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Vermiremediation Potentials of *Lumbricus terrestris* and *Eudrilus euginae* in Heavy Metal Contaminated Soil from Mechanic, Welder workshop and Metallic Dumpsite

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Abstract

The need to reclaim contaminated soils is important in ensuring the sustainability of life and biodiversity. Food crops grown in heavy metal contaminated soils have the risk of passing the accumulated metals to man and such has been demonstrated to cause various diseases in man. In this study, we investigated the potentials to reclaim heavy metal contaminated soil using *Eudrilus euginae* and *Lumbricus terrestris.* The contaminated soils were obtained from mechanic, welder and metallic workshops and were grouped individually and as combinations. The soils were incubated with the earthworms and the quantities of heavy metals in soil were determined before and after the growth of the earthworms. The percentage of the heavy lost from soils at the end of the study was calculated to evaluate the ability of the earthworm to enhance the remediation of heavy metal contaminated soil. The presence of the earthworms led to more reduction of the levels of the heavy metals in the soils than natural attenuation. The presence of *L. terrestris* led to a lower reduction of Cd, Cr and Pb in the combined soil from the three sites but a greater reduction of Zn and Ni from such soil. The presence of *E. euginae* led to greater loss of all the heavy metals in the soil from the welder workshop and greater loss of Cd, Pb, Ni and Zn in the soil the mechanic workshop than *L. terrestris*. The presence of *E. euginae* led to more loss of Cr, Pb, and Ni in combined soils of mechanic and welder workshops and mechanic workshop and metallic dumpsite. The findings of this study show that although the presence of both earthworms enhanced the remediation of heavy metals from the soils, the efficiency of the remediation is organism-specific and site-specific. Study on detailed mechanisms of enhancing heavy metals by earthworms is recommended.

Keywords: Auto-mechanic, *Eudrilus euginae,* Heavy metals, Metallic Dumpsite, *Lumbricus terrestris*, Vermiremediation.
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Introduction

Contamination of the environment by heavy metals has become a critical ecological and global health problem that elicits high apprehension. This is because heavy metals unlike other pollutants are not biodegradable and consequently not easily removed, reduced or detoxified in the environment (Bazrafshan, *et al.*, 2015). The term heavy metals refers to a group of metals and semi metals that have a relatively high density (greater than 5g/cm^3) when compared with other metals and are associated with contamination (Ukpong *et al.*, 2013). They occur naturally, mainly as trace elements and some of them: copper (Cu), cobalt (Co), manganese (Mn), zinc (Zn), nickel (Ni), iron (Fe), calcium (Ca), magnesium (Mg) have known physiological and biochemical functions in plants and animals while others like cadmium

(Cd), lead (Pb), mercury (Hg), arsenic (As) and chromium (Cr) have no established biological functions (Oves *et al*., 2016). Heavy metals increase in the environment through anthropogenic sources such as metallic dumping, application of fertilizers, pesticides, petrochemicals, biosolids and manure, municipal and industrial waste water, mining, smelting and coal combustion residues (Ukpong *et al*., 2013). Auto mechanic activities have also been identified as one of the major sources of heavy metals pollution (Adewole and Uchegbu, 2010). These anthropogenic activities accelerate nature's slowly occurring geochemical cycle of metals leading to the accumulation of one or more heavy metals above permissible limits in the ecosystems thereby posing a high risk to biodiversity and human health (Ekperusi and Aigbodion, 2015).

Heavy metals contamination adversely affects soil microbial population and diversity and subsequently the overall soil enzyme activities. In high concentrations, they are toxic to plants resulting in disorders in plant metabolism, oxidative stress, chlorosis, poor plant growth and low yield (Ashraf *et al*., 2011). More importantly, heavy metals in high concentrations become a potential threat to human health when taken up by plants and subsequently accumulated along the food chain (Xie *et al*., 2016). Duruibe *et al*. (2007) observed that the chronic ingestion of cadmium causes abdominal cramps, muscle weakness, pulmonary oedema and death while same level of ingestion for zinc causes impairment of growth and reproduction in addition to liver and kidney failure. Cadmium causes kidney lesions, mutation, cancer, and blood pressure increase (Sizmur and Hodson, 2009). According to Duruibe *et al*., (2007), the excessive human intake of copper leads to severe capillary damage, hepatic and renal damage, central nervous system irritation and depression and mercury even at low concentrations causes congenital malformations, pink disease and neurological disorders while Arsenic has been found to inhibit production of ATP and is carcinogenic. Lead poisoning leads to inhibition of haemoglobin synthesis, damage to cardiovascular system, pregnancy problems, gastrointestinal disorders, muscles and joint pains (Hu *et al*., 2017). According to Huang *et al*. (2009) chromium (VI) is known to be able to diffuse through cell membrane and oxidize biological molecules thereby deriving its ability as a potential carcinogen. Chromium and cadmium are two dangerous heavy metals; exposure to chromium causes lung and digestive organ cancer, severe diarrhea and nausea (Cefalu and Hu, 2004). Human exposure to nickel contamination causes the damage of the lungs, mucous membrane and nervous system (Argun *et al*., 2007).

The significant health risks on humans and the environmental damage caused by heavy metals necessitate the need for their removal from the environment. The quest for an environmentally sustainable approach towards the contaminated environments has led to the adoption of various bioremediation strategies such as vermiremediation for the remediation and restoration of contaminated environments. It uses earthworms to breakdown and clean up contaminated soils and has been found applicable to soils contaminated with heavy metals (Ekperusi and Aigbodion, 2015, Dada *et al.,* 2021). The relevance of this process hinges on the earthworm's ability to tolerate the presence of the contaminant and their ability to bioaccumulate and biodegrade both inorganic and organic contaminants (Pattanik and Reddy, 2011). Studies have shown that *Perionyx excavatus*, *Eisenia fetida*, *E. tetraedra, Lumbricus terrestris, L. rubellas, Aporrectoda tuberculata, Allobophora chlorotica* and *Lybiodrilus violaceus* are some earthworm species that can remove heavy metals from the soil (Sinha *et al*., 2010; Ekperusi *et al*., 2016 and Dada *et al.,* 2016).

Many available researches show the use of earthworms in remediating simulated contaminated environments (Njoku *et al*, 2016, Njoku *et al*, 2018, Dada *et al*. 2021) and the use of vermiremediation technology for reclaiming damaged or contaminated soil has gained increasing awareness. The report of Jolaoso *et al.* (2019) shows that soils of automobile mechanic workshop contain high levels / concentrations of heavy metals (Cd, As, Cu, Pb, Ni and Zn). Also, Mustapha *et al*. (2019) reported the high levels of heavy metals (Cu, Zn, Pb and Cr) in soils obtained from metallic dumpsite and mechanic workshop. Review of literature by Shahmansouri *et al.* (2005), Marcoparra *et al.* (2010), Pattnaik and Redy (2011), Dabke *et al.* (2013) and Dada *et al.*

(2021) have revealed little or no information on the vermiremediation of heavy metals in soils of automobile workshop, welder and metallic dumpsites.

This study evaluated the potentials of two earthworm species (*L. terrestris* and *E. euginae)* to enhance the clean-up of heavy metal soils from a mechanic workshop, welder workshop and metallic dumpsite. It determined and compared the individual abilities of the two earthworms to enhance the remediation of heavy metals contaminated soil. *L. terrestris* and *E. euginae* were chosen for their high rate of reproduction, high potential for waste decomposition (Hartenstein and Bisesi,1989; Birundha *et al*., 2013) and high resistance to chemical contaminants (Sinha *et al.,* 2010). The heavy metals of interest in this study are Cd, Cr, Ni, Pb and Zn because they are the most commonly found in polluted soils and have been identified to be toxic to a wide range of earthworm species (Spurgeon and Hopkin, 2000). The study will determine the level of contamination by each of the heavy metals in the soil samples from different contaminated sites and the amount of heavy metals lost from the soil at the end of study.

Materials and Methods

Sources and collection of materials

Contaminated soil samples were collected from sites in Lagos metropolitan city, southwestern Nigeria namely: welder's workshops and auto mechanic workshops at Agidingbi, Ikeja (6.60^0 $92'$ N and 3^0 49'E) and a metallic refuse dumpsite at Owode, Ikorodu road ($6.60^0 14'$ N and 3.40^0 $26'$ E). The method described in Njoku *et al*. (2016) was adopted for collecting and treating the soil. Soil from all the sites were collected at a depth of 4-5cm using a sterilized spatula and were kept in appropriately labelled polyethene bags. The soil samples were air dried for 3 days, mixed and passed through 2mm sieve to reduce particle size and kept for further analysis.

The earthworm species used were obtained from the University of Lagos Zoological/Botanical garden (6.3^0 $52'$ N and 3.23^0 $47'$ E). They were identified by an earthworm taxonomist from the Department of Zoology, University of Lagos using methods described by Owa (1992) as *Lumbricus terrestris* and *Eudrilus euginae*. They were allowed to acclimatize for 48 hours in moist soil before being placed in the heavy metals contaminated soil.

Experimental design

The soil samples were arranged in seven groups namely, Mechanics' workshop, welders' workshop, metallic dumpsite, a combination of mechanics' and welders' workshop soils, a combination of mechanics' workshop and metallic dumpsite soils, a combination of welders' workshop and metallic dumpsite soils and a combination of soil from mechanics' workshop, welders' workshop and metallic dumpsite. Each group was subdivided into three subgroups each. One portion represented the control (without earthworm), and the other two represented the experimental (with *E. euginae* and with *L. terrestris*), all treatments were replicated three times. Seven of each of the earthworm species were placed in each of the corresponding samples of the experimental group. Each container was filled with 300 g of soil. The set up was monitored regularly and 30 ml of water was added every 48 hours for 35 days to prevent the soils from drying out and also to avoid dehydrating the earthworms.

Analysis of samples

The soil samples were tested for heavy metals content before and after the vermiremediation period of 35days. Digestion of the soil for heavy metals content was done in accordance with Njoku *et al* (2020) procedure using a mixture of hydrochloric acid and perchloric acid (5:1). The filtrate and washings from the digest were diluted to 50ml with deionized water and kept for metal analysis. The filtrate from the digest were analysed for the concentrations of the heavy metals (Cd, Cr, Ni, Pb and Zn) using the inductively coupled plasma optical emission spectrophotometer (Agilent ICP-OES710 axial). The test results were validated with calibration curves obtained with certified metal standards (Accstandard Inc. USA) and quantification was obtained with Agilent Expert II software (USEPA, 1998). The values obtained were used to generate the following data:

The percentage loss of the heavy metals and the effect of the earthworm on the heavy metal content of the soil were calculated as were described by Njoku *et al* ((2018) using the formulae:

Percentage loss of heavy metals due to natural attenuation =

$$\frac{Initial\ heavy\ metal\ concentration - final\ heavy\ metal\ concentration\ without\ earthworm \times 100}{Initial\ heavy\ metal\ concentration}$$

The effect of earthworm on heavy metal concentrations in the soil =

$$\frac{Initial\ heavy\ metal\ concentration - final\ heavy\ metal\ concentration\ with\ earth\ worm\ \times 100}{Initial\ heavy\ metal\ concentration}$$

The Percentage loss of heavy metal due to earthworm = percentage loss of heavy metal in soil with earthworm minus percentage loss of heavy metal in soil without earthworm.

Statistical Analysis

The data obtained from the analyses of the heavy metal content of soil was statistically analyzed using two-way analysis of variance (ANOVA) to assess significant variation in the mean concentration of heavy metals in the soil of the two earthworm species and in the earthworm samples at a probability level of $P<0.05$. A correlation analysis was used to determine the association between the heavy metals in the soil samples. All the statistical analyses were done using SPSS version 26 software for windows.

Results.

Cadmium content of the contaminated soil samples and contributions of the earthworms to heavy metal loss.

Table 1 shows that there was a reduction of Cd from 1.18mg/kg to 0.22 mg/kg in soil with *E. euginae* and 0.13 mg/kg in soil with *L. terrestris* in the soil sample from mechanic workshop at the end of the study. For the soil from welder workshop, the Cd level reduced from 8.3mg/kg to 1.08mg/kg in the soil with *E. euginae* and 2.44 mg/kg for soil with *L. terrestris*. The Cd level in the soil from metallic dump reduced from 1.07 mg/kg to 0.30 mg/kg in the soil with *E. euginae* and 0.28 mg/kg in the soil with *L. terrestris*. In combined soil of the mechanic and welder workshops, the Cd level reduced from 3.47 mg/kg to 1.07mg/kg in the soil with *E. euginae* and 0.88mg/kg in the soil with *L. terrestris* In the case of the combined soil of the mechanic workshop and metallic dump, the Cd level reduced from 2.03 mg/kg to 0.70 mg/kg in soil with *E. euginae* and 0.12 mg/kg in the soil with *L. terrestris*. In the combined soil of the welder workshop and metallic dump, the Cd level reduced from 3.47 mg/kg to 0.76 mg/kg in the soil with *E. euginae* and 0.23 mg/kg in the soil with *L. terrestris.* Cadmium level in the soil from a combination of all the sites reduced from 3.33 mg/kg to 0.60 mg/kg in the soil with *E. euginae* and 0.79 mg/kg in the soil with *L. terrestris*. At the end of the study, Cd level in the soils without earthworm were generally lower than the initial levels but higher than in the soils with earthworm except in the soils from mechanic workshop and soil from a combination of mechanic and metallic sites in which the soil with *E. euginae* had more Cd than the soil without earthworm. Final Cd level was lower in the soil with *L. terrestris* than in the soil with *E. euginae* in the soil samples except in the soil from welder's workshop and the combination of soil from the three sites.

Table 1: Cadmium levels in the soil samples (mg/kg) at the beginning and the end of the study

Soil sample from	Earthworm species	Initial Cd level In soil	final Cd level in soil without earthworm	final Cd level in soil with earthworm	% loss in soil without earthworm	% loss in soil with earthworm	% loss due to earthworm
Mechanic workshop	*E. euginae*	1.18± 0.00	0.18±0.33	0.22 ± 0.02	84.74	81.35	- 3.39
	L. terrestris	1.18 ± 0.23	0.18±0.33	0.13 ± 0.02	84.74	88.60	3.86

Welder workshop	*E. euginae*	8.31 ± 0.00	2.76 ±0.02	1.08 ± 0.11	66.78	87.00	20.22
	L. terrestris	8.31 ± 2.59	2.76 ±0.02	2.44 ± 0.01	66.78	70.63	3.35
Metallic dump	*E. euginae*	1.07 ± 0.32	0.30 ±0.05	0.30 ± 0.01	71.96	71.96	0.00
	L. terrestris	1.07 ± 0.32	0.30 ±0.05	0.28 ± 0.03	71.96	73.88	1.92
Mechanic and Welder	*E. euginae*	3.47 ± 0.21	1.28 ±0.33	1.07 ± 0.10	63.11	69.16	6.05
	L. terrestris	3.47 ± 0.21	1.28 ±0.33	0.88 ± 0.02	63.11	74.64	11.53
Mechanic and Metallic	*E. euginae*	2.03 ± 0.22	0.12 ±0.01	0.70 ± 0.85	94.09	65.55	- 28.54
	L. terrestris	2.03 ± 0.22	0.12 ±0.01	0.12 ± 0.01	94.09	94.09	0.00
Welder and Metallic	*E. euginae*	3.47 ± 1.02	1.27 ±0.10	0.76 ± 0.11	67.71	78.10	10.39
	L. terrestris	3.47 ± 1.02	1.27 ±0.10	0.23 ± 0.03	67.71	93.37	25.66
Mechanic, welder and Metallic	*E. euginae*	3.33± 0.20	0.82 ±0.11	0.60 ± 0.06	75.38	81.98	6.60
	L. terrestris	3.33 ± 0.20	0.82 ±0.11	0.79 ± 0.06	75.38	76.28	0.90

Chromium content of the contaminated soil samples and contributions of the earthworms to chromium loss.

Table 2 shows the Cr levels in the different soil samples at the beginning and at the end of the experiment. The Cr level reduced from 0.63 mg/kg to 0.01 mg/kg in soils with *E. euginae* and *L. terrestris* respectively in the mechanic workshop soil samples. For the soil from the welder workshop, the Cr level reduced from 1.21 mg/kg to 0.01 mg/kg in the soil with *E. euginae* and 0.04 mg/kg in the soil with *L. terrestris.* The Cr level in the soil from metallic dump reduced from 0.01 mg/kg to 0.00 mg/kg in the soil with *E. euginae* and remained the same in the soil with *L.terrestris*. In the combined soil of mechanic and welder workshops, the Cr level reduced from 1.05 mg/kg to 0.00 mg/kg in the soil with *E. euginae* and 0.02 mg/kg in the soil with *L. terrestris*. For the combined soil of mechanic workshop and metallic dump, the Cr level reduced from 0.50 mg/kg to 0.00 mg/kg in the soil with *E. euginae* and 0.01 mg/kg in the soil with *L. terrestris*. In the combined soil of the welder workshop and metallic dump, the Cr level reduced from 2.00 mg/kg to 0.00 mg/kg in the soil with *E. euginae* and 0.02 mg/kg in the soil with *L.terrestris*. In the soil from the combination of all the sites, the Cr level reduced from 0.51 mg/kg to 0.00 mg/kg in the soil with *E. euginae* and 0.02 mg/kg in the soil with *L. terrestris*. At the end of the study, Cr levels for soils without earthworm were lower than the initials except in the soil from the metallic dump site. Soils with *E. euginae* had lower level of Cr than soils with *L. terrestris* in all the soil samples except in the soil from mechanic workshop where the soils with the earthworms had equal level of Cr.

Table 2: Chromium levels in the soil samples (mg/kg) at the beginning and the end of the study

Soil sample	Earthworm species	Initial Cr level In soil	final Cr level in soil without earthworm	final Cr level in soil with earthworm	% loss without earthworm	% loss with earthworm	% loss due to earthworm
Mechanic workshop	*E. euginae*	0.63±0.00	0.01±0.00	0.01±0.00	98.41	98.41	0.00
	L. terrestris	0.63± 0.21	0.01± 0.00	0.01±0.00	98.41	98.41	0.00
Welder workshop	*E. euginae*	1.21± 0.00	0.24± 0.14	0.01±0.00	80.16	99.17	19.01
	L. terrestris	1.21± 0.33	0.24± 0.08	0.04±0.01	80.16	96.70	16.54

Metallic dump	*E. euginae*	0.01± 0.00	0.01± 0.01	0.00±0.00	0.00	100	100
	L. terrestris	0.01± 0.00	0.01± 0.00	0.01± 0.00	0.00	0.00	0.00
Mechanic and Welder	*E. euginae*	1.05± 0.00	0.10± 0.01	0.00 ± 0.00	90.47	100	9.53
	L. terrestris	1.05± 0.40	0.10± 0.00	0.02 ± 0.00	90.47	98.00	7.53
Mechanic and Metallic	*E. euginae*	0.50± 0.00	0.01± 0.00	0.00 ± 0.00	98.00	100	2.00
	L. terrestris	0.50± 0.02	0.01± 0.00	0.01 ± 0.00	98.00	98.00	0.00
Welder and Metallic	*E. euginae*	2.00± 0.00	0.36± 0.06	0.00 ± 0.00	82.00	100	100
	L. terrestris	2.00± 1.43	0.36± 0.04	0.02 ± 0.00	82.00	90.00	8.00
Mechanic, welder and Metallic	*E. euginae*	0.51± 0.00	0.05± 0.02	0.00 ± 0.00	90.09	100	9.91
	L. terrestris	0.51± 0.01	0.05± 0.01	0.02 ± 0.00	90.09	96.07	5.98

Lead content of the contaminated soil samples and contributions of the earthworms to lead loss.

Table 3 shows that in the soil from the mechanic workshop, the Pb level reduced from 12.20 mg/kg to 0.18 mg/kg in the soil with *E. euginae* and 0.12 mg/kg in the soil with *L. terrestris*. The initial Pb level in the soil sample from welder workshop (37.48 mg/kg) reduced to 0.02 mg/kg in the soil with *E. euginae* and 3.14 mg/kg in the soil with *L. terrestris*. The Pb level in the metallic dump soil reduced from 11.24 mg/kg to 0.01mg/kg in the soil with *E. euginae* and 0.18 mg/kg in the soil with *L. terrestris*. Pb in combined soil from mechanic and welder workshops reduced from 12.71 mg/kg to 0.01 mg/kg in the soil with *E. euginae* and 2.05 mg/kg in the soil with *L. terrestris*. In the combined soil from metallic and mechanic workshops, the Pb level reduced from 3.65 mg/kg to 0.02 mg/kg and 0.1 mg/kg in soils with *E. euginae* and *L. terrestris* respectively. In the soil from combination of welder and metallic dumpsite, the Pb level reduced from 15.23 mg/kg in the soil with *E. euginae* and 0.07 mg/kg in the soil with *L. terrestris*. In the case of the combined soil of mechanic, welder and metallic dumpsite, the Pb level reduced from 10.85 mg/kg to 0.1 mg/kg and 0.74 mg/kg in the soil with *E. euginae* and *L. terrestris* respectively. At the end of the experiment, the Pb level reduced in all soil samples without earthworm and with earthworm. Soils with *E. euginae* had lower level of Pb than soil with *L. terrestris* in most soil samples except soil from mechanic workshop.

Table 3: Lead Levels in soil samples (mg/kg) at the beginning and the end of the study.

Soil sample	Earthworm species	Initial Pb level in soil	Final Pb level in soil without earthworm	Final Pb level in soil with earthworm	% loss without earthworm	% loss with earthworm	% loss due to earthworm
Mechanic workshop	*E. euginae*	12.20± 0.00	1.61±0.42	0.18±0.17	86.73	98.52	11.79
	L. terrestris	12.20± 4.51	1.61±0.28	0.12 0.02	86.80	99.03	12.30
Welder workshop	*E. euginae*	37.48± 0.00	6.70±0.00	0.02±0.00	82.13	99.94	17.81
	L. terrestris	37.48±10.39	6.69±0.03	3.14±0.19	82.13	91.61	9.48
Metallic dump	*E. euginae*	11.24± 0.00	0.20±0.07	0.01±0.00	98.26	99.92	1.66
	L. terrestris	11.24± 4.03	0.20±0.04	0.18±0.03	98.26	98.43	0.17
Mechanic and	*E. euginae*	12.71± 0.00	4.44± 1.22	0.07±0.05	65.06	99.45	34.39

Welder	*L. terrestris*	12.71± 2.71	4.88± 0.70	2.05±0.08	61.60	83.87	22.27
Mechanic and Metallic	*E. euginae*	3.65 ± 0.00	0.87± 0.15	0.02±0.03	76.16	99.45	23.29
	L. terrestris	3.65 ± 0.20	0.87± 0.08	0.01± .00	76.16	99.72	22.96
Welder and Metallic	*E. euginae*	15.23± 0.00	4.32± 1.15	0.01±0.00	71.63	99.93	28.30
	L. terrestris	15.23± 2.30	4.32± 0.66	0.07±0.02	71.63	99.54	28.11
Mechanic, welder and Metallic	*E. euginae*	10.85± 0.00	3.31± 0.59	0.10±0.00	69.49	99.07	29.58
	L. terrestris	10.85± 1.56	3.31± 0.34	0.74±0.11	69.49	93.17	23.68

Nickel content of the contaminated soil samples and contributions of the earthworms to nickel loss.

Table 4 shows that in the soil sample from the mechanic workshop, Ni level reduced from 12.02 mg/kg to 0.18 mg/kg in the soil with *E. euginae* and 0.22 mg/kg in the soil with *L. terrestris*. In the soil from the welder's workshop, the Ni level reduced from 5.44 mg/kg to 0.18 mg/kg and 0.15 mg/kg for soil with *E. euginae* and *L. terrestris* respectively. The soil from metallic dumpsite has its Ni content reduced from 0.54 mg/kg to 0.31 mg/kg for the soil with *E. euginae* and 0.26 mg/kg for the soil with *L. terrestris*. In the combination of soil from mechanic and welder workshops, the Ni level reduced from 4.88 mg/kg to 0.17 mg/kg and 0.21 mg/kg in the soils with *E. euginae* and *L. terrestris* respectively. In the combined soil from mechanic workshop and metallic dumpsite, the Ni level reduced from 3.75 mg/kg to 0.19 mg/kg for the soil with *E. euginae* and 0.10 mg/kg for the soil with *L. terrestris*. In the combination of soil from the welder workshop and metallic dumpsite, the Ni level reduced from 2.10 mg/kg to 0.16 mg/kg in the soil with *E. euginae* and 0.14 mg/kg in the soil with *L. terrestris*. In the case of the combination of soil from the three sites, the Ni level reduced from 3.5 mg/kg to 0.10 mg/kg for soil with *E. euginae* and 0.23 mg/kg for soil with *L. terrestris*. Final Ni levels in all soils without earthworm were lesser than the initials. In the metallic dump soil and combination of mechanic and metallic soils, soil with *E. euginae* had higher Ni content than the soil without earthworm. In four soil samples out of the seven (mixture of soil from metallic dump and welder workshop, mixture of soil from the 3 sites, soil from mechanic workshop and metallic dump site) the soil with *L. terrestris* had lower Ni level than the soil with *E. euginae* while in the other three samples, soil with *E. euginae* had lower Ni level.

Table 4 Nickel levels in the soil samples (mg/kg) at the beginning and the end of the study.

Soil sample	Earthworm species	Initial Ni level in soil	Final Ni level in soil without earthworm	Final Ni level in soil with earthworm	% loss without earthworm	% loss with earthworm	% loss due to earthworm
Mechanic workshop	*E. euginae*	12.02±0.00	0.21± 0.09	0.18± 0.05	98.21	98.54	0.33
	L. terrestris	12.04± 5.30	0.26± 0.05	0.22± 0.05	97.83	98.17	0.34
Welder workshop	*E. euginae*	5.44 ± 0.00	0.41± 0.26	0.18± 0.11	92.44	96.79	4.35
	L.terrestris	5.44 ± 2.00	0.41 ± 0.15	0.15± 0.04	92.39	97.24	4.85
Metallic dump	*E. euginae*	0.54 ± 0.00	0.29 ± 0.07	0.31± 0.03	46.29	42.59	-3.70
	L.terrestris	0.54 ± 0.07	0.29 ± 0.04	0.26± 0.02	46.85	52.78	5.93
Mechanic and Welder	*E. euginae*	4.88 ± 0.00	0.42± 0.37	0.17± 0.06	91.39	96.51	5.12
	L. terrestris	4.88 ± 0.01	0.42 ± 0.21	0.21± 0.05	91.39	95.96	4.17

Mechanic and Metallic	*E. euginae*	3.75 ± 0.00	0.11 ± 0.01	0.19± 0.02	97.06	94.93	-2.83
	L. terrestris	3.75 ± 0.41	0.11 ± 0.07	0.10± 0.23	97.06	97.33	0.27
Welder and Metallic	*E. euginae*	2.10 ± 0.00	0.30 ± 0.15	0.16± 0.11	85.71	92.38	6.67
	L.terrestris	2.10 ± 0.04	0.30 ± 0.09	0.14± 0.03	85.71	93.33	7.62
Mechanic, welder and Metallic	*E. euginae*	3.50 ± 0.00	0.30 ± 0.19	0.10± 0.00	91.42	97.14	5.72
	L. terrestris	3.50 ± 0.01	0.30 ± 0.11	0.23± 0.03	71.42	93.42	2.00

Zinc content of the contaminated soil samples and contributions of the earthworms to zinc loss.

Table 5 shows that at the beginning of the study, Zn level in the soil from mechanic workshop (7.57 mg/kg) reduced to 2.12 mg/kg and 0.87 mg/kg for the soil with *E. euginae* and *L. terrestris* respectively. For the welder workshop soil sample, the Zn level reduced from 11.04 mg/kg to 4.46 mg/kg in the soil with *E. euginae* and 5.28 mg/kg in the soil with *L. terrestris*. In the soil from metallic dumpsite, the Zn level reduced from 7.42 mg/kg to 2.46 mg/kg in the soil with *E. euginae* and 1.09 mg/kg in the soil with *L.terrestris*. In the combination of soil from mechanic and welder workshops, the Zn level reduced from 7.52 mg/kg to 2.51 mg/kg and 2.77 mg/kg for soil with *E. euginae* and *L. terrestris* respectively. In the case of the combination of soil from mechanic and metallic sites, the Zn level reduced from 5.28 mg/kg to 2.05 mg/kg in the soil with *E. euginae* and 0.95 mg/kg in the soil with *L. terrestris*. The Zn level in the soil from combination of welder and metallic sites reduced from 6.76 mg/kg to 3.10 mg/kg and 2.95 mg/kg for the soils with *E. euginae* and *L. terrestris* respectively. In the combination of soil from the three sites, the Zn level reduced from 7.27 mg/kg to 3.08 mg/kg for the soil with *E. euginae* and *L. terrestris* respectively. Final Zn level in soils without earthworm were all lower than the initials. Soil without earthworm had lower Zn level (1.38 mg/kg) than soil with *E. euginae* (2.12 mg/kg) in the soil from mechanic workshop. In the soil samples, soil with *L.terrestris* had lower Zn level than soil with *E. euginae* except in the soil from welder workshop.

Table 5: Zinc levels in the soil samples (mg/kg) at the beginning and the end of the study

Soil sample	Earthworm species	Initial Zn level in soil	Final Zn level in soil without earthworm	Final Zn level in soil with earthworm	% loss without earthworm	% loss with earthworm	% loss due to earthworm
Mechanic workshop	*E. euginae*	7.57± 0.00	1.38± 0.54	2.12± 0.72	80.84	71.99	-8.85
	L. terrestris	7.57± 1.08	1.38± 0.31	0.87± 0.01	80.84	88.50	7.66
Welder workshop	*E. euginae*	11.04±0.00	5.28± 0.51	4.46± 0.88	52.17	59.60	7.43
	L. terrestris	11.04±6.01	5.99± 0.20	5.28± 0.29	45.74	52.17	6.43
Metallic dump	*E. euginae*	7.42± 0.00	2.70± 0.72	2.46± 0.76	63.61	66.84	3.23
	L. terrestris	7.42± 4.06	2.69± 0.41	1.09± 0.06	63.74	85.30	21.56
Mechanic and Welder	*E. euginae*	7.52± 0.00	2.78± 0.68	2.51± 1.41	63.03	66.62	3.95
	L. terrestris	7.52± 1.9	3.67± 0.34	2.77± 0.39	51.19	63.16	11.97
Mechanic and Metallic	*E. euginae*	5.28± 0.00	2.04± 0.08	2.05± 0.23	61.36	61.20	- 0.16
	L. terrestris	5.28± 1.73	2.03± 0.04	0.95± 0.07	61.35	82.01	20.52
Welder and Metallic	*E. euginae*	6.76± 0.00	3.63± 0.46	3.10± 0.17	46.39	54.14	8.13
	L. terrestris	6.76± 3.09	3.63± 0.26	2.95± 0.06	46.30	56.36	10.06

Mechanic, welder and Metallic	*E. euginae*	7.27± 0.00	3.09± 1.01	3.08± 0.28	57.49	57.63	0.14
	L. terrestris	7.27± 2.91	3.12± 0.12	3.08± 0.58	57.08	57.63	0.55

Contributions of the earthworms in percentage loss of heavy metals in the different soil samples

Figure 1 shows the contribution of the earthworms in the loss of heavy metals in the soil sample from a combination of the three contaminated sites. The percentage loss of Cd, Cr, Pb and Ni due to *E. euginae* activities was higher than loss due to *L. terrestris*. *L. terrestris* had no effect (0%) on the removal of nickel from the soil

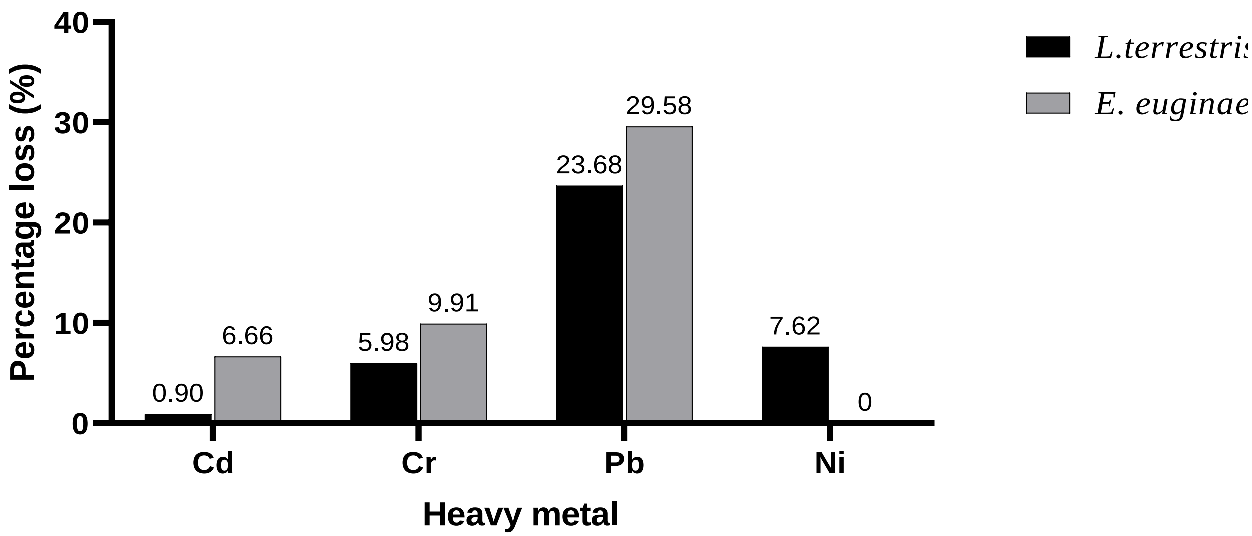
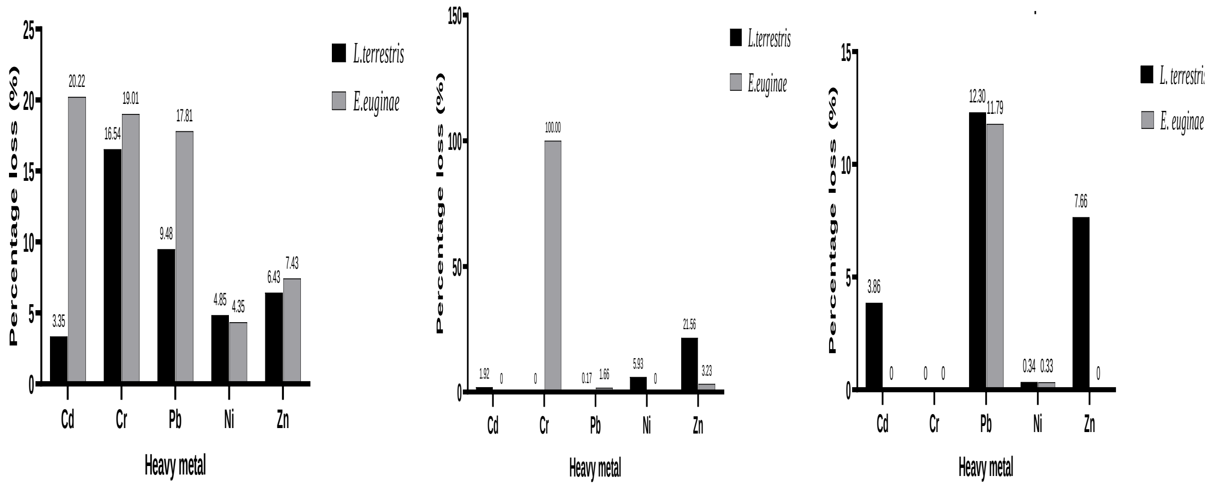


Fig. 1: Percentage loss of heavy metals in the soil combination from mechanic, welder and Metallic dumpsite

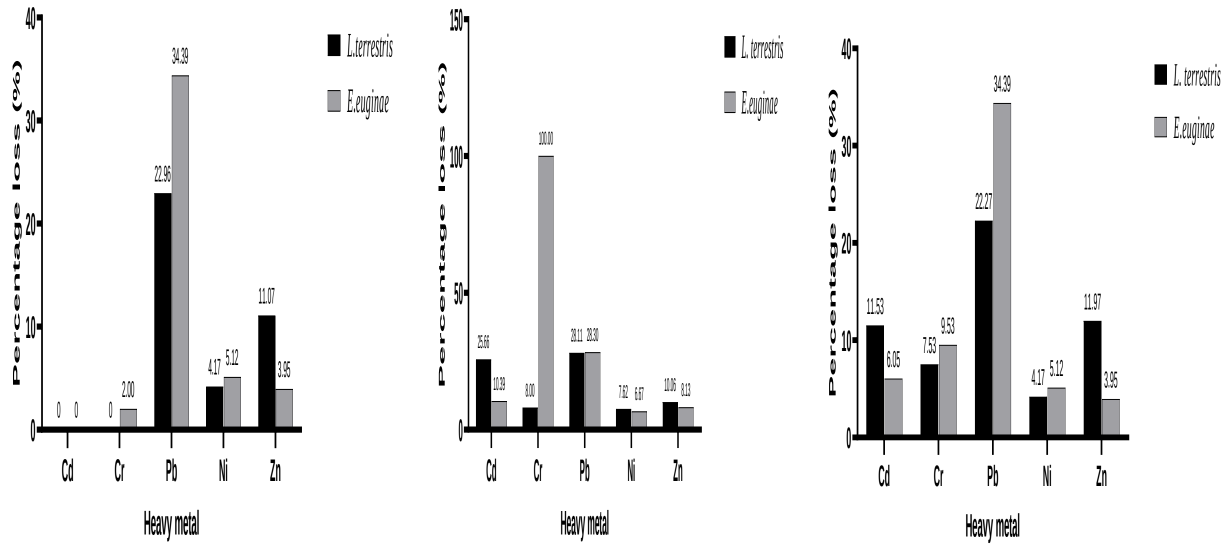
Figure 2 shows the contribution of the earthworms in the loss of heavy metals in the soil from welder's workshop, mechanic workshop and metallic dumpsite. In the welder's workshop (A), the percentage loss of the heavy metals due to the activities of *E. euginae* was higher than that of *L. terrestris* except in the loss of Ni where percentage loss by *L. terrestris* was slightly higher. The loss of Cd with *E. euginae* in this soil sample was the highest (20.22%) recorded among other heavy metals. In the case of the soils from the metallic dump site (B), loss of heavy metals due to earthworm was generally low but there was 100% loss of Cr due to *E. euginae*. More Zn, Ni and Cd were lost due to the activities of *L. terrestris* than *E. euginae.* , In the soil from the mechanic workshop (C), there was higher percentage loss of Pb, Zn and Cd due to *L. terrestris* than *E. euginae*. However, loss of Cr was not due to any of the earthworms.



A B C

Fig. 2: Percentage loss of heavy metals in the soil from welder's workshop (A) and metallic dumpsite (B) and mechanic workshop (C)

Figure 3 shows the contribution of the earthworms in the loss of heavy metals in the combination of soils from the different sites. There was higher percentage loss of Pb, Cr and Ni due to *E. euginae* than *L. terrestris* while higher percentage loss of Zn and Cd was due to *L. terrestris* than *E. euginae* (A). In the combined soil of welder and mechanic workshop (B), there was higher percentage loss of Pb, Ni and Cr in the soil with *E. euginae* than *L. terrestris* while higher percentage loss of Zn was observed in the soil with *L. terrestris*. The loss of Cd was not affected by any of the earthworms. In the combined soil of welder's workshop and metallic dump site (C),. there was 100% loss of Cr due to *E. euginae* while *L. terrestris* led to higher percentage loss of Cd, Ni and Zn than *E. euginae*. Both earth worms affected the loss of Pb equally.



A B C

Fig 3: Percentage loss of heavy metals in the combination of soils from mechanic and welders workshop (A) and mechanic workshop and metallic dump site (B) and welder's workshop and metallic dump site (C)

Discussion

The aim of this study was to evaluate the performance of *E. euginae* and *L. terrestris* in vermiremediation of heavy metal contaminated soil. We observed a decrease in the heavy metals content of all the contaminated soil samples at the end of the study period of 35 days which corresponded with the results obtained by Marco Parra *et al*. (2010), Pattnaik and Reddy (2011), Dabke (2013), Dada *et al*. (2016) and Shameena and Chinnamma (2018) in which there was significant reduction of heavy metals in the contaminated soils in the presence of earthworms. Reduction of heavy metals in contaminated soils can be attributed to the fact that earthworms have an effect on metal speciation in soils, changing the bio accessibility and bioavailability of the metals. They can bio-accumulate and bio-transform heavy metals and clean up the contaminated soil (Li *et al*., 2010, Dabke, 2013, Usmani and Kumar, 2015). According to Dada *et al*. (2016) earthworms remediate metals from soil through dermal absorption, intestinal uptake and subsequent accumulations into their bodies. These earthworms cope with the metal burden by excreting some of these metals through calciferous glands while the un-excreted is removed through the induction of metallothioneins and subsequent sequestration in structures such as waste nodules and chloragogen (fatty cells in the gut wall). The ingested soil contaminated by heavy metals may have been processed in the gut of the earthworms and on egestion as worm casts may have a different pH, bacterial count and dissolved organic carbon content all of which may modify the organic matter sorbed metals into less harmful products or into forms that are bio assessable and hence removed from the soil. Earthworms are highly resistant to chemical inorganic pollutants in the soil and are able to carry out chemical element transformations to replace them with less harmful products (Usmani and Kumar, 2015). As was noted by Dabke (2013), earthworms usually remove heavy metals from the soil through bioaccumulation and stimulated microbial remediation. Thus, the enhanced removal of metals which we observed in soils with the earthworm could have occurred through any of the processes.

In all the soil samples, *L. terrestris* contributed across board more to the loss of Cd, Ni and Zn than *E. euginae*. Results from earlier studies have shown that effective removal of heavy metals from the soil is an indication that the earthworm is an efficient accumulator of the heavy metal (Panday *et al*. 2014). On this note, *L. terrestris* may be a better accumulator of Cd, Ni and Zn than *E. euginae* leading to subsequent better ability to influence the loss of these metals in the contaminated soil. It could also be that *L. terrestris* stimulated more bacterial populations than *E. euginae* in these soil samples through excretion of urine, mucus and other stimulating substances. *L. terrestris* may have affected metal availability more than *E. euginae* by grazing more efficient group of soil microorganisms in metal cycling in the soil. The difference in ability to accumulate Zn, Cd and Ni and subsequently, remediation from the soil is a species specific feature due to variation in intake and metabolism of the metals in the earthworms and also in the way the earthworm affects the soil physicochemical properties. These soil conditions are major determinants of the bioacceessibility of the heavy metals for removal. *L. terrestris* may have enhanced greater loss of Cd, Zn and Ni better than *E. euginae* through effective production of heavy metal chelating metallophores to elevate pH and water soluble heavy metals. The interactions between heavy metals and earthworms depend on the earthworm species (Suthar *et al*. 2008), the metal, and the physical and chemical properties of the soil. *L. terrestris* may have influenced the soil properties better than *E. euginae* for instance it may have increased the amount of organic matter in the composting material.. It may also be possible that casts of *L. terrestris* contains hormones and enzymes that stimulate soil microbes that are very relevant in removal of Cd, Zn and Ni more than that of *E. euginae*. According to Wen *et al*. (2004), increased biomass of microorganisms in earthworm casts denotes availability of the metal for removal.

E. euginae contributed to higher percentage loss of: Cr, Pb and Cd in the soil from the combination of the three sites, Cd, Pb, Cr and Zn in the soil from welder's workshop, Cr (100%) in the soil from metallic dump site, Pb, Cr and Ni in the soil of the combination of mechanic and welders shop, Cr, Pb and Ni in the combination of soil from metallic dumpsite and mechanic workshop and Cr (100%) in the soil of welder and mechanic workshop. This may also be the reason for lower levels of these heavy metals in the soil with *E. euginae* in these soil samples. *E. euginae*, did not contribute to any loss of Cd in the soil from mechanic workshop and a combination of mechanic and metallic soil. This may have been caused by the toxicity of Cd (Otmani *et al* 2018) which may have affected the growth and survival tendencies of the earthworm in the contaminated soil samples and hence its inability to remediate Cd. According to Ekperusi *et al*. (2016), earthworms may selectively respond against a heavy metal because of its toxicity in the contaminated environment. Also according to Nahmani *et al*. (2007), heavy metals have been shown to reduce growth and survival in earthworms and hence low capacity to remove cadmium from the soil. This trend may also be linked to the fact that the form of cadmium present in the soil was not readily available for uptake by *E. euginae* or the earthworm has a low threshold for heavy metals when compared with *L. terrestris*.

The observed greater loss of Pb in most soil samples with *E. euginae* than in soils with *L. terrestris* suggests the differential abilities of the earthworms used to facilitate heavy metals removal from soil. This is similar to the findings of Sinha *et al* (2010) and Njoku *et al* (2018) who reported differences in the amounts of pollutants lost from soils incubated with different earthworms. Dada *et al* (2021) stated that the main mechanism of heavy remediation by earthworms is via dermal absorption. Therefore the differential losses of lead in the different soils due to the presence of the earthworms we used in this study can be attributed to the varying abilities of the earthworms to absorb and accumulate the metal. This result corroborates the findings of Suthar and Singh (2009) and Parihar *et al.* (2019) which showed high accumulation of Pb *by E. euginae* which signifies higher removal from the substrate.

Conclusion

The result of this study shows that *L. terrestris* has a high propensity to remove Cd, Zn and Ni from the soil than *E. euginae* while *E. euginae* has the ability to remove Cr and Pb better than *L. terrestris* can do. This study further confirms that the remediation of heavy metals could be species specific and that *L. terrestris* and *E. euginae* can be used for soil restoration from specific types of heavy metal toxicity.

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