 0.052	0.87 ± 0.091	2.18 ± 0.143
315. 4 ± 11.06	247.2 ± 3.211	110.8 ± 6.233	21.3 ± 3.001	1.67 ± 0.332	63.7 ± 1.601	2.18 ± 0.013	3.9 ± 0.156	0.78 ± 0.045	1.08 ± 0.144
302.8 ± 0.016	239.3 ± 13.113	89.7 ± 4.701	19.4 ± 2.501	2.18 ± 0.045	82.2 ± 1.980	2.04 ± 0.118	4.3 ± 0.142	0.62 ± 0.043	1.99 ± 0.158
281.7 ± 8.067	247.7 ± 9.026	87.4 ± 9.072	11.8 ± 1.013	1.92 ± 0.231	48.4 ± 0.012	1.98 ± 0.044	1.7 ± 0.045	ND	
338.3 ± 3.550	251.1 ± 2.056	105.6 ± 6.542	13.8 ± 0.974	1.82 ± 0.671	51.1 ± 7.071	1.82 ± 0.128	3.5 ± 0.144	0.47 ± 0.022	1.22 ± 0.451
316.2 ± 0.042	293.7 ± 6.151	94.8 ± 1.677	12.6 ± 1.287	2.31 ± 0.813	66.2 ± 1.089	3.07 ± 1.248	3.1 ± 1.479	0.37 ± 0.062	1.67 ± 0.239
292.7 ± 11.02	263.6 ± 5.662	119.5 ± 7.844	18.5 ± 1.455	1.92 ± 0.318	65.7 ± 8.240	2.11 ± 1.781	1.6 ± 0.487	0.55 ± 0.071	1.56 ± 0.623
304.9 ± 6.153	237.7 ± 8.005	95.6 ± 5.446	17.4 ± 0.675	1.88 ± 0.612	81.3 ± 0.071	3.69 ± 1.044	2.3 ± 0.113	0.49 ± 0.065	1.78 ± 0.452
275.3 ± 10.63	245.6 ± 4.001	88.2 ± 1.441	12.9 ± 1.677	1.19 ± 0.033	50.3 ± 0.711	1.81 ± 0.032	4.7 ± 0.581	0.44 ± 0.078	1.62 ± 0.512
328.1 ± 2.371	293.5 ± 1.233	102.7 ± 5.671	20.3 ± 1.007	1.14 ± 0.041	47.9 ± 0.057	1.45 ± 0.045	3.8 ± 0.078	0.34 ± 0.067	1.77 ± 0.431
7500*	NA	4300*	840*	85*	NA	NA	NA	NA	NA
7500**	NA	4300**	840**	85**	NA	NA	NA	NA	NA
2750***	NA	750***	400***	20***	NA	NA	NA	NA	NA

Data is on dry weight basis
Values are in the form of mean ± standard error
Max: maximum acceptable limits (* United States, **European Union and ***South Africa) (US-EPA 1983, Gendebien *et al.* 1999; Vivier, 1994).
NA; Standards Not Available
ND; Not Detected

characteristics of metallothioneins and also sequestration of cadmium by isomers of proteins (Suzuki *et al.*, 1980; Morgan *et al.*, 1999). This could also be the case with *T. tubifex*. The inability of *T. tubifex* to accumulate the other heavy metals could be attributed to its high metabolic and defecation rate. For example, Volpers and Neumann (2005) found that *T. tubifex*, had a defecation rate (which is correlated with metabolic rate) always slightly higher than that of *Limnodrilus hoffmeisteri* (another Tubificid species).

CONCLUSION

The study generally found that the concentration of heavy metals in *T. tubifex* was lower than that of sewage sludge. The results showed that *T. tubifex* cannot be used as an indicator of general heavy metal pollution in sewage sludge except for cadmium. The study recommended that the local assembly should look at possible ways of monitoring heavy metals in sewage sludge. Guidelines should also be devised for the utilization of sewage sludge in Malawi in line with the quality of the sludge.

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