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Research Article

COBALT, CHROMIUM AND LEAD HEAVY METALS ACCUMULATION FROM ANIMAL DUNG, SOIL AND RICE GRAIN THROUGH VERMIC-ACTIVITY BY *LAMPITO MAURITII* KINBERG

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ABSTRACT

Heavy metals are a severe concern that causes a variety of health issues in both human beings and animals. They also have an impact on the variety of soil organisms and the ecosystem. The inoculation of earthworms in their natural environments can assist in monitoring heavy metal concentrations. The earthworm *Lampito mauritii* is a significant species for bioaccumulating highly toxic metals from soil and organic wastes for vermicomposting. This research aims to investigate the concentrations of heavy metals such as cobalt (Co), chromium (Cr), and lead (Pb) found in various animal dungs, earthworm *Lampito mauritii* body tissues before and after vermicomposting, soil, and rice grains (*Oryza sativa* L.). Compared to the initial feed mixture of various animal dungs, it was found that the Co, Cr, and Pb heavy metal concentrations decreased ($P < 0.05$) in the final vermicompost. When the soil was mixed with cow dung vermicompost that had been inoculated with the earthworm *Lampito mauritii*, the highest concentrations of cobalt (Co) and chromium (Cr) were found to decrease by nearly 44.03% and 17.08%, respectively, while the maximum concentration of lead (Pb) was significantly reduced by 32.46% when the soil was mixed with goat dung. Heavy metal concentrations in rice grains were measured both before and after harvesting. When the soil was combined with goat dung vermicompost, the highest concentrations of Co, Cr, and Pb were found to decrease. Before and after crop harvesting, heavy metal concentrations were also investigated in the earthworm body in soil and soil with vermicompost of different animal dungs. When soil and cow dung were combined, the highest amount of cobalt (Co) was found to increase by 29.91% in the earthworm *Lampito mauritii* body, whereas the highest concentrations of chromium (Cr) and lead (Pb) were found to increase by 18.05% and 30.22%, respectively, in the earthworm body when soil and buffalo dung were combined. For the protection of both the health of humans and the environment, vermicomposting and the utilization of *Lampito mauritii* are considered to be efficient methods for extracting these harmful metals from rice field soil.

Keywords: Accumulation, Heavy Metals, *Lampito mauritii*, Rice Grain (*Oryza sativa* L.), Vermibiotechnology.

INTRODUCTION

Rice (*Oryza sativa* L.) is a group of the family Poaceae and a staple food for most of India (Singh and Bhartiya, 2020). Since it accounts for 20% of global daily calorie consumption, rice is essential for human nutrition (Buffon *et al*., 2021). Southeast Asian nations, including India, Bangladesh, and Nepal, largely depend on rice as a staple grain (Shome and Upadhyay, 2023). Almost 90% of the world's population takes rice (*Oryza sativa* L.), which fulfills a significant amount of their nutritional energy needs (Jana *et al*., 2020). India produces 116.42 million tonnes of rice per year on 43.79 million hectares of land (Anonymous, 2019). After West Bengal, Uttar Pradesh has become the second-largest producer of rice, with a total agricultural area of 5.75 million hectares, a yield of 15.54 million metric tonnes, and a productivity efficiency of 2.70 metric tonnes per hectare (Anonymous, 2019). It is predicted that by 2030, there will be more than 8 billion people on earth, necessitating a 40% increase in paddy production to meet increased nutritional requirements (Yadav *et al*., 2018; Jena *et al*., 2018). To enhance crop

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productivity and maximize profit to satisfy the expanding demand for rice, rice farmers have increasingly used synthetic fertilizers and other agrochemicals, which include heavy metals like arsenic, cadmium, chromium, and lead (Struger and Fletcher, 2007). The ability of rice crops to absorb heavy metals from both artificial and natural sources has raised concerns (Zulkafflee *et al*., 2022). It is very concerning since rice contains various carcinogenic metals, including Cd, Cu, Pb, and Hg (McLaughlin and Singh, 1999). Rice's significant harmful effects include a higher risk of cardiovascular disease, diabetes, maybe a stroke, obesity, and so on, while the detrimental consequences include liver problems, sore mouth, diarrhea, and modifications in blood pressure (Pateriya *et al*., 2021).

India produces 320 million metric tonnes of agricultural residues each year (Suthar *et al*., 2005). The management of organic waste, specifically the processing of animal manure on farms, was a significant issue. If biological wastes are not managed effectively, they pose a severe threat to society and have several negative consequences for both humans and the environment (Singh and Bhartiya, 2020). In India and other developing nations, using animal dung to improve agricultural yields is a prevalent practice. Worldwide, cattle farms produce a significant amount of manure, so it's important to use the right management approaches to minimize any detrimental effects on the environment (Burton and Turner, 2003). Vermicomposting, which converts animal wastes into compost through microbial activities, is considered one of the easiest methods for properly managing raw dairy waste and reducing environmental contamination problems caused by land disposal (Alwaneen, 2016). A group of metals and metalloids are referred to as "heavy metals" if their atomic weight is more than or comparable to 4000 kg/m^3 (Edelstein and Ben-Hur, 2018). As there is no suitable technique for eliminating toxins, contamination with toxic metals in the ecosystem is extremely detrimental to humans and other living organisms, even at low concentrations (Mekonen and Habte, 2022). Large concentrations of different heavy metals have the potential to degrade soil, reduce crop productivity, and result in inadequate agricultural output. Several studies on heavy metal contamination in soil have been conducted in recent years (Ma *et al*., 2022). Heavy metals in soil include a wide variety of physiologically detrimental and highly toxic elements, including cobalt (Co), cadmium (Cd), lead (Pb), chromium (Cr), and arsenic (As) (Abdullahi *et al*., 2021).

According to research, there is a strong association between the concentrations of potentially harmful compounds in rice and those found in the soil (Liu *et al*., 2005). The human body can be affected by cobalt in both positive and negative ways. Although large-scale environmental exposures to cobalt can be fatal, low amounts of the metal frequently have no negative consequences (Domingo, 1989). The human body does not require lead (Pb), and elevated Pb ingestion can have negative effects on circulatory, immune, endocrine, and neurological functions. Chromium in surplus is detrimental to plants because it adversely affects their biological processes and penetrates the food web when these plant components are consumed. For instance, the plant absorbs cadmium (Cd) from the soil through its roots. Lead (Pb) is a heavy metal that plants obtain from airborne dust (Malav *et al*., 2020). Research on the impacts of Pb, Cr, Hg, and Cd on rice, tomato, garlic, and maize reported shorter shoots, less effective nutrient absorption, reduced germination rates, and a decline in plant protein levels (Gautam *et al*., 2016). The environment is becoming more contaminated, and remediating the pollutants using physicochemical methods is quite expensive (Halder and Anirban, 2022). Consequently, it is necessary to use the vermicomposting process as a strategy to remove contaminants.

Through the mechanism of vermicomposting, organic matter is transformed into manure with the aid of earthworms. Vermicomposting mostly uses *Lampito mauritii*, *Drawida willsi*, *Dichogaster bolani*, and *Pheretima elongata*, in contrast to such desirable species (Kaviraj and Sharma, 2003). Vermicomposting is a phenomenal method for disinfecting contaminated soils because it not only assists in minimizing waste but also enriches the soil. Through bioaccumulation and transformation into non-toxic states, the vermicomposting practice is used to detoxify heavy metal-contaminated soil (Jain *et al*., 2004). Due to their continual reproduction, efficient cocoon formation, quick development, and great hatching ability, the Indian earthworm species *Perionyx excavatus*, *Dichogaster modigilani*, and *Lampito mauritii* are appropriate for vermicomposting (Bhattacharya and Chattopadhyay, 2002). Earthworms may accumulate toxic metals in the cells of their yellow tissue and have the capacity to survive and function well in surroundings that are contaminated with metals (Lukkari *et al*., 2004). Earthworms are a great biological indicator for assessing soil contamination (Singh and Fatima, 2022). The bioaccumulation levels of different metals varied, but there was a substantial link between the heavy metal concentration in soil and earthworms. This could be due to the different metabolic activities that metals have on earthworms (Lukkari *et al*., 2006). A higher concentration of toxic heavy metals, including Co, Cr, Pb, Cu, Cd, and Zn, can be absorbed by earthworms (Vinoth *et al*., 2014). A species of earthworm referred to as *Lampito mauritii* can serve as a more accurate indicator of metal toxicity due to bioaccumulation since it has a higher level of heavy metals in its internal organs (Hobbelen *et al*., 2006). Maity *et al*. (2008) investigated the effects of the earthworm *Lampito mauritii* on the mobility of Pb^{2+} and Zn^{2+} in the soil and the possibilities for the soil's breakdown in the presence of these metals, and they suggested employing *Lampito mauritii* to rehabilitate metal-contaminated soil.

In the present investigation, the amounts of several heavy metals, particularly cobalt, chromium, and lead, were analyzed in earthworm *Lampito mauritii* body tissue and rice grains, as well as in vermicomposts produced from various mixtures of animal (buffalo, cow, and goat) dungs in both the initial feed mixture and the final vermicompost during vermicomposting by earthworm *Lampito mauritii*.

MATERIALS AND METHODS

Animal Waste Collection

Animal waste (cow, buffalo, and goat dung) was obtained from several farmhouses in Gorakhpur.

Collection of Earthworms

In the Vermiculture Research Laboratory of the Department of Zoology at D.D.U. Gorakhpur University, Gorakhpur, earthworms of the anecic species *Lampito mauritii* were cultured. The earthworms were raised in a laboratory environment with stable aeration and temperature ranges between 20°C and 30°C. For appropriate development and survival of the earthworms, the moisture was maintained between 50 and 70% RH.

Procedures for Vermicomposting

Utilizing the Bhartiya and Singh (2012) method, vermicomposting was performed on the surface of the cemented earth. Various mixtures of animal waste in the ratios of 1:1, 1:2, and 1:3 with municipal solid wastes and kitchen wastes were performed. Each vermibed was 3 m × 1 m × 9 cm in size. Each bed was soaked and inoculated with 1 kilogram of cultured *Lampito mauritii* after the vermibed construction process. The vermibeds were watered regularly for up to 40 or 50 days while being covered in jute packets to sustain the appropriate amount of moisture. During three weeks, the assortment of beds was manually turned up at intervals of one week. Vermicompost looks like granular tea develops on the surface of the bed after 50 to 60 days. The prepared vermicomposts and inoculated earthworms were used for experiments.

Crop Cultivation Experimental Setup

At the research area of the Zoology Department of D.D.U. Gorakhpur University, Gorakhpur, six beds (1 m x 1 m) for rice crops were chosen. On each bed of soil, one kilogram of vermicompost made from various animal waste was properly combined. Afterward, 50 mature earthworms (*Lampito mauritii*) were inoculated into each bed. Apart from the use of pesticides and fertilizers, general agro-practices were used in the field. *Oryza sativa* (BPT-5204) was raised because of its commercial significance as a necessary diet for human health.

Analysis of Heavy Metals

Heavy metal assessment in the initial feed combination, vermicompost, soil, and crop grains

The method of Maboeta (2003) was employed to determine the level of heavy metals (Co, Cr, and Pb) in the initial feed combination, vermicompost, soil (before earthworm inoculation and during crop harvest), as well as in rice (*Oryza sativa*) (BPT-5204) seed grains. To prepare the necessary samples, 1 gram of the initial feed combination, vermicompost, and soil (before inoculation and after crop harvest) were collected. These samples were placed on a hot plate and heated for four hours between 90 and 100 °C while being treated for digestion with an excessive amount of nitric acid (1:1). The digestion was carefully controlled to avoid the sample drying out. After digestion, each sample was filtered using Whatman No. 41 filter paper and placed in a 100-ml flask before being introduced to a flame atomic absorption machine (Shimadzu, Model AA-7000) to detect the concentration of heavy metals.

Examination of the earthworm's body tissue for heavy metals

Using the methodologies of Katz and Jennies (1983) and Bhartiya and Singh (2012), the heavy metals in the earthworm body tissue were digested. Earthworms were burned to ash after being dried, smashed, and heated. In a 15-ml test tube, 55% nitric acid was then added after the ash had been put inside. Twelve hours were devoted to letting this solution remain at room temperature. Following this, the sample was heated for two hours at a temperature between 40 and 60 °C, and the solution was chilled down to room temperature. 1 ml of 70% perchloric acid was included in the sample after it was once again heated to 90-95 °C. Before adding 5 ml of distilled water, the sample was set aside to cool down. Samples were heated to 130°C once more until white vapors began to release. The sample was eventually chilled before being micro-filtered. After being filtered through Whatman No. 41 filter paper and transferred to 100-ml flasks in glass vials, the solution was analyzed by flame atomic absorption.

Statistical Analysis

Data are presented as the mean ± SD of 6 replicates for all variables. A students 't'-test was used to evaluate whether there was a significant ($P < 0.05$) variance between the initial and final vermicompost, the earthworm *Lampito mauritii* body during inoculation and after vermicompost, the harvesting of rice (*Oryza sativa*) grain before and after the experiment, as well as in the earthworm body (Sokal and Rohlf, 1973).

RESULTS AND DISCUSSION

Once the earthworm *Lampito mauritii* was inoculated, the concentrations of the heavy metals cobalt (Co), chromium (Cr), and lead (Pb) in the final vermicompost were much lower than they had been in the initial feed mixture (Table 1). Cobalt (Co), chromium (Cr), and lead (Pb) concentrations all considerably decreased when combined with buffalo dung, exhibiting maximum decreases of 70.20%, 64.20%, and 72.41%, respectively (Figure 1-3). Table 2 illustrates the accumulation of heavy metals (cobalt, chromium, and lead) in earthworm (*Lampito mauritii*) tissues following vermicomposting of several animal dungs. Cobalt (Co) and chromium (Cr) concentrations considerably increased, with the highest increases of 43.75% and 9.40% in vermibed buffalo dung and cow dung, respectively (Figures 4 and 5).

Table 1. Cobalt, Chromium, and Lead concentrations (mg/kg) in various combinations of animal dung in the initial feed mixture and final vermicompost by the earthworm *Lampito mauritii*.

Particulars	Heavy Metals (mg/kg)								
	Cobalt (Co)			Chromium (Cr)			Lead (Pb)		
	IFM	FV	% Decrease	IFM	FV	% Decrease	IFM	FV	% Decrease
Buffalo Dung (BD)	1.728 ± 0.005	0.515 ± 0.004*	70.20	1.204 ± 0.002	0.431 ± 0.003*	64.20	2.976 ± 0.004	0.821 ± 0.005*	72.41
Cow Dung (CD)	0.863 ± 0.003	0.351 ± 0.003*	59.32	1.345 ± 0.004	0.603 ± 0.004*	55.16	1.151 ± 0.002	0.407 ± 0.003*	64.63
Goat Dung (GD)	0.263 ± 0.003	0.120 ± 0.002*	54.37	0.833 ± 0.002	0.419 ± 0.002*	49.70	0.707 ± 0.004	0.325 ± 0.003*	54.03

IFM= Initial feed mixture, FV= Final vermicompost, BD= Buffalo dung, CD= Cow dung, and GD= Goat dung
Each value is the Mean ± SD of six replicates.
Significant P<0.05 't' test between initial feed mixture and final vermicompost.

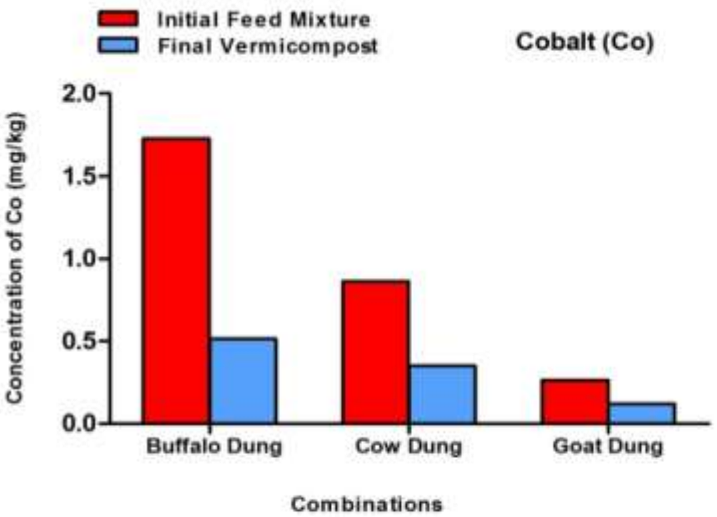

Figure 1. Concentration of Cobalt (Co) (mg/kg) in different combinations of animal dung within the initial feed mixture and final vermicompost.

Table 2. Cobalt, Chromium, and Lead concentrations (mg/kg) in the earthworm *Lampito mauritii* body following vermicomposting of various animal dungs.

Particulars	Heavy Metals (mg/kg)								
	Cobalt (Co)			Chromium (Cr)			Lead (Pb)		
	LmBC	LmBFV	% Increase	LmBC	LmBFV	% Increase	LmBC	LmBFV	% Increase
Buffalo Dung (BD)	1.185 ± 0.003	2.107 ± 0.004*	43.75	8.123 ± 0.004	8.912 ±0.002*	8.85	3.810 ± 0.002	5.515 ± 0.005*	30.91
Cow Dung (CD)	1.185 ± 0.003	1.421 ± 0.003*	16.60	8.123 ± 0.004	8.966 ± 0.001*	9.40	3.810 ± 0.002	4.617 ± 0.003*	17.47
Goat Dung (GD)	1.185 ± 0.003	1.357 ± 0.004*	12.67	8.123 ± 0.004	8.412 ± 0.003*	3.43	3.810 ± 0.002	4.111 ± 0.002*	7.32

BD= Buffalo dung, CD= Cow dung, and GD= Goat dung
LmBC= earthworm *Lampito mauritii* body (control), LmBFV= earthworm *Lampito mauritii* body in final vermicompost.
Each value is the Mean ± SD of six replicates.
Significant P<0.05't' test between earthworm body (control) and earthworm body after prepared final vermicompost.

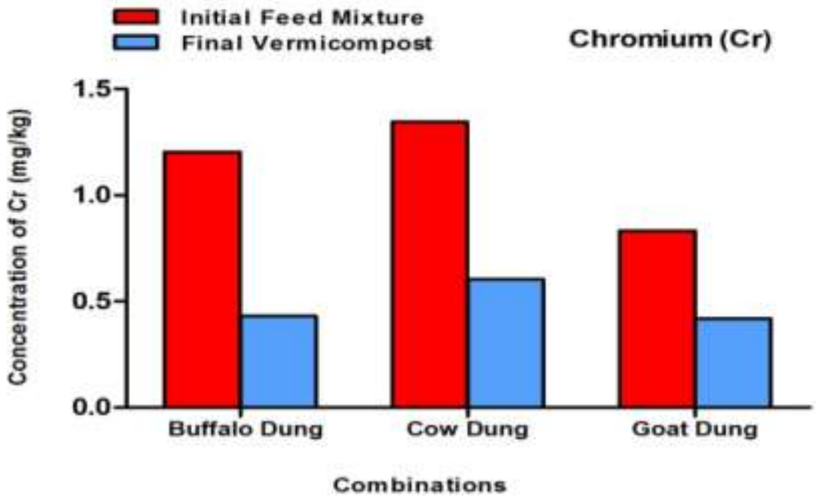

Figure 2. Concentration of Chromium (Cr) (mg/kg) in different combinations of animal dung within the initial feed mixture and final vermicompost.

Whereas, the 30.91% increased Pb concentration was observed in *Lampito mauritii* body in a combination of buffalo dung during vermicomposting (Figure 6). Once the rice crop (*Oryza sativa* L.) was harvested, the concentrations of cobalt, chromium, and lead changed considerably (P < 0.05 t-test) in the soil and soil that had been inoculated with earthworms (*Lampito mauritii*) in various combinations of vermicompost from different animal dungs (Table 3-5). When cow dung vermicompost was added to the soil and earthworm *Lampito mauritii* was inserted, the maximum concentration of cobalt (Co) was observed to decline (44.03% decreased, from 2.891 ± 0.012 to 1.618 ± 0.009 mg/kg). After harvesting rice plant grain from the soil, the concentration of cobalt was also determined. The mixture of soil and goat dung vermicompost resulted in the greatest decline in cobalt (Co) percent (100%, 0.284 ± 0.007 to below the detection limit).When soil and cow dung vermicompost were mixed, the greatest concentration of cobalt (Co) was significantly increased in the inoculated earthworm *Lampito mauritii* tissues (29.91%, 6.180 ± 0.008 to 8.818 ± 0.005 mg/kg) (Table 3, Figure 7).

The data in Table 4 demonstrated that the combination of soil and cow dung vermicompost that had been inoculated with the earthworm *Lampito mauritii* had the largest reduction in the level of chromium (Cr) (17.08%, decreased from 10.325 ± 0.010 to 8.561 ± 0.007 mg/kg). Chromium (Cr) concentrations have also been observed in rice grains. The highest reduction in chromium content (89.16%, from 1.357 ± 0.004 to 0.147 ± 0.006 mg/kg) was obtained when the soil was combined with vermicompost prepared from goat dung inoculated with earthworm *Lampito mauritii*. When the soil was mixed with buffalo dung vermicompost, the maximum concentration of Cr was elevated in inoculated earthworm *Lampito mauritii* tissues (18.05%, 5.910 ± 0.010 to 7.212 ± 0.007 mg/kg)(Figure 8).

Table 5 and Figure 9 show that the lead (Pb) contents in the soil, soil with vermicompost of different animal dungs, and soil inoculated with earthworm *Lampito mauritii* in their different combinations were significantly (P < 0.05 t-test) decreased after the harvest of a rice crop. The highest Pb concentration was reduced by 32.46% when the soil was mixed with goat dung vermicompost and inoculated with earthworm *Lampito mauritii* (from 4.959 ± 0.010 mg/kg to 3.349 ± 0.006 mg/kg). Lead concentrations were also reported in rice grain. The highest reduction in Pb level (89.20%, from 0.250 ± 0.012 to 0.027 ± 0.005 mg/kg) was obtained using soil and vermicompost prepared from goat dung inoculated with an earthworm (*Lampito mauritii*). Whereas in comparison, the highest lead (Pb) concentration in the inoculated earthworm, *Lampito mauritii*, increased significantly when soil and vermicompost prepared from buffalo dung were combined (30.22%, 8.061 ± 0.004 to 11.553 ± 0.011 mg/kg).

Vermitechnology is the research that uses earthworms to boost crop production, ease environmental problems, and manage other societal problems (Dada *et al*., 2015; Dada *et al*., 2016). Due to its superior metal uptake capabilities, vermicompost is used as a bioremediation technique to remove metallic ions such as lead (Pb), cadmium (Cd), nickel (Ni), and chromium (Cr) from organic residues (He *et al*., 2017; Zhu *et al*., 2017). Consequently, manure made of organic substances (compost, animal dung, sewage sludge, etc.) can contain heavy metals (Samarajeewa, 2022). When earthworms are feeding, buffalo manure serves as an excellent feed. Depending on the concentration differential between pollutants in the soil-saturated region and the earthworm's body fluid, earthworms' instant physiological reactions to pollutant remediation include the digestion of harmful compounds from the soil while transiting through their intestines. Vermicompost was implemented as an adsorption medium to remediate wastewater contaminated with nickel, chromium, cobalt, and lead (Pb).

Table 3. Cobalt (Co) concentration (mg/kg) in experimental soil, rice grain, and earthworm body before and after rice crop harvesting when the soil was combined with vermicompost of various animal dungs and inoculated with the earthworm *Lampito mauritii*.

Particulars	Concentration of Cobalt (mg/kg)								
	In experimental soil			In rice crop grains			In earthworm body		
	Before sowing	After harvesting	% Decrease	Before sowing	After harvesting	% Decrease	Before sowing	After harvesting	% Increase
Soil control	2.861 ± 0.010	2.532 ± 0.007	11.50	0.284 ± 0.007	0.110 ± 0.006*	61.27	-	-	-
Soil+VC of BD+Lm	2.878 ± 0.006*	1.616 ± 0.005*	43.85	0.284 ± 0.007	0.047 ± 0.007*	83.45	6.180 ± 0.008	7.715 ± 0.008*	19.89
Soil+VC of CD+Lm	2.891 ± 0.012*	1.618 ± 0.009*	44.03	0.284 ± 0.007	0.035 ± 0.005*	87.67	6.180 ± 0.008	8.818 ± 0.005*	29.91
Soil+VC of GD +Lm	2.882 ± 0.011*	1.622 ± 0.012*	43.71	0.284 ± 0.007	BDL	100.00	6.180 ± 0.008	8.739 ± 0.011*	29.28

VC = vermicompost, BD = buffalo dung, CD = cow dung, GD = goat dung, Lm = earthworm *Lampito mauritii*, BDL = Below Detection Limit

Values are expressed as Mean ± SD (six replicates).
*Differences between mean values of concentration of heavy metals in the soil, soil with different animal dungs vermicompost, earthworm body as well as before sowing and after harvesting of rice crop grain are significant at $P<0.05$ (t-test).

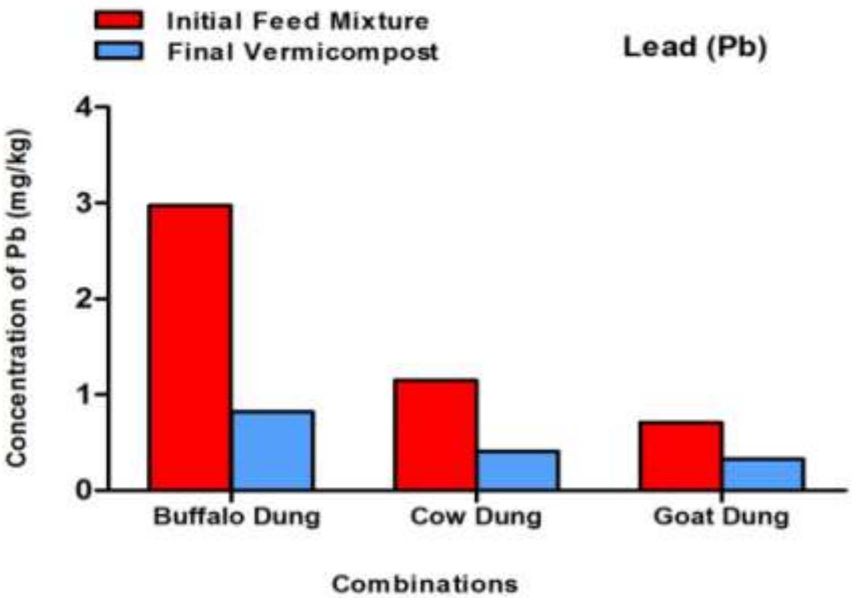

Figure 3. Concentration of Lead (Pb) (mg/kg) in different combinations of animal dung within the initial feed mixture and final vermicompost.

Table 4. Chromium (Cr) concentration (mg/kg) in experimental soil, rice grain, and earthworm body before and after rice crop harvesting when the soil was combined with vermicompost of various animal dungs and inoculated with the earthworm *Lampito mauritii*.

Particulars	Concentration of Chromium (mg/kg)								
	In experimental soil			In rice crop grains			In earthworm body		
	Before sowing	After harvesting	% Decrease	Before sowing	After harvesting	% Decrease	Before sowing	After harvesting	% Increase
Soil control	10.305 ± 0.008	9.466 ± 0.012	8.14	1.357 ± 0.004	0.849 ± 0.009*	37.43	-	-	-
Soil+VC of BD+Lm	10.330 ± 0.011*	8.569 ± 0.005*	17.04	1.357 ± 0.004	0.718 ± 0.012*	47.09	5.910 ± 0.010	7.212 ± 0.007*	18.05
Soil+VC of CD+Lm	10.325 ± 0.010*	8.561 ± 0.007*	17.08	1.357 ± 0.004	0.160 ± 0.010*	88.20	5.910 ± 0.010	6.178 ± 0.011*	4.33
Soil+VC of GD +Lm	10.320 ± 0.006*	8.563 ± 0.009*	17.02	1.357 ± 0.004	0.147 ± 0.006*	89.16	5.910 ± 0.010	6.847 ± 0.012*	13.68

VC = vermicompost, BD = buffalo dung, CD = cow dung, GD = goat dung, Lm = earthworm *Lampito mauritii*

Values are expressed as Mean ± SD (six replicates).
*Differences between mean values of concentration of heavy metals in the soil, soil with different animal dungs vermicompost, earthworm body as well as before sowing and after harvesting of rice crop grain are significant at P<0.05 (t-test).

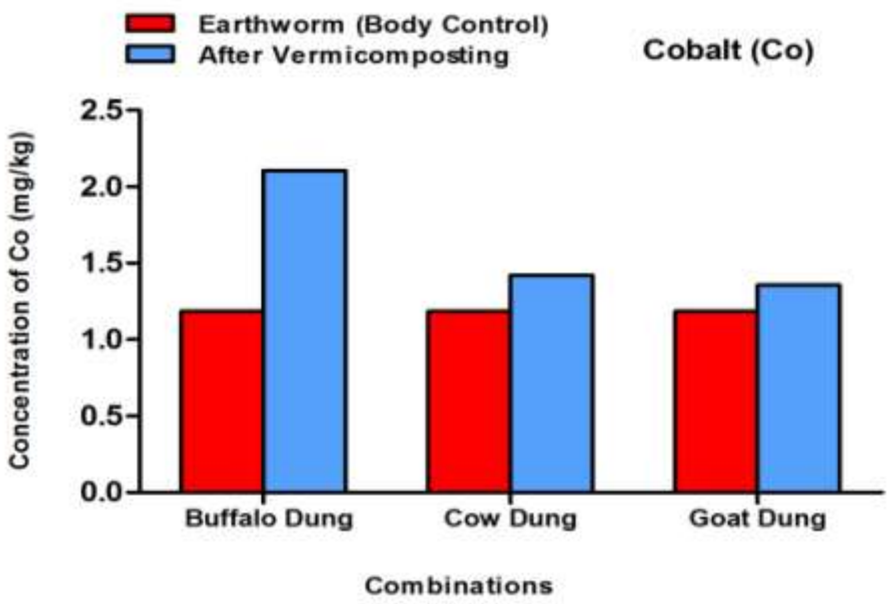

Figure 4. Concentration of Cobalt (Co) (mg/kg) in earthworm *Lampito mauritii* body before inoculation in vermibeds and after vermicomposting of different combinations of animal dungs.

Table 5. Lead (Pb) concentration (mg/kg) in experimental soil, rice grain, and earthworm body before and after rice crop harvesting when the soil was combined with vermicompost of various animal dungs and inoculated with the earthworm *Lampito mauritii*.

Particulars	Concentration of Lead (mg/kg)								
	In experimental soil			In rice crop grains			In earthworm body		
	Before sowing	After harvesting	% Decrease	Before Sowing	After harvesting	% Decrease	Before sowing	After harvesting	% Increase
Soil control	4.953 ± 0.003	4.622 ± 0.011	6.68	0.250 ± 0.012	0.389 ± 0.008*	-55.60	-	-	-
Soil+VC of BD+Lm	5.140 ± 0.007*	3.545 ± 0.009*	31.03	0.250 ± 0.012	0.041 ± 0.006*	83.60	8.061 ± 0.004	11.553 ± 0.011*	30.22
Soil+VC of CD+Lm	5.024 ± 0.003*	3.470 ± 0.005*	30.93	0.250 ± 0.012	0.065 ± 0.007*	74.01	8.061 ± 0.004	10.226 ± 0.007*	21.17
Soil+VC of GD +Lm	4.959 ± 0.010*	3.349 ± 0.006*	32.46	0.250 ± 0.012	0.027 ± 0.005*	89.20	8.061 ± 0.004	9.179 ± 0.010*	12.17

VC = vermicompost, BD = buffalo dung, CD = cow dung, GD =goat dung, Lm = earthworm *Lampito mauritii*

Values are expressed as Mean ± SD (six replicates).

*Differences between mean values of concentration of heavy metals in the soil, soil with different animal dungs vermicompost, earthworm body as well as before sowing and after harvesting of rice crop grain are significant at $P<0.05$ (t-test).

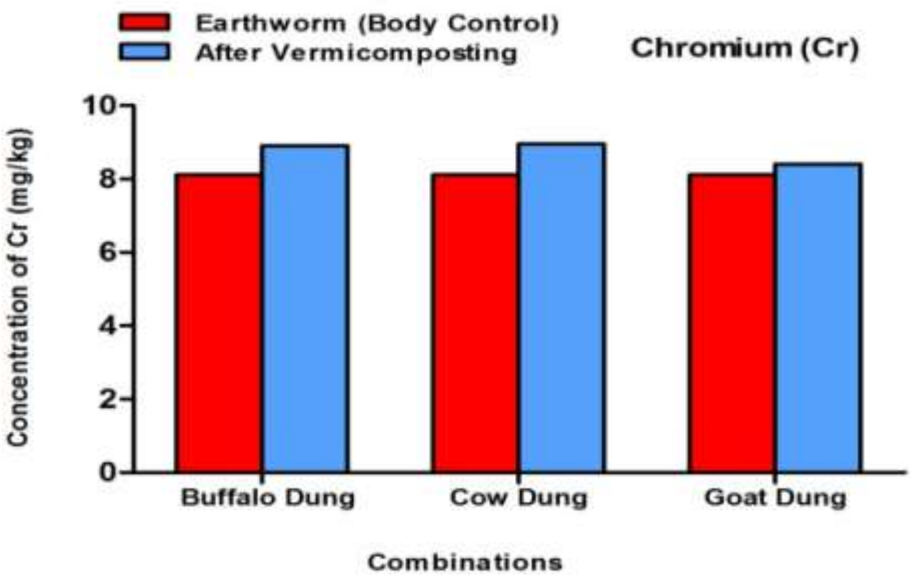

Figure 5. Concentration of Chromium (Cr) (mg/kg) in earthworm *Lampito mauritii* body before inoculation in vermibeds and after vermicomposting of different combinations of animal dungs.

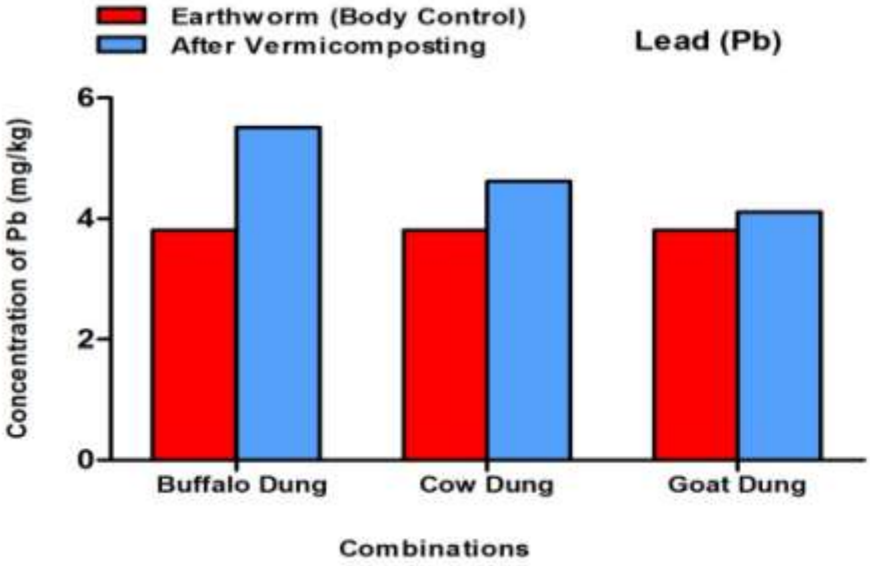

Figure 6. Concentration of Lead (Pb) (mg/kg) in earthworm *Lampito mauritii* body before inoculation in vermibeds and after vermicomposting of different combinations of animal dungs.

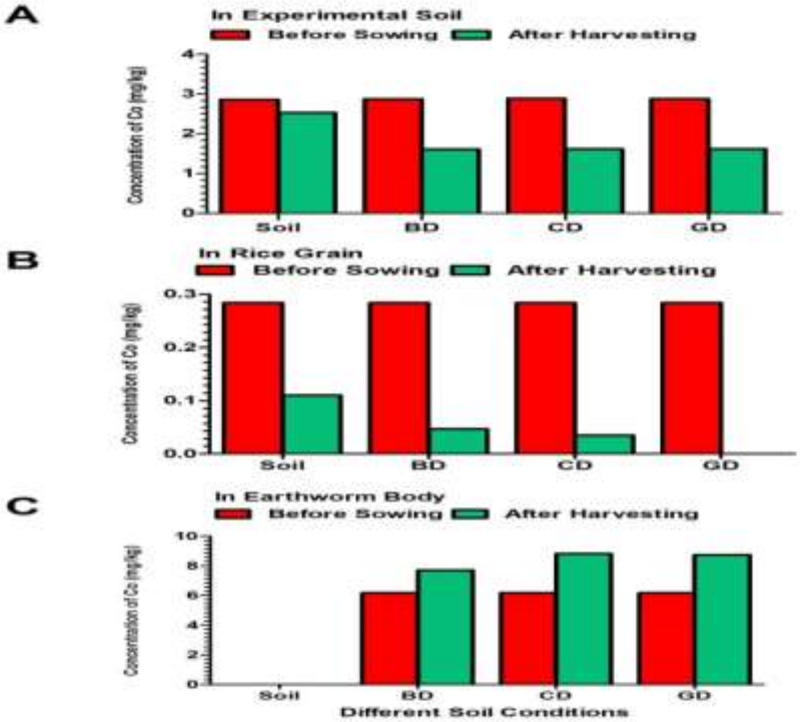

Figure 7. Cobalt (Co) concentration (mg/kg) in experimental soil (A), rice grain (B), and earthworm body tissue (C) before and after the rice crop was harvested. The soil had been mixed with vermicompost made from various animal dungs (BD=buffalo dung, CD=cow dung, GD=goat dung), and an earthworm called *Lampito mauritii* had been inoculated.

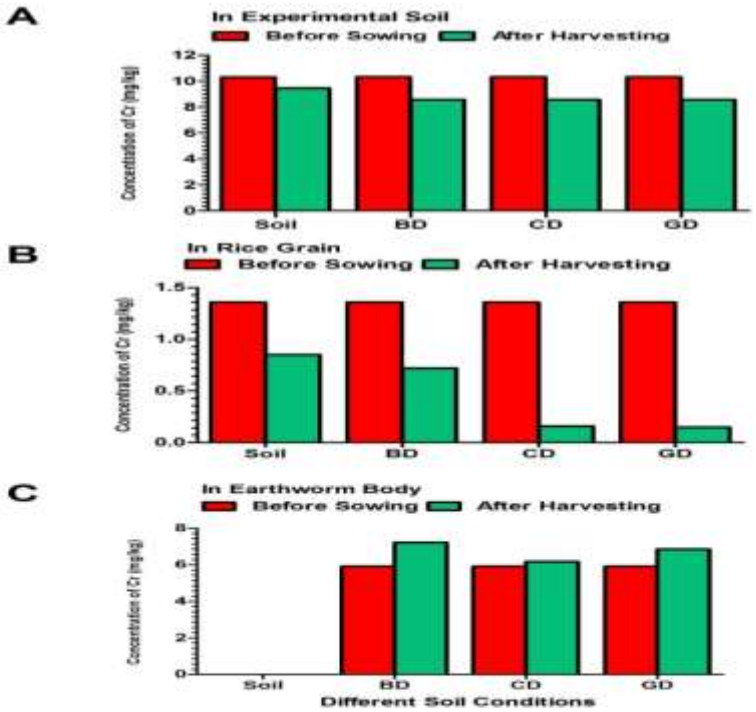

Figure 8. Chromium (Cr) concentration (mg/kg) in experimental soil (A), rice grain (B), and earthworm body tissue (C) before and after the rice crop was harvested. The soil had been mixed with vermicompost made from various animal dungs (BD=buffalo dung, CD=cow dung, GD=goat dung), and an earthworm called *Lampito mauritii* had been inoculated.

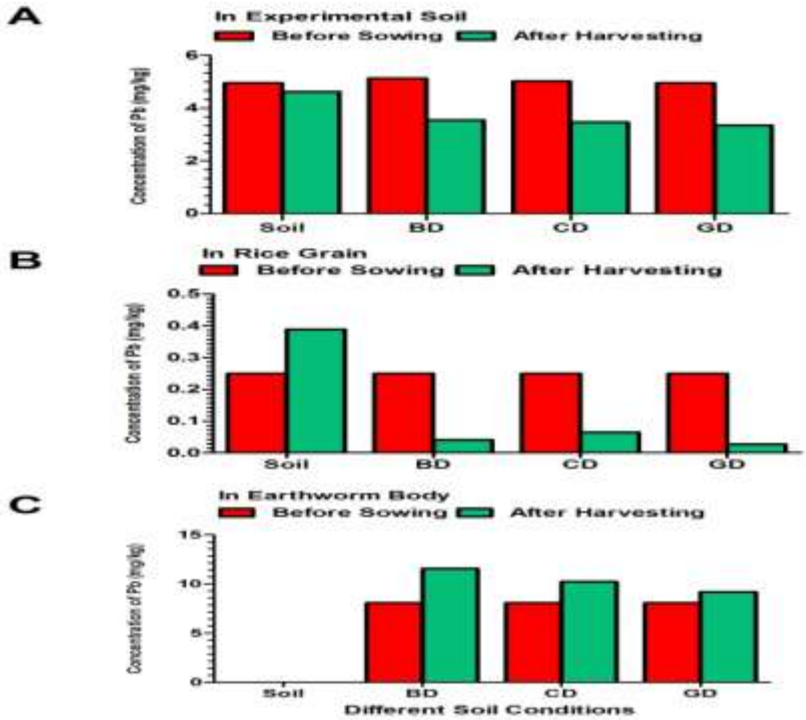

Figure 9. Lead (Pb) concentration (mg/kg) in experimental soil (A), rice grain (B), and earthworm body tissue (C) before and after the rice crop was harvested. The soil had been mixed with vermicompost made from various animal dungs (BD=buffalo dung, CD=cow dung, GD=goat dung), and an earthworm called *Lampito mauritii* had been inoculated.

Heavy metal contamination of the environment has grown to be a substantial danger to human health on a global scale as a result of humans' dependence on industrialization (Donald *et al*., 2022). Many factors, including plant species, soil conditions, crop preference, and metal tolerance, determine the degree of heavy metals that accumulate in crops (Aktaruzzaman *et al*., 2014). Heavy metals are highly antagonistic with plants for adsorption and subsequent uptake, which hinders the uptake of other important metals by plants. This is in addition to their enhanced mobility and leaching capability in low-pH soil (Campillo-Cora *et al*., 2020). Because of soil contamination, food crops could potentially expose individuals to trace substances that are not required. Rice, the principal nutritional grain in many Asian nations, is now prone to heavy metal poisoning as a consequence of the widespread use of metal-enriched agrochemicals, excessive water consumption, and sewage sludge (Fangmin *et al*., 2006). The following heavy metals were found to be transferred in the sequence mentioned as per soil to rice: Zinc is more abundant than copper, chromium, cobalt, cadmium, lead, iron, arsenic, and nickel. Major public health considerations surround the accumulation of toxic heavy metals in rice (Hasan *et al*., 2022).

Significant concentrations of cobalt are highly toxic to crops. Cobalt (Co) causes pale-colored leaves, discolored veins, leaf loss, and iron deficiencies in plants (Hu *et al*., 2021). When cultivated in soils with cobalt concentrations between 25 and 50 mg/kg, rice crops (*Oryza sativa*) would exhibit hazardous effects (Kitagishi and Yamane, 1981). The food chain and human health will be affected because the concentration of chromium absorbed in rice plant grains exceeds the allowable range (Majhi and Samantaray, 2020). When soil contains a significant amount of chromium, rice cannot grow well and produces fewer grains (Qiu *et al*., 2010). Although the majority of the chromium in rice has been deposited in the roots, a substantial amount can still be transported into tissues, such as grains, posing a health concern for humans (Qiu *et al*., 2011). By using vermicompost and farm yard manure, the toxicity of chromium on rice grains may be decreased more efficiently (Koka *et al*., 2019). Elevated lead (Pb) levels in the plants decrease rice biomass, inhibit photosynthesis and pigment formation, obstruct mineral absorption, and disrupt rice respiratory functions (Ashraf *et al*., 2015). Muktamar *et al*. (2023) report that vermicompost might effectively immobilize lead and cadmium at a value of 30 Mg ha^{-1} in polluted soils.

Earthworm survivability in contaminated soil conditions depends on the induction of metallothionein, which is necessary for metal remediation. Earthworms can not only accumulate and ingest heavy metals from the soil but also modify the heavy metal composition of the final product (He *et al*., 2016). Earthworms have distinctive chloragogenous cells in their posterior area that may absorb and deposit a variety of toxic metal ions. Soobhany *et al*. (2015) reported that the following relationships existed between the reduction in bioaccumulation parameters and the vermiaccumulation of several heavy metals: Ni, Cu, Co, Cr, and Zn occur after Cd. *Lumbricus rubellus* was used for 90 days to rehabilitate disturbed soil in Malaysia that was contaminated by toxic elements (Cu, Mn, Pb, Fe, Cr, Ni, Zn, and As) (Cheng-Kim *et al*., 2016). According to Wang *et al*. (2018), Cd, Cu, Pb, and Zn contamination was found in areas (soil systems) close to mining sites. Earthworm species of the *Amynthas heterochaetus*, *Metaphire californica*, *Amynthas pecteniferus*, and *Amynthas homochaetus*, as well as others, were common in these areas. These earthworm species were capable of rehabilitating soils that had been contaminated by metals and accumulated significant amounts of Cd, Cu, Pb, and Zn. According to Singh and Bhartiya (2020), using vermicompost and inoculating rice field soil with *Eisenia fetida* can help eliminate potentially hazardous metals from rice grains. By sequestering the potentially toxic metals, metallothioneins assist *Lampito mauritii* in surviving in soil that has been polluted with metal ions (Maity *et al*., 2011). *Lampito mauritii* can maintain significant amounts of metals, including toxic metals, in their tissues without undergoing any negative physiological consequences.

Heavy metal concentrations, including cobalt (Co), chromium (Cr), lead (Pb), nickel (Ni), and cadmium (Cd), are significantly reduced in diverse compositions of animal dung with municipal solid waste after vermicomposting by the earthworm *Lampito mauritii* (Fatima and Singh, 2023). Heavy metal concentrations, including Cd, Cu, Cr, Pb, and Zn that could be removed with DTPA, were higher in the interior tissues of *Lampito mauritii* and *Drawida sulcata* (Yuvaraj *et al*., 2021). The current investigation shows that heavy metal concentrations (Co, Cr, and Pb) in the earthworm *Lampito mauritii* body from an agricultural field of rice (*Oryza sativa*) were significantly ($P < 0.05$) enhanced due to metal deposition in their body tissue. The bioaccumulation of metals in the *Lampito mauritii* body tissues caused these highly toxic metals to be eliminated from both the cultivated field soil and the final vermicompost.

CONCLUSION

The earthworm *Lampito mauritii* was a suitable species for bioaccumulating heavy metals from various animal (buffalo, cow, and goat) dung during the creation of vermicompost as well as for reducing harmful metal concentrations from rice grains. As a consequence, it significantly decreased heavy metals in produced rice (*Oryza sativa*) grains compared to before sowing in soil rice seed grains and increased metal levels in its body during vermic-activity. Hence, we can conclude that vermibiotechnology is an effective way to remove heavy metals from soil and other organic wastes while safeguarding both the health of people and the environment.

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