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Periphyton and Earthworms as Biological Indicators of Metal Pollution in Streams of Blantyre City, Malawi

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1. Introduction

Pollution of water and soils arises from overburdens of mines, application of fertilizers and pesticides, industrial effluents and sewage sludge (Alloway & Ayres, 1997), among others. In heavily contaminated soils and water, there is a decrease in the population, growth and function of biota. In most cases all biological indices of environmental health (fish, invertebrates and algae) decline as pollution intensity increases (Cuffney et al., 2000; Hill et al., 2000; Khan, 1990). The identification of plant and animal species with the ability to accumulate metals is therefore of interest for the purposes of environmental monitoring (Chukwuma, 1998; Manly, 1996).

Earthworms and periphyton (attached algae) have been utilized as indicators of pollution of soils and water with metals (Ireland, 1983; Holan et al., 1993; McCormick & Cairns, 1994; Ramelow, 1987; Jin-fen et al., 2000). Despite being very small, experiments unequivocally demonstrate that algae sequester heavy metals by complexation to phytochelatins (Gekeler et al., 1988), which is an identical mechanism as higher plants. In the context of biomonitoring, earthworms act as quantitative monitors of total-soil metal and also estimators of ecologically significant soil metal concentration (Morgan & Morgan, 1988). Earthworms are important components of the soil system mainly because of their favourable effects on soil structure and function which include increasing soil fertility by formation of an organic matter layer in topsoil. The most widely studied earthworm species are *Eisenia fetida*, *Eisenia Andrei, Lumbricus terrestris* and *Lumbricus rubellus* (Georgescu & Weber, 2007).

In Malawi, several studies have confirmed the presence of heavy metals in water and soils. Kadewa et al. (2001) found levels of copper, cadmium and chromium in soils fertilized by sewage sludge from Soche waste water treatment plant in Blantyre to be higher than the range for critical concentration for sludge amended soils. Sajidu et al. (2007) found that the levels of lead, cadmium, iron, manganese, zinc, chromium and nickel in streams in the city of Blantyre were much higher than World Health Organisation (WHO) safe limits for drinking water in all sampled streams after they had passed through industrial areas. Lakudzala et al. (1999) found that at some points on Mudi, Likhubula and Shire Rivers, the iron and lead levels exceeded WHO guideline limits. However, not much has been done on the use of biological indicators to assess the state of the environment. In addition, studies that assess levels of heavy metals in biota are lacking because they only target either invertebrates or aquatic plants only. Compounds of heavy metals in earthworms may be transferred to other species at higher trophic levels and may be lethal to earthworm consumers (Hui, 2002; Ireland and Richards, 1977; Ma, 1982; Vyas et al., 2000). Monitoring programs, with a well-founded scientific base and defined management outcomes, using biological indicators (such as algae, fungi, earthworms and other microorganisms), will expand our knowledge of river/aquatic function (Burns & Ryder, 2001; Khosmanesh et al., 1996). This work reports on the levels of potentially harmful elements in the streams, wastewater and stream bank soils. In addition, aspects of metal accumulation in earthworms and green algae and their potential for biomontitoring are presented.

2. Materials and methods

2.1 Study area

Malawi is situated in South East Africa (Fig. 1a). This study was conducted in the City of Blantyre (Fig. 1b), the commercial and industrial city of Malawi. The City has eight designated industrial areas namely *Makata*, Ginnery corner, *Maselema*, *Limbe*, *Chirimba*, south *Lunzu*, *Maone* and *Chitawira*, with south *Lunzu* still under development (Fig. 1c). All the industrial sites are located along the banks of the main streams in the City. *Makata* industrial site lies between *Mudi* and *Nasolo* streams, Ginnery corner is along *Mudi* stream, *Maselema* is along *Naperi* River and *Chirimba* is along *Chirimba* stream (Fig. 1c). The sampling points fell into two major categories, which were; streams and wastewater treatment plants (WWTP). The waste water treatment plants were included because their effluent is released into the streams. Most of these streams pass through the major industrial areas except for Namangunda, which passes by a dumpsite and Michiru, which originates from a forest reserve and does not pass through the industrial sites (Fig. 1c).

2.2 Sample collection

All samples were collected in wet (November - February) and dry (July - October) seasons, to capture the effects of seasonal variation, from the selected streams and WWTPs in Blantyre City (Fig. 1). A total of eighteen periphyton (algae) samples were collected for each season. The samples were collected in 100 mL plastic bottles (Diatoms for Assessing River Ecological Status (DARES), 2004). The algae samples were chilled in a refrigerator pending analysis (New South Wales (NSW), 2002). The periphyton was identified as *Spirogyra aequinoctialis.*

Water samples were collected at an area where samples of *S. aequinoctialis* were collected. A total of forty three (43) water samples were collected for each season. Grab sampling method was used in the collection of water samples both upstream and downstream of a designated industrial area. At each sampling point, water samples were collected in triplicates for heavy metal analysis and a single sample for pH analysis. The samples were collected and stored in 1 L pre-cleaned new polyethylene bottles. Water samples for determination of metal were acidified to pH < 2 by adding concentrated nitric acid (Analytical Reagent (AR)) (American Public Health Association (APHA), 2005).

A total of forty-six (46) earthworm and soil samples were collected in both seasons. The earthworms were collected in 400 mL plastic bottles (at least three individuals per sampling site) into which a few holes were poked on the lid (Ecological Monitoring and Assessment Network (EMAN), 2004). Earthworm casts were used to find possible earthworm locations. Soil (stream sediment) samples were collected where earthworms were found using a soil auger. Soil samples were collected within the top soil range (0-20 cm) since most of the earthworms were found in this region. Soil horizons could not be distinguished in all these sampling points. Five augerings were collected at each site and were mixed in a bucket before sub sampling (quartering) (Anderson and Ingram, 1993). The samples were collected in plastic bags.

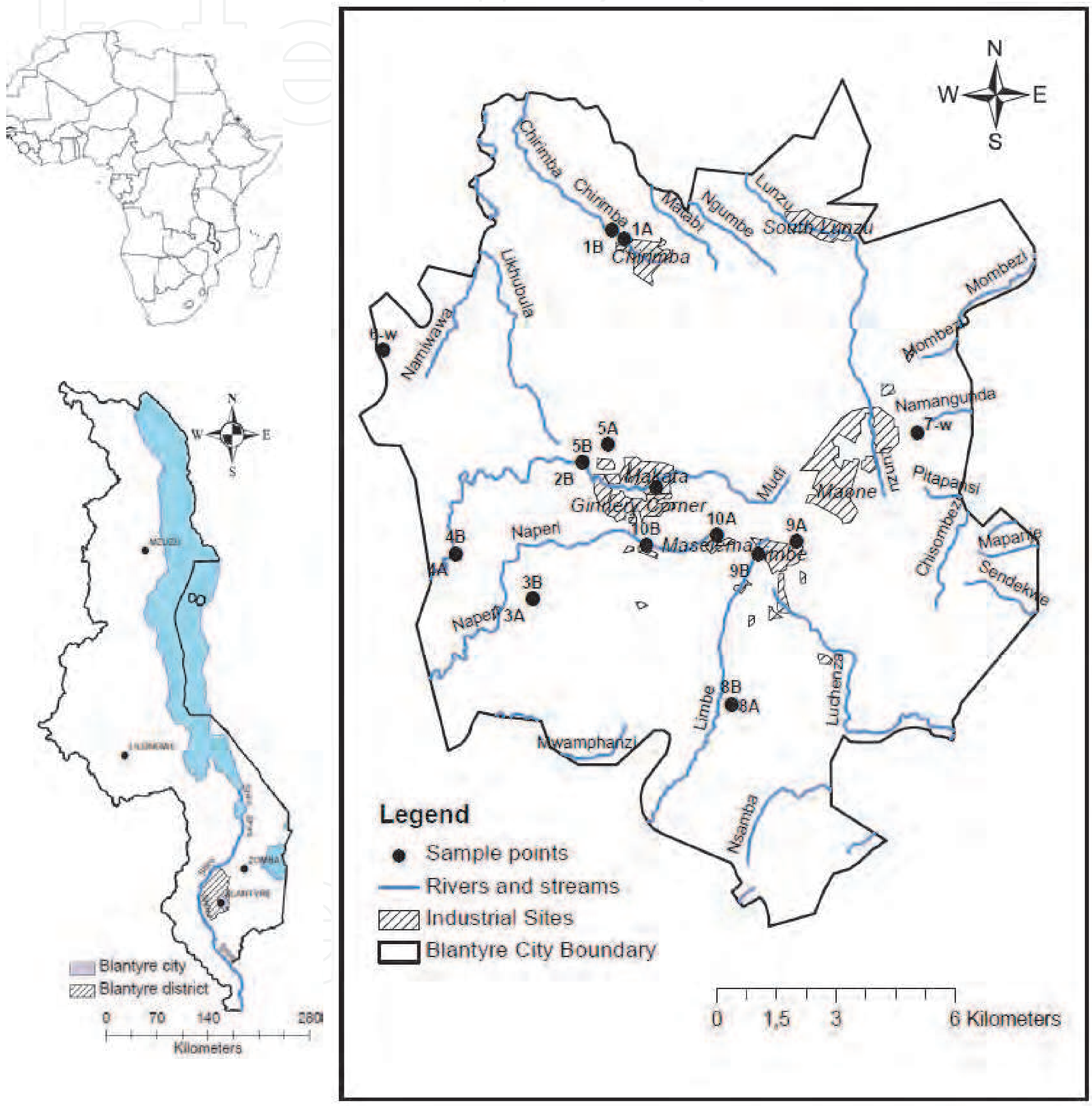

Fig. 1. Maps showing the location of Malawi in Africa, Blantyre city in Malawi and sampling points in the City of Blantyre. Sample IDs are explained in Table 1.

2.3 Analysis of water and wastewater samples

pH was measured immediately after sampling using Orion Research digital ionalyzer 601A and Metrohm 744 pH meters (ISO 10523-1:1994). Water samples were digested using concentrated nitric acid (AR). 50 mL of the sample was transferred to a beaker to which 5 mL concentrated nitric acid was added and brought to a boil on a hot plate to the lowest volume possible (15 to 20 mL). Filtration was done after digestion. The filtrate was then diluted to volume with distilled water in a 50 mL volumetric flask (APHA, 2005). Total concentrations of Mn, Cd, Cr, Cu, Pb, Ni, Fe, Zn were determined using flame atomic absorption spectroscopy (Perkin Elmer, Analyst 700; APHA, 2005).

2.4 Analysis of soil samples

The soil samples were air dried, ground in a mortar and passed through a 2 mm sieve. 5.0 g of the dry sieved soil sample was heated with 10 mL concentrated nitric acid (AR) for 45 minutes. The soil samples were then dried, re-dissolved in 5 mL aqua regia (3:1 conc. HCl (AR) and conc. nitric acid (AR)) and filtered. Total concentrations of Mn, Cd, Cr, Cu, Pb, Ni, Fe, Zn were determined using flame atomic absorption spectroscopy (Perkin Elmer, Analyst 700 and Buck Scientific AAS model 200A; Bamgbose et al., 2000). The total metal concentrations were expressed as mg/kg dry weight of soil (mg/kg dw).

Soil organic matter was determined using the Walkley – Black method (Walkley and Black, 1934). Briefly, the soil samples were ground using a mortar and then passed through a 0.5 mm sieve. 1.00 g soil was mixed with 10 mL of 1N potassium dichromate (AR) solution and 15 mL concentrated sulphuric acid (AR), whilst shaking. The mixture was then shaken for a further one minute and left to stand for thirty minutes. Then 150 mL distilled water and 5 mL concentrated phosphoric acid (AR) were added whilst shaking. After cooling, the mixture was titrated against 0.5 N ferrous ammonium sulphate (AR) solution, with 1 mL diphenylamine indicator. The colour change was from deep blue to dark green. Similarly triplicates of blank titrations were carried out. Where the volume of 0.5 N ferrous ammonium sulphate (AR) solutions was less than 3 mL, the determinations were repeated using 0.5 g soil. The percentage organic matter was calculated using the following equation:

$$\%OM = 1.729 \text{ x } 0.0031 \text{ x } 100 \text{ x } F \text{ x } \left[\frac{Me_{K_2Cr_2O_7} - Me_{Fe(NH_4)_2.6H_2O}}{\text{mass (g) of air dried soil}} \right] \quad (1)$$

where, F = Correction factor (1.33) and Me = Normality of solution $\times$ volume (mL) of solution used.

Soil pH was determined using glass electrode pH meters (model 601A Orion Research digital ionalyzer and model 744 Metrohm) in a 1:5 (V/V) of soil in water (pH-H_2O).

2.5 Analysis of metals in earthworm samples

The earthworms were identified as *Aporrectodea icteria* after being rinsed with distilled water. Only reproductively mature earthworms were identified because of presence of a clitellum. Then they were placed on moist filter papers and put in glass Petri dishes (one individual per dish) and kept at 10^o C for 24 hrs in order to purge the soil in the gut. They were then rinsed slightly with distilled water and then stored frozen and then freeze-dried. Gut contents remaining in some earthworms were removed manually. 3.0g of thawed and dried earthworm sample was heated with 2 mL concentrated HNO_3 (AR), filtered and made up to 50 mL with distilled water. The metal contents were determined by running samples on AAS as for the soil samples. The metal concentrations were expressed as mg/kg dry weight of earthworm (mg/kg dw).

2.6 Analysis of metals in *S. aequinoctialis* samples

Periphyton samples were air dried (Hoffman, 1996). The air dried samples were then dry-ashed in a furnace after adding nitric (AR) and hydrochloric acid (AR) (Association of Official Analytical Chemists (AOAC), 1990). Thereafter the sample was made up to 50 mL with distilled water in a volumetric flask. The samples were prepared in triplicates and blank and standard samples were used to check accuracy of analysis. The concentration of heavy metals was determined by running samples on AAS (Perkin Elmer, Analyst 700).

2.7 Quality control

To ensure quality control of sampling and analysis, a number of procedures were followed. Firstly the sampling devices were carefully chosen so that they should not contaminate the samples. If the same apparatus were to be used for the next sampling process, they were thoroughly cleaned and rinsed with distilled water. For water samples in which heavy metals were to be determined, acidification was done to pH less than 2 to avoid adsorption of metals on the sides of the sampling containers. In the measurement of volume a pipette which is more accurate was used rather than measuring cylinders. Soil samples were prepared away from the rest of the samples since dust could easily contaminate the other samples. All samples for heavy metal analysis were determined in triplicates. Analytical reagents were used for all procedures rather than general purpose reagents. In addition, the following minimum laboratory quality control measures (United States Environmental Protection Agency (US-EPA), 2010) for the instruments used in the analysis and also samples were followed;

a. Initial calibration; This was done prior to analysis of samples (minimum three concentration levels for every compound and an instrument blank).
b. Continuing calibration; This was done once per 10 samples (mid-level standard containing all compounds) and a continuing calibration blank.
c. Method blank; This was done once per digestion or extraction set.
d. Soil and water samples were preserved at $4^{o}C$ and analysed within 28 days.

3. Results

3.1 Metal concentrations in stream water, wastewater and *S. aequinoctialis*

Table 1 provides the levels of determined metals in stream water and wastewater samples. The corresponding World Health Organization (WHO) drinking water guidelines (WHO, 2006) and the Malawi Standard (MS 214) (MBS, 2005) for the parameters are also included in Table 1. The levels of metals determined in *S. aequinoctialis* samples are provided in Table 2. In *S. aequinoctialis* samples, concentrations of manganese, cadmium and copper were significantly higher ($p < 0.05$) in the dry season than in the rainy season. There were, however, no significant seasonal differences in the levels of lead, zinc, chromium and nickel.

Chromium and copper were not detected in all samples in the rainy season, but they were measured in levels of up to 0.419 mg/L and 0.076 mg/L, respectively, in the dry season (Table 1). For both seasons, the determined levels of zinc and copper were below MS 214 and WHO water quality guidelines, whereas levels of nickel and cadmium were above these guidelines (Table 1). 17% of the samples had chromium levels above the MS 214 (0.05-0.1 mg/L) and WHO (0.05 mg/L) water quality standards. For lead, 44 % and 61 % of the sampled points contained lead levels above MS 214 (0.01 – 0.05 mg/L) and WHO (0.01 mg/L) drinking water standards in the rainy and dry seasons, respectively. In the case of manganese, water quality standards were only exceeded at Mangunda stream, in the rainy season. In the dry season, however, 83% and 17% of the sampling points showed manganese levels above the MS 214 and WHO drinking water quality guidelines, respectively (Table 1).

3.2 Metal content in soils and *A. icteria*

Table 3 and Table 4 provide results of soil and earthworm sample analyses, respectively. The metal content in the assessed soil sites is low in comparison to guideline values in several European countries. Levels of Cd (rainy season), Pb, Cr, Cu, Zn and Ni (both seasons) were below their respective England toxic limits (0.06 mg/kg for Cd, 10 mg/kg for Pb, 50 mg/kg for Zn , 20 mg/kg for Cr and 40 mg/kg for Ni; Bohn et al, 1985), Swiss guide levels (0.8 mg/kg dry soil for Cd, 50 mg/kg dry soil for Pb; OIS, 1998) and the Netherlands target levels (85 mg/kg for Pb, 36 mg/kg for Cu, 140 mg/kg for Zn, 100 mg/kg for Cr, 35 mg/kg for Ni; Alloway and Ayres, 1997). However, for the dry season, 33% of the soil samples were above the England toxic level (0.06 mg/kg; Bohn et al., 1985).

The internal concentrations of Cd, Cu, Pb, Zn and Cr were below the levels that show significant changes in (sub-) lethal endpoints for earthworms (see e.g. Langdon et al, 2001; Spurgeon and Hopkin, 1999; Spurgeon et al, 2000). There were significantly higher concentrations of Cd in *A. icteria* than in the soils, but significantly lower values of Mn, Fe, Pb, Cr, Zn and Cu in the earthworm than soils ($p < 0.05$). There was no significant difference in the concentrations of Ni in soils and earthworms ($p > 0.05$). The effect of seasonality varies among the studied metals. In the soils, levels of Mn were significantly higher in dry season than the rainy season ($p < 0.05$), but there were no significant differences between the seasons for the values of total soil concentrations of Cd, Cu, Zn, Pb, Cr and Ni ($p > 0.05$). pH was significantly higher in the rainy season than the dry season ($p < 0.05$), but there were no significant differences in soil OM content between the seasons ($p > 0.05$). In *A. icteria*, levels of Cd and Cr were significantly higher in dry season than the rainy season ($p < 0.05$), but there were no significant differences between the seasons for the values of concentrations of Mn, Cu, Zn, Pb, Ni and Ca ($p > 0.05$).

4. Discussion

4.1 Potential sources of metal pollution

Pearson correlations were calculated to find empirical inter-relationships between the chemical parameters. Correlation between chemical parameters may indicate similar origins or conceptual relationships, as well as common governing factors. In the soil samples, concentrations of Cr were significantly correlated with Zn, Cu and Pb in the rainy season and with Pb in the dry season (Table 5). The strong association of these metals with each other indicates their anthropogenic origin. Organic matter content strongly affects the soil content of Cd, Zn (rainy season) and Cr (Table 5).

The presence of heavy metal pollution in the streams of Blanytre City has been reported upon by Sajidu et al (2007) and Kuyeli (2007) and both studies pointed at industrial activities as the possible sources of pollution. Kuyeli (2007) reported Cd in effluent from printing (0.034 mg/l), textiles (0.034 mg/l), motor oil (0.025 mg/l), battery (0.019 mg/l) and abattoir industry (0.06 mg/l) in the dry season; Cr in effluent from match stick production (41.59 mg/l in the dry season and 56.12 mg/l in the rainy season); Cu in the range 0.026 mg/l (battery manufacturer) to 2.00 mg/l (Paint industry); Zn in effluent from battery manufacturer (30.83 mg/l) and match stick production (15.51 mg/l) in the rainy season and 18.97 mg/l (match stick), 13.9 mg/l (battery) and 14.4 mg/l (fertiliser manufacturer) in the dry season; Pb in paint (1.29 mg/l), printing (2.60 mg/l) in the dry season and match stick (0.465 mg/l) and printing (0.233 mg/l) in the rainy season. Sajidu et al (2007) reported a significant increase in the levels of Pb, Cd, Cr, Fe, Cu, Ni and Mn in the same Blantyre streams after passing through an industrial site. The results from this study show enrichment of the heavy metals (Zn, Cd, Cr and Pb) in most streams over that of Michiru stream, which is in a forest reserve. In Malawi, cadmium is present in coatings on steel and also in batteries and potassium dichromate ($K_2Cr_2O_7$) is used as a raw material for producing match-heads. Copper compounds are used in textile, print and paint industry for pigmentation whereas Pb is used as a pigment, dispersing and drying agent in the print and paint industry. In match stick production lead oxide is used to give the scarlet colour of the match.

4.2 Accumulation of metals in *S. aequinoctialis*

Calculated bioconcentration factors (BCF) show that *S. aequinoctialis* accumulated heavy metals in the order Mn> Zn>Cu> Pb (Table 6). *S. aequinoctialis* had significantly higher ($p < 0.05$) levels of lead, copper, zinc and manganese than the corresponding water samples, in both seasons. There were no significant differences in levels of chromium between the algae samples and water samples whereas the differences were season dependent for the other metals. Water samples had high cadmium levels in the rainy season while in the dry season the levels were higher in *S. aequinoctialis*. For nickel, water samples indicated significantly higher nickel levels than *S. aequinoctialis* ($p < 0.05$), in the rainy season, but there was no significant difference in the dry season ($p > 0.05$).

There were strong correlations between water and algae metal contents for Cu ($r = 0.73$; $p < 0.05$; Fig. 2a) and Cr ($r = 0.65$; $p < 0.05$) in the rainy season. A low correlation for Mn ($r = 0.40$; $p < 0.05$) was also obtained for the dry season. There were no correlations for the other metals. There is an established consensus in the literature that brown and green algae are capable of biosorption of metals from their environment (Davis et al., 2003; Rajfur et al., 2010). They have thus been used in biomonitoring of heavy metals mostly in marine environments (Filho et al., 1999; Żbikowski et al., 2007; Akcali and Kucuksezgin, 2011). This study is in agreement with these studies and adds to the knowledge of heavy metal accumulation of green algae in a fresh water environment. Heavy metal levels in algae species are dependent both on environmental parameters (salinity, temperature, pH, light, oxygen, nutrient concentrations, complexing agents) and on the structural differences among the algae species (Garnharm et al., 1992; Favero et al., 1996).

4.3 Metal accumulation in *A.icteria*

Concentrations of Cu, Zn (rainy season) and Cd (dry season) in the soil were significantly correlated with the concentrations in *A.icteria* (Fig. 2b-d); Table 5). These metals also show correlations of varying strength with soil OM content (Table 5). The calculated bioconcentration factors (BCF) show that *A. icteria* accumulated heavy metals in the order Cd > Zn = Ni > Pb > Cu = Cr (Table 7), consistent with data from other similar studies (e.g.

		Wet Season								Dry Season							
ID	Sampling Area	pH	Mn	Cd	Cu	Zn	Pb	Cr	Ni	pH	Cd	Mn	Cu	Zn	Pb	Cr	Ni
1A	Chirimba at Cori	7.53	[1]ND	0.073 ±0.005	ND	0.502 ±0.056	0.037 ± .001	ND	0.398 ±0.013	6.81	0.037 ±0.005	0.035 ±0.014	0.046 ±0.037	0.295 ±0.240	0.026 ±0.015	ND	0.420 ±0.009
1B	Chirimba at Machinjiri	7.22	ND	0.073 ±0.004	ND	0.558 ±0.164	0.035 ±0.002	ND	0.391 ±0.004	7.05	0.041 ±0.003	0.155 ± 0.07	0.023 ±0.002	0.148 ±0.047	0.108 ±0.004	ND	0.405 ±0.004
2A	Mudi at MDI	7.54	0.060± 0.028	0.111 ±0.031	ND	1.494 ±0.002	0.038 ±0.014	ND	0.329 ± 0.04	7.58	0.052 ±0.137	0.244 ± 0.01	0.006 ±0.022	0.116 ±0.057	0.079 ±0.014	ND	0.349 ±0.103
2B	Mudi at SRN	7.39	ND	0.085 ±0.003	ND	2.614 ±.521	0.064 ±0.048	ND	0.347 ±0.006	7.19	0.047 ±0.011	0.178 ± 0.04	0.045 ±0.109	0.102 ±0.019	0.091 ±0.043	0.395 ±0.085	0.573 ±0.034
3A	Soche WWTP raw	7.62	ND	0.089 ±0.003	ND	0.703 ±0.183	0.042 ±0.016	ND	0.387 ±0.016	7.21	0.111 ±0.013	0.365 ±0.027	0.065 ±0.892	0.233 ±0.031	0.110 ±0.007	ND	0.234 ±0.008
3B	Soche WWTP effluent	7.79	ND	0.087 ±0.003	ND	0.711 ±0.187	0.058 ±0.013	ND	0.392 ±0.007	7.25	0.087 ±0.009	0.384 ±0.029	0.054 ±0.003	0.195 ±0.157	0.014 ±0.010	ND	0.101 ±0.003
4A	Blantyre WWTP raw	7.2	ND	0.081 ±0.002	ND	0.674 ±0.034	0.047 ±0.011	ND	0.426 ±0.029	6.98	0.092 ±1.002	0.435 ±0.011	0.076 ±0.153	0.173 ±0.038	0.061 ±0.006	0.297 ±0.058	0.505 ±0.007
4B	Blantyre WWTP effluent	7.55	ND	0.082 ±0.003	ND	0.742 ±0.111	0.034 ±0.008	ND	0.409 ±0.030	7.04	0.075 ±0.018	0.453 ±1.034	0.044 ±0.113	0.135 ±0.061	0.052 ±0.032	0.014 ±0.013	0.317 ±0.003
5A	Nasolo at BNC	7.53	ND	0.082 ±0.004	ND	1.079 ±0.134	0.069 ±0.039	ND	0.497 ±0.007	7.13	0.079 ±0.017	0.42 ±0.029	0.038 ±0.293	0.133 ±0.012	0.092 ±0.041	0.025 ±0.004	0.365 ±0.008
5B	Nasolo at SRN	8.8	ND	0.08 ± 0.008	ND	0.951 ±0.133	0.074 ±0.015	ND	0.451 ±0.091	5.98	0.098 ±1.016	0.457 ±0.018	0.064 ±1.113	0.159 ±0.050	0.048 ±0.011	0.036 ±0.011	0.515 ±0.110
6	Michiru	5.99	ND	0.086 ±0.002	ND	0.526 ±0.038	0.012 ±0.003	ND	0.413 ±0.024	7.37	0.014 ±0.001	0.056 ±0.001	ND	0.139 ±0.032	ND	ND	0.113 ±0.001
7	Mangunda	7.79	0.530 ±0.121	0.09 ± 0.003	ND	0.503 ±0.066	0.098 ±0.014	ND	0.394 ± 0.02	7.32	0.102 ±0.007	0.489 ±0.006	0.013 ±1.018	0.151 ±0.069	0.102 ±0.017	0.419 ±0.003	0.578 ±0.012
8B	Limbe WWTP effluent	10.13	ND	0.077 ± .002	ND	0.675 ± 0.09	0.074 ±0.002	ND	0.349 ±0.032	9.68	0.065 ±0.104	0.511 ±0.015	ND	0.291 ±0.285	ND	ND	0.236 ±0.065
8A	Limbe WWTP raw	7.4	ND	0.08 ± 0.004	ND	0.629 ±0.056	0.065 ±0.013	ND	0.215 ±0.035	6.84	0.018 ±2.101	0.626 ±0.041	0.013 ±1.018	0.264 ±0.113	0.04 ±0.001	ND	0.305 ±0.008
9A	Limbe at Mpingwe	7.19	ND	0.07 ± 0.002	ND	0.562 ±0.019	0.033 ±0.019	ND	0.433 ±0.044	7.18	0.049 ±0.131	0.0614 ±1.114	ND	0.403 ±0.332	0.23 ± 0.019	ND	0.155 ±0.019
9B	Limbe at Highway	7.46	ND	0.072 ±0.004	ND	0.633 ±0.116	0.089 ±0.006	ND	0.416 ±0.012	7.12	0.168 ±0.008	0.095 ±0.180	0.016 ±0.007	0.172 ±0.028	0.083 ±0.015	0.037 ±0.016	0.434 ±0.264
10A	Naperi at Rainbow	7.06	ND	0.081 ±0.003	ND	0.714 ±0.103	0.011 ±0.002	ND	0.432 ±0.009	7.28	0.585 ±0.012	0.089 ±1.982	ND	0.143 ±0.035	0.039 ±0.012	ND	0.475 ±0.106
10B	Naperi at Moi	7.01	ND	0.082 ±0.001	ND	0.621 ±0.064	0.038 ±0.004	ND	0.318 ±0.004	7.19	0.464 ±0.004	0.092 ±1.089	0.025 ±1.015	0.119 ±0.002	0.057 ±0.011	ND	0.405 ±0.011
	[2]MS 214		0.05- 0.1	0.003 – 0.005	0.5 – 1	3.0– 5.0	0.01– 0.05	0.05– 0.1	0.05 –0.15								
	[3]WHO		0.5	0.003	2	3	0.01	0.05	0.02								

[1]Not detected
[2] Malawi Bureau of standards (Standards for drinking water)
[3] World Health Organisation (Standards for drinking water)
Values are in the form of mean ± standard deviation

Table 1. Levels of Ni, Cu, Fe, Pb, Cr, Cd, Mn and Zn in water samples (concentrations are in mg/L)

Sampling point	Rainy Season							Dry season						
	Mn	**Cd**	**Cu**	**Zn**	**Pb**	**Cr**	**Ni**	**Mn**	Cd	**Cu**	**Zn**	**Pb**	**Cr**	**Ni**
Chirimba at Cori	3.185 ±0.931	0.018 ±0.135	0.563 ±0.067	2.828 ±0.231	ND	0.087 ±0.056	0.443 ±0.088	3.351 ±0.541	0.291 ±0.013	0.313 ±0.065	3.035 ±0.125	0.194 ±0.068	0.013 ±0.001	0.028 ±0.013
Chirimba at Machinjiri	1.903 ±0.284	0.029 ±0.013	0.129 ±0.009	1.393 ±.333	0.132 ±0.031	0.057 ±0.023	0.019 ±0.071	0.522 ±0.123	0.054 ±0.001	0.154 ±0.124	0.351 ±0.123	0.121 ±0.063	0.036 ±0.011	0.416 ±0.032
Mudi at MDI	5.641 ±0.963	ND	0.091 ±0.035	1.16 ± 0.611	0.198 ±0.132	0.036 ±0.002	0.146 ±0.003	4.875 ±1.112	0.362 ±0.041	0.171 ±0.023	1.258 ±0.047	0.704 ±0.126	0.045 ±0.016	ND
Mudi at SRN	1.782 ±0.491	ND	0.223 ±0.029	2.734 ±0.328	0.266 ±0.204	0.335 ±0.057	ND	13.521 ±1.088	0.171 ±0.008	0.105 ±0.041	2.263 ±1.334	0.972 ±0.012	0.663 ±0.031	0.233 ±0.036
Soche WWTP raw	1.438 ±0.196	ND	0.374 ±0.064	2.993 ±0.640	0.174 ±0.100	ND	ND	4.203 ±0.805	0.836 ±0.078	0.196 ±0.031	2.603 ±0.072	0.782 ±0.013	0.024 ±0.007	0.073 ±0.012
Soche WWTP effluent	0.586 ±0.168	ND	0.299 ±0.052	3.270 ±0.149	ND	ND	ND	0.281 ±0.142	0.142 ±0.031	0.175 ±0.136	2.621 ±1.209	0.042 ±0.001	ND	ND
Blantyre WWTP raw	0.432 ±0.075	ND	0.826 ±0.237	2.018 ±0.512	ND	0.431 ±0.137	ND	3.862 ±0.335	0.912 ±0.012	1.804 ±0.201	6.188 ±0.527	0.186 ±0.093	0.514 ±0.003	0.025 ±0.002
Blantyre WWTP effluent	0.731 ±0.406	ND	0.265 ±0.039	1.241 ±0.222	ND	0.029 ±0.003	ND	7.393 ±2.654	0.044 ±0.018	0.614 ±0.335	2.149 ±0.893	0.224 ±0.016	0.035 ±0.019	0.016 ±0.014
Nasolo at BNC	1.725 ±0.533	0.024 ±0.403	0.113 ±0.012	2.289 ±0.472	0.702 ±0.076	0.028 ±0.001	ND	4.213 ±1.018	0.468 ±0.031	0.056 ±1.032	4.426 ±1.244	0.423 ±0.072	0.075 ±0.041	ND
Nasolo at SRN	2.333 ±1.452	0.035 ±1.062	0.122 ±0.095	1.915 ±0.707	0.965 ±0.076	ND	ND	5.061 ±0.198	0.022 ±0.142	1.498 ±0.417	1.744 ±0.124	0.071 ±0.031	ND	0.061 ±0.012
Michiru	3.817 ±0.601	ND	0.002 ±0.012	0.202 ±0.159	ND	ND	ND	2.399 ±0.544	0.393 ±0.017	0.605 ±0.386	0.203 ±0.091	ND	ND	ND
Mangunda	3.968 ±1.098	0.022 ±1.463	0.026 ± 0.02	0.594 ±0.595	0.523 ±0.005	ND	ND	12.421 ±1.711	0.586 ±0.047	0.326 ±0.026	0.922 ±0.024	0.376 ±0.012	0.153 ±0.055	0.421 ±0.026
Limbe WWTP effluent	0.860 ±0.456	ND	0.229 ± 0.07	2.855 ±0.039	0.141 ±0.016	ND	ND	0.793 ± .117	0.796 ±0.141	0.223 ±0.041	0.726 ±0.124	ND	0.043 ±0.012	0.051 ±0.013
Limbe WWTP raw	2.769 ±1.586	ND	0.092 ±0.016	0.936 ±0.678	0.162 ±0.102	ND	ND	2.065 ±0.408	0.082 ±0.013	2.302 ±0.135	0.459 ±0.073	0.323 ±0.094	0.061 ±0.004	0.035 ±0.004
Limbe at Mpingwe	3.599 ±1.586	ND	0.029 ± 0.02	0.923 ±0.767	ND	ND	ND	16.132 ±1.527	0.074 ±0.034	0.551 ±0.310	0.496 ±0.043	0.406 ±0.072	ND	ND
Limbe at Highway	3.950 ±0.998	ND	0.09 ± 0.036	2.436 ±0.378	0.351 ±0.076	ND	ND	4.405 ±1.203	0.428 ±0.153	0.123 ±0.052	1.751 ±0.381	0.475 ±0.024	ND	0.063 ±0.013
Naperi at Rainbow	4.634 ±1.289	0.016 ±0.217	0.225 ± 0.04	1.774 ±0.308	0.263 ± 0.132	ND	ND	3.401 ± 0.467	0.039 ± 0.019	0.596 ± 0.102	5.358 ± 1.134	0.461 ± 0.068	0.011 ± 0.002	ND
Naperi at Moi	1.913 ± 0.2	ND	0.077 ±0.037	0.230 ±0.005	0.14 1±0.011	0.016 ±0.007	ND	7.164 ±0.842	0.116 ±0.042	1.418 ±0.426	0.988 ±0.403	0.037 ±0.008	0.063 ±0.014	ND

ND – not detected (below detection limit)

Table 2. Levels of heavy metals in *S. aequinoctialis* (in mg/kg dry weight)

Site	Dry season (mg/kg dw; x ± SD)									Rainy season (mg/kg dw; x ± SD)								
	pH	%OM	Mn	Ni	Cr	Cd	Pb	Cu	Zn	pH	%OM	Mn	Ni	Cr	Cd	Pb	Cu	Zn
Chirimba (A)	7.3	1.971 ±0.313	12.40 ±0.88	0.64 ±0.07	0.49 ±0.02	0.01 ±1.05	1.38 ±0.25	0.74 ±0.14	3.15 ±0.44	7.93	3.101 ±0.102	17.89 ± 0.14	0.001 ±0.001	1.24 ±0.35	0.04 ±0.05	0.55 ± .61	1.69 ±0.14	5.03 ± .24
Chirimba (B)	6.27	4.789 ±0.303	10.11 ±0.33	0.44 ± .23	0.15 ±1.06	0.02 ±0.04	2.48 ±0.31	1.63 ±0.04	3.17 ±0.49	6.94	4.759 ±0.327	15.70 ± 0.54	1.52 ± 0.18	1.28 ±0.29	ND	1.42 ± .27	1.62 ±0.40	9.54 0.51
Mudi (A)	7.22	5.674 ±0.379	14.81 ±0.91	2.94 ±0.86	5.62 ±1.09	0.16 ±0.04	3.49 ±0.67	1.31 ±0.16	5.22 ±0.53	6.73	4.455 ±1.764	13.98 ± 0.13	2.53 ± 0.18	4.42 ±0.13	ND	2.44± 0.41	3.35 ±0.84	13.94 ±0.83
Mudi (B)	6.62	0.880 ±0.658	14.60 ±0.82	0.05 ±0.01	0.93 ±0.06	0.07 ±1.01	1.75 ±0.44	7.31 ±2.32	0.26 ±0.20	6.95	0.588 ±0.428	10.26 ± 1.03	0.93 ± 0.34	2.37 ±0.92	ND	2.95 ±0.88	0.90 ±0.05	5.17 ±0.37
Michiru stream	6.63	2.732 ±0.668	14.40 ±0.32	1.45 ±0.37	1.09 ±0.15	ND	0.03 ±0.18	2.47 ±0.45	3.27 ±1.00	6.47	2.727 ±0.680	10.31 ± 2.92	2.89 ± 0.52	2.60 0.56	ND	2.32 ±0.37	0.41 ±0.22	3.31 ±0.30
Naperi (A)	6.52	4.021 ±0.392	12.85 ±0.86	0.86 ±0.11	2.81 ±0.29	0.03 ±1.01	0.22 ±0.09	7.11 ±1.42	2.16 ±0.59	7.03	4.082 ±1.184	13.73 ± 0.85	2.46 ± 0.65	2.85 ±1.24	ND	1.84 ±0.26	1.48 ±0.36	5.52 ±1.09
Naperi (B)	6.67	0.559 ±0.304	13.34 ±0.34	4.32 ± .56	1.35 ±0.30	ND	2.26 ±0.31	3.28 ±0.97	1.57 ±0.50	7.26	3.921 ±0.302	14.15 ± 0.41	1.09 ± 0.25	1.80 0.42	ND	1.77 ±0.19	1.90 ±0.16	6.29 ±0.19
Blantyre WWTP	6.33	7.755 ±0.778	27.43 ±0.95	2.54 ±0.46	8.19 ±0.70	0.13± 0.05	3.04 ±0.09	0.90 ±0.05	3.22± 0.52	6.47	9.266 ± .404	14.18 ± 0.50	1.15 ± 0.19	6.83 ±1.67	ND	2.46 ±0.23	5.87 ±0.86	17.45 ±0.92
Nasolo s(A)	6.89	3.310 ±0.042	16.87 ±0.71	0.07 ± .12	1.99 ±1.51	ND	0.31 ±0.25	1.71 ±0.47	6.26 ±1.30	7.21	2.460 ±0.858	10.64 ±1.050	0.41 ± 0.13	0.53 ±0.16	ND	1.97 ±0.20	0.75 ±0.17	7.65 ±1.84
Nasolo (B)	6.94	2.691 ± .445	12.01 ±2.15	0.97 ± .14	0.05 ±0.03	ND	0.04 ±0.15	0.31 ±0.03	7.04 ± .56	7.22	2.923 ± .806	11.31 ± 1.86	0.93 ± 0.12	0.54 0.47	ND	1.61 0.31	1.19 ± .15	11.09 0.21
Namangunda	6.37	9.357 ±0.525	9.00 ±0.18	4.14 ±0.37	3.48 ±0.58	0.13 ±2.03	0.71 ±0.09	10.13 ±0.98	1.44 ± .55	6.47	1.729 ± .228	10.96 ± 0.44	ND	ND	ND	0.51 ± .14	0.13 0.03	1.37 ± .09
Soche WWTP	6.31	5.834 ±0.179	26.78 ±1.89	1.19± 0.07	1.91 ±0.81	0.04 ±0.02	1.17 ±0.18	3.02 ±0.40	14.46 ±0.64	6.52	6.702 ±0.807	14.29 ± 0.23	0.80 ± 0.17	1.63 ±0.22	ND	1.47 0.034	3.18 ±0.13	16.18 ±0.45
Limbe WWTP	6.68	0.582 ±0.203	31.43 ±0.36	0.56 ±0.14	0.21 ±0.09	0.18 ±0.02	0.41 ±0.20	0.55 ±0.07	2.15 ±0.02	7.56	1.604 ±0.050	14.50 ± 0.14	1.40 ± 0.11	1.19 ±0.16	ND	1.16 ±0.23	1.67 ±0.15	3.83 ±0.64
Limbe stream (A)	6.5	1.866 ±0.134	17.43 ±0.23	1.82 ±0.18	0.07 ±0.15	ND	0.64± 0.08	0.12 ±1.14	2.58 ±0.15	6.93	0.873 ±0.832	10.84 ± 1.01	0.26 ± 0.06	0.37 ±0.54	ND	0.82 ±0.05	0.35 ±0.06	2.41 ±0.30
Limbe stream (B)	7.06	2.019 ±0.286	25.96 ±1.41	0.03 ±0.01	0.27 ±0.06	0.02± 0.01	1.15 ± .12	1.04 ±0.01	6.05 ±0.05	7.82	2.37 ± 0.731	12.24 ± 0.33	0.59 ± 0.16	1.37 ±0.72	ND	2.06 ±0.39	1.16 ±0.22	7.61± 0.52

x = mean value (n = 3); SD = standard deviation; ND = not detected (below detection limit)

Table 3. Metal concentrations in stream sediments soils and soils around WWTPs in the study area

Site	Dry season (mg/kg dw; $x \pm$ SD)							Rainy season (mg/kg dw; $x \pm$ SD)						
	Mn	**Ni**	**Cr**	**Cd**	**Pb**	**Cu**	**Zn**	**Mn**	**Ni**	**Cr**	**Cd**	**Pb**	**Cu**	**Zn**
Chirimba stream (A)	3.31 ±1.50	0.45 ±0.04	ND	0.17 ± 0.04	0.42 0.15	0.07 ± 0.06	4.30 ±0.08	5.24 ± 2.12	0.64 ±0.12	ND	0.123 ± 0.012	0.14 0.32	0.17 ± 0.16	3.67 ± 1.32
Chirimba stream (B)	4.66 ±0.75	0.93 ±0.02	ND	0.29 ± 0.01	0.27 0.02	0.14 ± 0.04	1.26 ±1.01	1.01 ± 0.13	0.30 ±0.09	ND	0.128 ± 0.016	0.12 0.50	ND	3.43 ± 0.27
Mudi stream (A)	4.29 ±2.50	0.74 ±0.03	0.018 ±0.003	0.43 ± 1.03	0.04 ±0.03	0.35 ± 0.03	0.70 ±0.26	2.36 ± 0.28	0.38 ±0.06	ND	0.144 ± 0.002	0.34 ±0.44	0.14 ± 0.07	4.08 ± 0.77
Mudi stream (B)	4.03 ± 1.69	0.13 ±0.01	ND	0.17 ± 0.01	0.19 ±0.01	0.24 ± 0.09	2.13 ± 0.11	6.39 ± 0.52	0.53 ± 0.17	ND	0.136 ± 0.007	ND	0.19 ± 0.05	3.71 0.31
Michiru stream	2.73 ±0.48	0.04 ±0.02	ND	0.14 ± 2.05	ND	0.005 ± 0.002	1.82 ± 0.14	1.94 ± 0.08	0.37 ± 0.02	ND	0.115 ± 0.004	ND	ND	3.00 ± 0.17
Naperi stream (A)	1.60 ±0.72	0.35 ±0.05	0.008 ± 0.001	0.12 ± 0.55	0.03 ± 0.11	0.128 ± 0.013	0.70 ± 0.27	3.69 ± 0.38	0.38 ± 0.02	ND	0.108 ± 0.014	ND	0.004 ± 0.001	4.03 ± 0.26
Naperi stream (B)	1.88 ±0.85	0.26 ±0.04	ND	0.22 ± 0.49	ND	0.099 ± 0.027	0.74 ± 0.20	3.16 ± 1.752	0.33 ± 0.08	ND	0.123 ± 0.013	ND	0.040 ± 0.009	3.16 ± 2.385
Blantyre WWTP	2.08 ±0.34	0.54 ±0.05	0.029 ± 0.012	0.17 ± 0.02	0.08 ± 0.39	0.202 ± 0.002	4.29 ± 0.11	3.25 ± 0.25	0.32 ± 0.04	ND	0.118 ± 0.006	ND	0.41 ± 0.22	4.82 ± 0.74
Nasolo stream (A)	4.02 ±0.27	0.25 ±0.04	ND	0.19 ± 0.02	0.45 ± 0.14	0.373 ± 0.065	1.90 ± 1.05	3.01 ± 0.68	0.30 ± 0.06	ND	0.119 ± 0.002	0.80 ± 0.18	0.05 ± 0.01	4.35 ± 0.79
Nasolo stream (B)	1.66 ±0.29	0.65 ±0.03	ND	0.50 ± 0.01	0.18 ± 0.02	0.136 ± .004	1.15 ± .08	2.16 ± 0.13	0.33 ± .03	ND	0.129 ± 0.003	0.48 ± 0.33	0.03 ± 0.01	2.78 ± 0.24
Mzedi Stream	3.83 ±0.81	0.46 ±0.04	0.031 ± .018	0.24 ± 0.85	0.48 ± 0.06	0.281 ± .041	5.11 ± .92	3.90 ± 1.46	0.29 ± .03	ND	0.121 ± 0.004	ND	ND	0.66 ± 0.12
Soche WWTP	5.89 ± 0.18	0.36 ±0.04	0.014 ± .006	0.33 ± 0.01	0.34 ± 0.05	0.044 ± .003	4.06 ± .04	4.39 ± 0.57	0.87 ± .44	ND	0.114 ± 0.010	ND	0.09 ± 0.07	5.27 ± 0.68
Limbe WWTP	7.58 ± 0.35	0.27 ±0.02	ND	0.55 ± 0.02	0.14 ± 1.03	0.265 ± .119	1.40 ± .32	9.62 ± 0.49	0.50 ± .03	ND	0.127 ± 0.019	ND	0.21 ± 0.16	2.71 ± 0.26
Limbe stream (A)	3.86 ± 0.55	0.05 ±0.04	ND	0.12 ± 0.27	0.02 ± 0.11	0.028 ± .006	1.51 ± .58	7.98 ± 0.58	0.35 ± .03	ND	0.109 ± 0.013	ND	0.03 ± 0.01	2.78 ± 0.27
Limbe stream (B)	1.92 ± 0.23	0.84 ±0.05	ND	0.19 ± 0.12	0.3 ± 1.09	0.155 ± .012	4.45 ± .44	4.19 ± 1.50	0.28 ± .07	ND	0.114 ± 0.009	ND	0.11 ± 0.04	3.98 ± 1.66

Table 4. Metal concentrations in *A.* icteria inhabiting stream- sediments and soils around WWTPs in Blantyre City, Malawi

	%OM	**pH**	$[Cd]_s$	$[Cd]_w$	$[Pb]_s$	$[Pb]_w$	$[Mn]_s$	$[Mn]_w$	$[Cu]_s$	$[Cu]_w$	$[Zn]_s$	$[Zn]_w$	$[Cr]_s$	$[Cr]_w$	$[Ni]_s$	$[Ni]_w$
%OM	1	-0.39	ND	-0.09	0.23	-0.68	0.49	-0.46	0.89**	0.49	0.84**	0.61*	0.69**	ND	0.17	0.17
pH	-0.40	1	ND	0.03	-0.25	-0.16	0.36	0.33	-0.25	-0.21	-0.28	0.02	-0.53*	ND	-0.51	0.016
$[Cd]_s$	0.25	-0.02	1	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
$[Cd]_w$	-0.03	0.18	0.63*	1	0.29	-0.29	0.12	-0.11	0.15	0.25	0.21	-0.08	0.16	ND	0.16	-004
$[Pb]_s$	0.28	0.02	0.20	0.04	1	0.54	-0.32	-0.31	0.33	0.31	0.41	0.53*	0.59*	ND	0.46	-0.15
$[Pb]_w$	0.18	0.10	-0.31	-0.097	-0.23	1	-0.91*	-0.045	0.45	-0.82	0.07	0.35	-0.28	ND	-0.19	-0.50
$[Mn]_s$	-0.08	-0.09	0.31	0.25	0.03	-0.20	1	-0.012	0.50	0.31	0.31	0.31	0.16	ND	-0.08	0.38
$[Mn]_w$	-0.02	-0.17	0.39	0.50	0.000	0.13	0.39	1	-0.17	0.15	-0.41	-0.16	-0.26	ND	0.39	0.35
$[Cu]_s$	0.35	-0.38	-0.02	-0.30	-0.13	0.23	-0.41	-0.09	1	0.73**	0.85**	0.63*	0.81**	ND	0.09	0.21
$[Cu]_w$	0.26	0.22	0.83**	0.33	0.24	0.12	0.05	0.26	0.18	1	0.42	0.31	0.72**	ND	0.007	0.092
$[Zn]_s$	0.21	0.03	-0.23	0.29	-0.08	0.25	0.36	0.19	-0.23	-0.15	1	0.72**	0.56*	ND	0.04	0.23
$[Zn]_w$	0.47	-0.07	-0.15	-0.25	0.026	0.61*	0.27	-0.007	-0.31	-0.04	0.22	1	0.48	ND	-0.08	0.41
$[Cr]_s$	0.71**	-0.15	0.53	-0.09	0.56*	-0.24	0.154	-0.17	0.18	0.41	-0.007	0.22	1	ND	0.46	-0.09
$[Cr]_w$	0.96**	-0.23	0.73	0.011	0.33	0.44	0.004	-0.03	0.15	0.50	-0.38	0.73	0.56	1	ND	ND
$[Ni]_s$	0.44	-0.20	0.60	-0.003	0.36	-0.08	-0.236	-0.19	0.09	0.04	-0.21	0.05	0.49	0.86	1	-0.21
$[Ni]_w$	0.36	0.20	-0.20	0.34	0.46	0.099	-0.04	-0.13	0.28	0.24	0.22	0.16	0.17	0.33	-0.06	1

** Correlation is significant at the 0.01 level; * Correlation is significant at the 0.05 level; ND = not determined

Table 5. Pearson's correlation coefficients of soil OM, pH-H_2O, metal soil and *A.icteria* metal content (Upper panel – rainy season Lower panel – dry season)

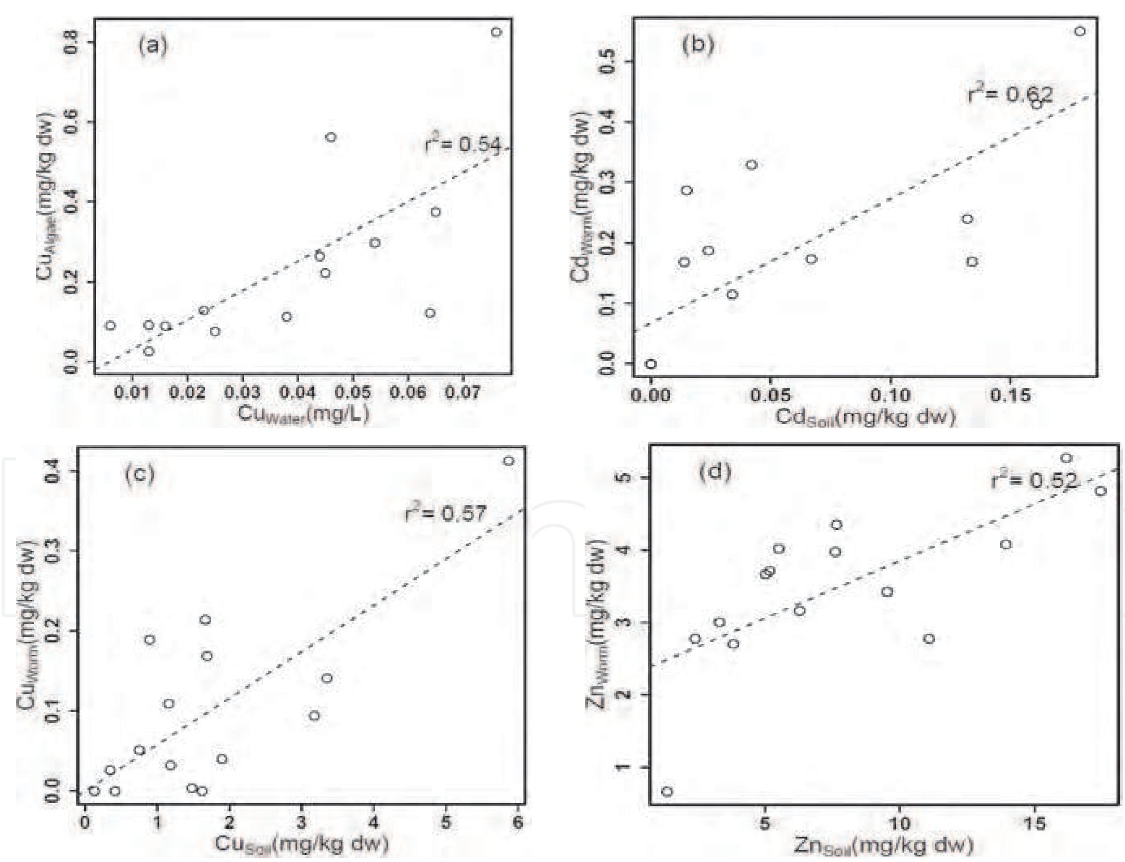

Fig. 2. Scatter plots showing relationship between metal content in *A.icteria* and sediments (*b* –*d*) and metal content in *S. aequinoctialis* and stream water (*a*). Dry season data is used for plot (b) and rainy season data is used for the other plots. No significant correlations were found for the other combinations of metals.

Site	Dry season							Rainy season						
	Mn	Ni	Cr	Cd	Pb	Cu	Zn	Mn	Ni	Cr	Cd	Pb	Cu	Zn
Chirimba at Cori	ND	0.07	ND	3.99	5.24	ND	6.05	91.00	1.05	ND	0.49	0.00	12.24	9.59
Chirimba at Machinjiri	ND	1.06	ND	0.74	3.46	ND	0.63	12.28	0.05	ND	0.71	1.22	5.61	9.41
Mudi at MDI	81.25	ND	ND	3.26	18.53	ND	0.84	23.12	0.42	ND	0.00	2.51	15.17	10.00
Mudi at SRN	ND	0.67	ND	2.01	15.19	ND	0.87	10.01	ND	0.85	0.00	2.92	4.96	26.80
Soche WWTP raw	ND	0.19	ND	9.39	18.62	ND	3.70	3.94	ND	ND	0.00	1.58	5.75	12.85
Soche WWTP effluent	ND	ND	ND	1.63	0.72	ND	3.69	1.53	ND	ND	0.00	0.00	5.54	16.77
Blantyre WWTP raw	ND	0.06	ND	11.26	3.96	ND	9.18	0.99	ND	1.45	0.00	0.00	10.87	11.66
Blantyre WWTP effluent	ND	0.04	ND	0.54	6.59	ND	2.90	1.61	ND	2.07	0.00	0.00	6.02	9.19
Nasolo at BNC	ND	0.00	ND	5.71	6.13	ND	4.10	4.11	ND	1.12	0.30	7.63	2.97	17.21
Nasolo at SRN	ND	0.14	ND	0.28	0.96	ND	1.83	5.11	ND	ND	0.36	20.10	1.91	12.04
Michiru	ND	ND	ND	4.57	0.00	ND	0.39	68.16	ND	ND	0.00	ND	ND	1.45
Mangunda	23.44	1.07	ND	6.51	3.84	ND	1.83	8.11	ND	ND	0.22	5.13	2.00	3.93
Limbe WWTP effluent	ND	0.15	ND	10.34	0.00	ND	1.08	1.68	ND	ND	0.00	ND	ND	9.81
Limbe WWTP raw	ND	0.16	ND	1.03	4.97	ND	0.73	4.42	ND	ND	0.00	4.05	7.08	3.55
Limbe at Mpingwe	ND	0.00	ND	1.06	12.30	ND	0.88	73.45	ND	ND	0.00	0.00	ND	2.29
Limbe at Highway	ND	0.15	ND	5.94	5.34	ND	2.77	23.51	ND	ND	0.00	4.23	5.63	14.16
Naperi at Rainbow	ND	0.00	ND	0.48	41.91	ND	7.50	7.92	ND	ND	0.18	6.74	ND	12.41
Naperi at Moi	ND	0.00	ND	1.41	0.97	ND	1.59	4.12	ND	ND	0.00	0.00	3.08	1.93

ND = not determined (because metal content was below detection limit in either water or algae)

Table 6. Bioconcentration factors (BCF) for rainy and dry seasons for *S. aequinoctialis* from the sampled streams in Blantyre City, Malawi.

Site	Dry season							Rainy season						
	Mn	Ni	Cr	Cd	Pb	Cu	Zn	Mn	Ni	Cr	Cd	Pb	Cu	Zn
Chirimba stream (A)	0.27	0.70	ND	12.07	0.31	0.10	1.36	0.29	ND	ND	2.98	0.24	0.100	0.73
Chirimba stream (B)	0.46	2.10	ND	19.13	0.11	0.09	0.40	0.06	0.19	ND	ND	0.08	ND	0.36
Mudi stream (A)	0.29	0.25	0.0032	2.66	0.01	0.27	0.13	0.17	0.15	ND	ND	0.14	0.042	0.29
Mudi stream (B)	0.28	2.46	ND	2.60	0.11	0.03	8.35	0.62	0.57	ND	ND	ND	0.211	0.72
Michiru stream	0.19	0.03	ND	ND	ND	0.00	0.56	0.19	0.13	ND	ND	ND	ND	0.91
Naperi stream (A)	0.12	0.41	0.0028	3.38	0.12	0.02	0.32	0.27	0.15	ND	ND	ND	0.003	0.73
Naperi stream (B)	0.14	0.06	ND	ND	ND	0.03	0.47	0.22	0.31	ND	ND	ND	0.021	0.50
Blantyre WWTP	0.08	0.21	0.0035	1.27	0.03	0.22	1.33	0.23	0.28	ND	ND	ND	0.070	0.28
Nasolo stream (A)	0.24	3.36	ND	ND	1.44	0.22	0.30	0.28	0.72	ND	ND	0.41	0.068	0.57
Nasolo stream (B)	0.14	0.67	ND	ND	4.44	0.43	0.16	0.19	0.35	ND	ND	0.30	0.027	0.25
Mzedi Stream	0.43	0.11	0.0089	1.84	0.67	0.03	3.56	0.36	ND	ND	ND	ND	ND	0.48
Soche WWTP	0.22	0.30	0.0073	7.83	0.29	0.01	0.28	0.31	1.08	ND	ND	ND	0.030	0.33
Limbe WWTP	0.24	0.49	ND	3.08	0.33	0.48	0.65	0.66	0.35	ND	ND	ND	0.128	0.71
Limbe stream (A)	0.22	0.03	ND	ND	0.03	0.24	0.58	0.74	1.34	ND	ND	ND	0.074	1.15
Limbe stream (B)	0.07	32.38	ND	7.83	0.26	0.15	0.74	0.34	0.48	ND	ND	ND	0.094	0.52

ND = not determined

Table 7. Bioconcentration factors (BCF) for rainy and dry seasons for *A.icteria* from the sampled stream-bank soils and soils around WWTPs in Blantyre City, Malawi.

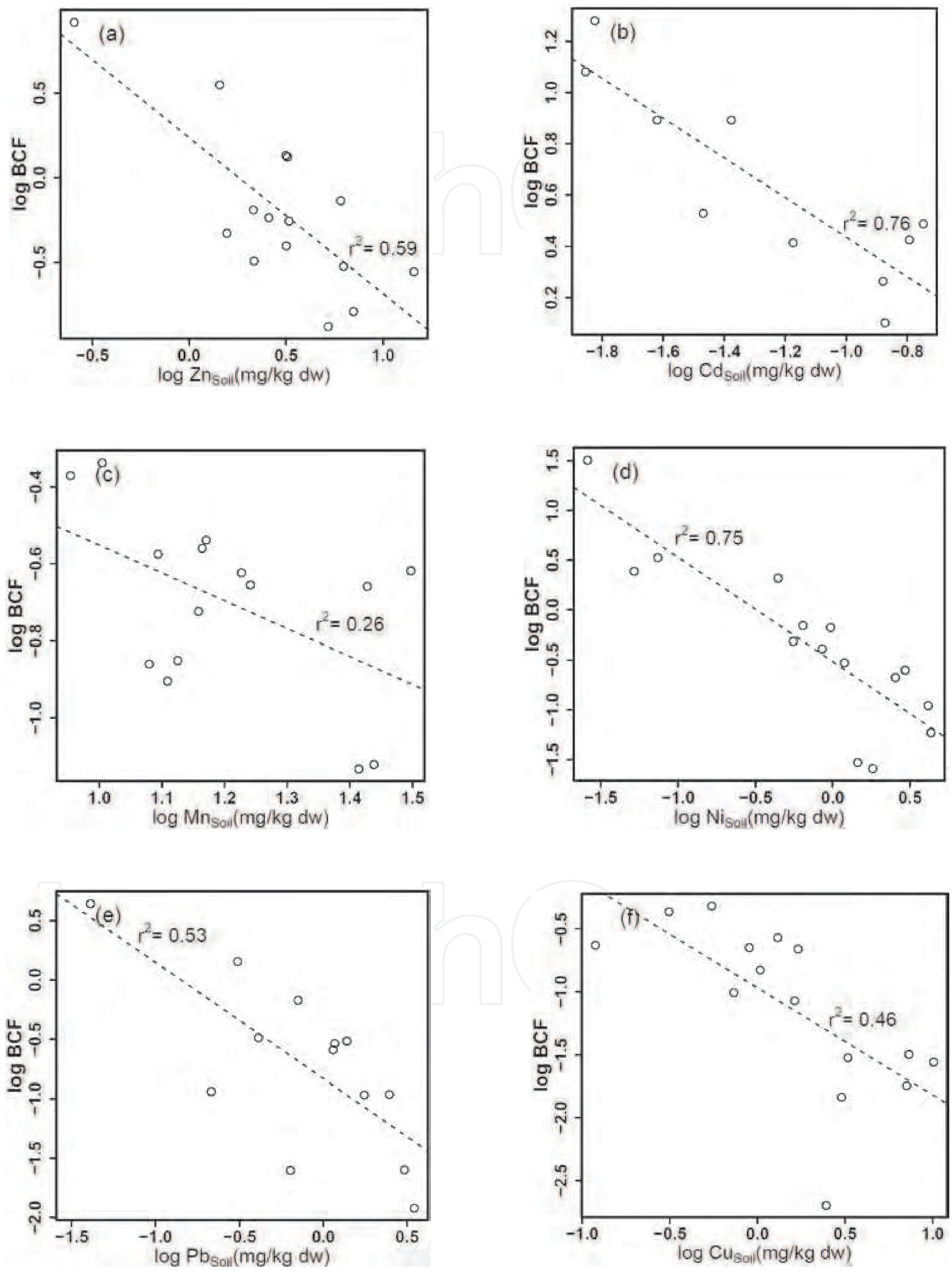

Fig. 3. Plots of log BCF against metal concentration in the soil using dry season data.

References		Species	Equation	Cd		Pb		Cu		Zn	
			#	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*
Heikens et al. (2001)	Bibliographic study	Lumbricidae (mixed)	1	0.39	1.1	0.62	-0.3	0.17	1.8	0.17	1.8
Ma et al. (2004)	Field data	Lumbricidae (mixed)	2	0.556	1.39	0.556	0.626	0.327	0.776	0.212	2.49
Neuhauser eta al. (1995)	Bibliographic studies	Lumbricidae (all species mixed)	3	0.66	1.21	0.74	0.05	0.27	2.09	0.27	2.09
Wright and Stringer (1980)	Soil quantity: field study	*Aporrectodea caliginosa*	4	0.32	0.33	0.9	-0.8	0.01	0.23	0.01	0.23
		Aporrectodea longa	5	0.3	-0.3	0.5	-0.1	0.1	2.5	0.1	2.5
		Aporrectodea rosea	6	0.5	0.7	0.5	-0.1	0.5	1.1	0.5	1.1

Table 8. Regression equations, Log M_{ew} = $a\log M_s + b$ for Lumbricidae, *Aporrectodea caliginosa, Aporrectodea longa, Aporrectodea rosea* and Cd, Cu, Pb and Zn from literature

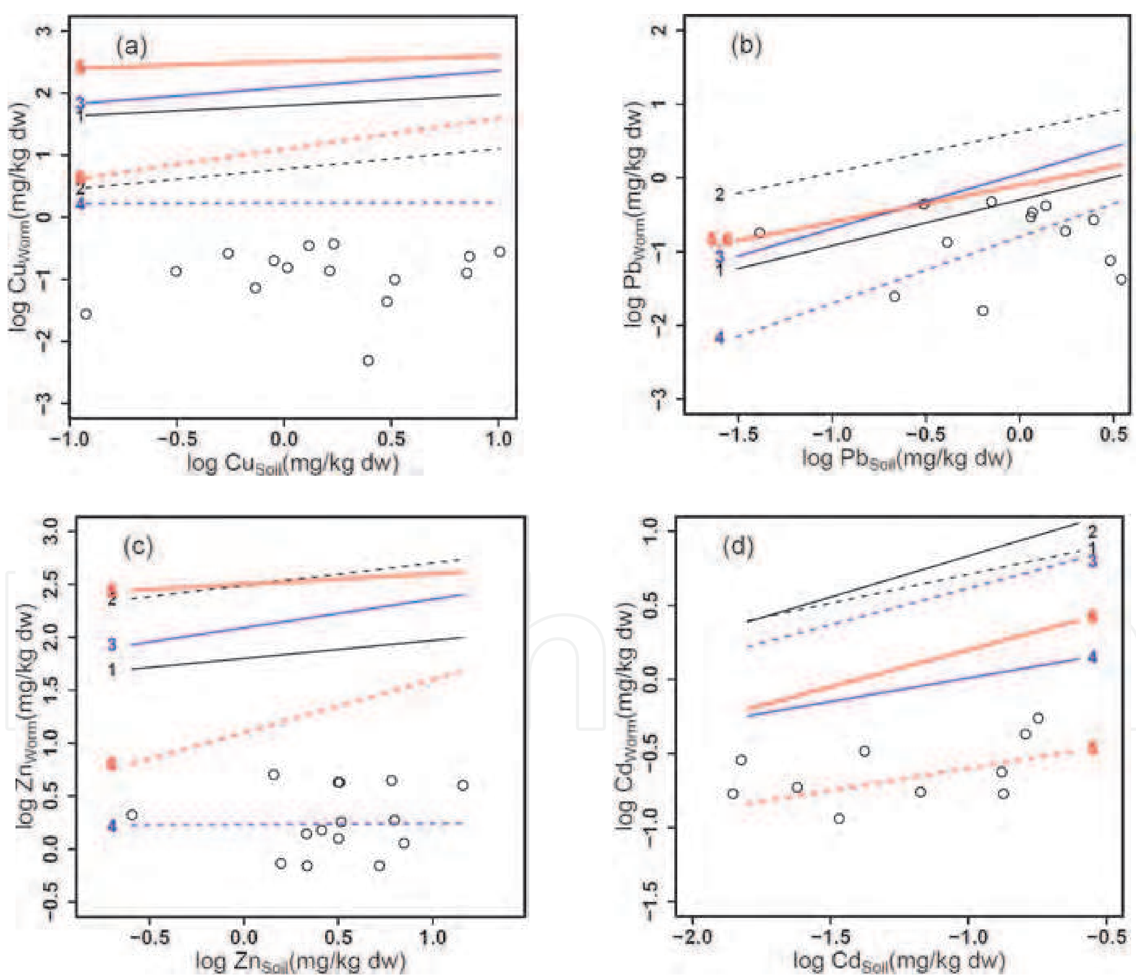

Fig. 4. Relationships between the internal Cd, Cu, Pb, Zn in earthworms and the total soil concentration, in comparison with regressions from the literature. The number on the lines corresponds to the number of regression model in Table 8, calculated using the soil data from this study. The dots are data from this study (*A.icteria*) for dry season.

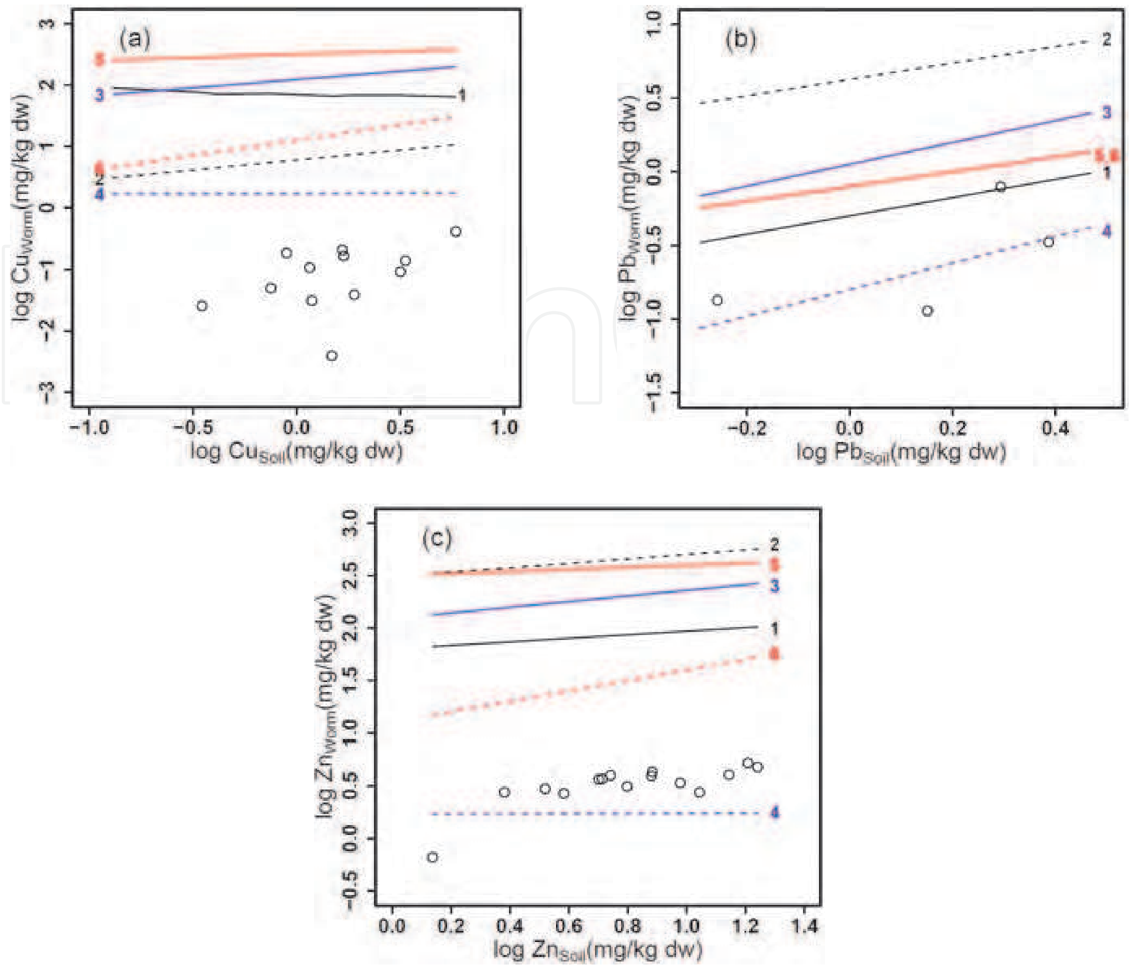

Fig. 5. Relationships between the internal Cd, Cu, Pb, Zn in earthworms and the total soil concentration, in comparison with regressions from the literature. The number on the lines corresponds to the number of regression model in Table 8, calculated using the soil data from this study. The dots are data from this study (*A.icteria*) for wet season.

Kamitani and Kaneko, 2007; Hsu et al., 2006; Dai et al, 2004; Ireland, 1983; Ma, 1982). The solubility of heavy metals in soil (pore) water is important for bioaccumulation by earthworms as the main pathways for chemical absorption are the skin (for soluble elements), gut transit and digestion (Weltje, 1998). The BCF order also reflects the affinity order for the specific adsorption of metal cations in soil: Pb > Cu > Zn > Cd. Cadmium tends to be more mobile in soils and therefore more available to earthworms than other heavy metals (Ma, 2004). Fig. 3 shows decreasing BCF with soil concentration of the metals indicating that bioconcentration depends on the metal concentrations in the soil and is greater at low soil concentrations. This implies that *A. icteria* exhibits metal regulation at high exposure rates (Neuhauser et al., 1995).

There is a significant consensus in the literature that bioaccumulation of heavy metals by earthworms is dependent on earthworm species and type of metal (see e.g. van Vliet et al., 2005, 2006; Vijver, 2007; Kamitani and Kaneko, 2007; Ernst et al., 2008).

Hence, the accumulation patterns of Cd, Cu, Zn and Pb in *A. icteria* (this study) were compared to those of other lumbricid earthworms, using the regression models from literature (Heikens et al., 2001; Neuhauser et al., 1995; Ma, 2004; Wright and Stringer, 1980) (see Table 8). Data for *A. icteria* is not readily available in the literature for comparison with our study. Figs 4 and 5 show that *A. icteria* (present study) accumulated relatively less Cu than the other species at the same soil concentration. However, similar accumulation levels were observed for Pb and Zn with *A. caliginosa* and for Cd with *A. longa* (Figs 4 and 5), even at our very low exposure levels. For all species, there is a metal dependent increase in body concentration with increasing soil concentration, following the order: Pb > Cd > Cu = Zn (Figs. 4 and 5). Cu and Zn are essential metals and are physiologically regulated by earthworms, resulting in a relatively constant body concentration with respect to soil metal concentrations (Panda et al., 1999; Heikens, et al., 2001; Morgan and Morgan, 1988; Lukkari, 2004). In contrast, Cd and Pb are non-essential metals and are not regulated resulting in metal increase with increasing soil concentrations (Spurgeon and Hopkin, 1999).

The plots in Figs. 4 and 5 also show that the regression models generated for all species of lumbricid earthworms would overestimate the amount of heavy metals in *A. icteria* living in the stream bank soils and soils around the WWTPs in Blantyre City. This result supports the general consensus that the degrees of heavy metal accumulation in earthworms show different, metal- and species-specific patterns. The observed differences in accumulation patterns are usually attributed to differences in metal kinetics of the earthworms, exposure route and food preference. In addition, it seems that the accuracy of the regression models is lost when they are generalised at family level but may be possible to relate accumulation patterns within the same genera. However, it should be noted that the comparison should be made with caution because metal availability is dependent on several environmental factors such as soil pH, cation exchange capacity, OM and Ca^{2+} (Christensen, 1989, Ma, 1982; Corp and Morgan, 1991; Peijnenburg et al., 1999a, 1999b). These factors may differ between studied field soils.

5. Conclusions

The study obtained concentrations of manganese, cadmium and lead in periphyton (*S. aequinoctialis*) in higher levels than in the corresponding water, implying that *S. aequinoctialis* accumulates these heavy metals. The results indicate the potential of periphyton as a biological indicator of heavy metal pollution. Heavy metals concentrations therefore measured in macroalgae species can give a picture of the quality of our surrounding environment. In addition, the levels of most of the heavy metals were higher than drinking water standards. It was also found that the general trend was that of high heavy metal values for water samples in the dry season than in the rainy season. The relatively low heavy metal levels in the rainy season were attributed to dilution. The study also showed that *A. icteria* can accumulate cadmium, but not lead and manganese. This work supports the published results that metal- and species-specific accumulation patterns of non-essential heavy metals in earthworms occur. In addition, the work extends the database to even very low exposure levels and therefore generates more information for *A. icteria*. Metal accumulation shows seasonal variations with significant correlations and multiple regression models between soil and internal metal content being more apparent in the rainy season. Similar accumulation levels observed between *A. icteria* and *A. caliginosa* for Pb and Zn and *A. longa* for Cd point to a possibility to relate accumulation patterns within the same genera, albeit metal specific. Further, it seems that the accuracy of regression models is lost when they are generalised at family level, making generalisations at that level difficult.

6. Acknowledgements

The authors would like to thank University of Malawi, The Polytechnic for funding this study. They are greatly indebted to Blantyre City Assembly for allowing them to collect samples in places that they use for their routine monitoring work. Thanks should also go to technical staff of Chancellor College Faculty of Science, all laboratory staff at Geological Surveys Department and National Herbarium and Botanical Gardens for their technical support.

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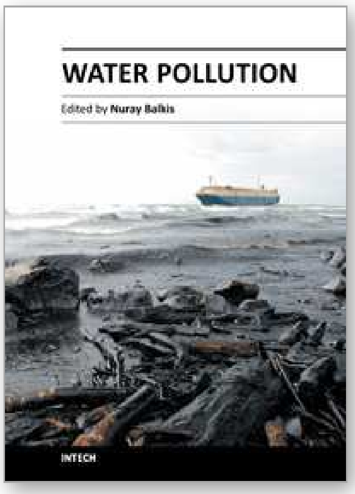

Water Pollution

Edited by Prof. Nuray Balkis

ISBN 978-953-307-962-2
Hard cover, 202 pages
Publisher InTech
Published online 24, February, 2012
Published in print edition February, 2012

Water pollution is a major global problem that requires ongoing evaluation and revision of water resource policy at all levels (from international down to individual aquifers and wells). It has been suggested that it is the leading worldwide cause of deaths and diseases, and that it accounts for the deaths of more than 14,000 people daily. In addition to the acute problems of water pollution in developing countries, industrialized countries continue to struggle with pollution problems as well. Water is typically referred to as polluted when it is impaired by anthropogenic contaminants and either does not support a human use, such as drinking water, and/or undergoes a marked shift in its ability to support its constituent biotic communities, such as fish. Natural phenomena such as volcanoes, algae blooms, storms, and earthquakes also cause major changes in water quality and the ecological status of water. Most water pollutants are eventually carried by rivers into the oceans.

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