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Full Length Research Paper

Uptake and elimination kinetics of heavy metals by earthworm (*Eudrilus eugenia*) exposed to used engine oil-contaminated soil

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Earthworm inoculation of petroleum hydrocarbon contaminated soil is thought to catalyze the bioremediation. Most bioremediation studies focus on the petroleum hydrocarbon content and not on the heavy metals. Here, the uptake kinetics of heavy metals by earthworm in used engine oil contaminated soil was investigated. The metals considered were zinc (Zn), copper (Cu), nickel (Ni), chromium (Cr), titanium (Ti) and manganese (Mn). Five different concentrations of used engine oil contaminated soil were considered: 12.5, 25, 50, 75 and 100 g/kg of soil. At the end of the 20 days study, the concentration of Zn, Cu, Ni, and Cr in the worms were found to have decreased (of which initial concentrations in the worms where higher than their concentrations in all used engine oil contaminated soils considered, that is, 12.5, 25, 50, 75, and 100 g/kg of soil), whereas Ti and Mn showed an increase (of which initial concentrations in the worms where lower than concentrations in all used engine oil contaminated soils considered, that is, 12.5, 25, 50, 75 and 100 g/kg of soil). The uptake and elimination rate constants of the metals were estimated using one-compartment model. The percentage drop in total petroleum hydrocarbon was also found to increase with increase in initial used engine oil concentrations.

Key words: *Eudrilus eugenia*, heavy metals, kinetics, one-compartment model, soil, used engine oil.

INTRODUCTION

In ecological risk assessment, it is widely recognized that earthworms are an important component of the soil biota. For this reason, much work has focused on metal accumulation and toxicity to earthworms (Nahmani et al., 2007) and the manure worm, *Eisenia fetida*, used as a standard earthworm. Surprisingly, little is known about the kinetics of the uptake and excretion of metals by earthworms, with the majority of available data focused on Cadmium (Cd), Copper (Cu), Lead (Pb) and zinc (Zn) (Spurgeon and Hopkin, 1999; Conder et al., 2002; Vijver et al., 2005) and other elements such as manganese (Mn), cesium (Cs), iodine (I) (Sheppard et al., 1997), arsenic (As), chromium (Cr), nickel (Ni) (Peijnenburg et al., 1999), and cobalt (Co) (Crossley et al., 1995). In broad terms, these studies show that the magnitude of

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Abbreviations: UEO, Used engine oil; **XRF,** X-ray fluorescence; **TPH,** total petroleum hydrocarbon.

accumulation and steady-state tissue metal concentrations are a function of either total or ''available'' metal concentration in the soil. The available metal concentrations are controlled by soil parameters such as pH, organic matter content, clay content, cation exchange capacity, and the concentration of Fe- and Al-oxyhydroxides.

Kinetic uptake and excretion data can inform researchers how likely the metal burden of an earthworm can reflect a steady-state concentration reached during exposure to metals in the soil the earthworm was immediately occupying prior to sampling, whether uptake rates impact on toxic effects (Van Straalen et al., 2005), how long toxicity tests should last in order for tissue metal concentrations to reach a steady state and whether earthworms are likely to excrete significant amounts of accumulated metal during depuration, which is important when determining metal concentrations. As stated by Van Straalen et al. (2005), the current need is for more studies to generate a sufficiently large data set so that reference values for uptake and elimination constants can be defined and used in bioavailability and ecological risk assessment studies.

Earthworms are key organisms in soil ecosystems and are known for their high uptake of metals (Nahmani et al., 2007). Considering their body size and ease of handling, they also are ideal organisms for assessing metal bioavailability.

From an ecological perspective, metal accumulation in earthworms is also important in that they are important prey to many amphibian, reptile bird, and mammalian species (OECD, 2004). Thus, metal accumulation may increase up the ladder of the food chain due to bioaccumulation.

The use of earthworms for bioremediation of petroleum hydrocarbon contaminated soil has generated interest (Schaefer, 2001; Callaham et al., 2002; Stom et al., 2003). Used engine oil (UEO) contamination may be hazardous to the environment as a result of petroleum hydrocarbon and heavy metal content of the oil. The occurrence of metals in used engine may be as a result of: (1) additives used in the production of engine oil, (2) wear from engine parts, (3) additives in gasoline, etc. In addition to these, in mechanic workshops, other activities that may result to elevated levels of heavy metals in the soil include; panel beating, welding, and servicing of batteries.

This paper is part of a larger project which aims to enhance the bioremediation of used engine oil contaminated soil by inoculation with earthworm. The specific aims of this study were to: (1) determine rate constants of uptake and excretion of metals by earthworms in metal contaminated soils to add to the existing small database on this subject, (2) relate uptake rates to properties of the soils, and (3) compare fitted model parameters to those already reported in the literature.

MATERIALS AND METHODS

Heavy metals uptake and elimination

The procedure followed was adopted from the OECD 317 (2010). Five different concentrations of used engine oil contaminated soil were prepared: 12.5, 25, 50, 75 and 100 g used engine oil/kg of soil. The mixtures were kept in clean plastic containers. The soil was made up to 60% water holding capacity and spiked with 20 g of cow dung. 20 earthworms (*Eudrilus eugenia* Kinberg) were added to each sample, and on days 0, 5, 10, 15,and 20, five earthworms were removed and prepared for X-ray fluorescence (XRF) analysis (to determine concentration of metals in the worms). The analysis of day 0 was done without introducing the worms to the contaminated soil so as to obtain the initial concentrations of the metals. Earthworms were introduced without prior conditioning in the laboratory. In preparing the worms for analysis, on the set days, the worms after being removed from the soil were placed on wet filter papers so as to void their guts for a period of 24 h. After which they were quickly killed by freezing at 0°C. The dead worms were then sun dried for 4 h before being transferred to an oven at 60°C for 12 h. Elemental analysis of the carcasses of the worms was then conducted using XRF (MiniPal PW4025, Philips Analytical). The soil samples were maintained at 60% water holding capacity throughout the test period by weighing everyday and adding make up deionized water when necessary. All samples were prepared in duplicates.

At the end of the study (day 20), the total petroleum hydrocarbon (TPH) of each treated soil sample was determined using gravimetric method of toluene cold extraction (Adesodun and Mbagwu, 2008): soil (10 g) was collected and dried at room temperature for 72 h. Toluene (10 ml) was then mixed with 5 g of the dried sample, covered and left to stand for 24 h. The supernatant was extracted using a syringe and fresh 10 ml of toluene was added. After 24 h, the supernatant was again removed and the soil sample was placed in an oven at 60°C to remove any remaining toluene. The weight of soil (M_1) was then determined. The TPH was calculated as:

$$\text{TPH (g/kg)} = \frac{5 - M_1}{5} \times 1000 \qquad (1)$$

Kinetic modeling and statistical analysis

One-compartment model of Van Straalen et al. (2005) was used to analyze the development of earthworm body concentrations with time, and to calculate uptake and elimination rate constants. Equation 2 was used for all elements.

$$C_{iworm}(t) = C_i(0)e^{-k_2 t} + \frac{a}{k_2}\left(1 - e^{-k_2 t}\right) \qquad (2)$$

Where, C_{iworm} (t) = metal concentration in the earthworm (µg/g dry weight) at time t (days), a = flux of metals into the earthworm (mg_{metal}/kg animal/day), k_2 = elimination rate constant (day^{-1}). $C_i(0)$ is the initial body concentration (µg/g dry weight), which was measured in the animals before exposure.

Other parameters of interest include: R=coefficient of correlation, C_e=external metal concentration (concentration in the soil, µg/g dry weight), k_1=uptake rate constant (from bulk concentrations (g soil/g animal/day), C_{SS}=calculated steady-state metal concentration, (mg/kg), t_{SS}=calculated time taken to reach steady state (days) and bioaccumulation factors (BAF)=(from bulk concentrations).

Table 1. Elemental composition of earthworm and used engine oil contaminated soil at time = t_o.

Element	Composition of element (%)					
	Worms at t = 0	Concentration of used engine oil contaminated soil (g UEO/kg soil)				
		12.5	25	50	75	100
Al	0.000	7.619	6.138	6.138	5.820	6.931
Si	0.000	32.977	31.483	31.856	30.782	30.689
P	0.000	0.292	0.187	0.000	0.000	0.179
S	0.000	0.080	0.104	0.080	0.080	0.284
K	0.000	3.369	4.216	4.307	4.482	4.008
Ca	14.510	0.399	0.428	0.401	0.429	0.539
Ti	0.527	0.857	1.121	1.067	1.198	1.180
V	0.028	0.039	0.062	0.053	0.051	0.052
Cr	0.178	0.030	0.055	0.047	0.075	0.041
Mn	0.000	0.060	0.0596	0.055	0.057	0.074
Fe	9.434	5.201	7.638	7.211	8.456	7.876
Ni	0.204	0.010	0.033	0.031	0.050	0.023
Cu	0.232	0.0111	0.042	0.041	0.063	0.029
Zn	0.739	0.003	0.030	0.034	0.043	0.034
Ga	0.000	0.004	0.024	0.000	0.024	0.023
Mo	0.000	0.000	0.000	0.133	0.400	0.000
Ba	0.475	0.000	0.332	0.385	0.466	0.260

Table 2. Elimination kinetics of Zn and Cu by the earthworm (*E. eugenia*) exposed to UEO contaminated soils.

Parameter	Zn					Cu				
	UEO concentration (g/kg soil)					UEO concentration (g/kg soil)				
	12.5	25	50	75	100	12.5	25	50	75	100
a	1268.70	1495.00	1424.30	108.45	93.82	674.77	6.50	589.91	9.46	42.93
k_2	4.40	4.40	4.20	0.28	0.26	4.12	0.08	4.22	0.09	-0.18
R^2	0.82	0.99	0.87	0.93	0.96	0.11	0.65	0.72	0.99	0.88
C_{ss}	280.00	337.50	330.00	380.00	362.50	170.00	NSS	140.00	NSS	NSS
C_e	3.21	29.72	33.73	43.37	33.73	11.18	42.33	40.73	63.10	28.75
k_1	394.87	50.31	42.22	2.50	2.78	60.34	0.15	14.48	0.15	1.49
BAF	89.74	11.43	10.05	8.85	10.55	14.64	1.99	3.43	1.70	-8.14
t_{SS} (days)	2.00	2.00	2.00	18.00	18.00	2	NSS	2	NSS	NSS

Experimental data were used to obtain values of a and k_2 using non-linear regression (MATLAB software). Subsequently, Equation 1 was used to calculate steady-state tissue metal concentrations for earthworms and the time taken to achieve it (t_{SS}). Steady-state concentrations were used to calculate bioaccumulation factors (the ratio of metal concentration in the earthworm to either bulk soil metal concentration). The flux of metals into the earthworm, a, is actually the product of an uptake rate constant k_1 (g soil/g animal/day) and the external metal concentration C_e (Nahmani et al., 2009), that is,

$$k_1 = \frac{a}{C_e} \quad (3)$$

RESULTS

The initial metal concentrations in the earthworms prior to exposure to the metal contaminated soil (that is, Ci(0)) as well as the elemental analysis of the various used engine oil contaminated soil are given in Table 1. Tables 2, 3, and 4 present the elimination kinetics for Zn, Cu, Ni, Cr, Ti, and Mn. Fits to Equation 2 are reported for elements which showed appreciable change in earthworm on introduction to used engine oil contaminated soil. Table 5 presents the result of the drop in TPH for the various initial concentration of used engine oil contaminated soil inoculated with 20 earthworms.

Figures 1 and 2 present the sample result of the

Table 3. Elimination kinetics of Ni and Cr by the earthworm (*E. eugenia*) exposed to UEO contaminated soils.

Parameter	Ni					Cr				
	UEO concentration (g/kg soil)					UEO concentration (g/kg soil)				
	12.50	25.00	50.00	75.00	100.00	12.50	25.00	50.00	75.00	100.00
a	759.62	582.38	618.20	9.36	-97.61	646.48	533.34	561.63	561.63	-22.69
k_2	4.16	4.17	4.19	0.08	-0.48	4.11	4.05	4.38	4.06	-0.13
R^2	0.01	0.49	0.37	0.82	0.60	0.01	0.27	0.20	0.68	0.44
C_{ss}	185.00	140.00	148.00	NSS	NSS	160.00	132.00	130.00	139.00	NSS
C_e	10.22	33.02	30.66	50.32	22.80	30.10	54.72	47.20	75.24	41.04
k_1	74.32	17.64	20.16	0.19	-4.28	21.48	9.75	11.90	7.47	-0.55
BAF	17.89	4.23	4.81	2.36	8.97	5.23	2.41	2.72	1.84	4.40
t_{SS} (days)	2.00	2.00	2.00	NSS	NSS	1.00	2.00	2.00	2.00	NSS

comparison between experimental and kinetics modeling using Equation 2.

DISCUSSION

Table 1 compares the concentration of metals in the earthworms to the concentration of metals in the various concentrations of used engine oil contaminated soil (12.5 to 100 g UEO/kg soil). The table shows that for most of the metals detected in the worms (apart from Vanadium (V), Mn, and Ti), the dry body burden of the worm (concentration in the dried carcasses of the earthworms) were higher than those in all contaminated soil concentrations. Only a marginal difference existed between the concentration of iron (Fe) and barium (Ba) in the earthworm and the used engine oil contaminated soil at t = 0. Based on these and should the metals in the soil be bioavailable, it is expected that elimination should take place for metals with higher concentration in the worm and accumulation for the metals with lower concentrations in the worms (V, Mn and Ti). Fe and Ba should remain relatively constant in the worms with time. These expected trends were confirmed and the elements that remained fairly constant where not modeled.

Accumulation kinetics for earthworms exposed to the contaminated soils could be described by one of three different patterns: some tissue metal concentration data showed an initial increase/decrease, followed by a leveling off over time. These data produced good fits to Equation 1, values for k_1 and k_2 were determined and steady-state metal concentrations could be predicted. The second group of data showed an increase/decrease in tissue metal concentration with time, but with no sign of any leveling off. For these data, elimination was negligible and biologically considered as zero. Consequently, it was not possible to predict a steady-state metal concentration, though accumulation rate constants were derived. The final group of data showed no accumulation or excretion of metals over the course of the experiment, and thus, the metal concentrations remained constant.

Values of uptake (k_1) and elimination (k_2) constants and metal uptake flux (a)

Values for k_1, k_2 and a showed significant variation. Whilst this would be expected for the uptake flux as it is a function of external metal concentration, k_1 and k_2 are in principle constants. Such variation has been reported by Peijnenburg et al. (1999) and Spurgeon and Hopkin (1999). One interpretation of this variation is therefore that the values of C_e used to derive the values of k_1 and k_2, that is, either bulk or soil solution metal concentration, do not accurately reflect the concentration of bioavailable metals that the earthworms were exposed to. Earthworm concentration data used to model accumulation kinetics are a function of the concentration of metal accumulated by the earthworm, and thus, the mass of the earthworm. In cases where earthworms have lost weight due to either (1) loss of condition in response to adverse soil conditions (e.g. low soil organic matter, coarse soil texture or high metal concentration) or (2) lack of food (in this experiment, food was deliberately added to mimic a bioremediation set up with organic amendment), this will impact on calculated metal concentrations, and thus, the derived uptake and elimination constants. The weight of worms used in this investigation fell within the range of 0.5 to 0.6 g and remained relatively constant through the test period.

The values of k_1 showed some few correlations with bulk soil metal concentration. The highest correlation was exhibited by Zn (r^2 = 0.9335), followed by Cu (r^2 = 0.5979), then Ni (r^2 = 0.5018). The k_1 values for Cr, Ti, and Mn showed poor correlations with bulk metal concentration ($r^2 < 0.32$). k_2 showed a poor correlation with bulk soil metal concentrations for all the metals ($r^2 <$ 0.37). This is consistent with theory which suggests that k_2 is a property intrinsic to the organism and not influenced by external factors. Values of "*a*" showed an insignificant positive correlation with bulk soil metal

Table 4. Uptake kinetics of Ti and Mn by the earthworm (*E. eugenia*) exposed to UEO contaminated soils.

Parameter	Ti					Mn				
	UEO concentration (g/kg soil)					UEO concentration (g/kg soil)				
	12.50	25.00	50.00	75.00	100.00	12.50	25.00	50.00	75.00	100.00
a	90.17	248.82	-11.15	264.61	62.84	7.25	25.02	12.83	298.80	41.07
k_2	0.10	0.26	-0.04	0.36	0.08	0.04	0.30	0.28	4.45	0.53
R^2	0.82	0.64	0.66	0.51	0.79	0.97	0.78	0.99	0.91	0.84
C_{ss}	NSS	975.00	NSS	745.00	NSS	NSS	83.00	46.00	76.90	78.00
C_e	856.86	1120.5	1066.58	1198.40	1180.42	59.60	59.60	54.95	56.50	74.30
k_1	0.11	0.22	-0.01	0.22	0.05	0.12	0.42	0.23	5.29	0.55
BAF	1.15	0.87	0.27	0.62	0.64	2.97	1.42	0.84	1.19	1.05
t_{SS} (days)	NSS	18.00	NSS	16.00	NSS	NSS	16.00	18.00	2.00	16.00

Table 5. TPH drop in used engine contaminated soil of various concentrations.

Concentration of UEO contaminated soil (g/kg)	Drop in TPH after 20 days (%)
12.50	-0.7574
25.00	-0.0159
50.00	3.7020
75.00	7.0142
100.00	13.8963

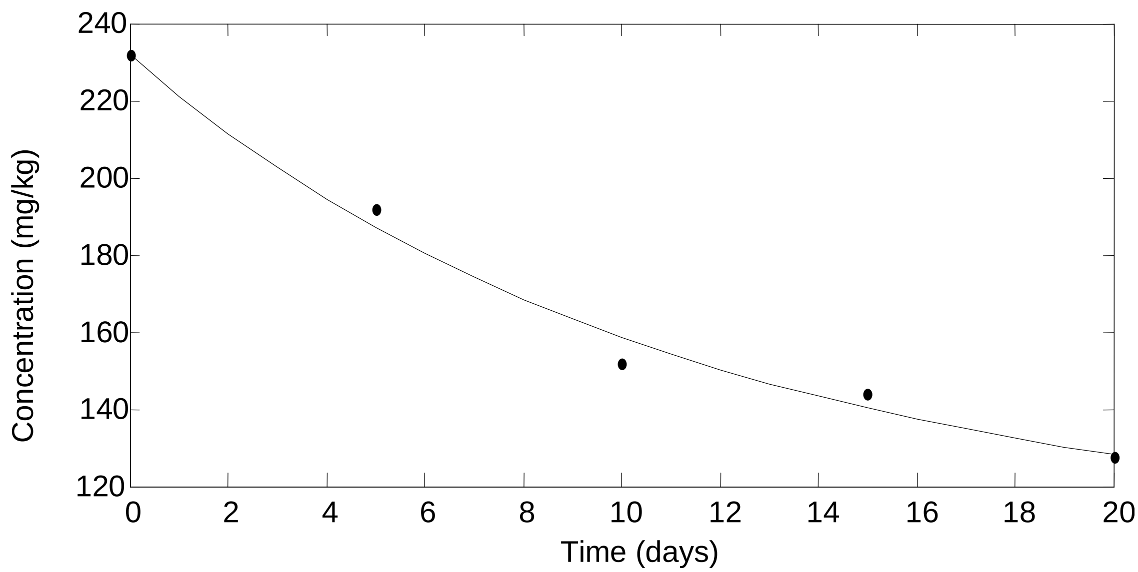

Figure 1. Modeling of Cu elimination kinetics for soil contaminated with 75 g/kg of UEO.

concentrations ($r^2 < 0.37$) for all the metals considered.

Time to reach C_{SS} (t_{SS})

Tables 2 to 4 show the time it is predicted to take for earthworm tissue metal concentrations to reach predicted steady-state concentrations. The times taken to reach steady state range from days one to 18 for the samples that recorded steady state. For Zn, a strong correlation ($r^2 = 0.9868$) was observed between t_{SS} and *a*, but the other elements, all having at least an instance where C_{SS} was not attained, did not show good correlation between *a* and t_{SS}.

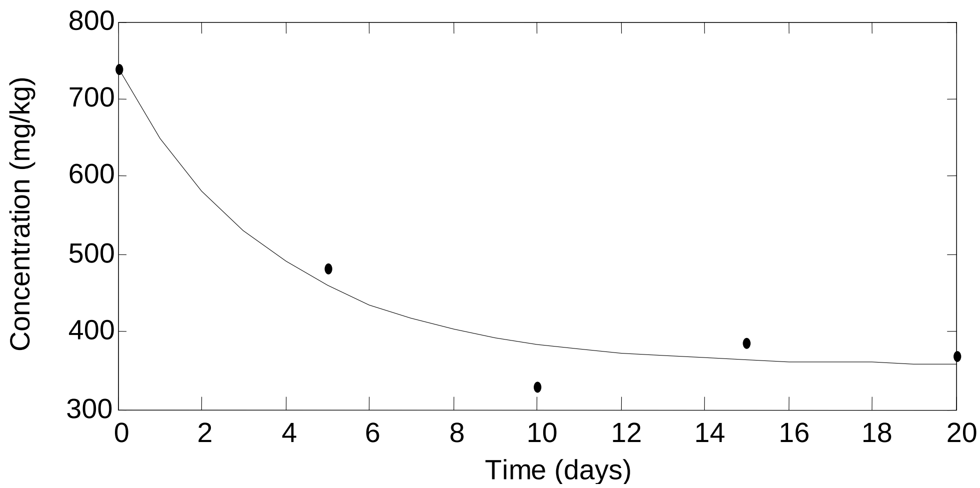

Figure 2. Modeling of Zn elimination kinetics for soil contaminated with 100 g/kg of UEO.

Result of TPH analysis

The percentage reduction in TPH for 12.5, 25, 50, 75, and 100 g used engine oil/kg of soil were found to be -0.7574, -0.0159, 3.702, 7.014, and 13.896%, respectively. The higher the contamination level, the greater the drop in TPH observed. Similar results have been reported by Schaefer (2001). Thus, earthworm assisted bioremediation of used engine oil contaminated soil may be more effective (accelerated) at higher contamination levels. The negative values in TPH drop (indicating an increase in TPH) may be attributed to the method of analysis used or a possible initial oxidation of the hydrocarbon resulting in molecules of higher molecular weight before eventually breaking down.

Conclusion

The main aim of this study, to generate accumulation-uptake and excretion kinetics data to add to the relatively poor data set currently in the literature, was partially achieved. The accumulation part of this study was successfully completed, but it proved impossible to obtain usable data from the excretion phase due to soil toxicity. Three of the 30 data sets obtained could be adequately modeled by one-compartment uptake models. Results were largely in agreement with those of previous reported studies. A note of caution has to be sounded, however, in that in this study as in other kinetic studies (Peijnenburg et al., 1999), earthworms lost weight over the duration of the experiment and this may impact on their fitness and their tendencies to accumulate and excrete metals. In this study, based on the five used engine oil contaminated soils levels, it proved impossible to determine relationships via regression between the fitted parameters, predicted steady-state concentrations and soil properties, and it was not possible to determine what soil properties governed whether earthworms did not accumulate metals, accumulated them to a steady state or accumulated them linearly. This, together with the limited success of applying existing relationships from the literature to our data suggests that predictive equations should be used with caution when applied to soils other than those from which they were derived. This is an important conclusion, because in theory, the rate constants calculated in uptake and excretion studies are indeed constants and can be used to predict accumulation in soils other than those from which the constants were derived. In addition, the results show that it would be difficult to run earthworm toxicity tests to a standard time designed to allow tissue metal concentrations to reach a steady state.

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