



Heavy Metal Remediation Potential of a Tropical Wetland Earthworm, *Libyodrilus violaceus* (Beddard)

E. O. Dada^{1*}, K. L. Njoku¹, A. A. Osuntoki² and M. O. Akinola¹

¹Department of Cell Biology and Genetics (Environmental Biology Unit), University of Lagos, Akoka, Yaba, Lagos, Nigeria

²Department of Biochemistry, Faculty of Basic Medical Sciences, College of Medicine, University of Lagos, Idi-Araba, Lagos, Nigeria

PAPER INFO

Paper history:

Received 20 January, 2016

Accepted in revised form 27 March 2016

Keywords:

Libyodrilus violaceus

Remediation

Pollution

contaminants

ABSTRACT

Wetlands play important strategic ecological and life-supporting roles. As a result, they are continuously exposed to pollutants inflow including heavy metals. The aim of this research was to evaluate the heavy metal remediation potential of a tropical wetland earthworm, *Libyodrilus violaceus*. *L. violaceus* were exposed to soils contaminated with different concentrations of heavy metals using natural optimal soil density of 18 worms per kg soil, for 12 weeks. Relative to their initial concentrations, there were significant reductions of Zn, Pb and Cd at the end of the experiment. From the soil contaminated with a combination of Zn, Pb and Cd, the highest significant ($p < 0.05$) Cd reduction of 8.08 mg/kg soil occurred in the 12th week, the highest significant ($p < 0.05$) Zn reduction of 37.47 mg/kg soil occurred in the 8th week, while Pb recorded the highest but not significant ($p > 0.05$) reduction of 19.07 mg/kg in the 12th week. The effect of addition of cow dung on the ability of *L. violaceus* to reduce heavy metals was generally low. *L. violaceus* is recommended as a good candidate for the remediation of low to moderate heavy metal contamination in wetland ecosystem.

doi: 10.5829/idosi.ijee.2016.07.03.06

INTRODUCTION

The problem of environmental pollution and contamination in this age of industrial and technological advancements calls for relentless, concerted and multi-faceted approach. Unlike in the time past when the effect of pollution was thought to be limited to the locality where pollutant level was high [1], the adverse effects of pollution are now known to be global and persistent [1, 2]. Wetland soils are not spared of the global pollution problem. Due to the strategic ecological and life-supporting roles they play, wetlands are continuously exposed to pollutants inflow including heavy metals [3, 4].

In situ biological remediation techniques are cheap and environment friendly alternatives to clean up moderately contaminated soils. Many research works aimed at investigating the potential of earthworms in soil heavy metals uptake and remediation have been carried out. However, with the exception of a few species such as *Eudrilus eugeniae* which is now globally distributed, majority of the earthworm species evaluated for

remediation potential are native to temperate region. The implication of this is that not much is known about the potential of the numerous tropical earthworms' capabilities in remediating contaminants, especially heavy metals. Moreover, less research attention is paid to wetland earthworms as most of the earthworm species studied for remediation potential are dry (friable) soil species whose availability in their natural habitats may be seasonal. Furthermore, majority of the earthworm species evaluated for remediation potential are epigeic (soil surface dwelling) species which may be less exposed to contaminants, including heavy metals, due to their behavioural, life cycle and feeding characteristics, than endogeic species which live in, and feed on mineral topsoil layer where heavy metals and other contaminants might have accumulated over a long period of time [5]. This study therefore attempted to look at the potential of a tropical, all-year-round available, endogeic (mineral topsoil dwelling), wetland earthworm, *L. violaceus*, in remediating heavy metal contaminated soil. The study will draw the attention of more researchers to other numerous tropical earthworm species with the aim of

* Corresponding author: E. O. Dada

E-mail: eodada@yahoo.com; eodada@unilag.edu.ng Tel. +2347030055768

tapping into their potential in solving environmental and other problems.

MATERIALS AND METHODS

Collection of test Samples

The soil (sandy loamy) and *L. violaceus* used for this study were collected from the main campus of the University of Lagos, Nigeria. The university is located on longitude 3° 24'E and latitude 6° 27'N within the Mainland area of Lagos. The soil was collected at 0 to 5.0 cm depth, air dried and passed through a 2mm sieve. *L. violaceus* were collected by digging and hand sorting at average depth of 5-22 cm. The earthworms were stabilized in soil collected from the same site for at least 24 hours before use. The earthworms were clitellate adults (for ease of identification and to reduce variability) with average live weight of 0.9 ± 0.25 g. The species was authenticated by an Earthworm Taxonomist, Professor S.O. Owa of Landmark University, Kwara State, Nigeria. The cow dung used for the study was obtained from a nomadic cattle herdsman settlement in Kwara State, Nigeria.

Test reagents

Nitrate salts of heavy metals were used, namely: zinc nitrate hexahydrate [$Zn(NO_3)_2 \cdot 6H_2O$], lead nitrate [$Pb(NO_3)_2$], and cadmium nitrate tetrahydrate [$Cd(NO_3)_4 \cdot 4H_2O$]. These salts were all analytical grade.

Preparations and analyses of test samples

Soil grain analysis

Determination of grading and particle size distribution of test soil was carried out in the Soil Mechanics Laboratory of the Department of Civil Engineering, University of Lagos, Lagos, Nigeria, using the method specified by the British Standard Institution (BSI) for Soil Tests for Civil Engineering Purposes, BS 1377: Part 2, [6].

Measurement of soil moisture content

The moisture content of test soil was measured according to method reported by Anderson and Ingram [7].

Preparation of earthworm for heavy metal determination

Two earthworms were rinsed in deionised water and gut-voided by placing each one in a watch glass lined with wet Whatman No. 1 filter papers for 24 hours. They were thereafter washed again with deionized water and kept in a freezer at -10°C pending further actions.

Digestion of samples

Soil sample was digested as in Akinola et al. [8]. Earthworm digestion was done according to Spurgeon and Hopkin [9].

Determination of heavy metals in digested samples

Digested samples were analysed for heavy metals (Zn, Pb, Cd) using Atomic Absorption Spectrometer (AAS) Perkin Elmer Analyte A 200 model.

Experimental Procedure

Preparation and contamination of test soil with heavy metals

Test soil was spiked with heavy metals to simulate natural field contamination. The spiking was done in 4 groups as follows: (1) Group A – Mixture of Zn, Pb, Cd; (2) Group B – Zn; (3) Group C – Pb; (4) Group D – Cd. Each group was divided into three treatments as follows: Treatment 1 (T1): Soil only (control); Treatment 2 (T2): Soil + worms; Treatment 3 (T3): Soil + worms + cow dung. Each treatment was replicated three times. Hence, there were 4 groups of 3 treatments each, replicated 3 times. Each container used had 7.5 cm radius and 12 cm depth. To each container was put 1,000 g of air dried, sandy loamy soil that had been passed through a 2 mm sieve [9, 10]. The soil in each container was spiked with metals (Zn, Pb, Cd) in combined or individual concentrations as the case might be, and left to age for 4 weeks. After the 4 weeks incubation period, the soils were dry to touch as a result of moisture loss. Consequently, 250 ml deionized water was added to the soil in each container and mixed again. In addition, 5 g of fermented cow dung [11] that had been prepared into paste was added to all treatments 3 (Soil + Worms + Cow dung) containers and mixed thoroughly with the soil. Three grams (3 g) of the metal contaminated soil was taken from each container to confirm the actual metal level by AAS which served as the initial metal level for the experiment. These concentrations were non-lethal to *L. violaceus* because no mortality was recorded at those concentrations when *L. violaceus* was preliminarily screened for heavy metal survival. Eighteen adult, clitellate *L. violaceus* that had been gut-voided and washed with distilled water were added to the 1,000 g soil in each container. This is the natural optimal number of *L. violaceus* in terms of productivity as found by Owa et al. [12]. The containers were covered with transparent perforated lids to prevent worms from escaping, allow sufficient air, and prevent excessive water loss. The experiment lasted for 12 weeks (84 days). In order to ensure adequate moisture for the set-ups, 100 ml distilled water was added to each container every 7 days. This was in addition to the initial moisture content of 35 ± 2 %. Soils and earthworms were sub-sampled every 4 weeks (28 days) for heavy metal contents.

Determination of heavy metals accumulation by *L. violaceus*

The heavy metals accumulated by *L. violaceus* were determined by subtracting the heavy metal background levels of *L. violaceus* (Zn: 10.22 mg/kg, Pb: 1.49 mg/kg,

Cd: 00.00 mg/kg) from the tissue-metals at weeks 4, 8, and 12 of the experiment.

Determination of ability of *L. violaceus* to reduce metals from soil

The heavy metal reducing ability of *L. violaceus* (metal reduction in soils contaminated with heavy metals) was determined by first subtracting the final soil metal level (at week 4, 8 or 12) from the initial metal level (at week 00). This was done for the soil of the control group (Treatment 1) and the soil treated with *L. violaceus* (Treatment 2). Thereafter, heavy metal reduction in the control soil (Treatment 1) was subtracted from heavy metal reduction in the soil treated with *L. violaceus* (Treatment 2). The percentage metal reduction was obtained by dividing metal reduction by the final metal level of the control soil (T1) for the week of sampling and multiplying by 100.

Determination of the effect of cow dung on the ability of *L. violaceus* to reduce heavy metals

The effect of cow dung on the ability of *L. violaceus* to reduce heavy metals in contaminated soil was determined by subtracting heavy metal reduction in the soil not treated with cow dung (Treatment 2) from heavy metal reduction in the soil treated with cow dung (Treatment 3). The percentage metal reduction due to addition of cow dung was calculated by dividing metal reduction due to addition of cow dung by the final metal level of Treatment 3 (T3) soil and multiplying by 100.

Data analyses

Data obtained from the experiment were subjected to one-way analysis of variance (ANOVA). The mean values obtained were compared using pairwise comparison and least significant difference (LSD). All analyses were done using the Statistical Package for Social Sciences (SPSS) version 20.0.

RESULTS AND DISCUSSION

Physico-chemical properties of test soil

The result of the baseline physico-chemical study conducted on the air-dried test soil is presented in Table 1. Zinc, and Pb background levels were 2.94 mg/kg respectively, while Cd was not detected in the soil.

Heavy metal background levels of earthworm and cow dung

The background heavy metal analysis conducted on the *L. violaceus* and the cow dung used for the study (Table 2) indicated that *L. violaceus* had Zn and Pb background

levels of 10.22 and 1.49 mg/kg, respectively; while cow dung had Zn and Pb background levels of 3.56 and 0.47 mg/kg, respectively. Cadmium was neither detected in *L. violaceus* nor in the cow dung.

Initial heavy metal concentrations of soils used for the experiment

The initial heavy metal concentrations of the soil used for the experiment, as confirmed by AAS, are shown in Table 3. The initial concentrations the soils of Treatment 1, 2 and 3 for each metal were not significantly different ($p > 0.05$).

Heavy metal accumulation in the tissues of *L. violaceus*

The heavy metal accumulation pattern by *L. violaceus* in soils contaminated with different heavy metal combinations (Table 4) shows that the highest tissue level of each metal was found in *L. violaceus* exposed to soils contaminated with individual metals.

Heavy metal reduction in soil contaminated with a combination of Zn, Pb, Cd

From the soil contaminated with a combination of Zn, Pb and Cd (Table 5), Cd recorded the highest significant reduction ($p < 0.05$) of 3.80 mg/kg (18.15 %) and 8.08 mg/kg soil (33.67 %) in the 8th and 12th weeks, respectively. This was followed by Zn which also recorded a significant reduction ($p < 0.05$) of 37.47 mg/kg soil (18.52 %) and 25.77 mg/kg soil (12.14 %) in week 8 and week 12, respectively.

Heavy metal reduction in soil contaminated with Zn due to actions of *L. violaceus*

From the soil contaminated with Zn only, there were significant Zn reductions ($p < 0.05$) throughout the sampling periods. Zinc reduction for the 4th week was 18.18 mg/kg soil which corresponds to 8.74 % (Table 6).

Heavy metal reduction in soil contaminated with Pb

In the soil contaminated with Pb only (Table 7), there were significant ($p < 0.05$) Pb reductions of 18.04 and 20.18 mg/kg soil, corresponding to 3.38 and 3.81 % in the 8th and 12th weeks, respectively.

Heavy metal reduction from soil contaminated with Cd

Significant metal reductions due to the actions of *L. violaceus* occurred in the soil contaminated with Cd only (Table 8). The highest significant Cd reduction ($p < 0.05$)

TABLE 1. Background physico-chemical properties of air dried test soil

| % Sand | % Silt | % Clay | % TOC | % Moisture | pH | Heavy metals (mg/kg) | | | CEC (meg/100g) | | | |
|--------|--------|--------|-------|------------|------|----------------------|------|----|-----------------|----------------|------------------|------------------|
| | | | | | | Zn | Pb | Cd | Na ⁺ | K ⁺ | Mg ²⁺ | Ca ²⁺ |
| 69 | 14 | 14 | 2.78 | 7.59 | 6.90 | 2.94 | 0.24 | ND | 5.87 | 4.07 | 8.17 | 14.71 |

TOC = total organic carbon ND = not detected CEC = cation exchange capacity

TABLE 2. Heavy metal background levels of earthworm and cow dung used for the study

| Metal | Background metal level (mg/kg) | |
|-------|--------------------------------|----------|
| | <i>L. violaceus</i> | Cow dung |
| Zn | 10.22 | 3.56 |
| Pb | 1.49 | 0.47 |
| Cd | ND | ND |

ND = Not detected

TABLE 3. Initial heavy metal concentrations of soil used in vermiremediation

| Soil group and metal content | Metal | Initial concentration (mg/kg) | | |
|------------------------------|-------|-------------------------------|----------------|----------------|
| | | T1 | T2 | T3 |
| A (Zn,Pb, Cd) | Zn | 206.18 ± 13.71 | 211.17 ± 14.00 | 202.90 ± 12.73 |
| | Pb | 557.77 ± 14.15 | 537.77±15.58 | 535.80 ± 22.55 |
| | Cd | 23.17 ± 0.68 | 23.01±1.00 | 21.11 ± 2.02 |
| B (Zn) | Zn | 208.85 ± 9.98 | 220.24 ± 0.15 | 212.40 ± 15.61 |
| C (Pb) | Pb | 553.88 ± 5.58 | 540.52 ± 30.62 | 550.23 ± 10.14 |
| D (Cd) | Cd | 22.45 ± 1.40 | 24.08 ± 2.36 | 23.43 ± 1.44 |

T1= Soil only (control) T2 = Soil + worms T3 = Soil + worms + cow dung

Values for initial concentration are mean of triplicate analysis + SD

TABLE 4. Heavy metal levels in the tissues of *L. violaceus* exposed to soils contaminated with different combinations of metals at 4th, 8th and 12th weeks of exposure

| Group | Metal | Metal level in soil (mg/kg) | | Metal level in Lv (mg/kg) ± SD(4weeks) | | Metal level in Lv (mg/kg) ± SD (8weeks) | | Metal level in Lv (mg/kg) ± SD (12weeks) | |
|-----------------|-------|-----------------------------|---------------|--|---------------|---|---------------|--|---------------|
| | | T2 | T3 | T2 | T3 | T2 | T3 | T2 | T3 |
| A (Zn,Pb,Cd) | Zn | 211.17 ±14.00 | 202.90 ±12.73 | 527.64 ±11.31 | 465.07 ±50.48 | 719.18 ±18.09 | 698.37 ±17.27 | 716.00 ±79.08 | 758.94 ±94.94 |
| | Pb | 537.77 ±15.58 | 535.80 ±22.55 | 442.05 ±26.40 | 349.55 ±12.44 | 716.24 ±17.10 | 680.95 ±14.83 | 696.14 ±17.07 | 789.65 ±20.74 |
| | Cd | 23.01 ±1.00 | 21.11 ±2.02 | 64.40 ±18.78 | 68.31 ±31.78 | 70.45 ±21.00 | 64.35 ±9.36 | 137.65 ±21.09 | 133.04 ±13.16 |
| B (Zn) | Zn | 220.24 ± 0.15 | 212.40 ±15.61 | 454.26 ±27.91 | 457.94 ±28.76 | 774.57 ±75.84 | 812.71 ±15.20 | 881.50 ±55.23 | 884.37 ±44.63 |
| C (Pb) | Pb | 540.52 ± 0.62 | 550.23 ±10.14 | 530.97 ±23.25 | 530.23 ±43.74 | 871.93 ±10.77 | 957.34 ±13.54 | 884.56 ±68.40 | 989.68 ±21.61 |
| D (Cd) | Cd | 24.08 ± 2.36 | 23.43 ± 1.44 | 69.31 ±49.95 | 62.79 ±33.88 | 111.18 ±17.39 | 138.20 ±44.47 | 169.71 ±42.81 | 191.54 ±9.67 |

T2 = Soil + Worms only T3 = Soil + Worms + Cow dung

Lv = *Libyodrilus violaceus*

of 5.37 mg/kg soil corresponding to 23.81 % occurred at the 12th week.

Effects of addition of cow dung on the ability of *L. violaceus* to reduce heavy metals

The results as shown in Table 9 indicated that the effect of addition of cow dung on the ability of *L. violaceus* to reduce heavy metals in soil was generally low. Heavy

metal accumulation by *L. violaceus* was lowest in the 4th week, increased in the 8th week and seemed to stabilize in the 12th week. Such a trend of accumulation suggests that homeostatic balance was achieved in the 8th week. The increase in tissue-metal levels of *L. violaceus* from the 4th to 8th week was probably related to the continuous availability of metals in the soils, since tissue-metal level is a reflection of metal availability [13]. Morgan [14]

indicated that elevated environmental heavy metal concentrations result in dispersion of the metals into the various tissues of earthworms. The ability of *L. violaceus* to accumulate high levels of heavy metals in this study can be partly linked to its feeding and ecological habits. Endogeic earthworms, such as *L. violaceus*, are geophagus, consuming large quantities of soil in order to meet their organic matter needs [15]. Endogeic earthworms are therefore more exposed to soil contaminants, including heavy metals, through dermal and intestinal routes. Endogeic earthworms have been reported to accumulate certain metals more than epigeic and anecic species inhabiting the same habitat [14]. The

high metal concentrations in the tissue of *L. violaceus* found in this study also conform to the findings of Dai et al. [5].

From the results, there were heavy metal reductions attributable to the presence of *L. violaceus* in every soil group, though these may be considered low relative to other works [13, 16]. In this study, relatively lower soil metal reductions observed may be related to the concentrations of the soil metals used. Moreover, the natural optimal soil density of earthworms used in the study may be another contributory factor for the relatively low soil metal reductions. According to Schaefer and Juliane [11], the density of earthworms used

TABLE 5. Heavy metal reduction in soil contaminated with a combination of Zn, Pb, Cd

| Metal | Initial (00 week) metal level (mg/kg) ± SD | | Period | Final metal level (mg/kg) ± SD | | Metal reduction (mg/kg) | | Metal reduction (mg/kg) due to actions of Lv (T2-T1) | % metal reduction due to actions of Lv |
|-------|--|------------------|-------------|--------------------------------|--------------------------------|-------------------------|-------|--|--|
| | T1 | T2 | | T1 | T2 | T1 | T2 | | |
| Zn | 206.18 ±13.71 | 211.17 ±14.00 | 4 Weeks | 205.35 ±10.72 | 205.51 ±8.08 | 0.83 | 5.66 | 4.83 | 2.35 |
| Pb | 557.77 ±14.15 | 537.77 ±15.58 | | 558.87 ±12.50 | 537.54 ±23.49 | -1.1 | 0.23 | -0.87 | -0.01 |
| Cd | 23.17 ±0.68 | 23.01 ±1.00 | | 22.49 ±0.43 | 20.66 ±0.92 | 0.68 | 2.35 | 1.67 | 7.43 |
| Zn | 206.18 ±13.71 | 211.17 ±14.00 | 8 Weeks | 205.58 ±6.78 | 173.10 ^{rs} ±23.73 | 0.60 | 38.07 | 37.47 | 18.52 |
| Pb | 557.77 ±14.15 | 537.77 ±15.58 | | 522.33 ^{rs} ±28.19 | 499.10 ±28.11 | 35.44 | 38.67 | 3.23 | 0.62 |
| Cd | 23.17 ±0.68 | 23.01 ±1.00 | | 20.94 ^r ±0.82 | 16.98 ^r ±2.75 | 2.23 | 6.03 | 3.80 | 18.15 |
| Zn | 206.18 ±13.71 | 211.17 ±14.00 | 12 Weeks | 212.19 ±3.92 | 191.41 ±5.20 | -6.01 | 19.76 | 25.77 | 12.14 |
| Pb | 557.77 ±14.15 | 537.77 ±15.58 | | 544.57 ±10.29 | 505.50 ±12.89 | 13.20 | 32.27 | 19.07 | 3.50 |
| Cd | 23.17 ±0.68 | 23.01 ±1.00 | | 24.00 ^s ±1.40 | 15.76 ^r ±2.70 | -0.83 | 7.25 | 8.08 | 33.67 |

T1 = Soil only (Control) T2 = Soil + worms Lv = *Libyodrilus violaceus*

r = mean difference is significant at $p < 0.05$ relative to the initial metal level (LSD)

s = mean difference is significant at $p < 0.05$ relative to the metal level for the immediate preceding period (LSD)

TABLE 6. Heavy metal reduction in soil contaminated with Zn

| Metal | Initial (00 week) metal level (mg/kg) ± SD | | Period | Final metal level (mg/kg) ± SD | | Metal reduction | | Metal reduction (mg/kg) due to actions of Lv (T2-T1) | % Metal reduction due to actions of Lv |
|-------|--|-----------------|-------------|--------------------------------|-------------------------------|-----------------|-------|--|--|
| | T1 | T2 | | T1 | T2 | T1 | T2 | | |
| Zn | 208.85 ±9.98 | 220.24 ±0.15 | 4 Weeks | 208.06 ±10.62 | 201.27 ^r ±13.18 | 0.79 | 18.97 | 18.18 | 8.74 |
| Zn | 208.85 ±9.98 | 220.24 ±0.15 | 8 Weeks | 201.12 ±0.50 | 188.63 ^r ±4.14 | 7.73 | 31.61 | 23.88 | 11.87 |
| Zn | 208.85 ±9.98 | 220.24 ±0.15 | 12 Weeks | 200.16 ±3.62 | 186.74 ^r ±10.86 | 8.69 | 33.50 | 24.81 | 12.40 |

T1 = Soil only (Control) T2 = Soil + worms Lv = *Libyodrilus violaceus*

r = mean difference is significant at $p < 0.05$ relative to the initial metal level (LSD)

TABLE 7. Heavy metal reduction in soil contaminated with Pb

| Initial (00 week) metal level (mg/kg) ± SD | | | Final metal level (mg/kg) ± SD | | | Metal reduction (mg/kg) | | Metal reduction (mg/kg) due to actions of Lv (T2-T1) | % Metal reduction due to actions of Lv |
|--|-----------------|------------------|--------------------------------|-------------------------------|------------------|-------------------------|-------|--|--|
| Metal | T1 | T2 | Period | T1 | T2 | T1 | T2 | | |
| Pb | 553.88 ±5.58 | 540.52 ±30.62 | 4 Weeks | 552.17 ±8.16 | 518.18 ±58.58 | 1.68 | 22.34 | 20.66 | 3.74 |
| Pb | 553.88 ±5.58 | 540.52 ±30.62 | 8 Weeks | 533.90 ^r ±15.01 | 502.50 ±7.04 | 19.98 | 38.02 | 18.04 | 3.38 |
| Pb | 553.88 ±5.58 | 540.52 ±30.62 | 12 Weeks | 529.43 ^r ±4.78 | 495.89 ±17.08 | 24.45 | 44.63 | 20.18 | 3.81 |

T1 = Soil only (Control) T2 = Soil + worms Lv = *Libyodrilus violaceus*r = mean difference is significant at $p < 0.05$ relative to the initial metal level (LSD)**TABLE 8.** Heavy metal reduction in soil contaminated with Cd

| Initial (00 week) metal level (mg/kg) ± SD | | | Final metal level (mg/kg) ± SD | | | Metal reduction (mg/kg) | | Metal reduction (mg/kg) due to action of Lv (T2-T1) | % metal reduction due to action of Lv |
|--|----------------|----------------|--------------------------------|----------------|-----------------------------|-------------------------|------|---|---------------------------------------|
| Metal | T1 | T2 | Period | T1 | T2 | T1 | T2 | | |
| Cd | 22.45 ±1.40 | 24.08 ±2.36 | 4 weeks | 21.78 ±1.64 | 21.05 ±2.60 | 0.67 | 3.03 | 2.36 | 10.84 |
| Cd | 22.45 ±1.40 | 24.08 ±2.36 | 8 weeks | 22.17 ±2.06 | 19.14 ^r ±1.05 | 0.28 | 4.94 | 4.66 | 21.02 |
| Cd | 22.45 ±1.40 | 24.08 ±2.36 | 12 weeks | 22.55 ±1.32 | 18.81 ^r ±1.45 | -0.10 | 5.27 | 5.37 | 23.81 |

T1 = Soil only (Control) T2 = Soil + worms Lv = *Libyodrilus violaceus*r = mean difference is significant at $p < 0.05$ relative to the initial metal level (LSD)**TABLE 9.** Effects of addition of cow dung on the ability of *L. violaceus* to reduce heavy metals in contaminated soil

| Soil group and metal content | Metal | Metal reduction (mg/kg) and relative % metal reduction | | |
|------------------------------|-------|--|----------------------------|---------------------------|
| | | 4 Weeks | 8 Weeks | 12 Weeks |
| A (Zn, Pb, Cd) | Zn | 0.19 (0.10%) | -6.64 (-3.87%) | 4.04 (2.26%) |
| | Pb | 9.69 (1.84%) | 51.44 ^r (9.54%) | 35.39 (7.56%) |
| | Cd | 0.57 (3.13%) | -1.40 (-8.50%) | -3.13 (-18.42%) |
| B (Zn) | Zn | 8.58 ^r (4.64%) | 12.94 ^r (7.71%) | 3.88 ^r (2.22%) |
| C (Pb) | Pb | -2.34 (-0.44%) | 21.54 ^r (4.39%) | 8.56 ^r (1.72%) |
| D (Cd) | Cd | 1.06 ^r (5.48%) | 1.19 ^r (6.88%) | 4.71 (-2.99%) |

r = mean difference is significant ($p < 0.05$) when compared with the control.

in the bioremediation of contaminated sites probably need to exceed those found under normal field conditions. Therefore, heavy metal reduction by *L. violaceus* may be improved by increasing the number of earthworms to soil ratio beyond the natural optimal soil density of 18 worms per kg soil used or by co-application of vermiremediation with phytoremediation.

Heavy metal accumulation in the tissues of *L. violaceus* tended to increase with time. In the same vein, metal reductions in soils due to actions of *L. violaceus* were generally higher in weeks 8 and 12. This pattern of results suggests that when *L. violaceus* is employed for remediation in the field, adequate time is required to

achieve optimal heavy metal reduction. In addition, it may be necessary to constantly monitor soil metal levels in order to establish the period of peak metal reductions. When such a climax is established, there may probably be a need to harvest the earthworm stock and then re-seed fresh worms so as to ensure continuous metal reductions or removals. Earthworm harvest may be done by digging and hand sorting, use of vermifuge to serve as skin irritants, or by passing a low electric current into the soil which will make earthworms run out of their burrows [17]. The latter option is however the most preferred as it has less side effects, and is less labour intensive. It was also found in this study that the effect of cow dung on the

ability of *L. violaceus* to reduce heavy metals was generally low. These results agree with the findings of Hickman and Reid [18] who found that application of additives made only minimal or no difference in remediation using earthworms. It may therefore not be compulsory to supplement the soil organic matter when *L. violaceus* is used in remediation.

Literature indicate that the desirable traits required for considering any earthworm species for vermiremediation include ecological exposure to contaminants, contaminant tolerance and accumulation ability [2, 11, 16]. Results from this study indicate that *L. violaceus* possesses the potential to tolerate and accumulate heavy metals. Its feeding and ecological habits make it potentially exposed to heavy metals. *L. violaceus* is therefore recommended as a good candidate for the remediation of low to moderate heavy metal contamination in wetland ecosystem. As with other animals used in remediation, remediation with *L. violaceus* can be achieved through: (1) harvesting of wild earthworm populations, (2) continuous seeding, culture, and harvest of earthworms, and (3) supplementation or maintenance of wild populations, which might lead to stabilization or immobilization of heavy metal contaminants [19]. Harvested earthworms can be depurated of heavy metals, after which the metals can be recycled or kept away safely, and the depurated worms processed for economic use.

CONCLUSION

Results from this study have indicated that *L. violaceus* possesses the potential to remediate low to moderate soil heavy metals. Since attention is now shifting towards biological solutions to pollution problems, it is recommended that more research attention should be paid to other tropical earthworm species to tackle soil pollution challenges. Vermiremediation will promote ecofriendly rehabilitation of polluted soil since it is an in situ application that does not require mechanical excavation of top soil.

REFERENCES

- Espinosa-Reyes, G., C.A. Ilizaliturri-Hernández, D.J. González-Mille, F.D.-B. Martínez and J. Mejía-Saavedra, Exposure assessment to persistent organic pollutants in wildlife: the case study of coatzaacoalcos, Veracruz, Mexico 2012: INTECH Open Access Publisher.
- Sinha, R.K., G. Bharambe and D. Ryan, 2008. Converting wasteland into wonderland by earthworms—a low-cost nature's technology for soil remediation: a case study of vermiremediation of PAHs contaminated soil. *The Environmentalist*, 28(4): 466-475.
- Karpagavalli, M.S., P. Malini and A. Ramachandran, 2012. Analysis of heavy metals in dyeing wetland Pallikaranai, Tamil Nadu, India. *Journal of Environmental Biology*, 33(4): 757.
- van Dam, A.A., R.C. Kaggwa and J. Kipkemboi, 2006. Integrated pond aquaculture in Lake Victoria wetlands. *Integrated irrigation and aquaculture in West Africa: concepts, practices and potential*, 129.
- Dai, J., T. Becquer, J.H. Rouiller, G. Reversat, F. Bernhard-Reversat, J. Nahmani and P. Lavelle, 2004. Heavy metal accumulation by two earthworm species and its relationship to total and DTPA-extractable metals in soils. *Soil Biology and Biochemistry*, 36(1): 91-98.
- Institution, B.S., BS 1377, 1990: United Kingdom. p. 39-44.
- Ana, G., M.K. Sridhar and G.O. Emerole, 2009. A comparative assessment of soil pollution by polycyclic aromatic hydrocarbons in two Niger Delta communities, Nigeria. *Afr J Pure Appl Chem*, 3(3): 31-41.
- Akinola, M., K. Njoku and B. Ekeifo, 2008. Determination of lead, cadmium and chromium in the tissue of an economically important plant grown around a textile industry at Ibeshe, Ikorodu area of Lagos State, Nigeria. *Advances in Environmental Biology*: 25-31.
- Spurgeon, D. and S. Hopkin, 1999. Comparisons of metal accumulation and excretion kinetics in earthworms (*Eisenia fetida*) exposed to contaminated field and laboratory soils. *Applied Soil Ecology*, 11(2): 227-243.
- Giannis, A., E. Gidarakos and A. Skouta, 2007. Application of sodium dodecyl sulfate and humic acid as surfactants on electrokinetic remediation of cadmium-contaminated soil. *Desalination*, 211(1): 249-260.
- Schaefer, M. and F. Juliane, 2007. The influence of earthworms and organic additives on the biodegradation of oil contaminated soil. *Applied soil ecology*, 36(1): 53-62.
- Owa, S., G. Dedeke, O. Moreyibi, S. Morafa, B. Senjobi and A. Aladesida, 2010. Partitioning of chemical and physical effects of earthworms on growth performance of the vegetable *Amaranthus*. *Australian Journal of Basic and Applied Sciences*, 4(8): 3755-3761.
- Pattnaik, S. and M.V. Reddy, 2011. Heavy metals remediation from urban wastes using three species of earthworm (*Eudrilus eugeniae*, *Eisenia fetida* and *Perionyx excavatus*). *J Environ Chem Ecotoxicol*, 3(14): 345-356.
- Morgan, J.E. and A. Morgan, 1998. The distribution and intracellular compartmentation of metals in the endogeic earthworm *Aporrectodea caliginosa* sampled from an unpolluted and a metal-contaminated site. *Environmental Pollution*, 99(2): 167-175.
- Ruiz, M.P., M. Ramajo, J. Jesús, D. Trigo and D.D. Cosín, 2006. Selective feeding of the earthworm *Hormogaster elisae* (*Oligochaeta*, *Hormogastridae*) in laboratory culture. *European journal of soil biology*, 42: S289-S295.
- Shahmansouri, M., H. Pourmoghadas, A. Parvaresh and H. Alidadi, 2005. Heavy metals bioaccumulation by Iranian and Australian earthworms (*Eisenia fetida*) in the sewage sludge vermicomposting.
- Butt, K.R. and N. Grigoropoulou, 2009. Basic research tools for earthworm ecology. *Applied and Environmental Soil Science*, 2010.
- Hickman, Z.A. and B.J. Reid, 2008. Earthworm assisted bioremediation of organic contaminants. *Environment International*, 34(7): 1072-1081.
- Gifford, S., R.H. Dunstan, W. O'Connor, C.E. Koller and G.R. MacFarlane, 2007. Aquatic zooremediation: deploying animals to remediate contaminated aquatic environments. *TRENDS in Biotechnology*, 25(2): 60-65.

Persian Abstract

DOI: 10.5829/idosi.ijee.2016.07.03.06

چکیده

تالاب ها نقش مهم و استراتژیکی در بهبود شرایط زندگی و زیست محیطی ایفا می کنند. آن ها به طور مداوم در معرض آلاینده های حاوی فلزات سنگین قرار می گیرند. هدف از این پژوهش، بررسی پتانسیل اصلاح فلزات سنگین توسط یک کرم خاکی تالاب گرمسیری، *Libyodrilus violaceus*، بود. *L. violaceus* در خاک هایی که با غلظت های مختلف فلزات سنگین آلوده شده بودند، در طول ۱۲ روز قرار داده شد (دانشیته ی خاک طبیعی بهینه ۱۸ کرم به ازای هر کیلوگرم خاک بود). در پایان آزمایش کاهش قابل توجهی در مقدار Zn، Pb و Cd نسبت به غلظت های اولیه ی آنها مشاهده شد. از خاک آلوده شده با ترکیبی از مس، روی، سرب و کادمیوم، قابل ملاحظه ترین ($p < 0.05$) کاهش در مقدار Cd (8.08 mg/kg soil) در هفته ی دوازدهم اتفاق افتاد در حالی که قابل ملاحظه ترین ($p < 0.05$) کاهش در مقدار Zn (37.47 mg/kg soil) در هفته ی هشتم اتفاق افتاد. سرب بالاترین کاهش (۱۹.۰۷ mg/kg) را نشان داد ولی قابل ملاحظه نبود. ($p > 0.05$) به طور کلی، اثر اضافه کردن کود گاو روی توانایی کرم *L. violaceus* برای حذف فلزات سنگین قابل ملاحظه نبود. *L. violaceus* به عنوان یک کاندیدای مناسب برای اصلاح کم تا متوسط آلودگی فلزات سنگین در اکوسیستم تالاب توصیه می شود.
