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"Earthworm-Assisted Bioremediation of Lead-Contaminated Municipal Sludge and Its Impact on Soil Health"

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Abstract

Lead contamination in soil and municipal sludge poses serious environmental and public health challenges, necessitating sustainable remediation strategies. This study investigated the potential of Eisenia fetida in the bioremediation of lead-contaminated municipal sludge and its subsequent application for soil improvement. Four experimental treatments (A, B, C, and D) were established using different proportions of municipal sludge and cow dung, including positive and negative controls. Key physicochemical parameters, including pH, electrical conductivity (EC), total organic carbon (TOC), total nitrogen (TN), available phosphorus (P), C:N ratio, and lead concentration (mg/kg), were analyzed over a 60-day vermicomposting period.The results revealed a significant reduction in lead concentration across all treatments, with the highest reduction observed in sludge-amended treatments. Vermicomposting also led to a decrease in pH (8.0 to 5.07–7.43), EC, TOC, and C:N ratio, indicating enhanced stabilization and maturity of the compost. In contrast, essential nutrients such as nitrogen and phosphorus increased significantly, improving the overall quality of the vermicompost.

The findings demonstrate that earthworm-assisted vermicomposting is an effective and eco-friendly approach for the stabilization of heavy metals and enhancement of nutrient content in municipal sludge. This technique offers a sustainable solution for waste management and soil fertility improvement in agricultural applications.

***Keywords**:* Vermicomposting, Lead contamination, Municipal sludge, *Eisenia fetida*, Bioremediation, Soil health, Heavy metal, Organic waste management

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I. Introduction

Lead contamination in soil and municipal sludge poses significant risks to environmental and human health. Major sources of lead pollution include industrial emissions, mining activities, and improper disposal of waste materials such as batteries and electronic devices. As a non-biodegradable heavy metal, lead accumulates in soils and persists for long periods, leading to its entry into plants, water sources, and ultimately the food chain (Li *et al*., 2018). This widespread contamination results in increased exposure risks for both humans and wildlife. Human exposure to lead-contaminated soil occurs through multiple pathways, including inhalation of dust particles, ingestion of contaminated food, and direct skin contact. Chronic exposure to lead is associated with severe health effects such as neurological disorders, developmental issues in children, cardiovascular diseases, and renal dysfunction in adults. Therefore, remediation of lead-contaminated soils and sludge is essential to reduce these risks and protect ecological systems (Wani *et al*., 2015).

Conventional remediation techniques, including excavation and chemical treatments, are often expensive and environmentally disruptive. Consequently, there is growing interest in sustainable and eco-friendly alternatives such as bioremediation. Among these, the use of earthworms for treating contaminated sludge has gained considerable attention due to their ability to improve soil structure, enhance nutrient cycling, and influence contaminant dynamics through their feeding and burrowing activities (Song *et al*., 2017; Lacalle *et al*., 2020; Kumar *et al*., 2025).Vermiremediation, a specialized form of bioremediation involving earthworms, has emerged as a promising approach for improving soil quality and reducing contaminant levels (Shen *et al*., 2026). Earthworms facilitate the decomposition of organic matter, increase microbial activity, and enhance nutrient availability. Additionally, they may alter the mobility and bioavailability of heavy metals such as lead (Dada *et al*., 2021; Li *et al*., 2011).

The present study aims to evaluate the effect of earthworm (*Eisenia fetida*) amendment on the physicochemical properties of lead-contaminated municipal sludge. Key parameters analyzed include lead concentration, electrical conductivity, pH, total organic carbon, and nutrient content. The study further seeks to assess the potential of earthworm-assisted bioremediation as a sustainable and effective strategy for improving sludge quality and reducing heavy metal contamination.

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II. Materials and Methods

A. Sample Collection

Collection of Lead-Contaminated Sludge Samples, earthworms and cow dung: Sludge samples were collected from the collection site i.e. 8MLD Sewage treatment plant, Brahmapuri, Jaipur. After the collection, sludge samples were dried in natural habitat and conditioned for further treatment. Earthworms and cow dung were collected from Rajasthan Go Seva Sangh, Tonk road, Durgapura, Jaipur. Fresh cow dung was treated with cold water for one month so that the greenhouse gases present in cow dung, which are detrimental to earthworms, can be removed.

Fig.1Municipal sludge sample collected from 8 MLD sewage treatment plant, Brahmapuri, Jaipur

B. Experimental Design

1. Design of Experimental and Control Groups

The study was designed to evaluate the impact of earthworm amendment on lead-contaminated municipal sludge by comparing experimental groups (sludge amended with earthworms) to a control group (Positive and negative control).

- The experimental groups consisted of lead-contaminated municipal sludge samples amended with earthworms of the species *Eisenia fetida*.
- Each experimental group received a same density of earthworms (20 earthwoms in each group) to assess the effect of earthworm population size on the remediation process.

- *Eisenia fetida* were acclimatized to the municipal sludge conditions before the experiment to ensure their health and effectiveness.

Description of the Earthworm Species Used

For this study, the earthworm species *Eisenia fetida* chosen was for the amendment of lead-contaminated. This species is commonly known as the red wiggler or red worm and is a popular choice for bioremediation due to its adaptability to various organic matter conditions and high tolerance for heavy metal concentrations (Yadav *et al*., 2023). *Eisenia fetida* is known for its efficient burrowing and feeding activities, which can enhance soil structure and nutrient cycling.

Method of Introducing Earthworms to the Sludge

The introduction of earthworms to the lead-contaminated sludge was conducted in a controlled manner to ensure consistency across experimental groups:

- **Acclimatization**: Prior to introduction, earthworms were acclimatized to the sludge by gradually exposing them to lead-contaminated samples. This step was necessary to allow the earthworms to adjust to the conditions and minimize stress during the experiment.

- **Introduction Process**: Earthworms were introduced to the sludge samples at same densities to create different experimental groups.

- **Placement**: Earthworms were placed evenly across the surface of the sludge samples to facilitate consistent burrowing and feeding throughout the experiment.

- **Containment**: The sludge samples, along with the earthworms, were contained to prevent the earthworms from escaping and to maintain consistent conditions across groups.

- **Monitoring**: The earthworms' health and activity were monitored regularly throughout the study to ensure their continued survival and effective contribution to the remediation process.

By carefully managing the introduction of earthworms to the lead-contaminated sludge, the study aimed to assess the potential benefits of earthworm-assisted bioremediation and its impact on the physiochemical properties of the soil.

Experimental Groups:

Group A: In group A, sludge and cow dung were mixed in weight ratio 100:100.

Group B: In group B, sludge and cow dung mixed were in the weight ratio50:150.

Control Group:

The control group was set up in the same type of containment as the experimental groups to maintain consistency in environmental conditions.

Group C: In group C, only cow dung was used as a positive control.

Group D: In group D, only soil was used as a negative control.

Each experimental and control group was replicated to ensure statistical validity and account for any natural variability in the samples.

C. Variables Measured

The study measured a range of physiochemical properties to assess the impact of earthworms on lead-contaminated municipal sludge:

- **pH**: The pH of the sludge was measured to assess any changes in acidity or alkalinity due to earthworm activity. pH can influence the mobility and bioavailability of lead and other heavy metals in the soil.

- **Electrical conductivity**: The electrical conductivity of the sludge was measured to assess any changes in EC due to earthworm activity.

- **Lead Concentration**: The concentration of lead in the sludge was measured to determine whether earthworm amendment reduces lead levels. This parameter is critical for assessing the effectiveness of the remediation process.

- **Total Organic carbon**: The total organic carbon of the sludge was measured to determine any changes in carbon content caused by earthworms and also check changes in the C: N ratio.

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- **Total Nitrogen and Phosphorous:** Total nitrogen and Phosphorous of the sludge was assessed to evaluate the impact of earthworms on nutrient cycling and soil quality. Higher organic matter content can enhance soil fertility and support microbial activity.

Data Analysis:

- Data collected from the experimental and control groups were analyzed to assess the impact of earthworm amendment on the measured variables.
- The results were compared across same densities of earthworms in the experimental groups and with the control group to identify any significant differences.

Methods of Analysis

This dataset provides a characterization of lead-contaminated sludge samples in terms of specific physiochemical properties. The pH level of sludge was determined using a standard pH meter, following direct measurement in sludge or in slurry (Indian Standard 2720, 1987). Homogenized samples were used to assay electrical conductivity with a conductivity meter (Indian Standard14767, 1997). Lead concentrations have been measured by atomic absorption spectroscopy (AAS) after acid digestion. Volume organic carbon was measured by titration with potassium dichromate following oven drying of the sample (Indian Standard 2720, 1972). Drying was followed by digestion and distillation and the relatively simple Kjeldahl procedure was used to quantify total nitrogen (Indian Standard 14684, 1999).Phosphorus concentration was estimated on the basis of absorbance readings, measuring the samples spectrophotometrically (Standard operating procedure, 2016). The sum of C amount was divided by N content to calculate the C/N ratio (Indian Standard 16556, 2016).

The frequency and timing of measurements for each parameter were standardized across experimental and control groups to ensure consistency and reliability of data. These analyses provided comprehensive insights into the changes in the physiochemical properties of lead-contaminated sludge due to earthworm amendment, supporting the evaluation of earthworm-assisted bioremediation's effectiveness.

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D. Data Analysis

1. Statistical Methods Used for Data Analysis

The data collected from the assessment of physiochemical properties and lead concentration in the lead-contaminated sludge, both with and without earthworm amendment, were subjected to statistical analysis to determine the effects of earthworms on soil remediation.
A one-way analysis of variance (ANOVA) was done to examine the effects of the treatment. The treatments were compared using the Tukey test with a significance level of 0. 05. The statistical analysis was done with vassarstats.

III. Results and Discussion

A. Reproduction and growth of earthworm

The survival and reproductive performance of Eisenia fetida varied across different treatment groups (Table 1). A decline in survival was observed in treatments with higher proportions of municipal sludge, which may be attributed to reduced aeration and the release of toxic substances during sludge decomposition (Ludibeth *et al*., 2012). Similar observations have been reported in recent studies, where elevated concentrations of heavy metals and poor aeration negatively affected earthworm survival and activity (Shen *et al.*, 2026).

After 60 days of vermicomposting, the number of adult earthworms increased in all treatments except the negative control. Treatment C (cow dung) showed the highest number of adult earthworms (53 ± 3), followed by Treatment B (46 ± 1) and Treatment A (35 ± 3). In contrast, the control (Treatment D) recorded the lowest number (17 ± 2). However, the variation in adult earthworm numbers among treatments was not statistically significant ($p > 0.05$).

Juvenile production followed a similar trend. Treatment C showed the highest number of juveniles (23 ± 2), followed by Treatment A (18 ± 3) and Treatment B (14 ± 1), whereas the control exhibited minimal juvenile production (5 ± 1). These findings are consistent with recent reports indicating that nutrient-rich substrates enhance juvenile production and overall earthworm activity (Kumar *et al*., 2025).

Cocoon production was also highest in Treatment C (60 ± 2), followed by Treatment A (55 ± 2), Treatment B (35 ± 3), and Treatment D (25 ± 2). The higher cocoon production in treatments

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containing cow dung indicates that nutrient-rich substrates promote earthworm reproduction. Recent studies have also demonstrated that vermicomposting substrates rich in organic matter significantly improve cocoon production and reproductive efficiency (Xu *et al*., 2026).

Overall, the results suggest that the proportion of municipal sludge in combination with cow dung significantly influences the growth and reproductive performance of earthworms. Higher sludge concentrations may adversely affect survival, whereas balanced mixtures enhance reproductive efficiency.

Table 1. Survival and reproduction of *Eisenia fetida*
Mean ± SD (n=3)

Group	**Compost Bed material**	**Initial no. of earthworms before treatment**	**Final number of earthworms after 60 days of treatment**		
			Number of adult earthworms (Final)	Number of juvenile earthworms	Cocoons
A	Municipal sludge (CD+MS=100:100)	20	35±3	18±3	**55±2**
B	Municipal sludge (CD+MS=150:50)	20	**46±1**	14±1	35±3
C	Cow dung(Positive control)	20	**53±3**	23±2	**60±2**
D	Soil(Negative control)	20	17±2	5±1	25±2

B. Changes in Physiochemical Properties

1. Electrical conductivity:

Electrical conductivity (EC) is an important indicator of soluble salt content in soil amendments such as compost and vermicompost, and it reflects their suitability for soil fertility and agricultural productivity (Abd El-Rahim *et al*., 2021; Lim *et al*., 2014).

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In the present study, the EC of the mature vermicompost ranged from 0.37 to 1.10 mS/cm. A consistent decrease in EC was observed in all treatments following vermicomposting, including both control and earthworm-amended groups. This reduction indicates the stabilization of soluble salts and improvement in substrate quality.

The initial EC of municipal sludge was 1.74 mS/cm, which was higher than that of cow dung (0.62 mS/cm) but slightly lower than that of soil (1.83 mS/cm) (Table 2). Among the treatments, Treatment A exhibited an EC of 0.77 mS/cm, followed by Treatment C (positive control) at 0.525 mS/cm and Treatment B at 0.47 mS/cm. The highest EC was recorded in Treatment D (negative control) at 0.873 mS/cm (Figure 1).

The decrease in EC during vermicomposting may be attributed to the consumption and transformation of soluble salts by Eisenia fetida, as well as the leaching of dissolved salts during regular watering. Similar findings have been reported by Arora and Kaur (2019), who observed a decline in EC due to the utilization of soluble nutrients and mineral stabilization processes.

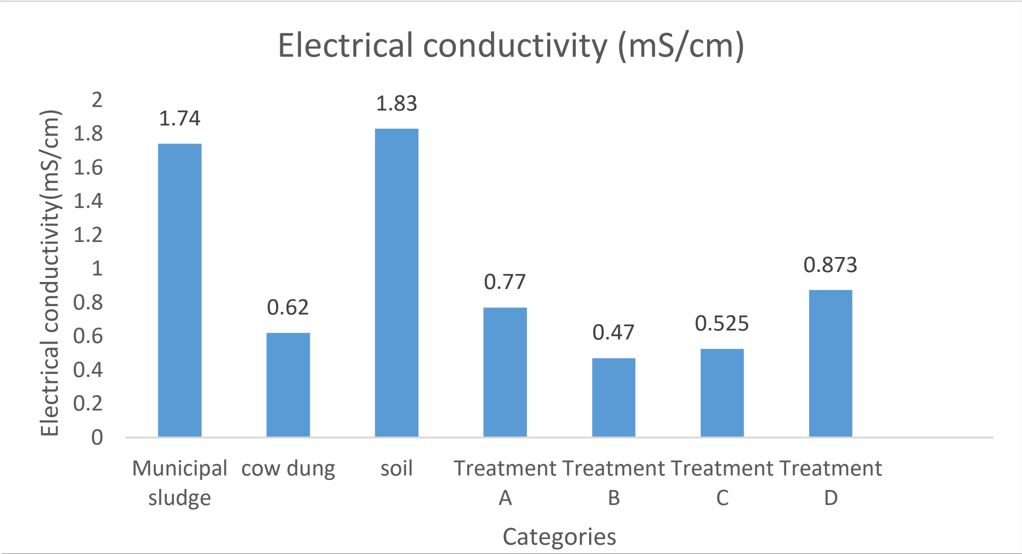

Figure 1: Comparison of electrical conductivity before and after vermicomposting

2. pH:

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pH is a critical parameter that determines the acidity or alkalinity of compost and vermicompost and plays an important role in soil fertility and plant growth (Majlessi *et al*., 2012).

In the present study, the initial pH of municipal sludge was alkaline (pH 8.0) at the beginning of the experiment (Table 2). After 60 days of vermicomposting, the pH decreased to a range of 5.07–7.43 across different treatments (Figure 2), indicating stabilization of the substrate.

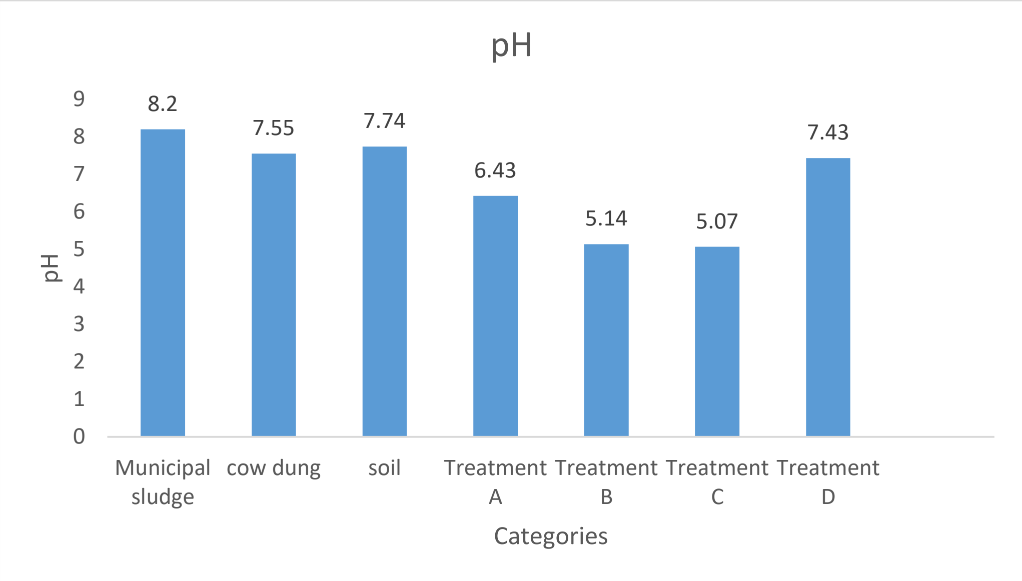

Figure 2: Comparison of pH before and after vermicomposting

Among the treatments, Treatment B (pH 5.14) and Treatment C (pH 5.07) exhibited significantly lower pH values compared to Treatment A, suggesting enhanced decomposition and earthworm activity. In contrast, Treatment D (negative control) showed minimal variation, with a final pH of 7.43, likely due to the absence of Eisenia fetida and limited biological activity.

The reduction in pH during vermicomposting can be attributed to the production of organic acids during the decomposition of organic matter by earthworms and associated microorganisms. Additionally, the release of CO_2, NH_3, and hydrogen ions during microbial processes such as nitrification contributes to the decline in pH. Similar findings have been

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reported by El-Sayed et al. (2022), who observed a decrease in pH due to organic acid formation and microbial activity during composting.

Total Organic Carbon (TOC):

Total organic carbon (TOC) plays a crucial role in sustaining microbial and earthworm activity during the vermicomposting process. It is an important parameter for evaluating the efficiency of organic matter degradation by earthworms compared to initial substrate conditions.

In the present study, a reduction in TOC was observed across all treatments after the introduction of earthworms, indicating active decomposition and mineralization of organic matter (Table 2; Figure 3). The initial TOC content was 21.21% in municipal sludge, while cow dung exhibited the highest value at 30.42%, followed by Treatment A at 28.38%. This suggests that cow dung amendment enhances the organic carbon content and supports microbial activity in the substrate.

Among the treatments, Treatment B (25.28%) and Treatment C (26.28%) showed the greatest reduction in TOC, indicating higher efficiency of Eisenia fetida in decomposing organic matter. In contrast, Treatment D (negative control) exhibited minimal reduction (1.82%), likely due to the absence of earthworm activity.

The decrease in TOC can be attributed to the metabolic activity of earthworms and microorganisms, which convert organic carbon into CO_2 through respiration, thereby reducing the overall carbon content. Additionally, increased earthworm population enhances the rate of decomposition and humification, as supported by previous studies (Na *et al.*, 2011; Ramnarain *et al.*, 2019).

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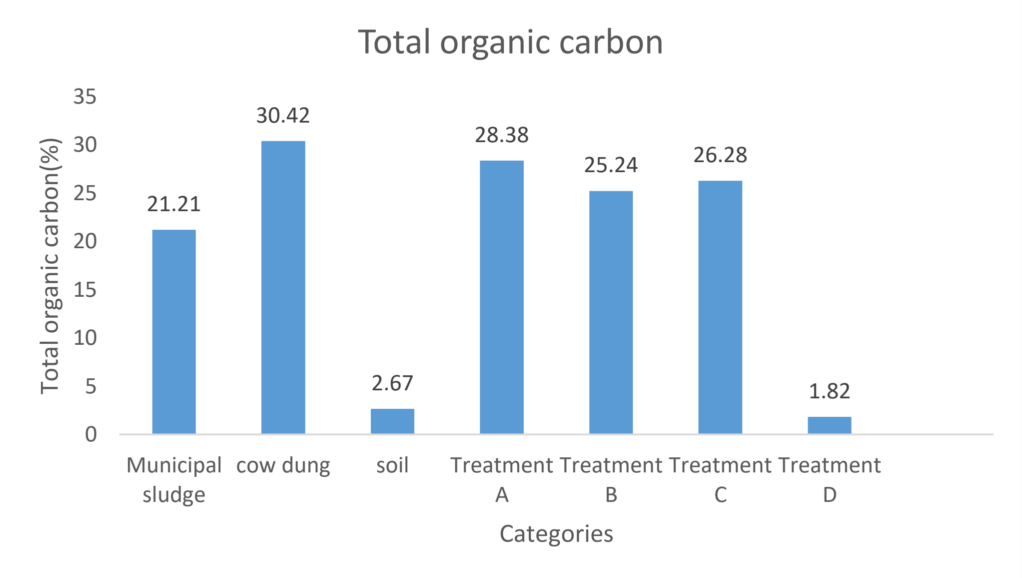

Figure 3: Comparison of Total organic carbon (TOC) before and after vermicompost

Total Nitrogen and Phosphorous:

The total nitrogen (TN) content increased in all treatments after vermicomposting, except for the negative control (Treatment D). In the negative control, only a slight increase of 0.91% was observed compared to the initial value (0.03%), indicating minimal biological activity. In contrast, the positive control (Treatment C) showed a substantial increase of 35.86% in nitrogen content (Table 2).

Among all treatments, the highest increase in nitrogen was recorded in Treatment A (43.94%), followed by Treatment B (33.42%) (Figure 4). The increase in nitrogen content can be attributed to the reduction in total organic matter during decomposition, where carbon is lost as CO_2, leading to a relative enrichment of nitrogen. Additionally, Eisenia fetida enhances nitrogen levels through digestion of organic substrates and the addition of nitrogenous excretory products, mucus, enzymes, and body fluids, which are released into the vermicompost as casts. The decomposition of dead earthworm tissues also contributes to nitrogen enrichment (Joseph, 2019).

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Potassium (K) content was also observed to increase during vermicomposting. This enhancement may be due to microbial enzymatic activity within the earthworm gut, which converts insoluble forms of potassium into soluble forms available for plant uptake. Similar observations have been reported by Zuhair (2011) and Wagmode *et al*. (2021).

Furthermore, total phosphorus (TP) content showed a significant increase after 60 days of vermicomposting (Table 2). Treatment A recorded the highest phosphorus content (28.65%), followed by Treatment C and Treatment B (25.64% each), while Treatment D exhibited the lowest value (5.25%) (Figure 5). The limited increase in phosphorus in the negative control may be attributed to reduced earthworm activity and lower microbial transformation in the absence of organic amendments.Overall, the increase in nitrogen, phosphorus, and potassium contents indicates improved nutrient availability and enhanced fertilizer quality of the vermicompost.

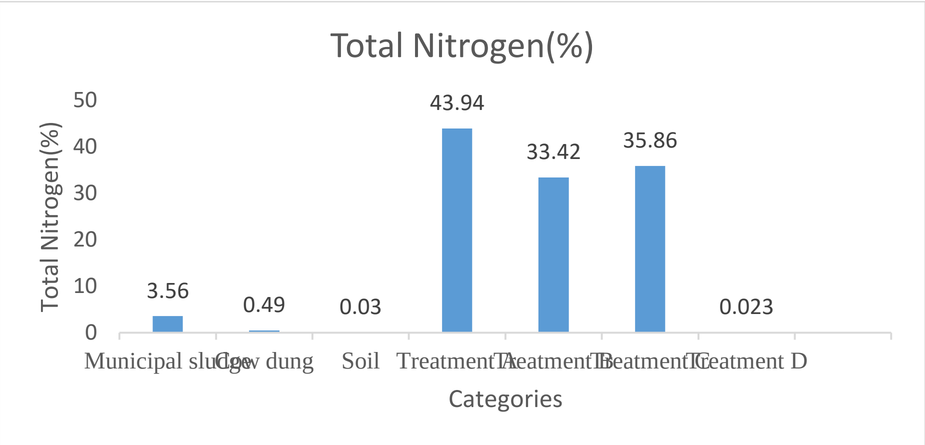

Figure 4: Comparison of Total nitrogen (TN) before and after vermicompost

According to Zuhair (2011), the potassium level in the vermicompost significantly increased as the vermicomposting duration extended. This increase could be due to the enzymatic activity of microbes in the earthworm digestive tracts, which might have converted insoluble potassium into a soluble form (Wagmode SY, 2021). Table 2 represents the changes in the total phosphorous content of municipal sludge and the control group after treatment. After 60 days, Treatment A had the highest total phosphorous content at 28.65%, followed by treatment C at 25.64%, Treatment B at 25.64%, and treatment D at 5.25%. The least

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phosphorous change was observed in treatment D because the growth of earthworms in the soil is reduced (Figure 5).

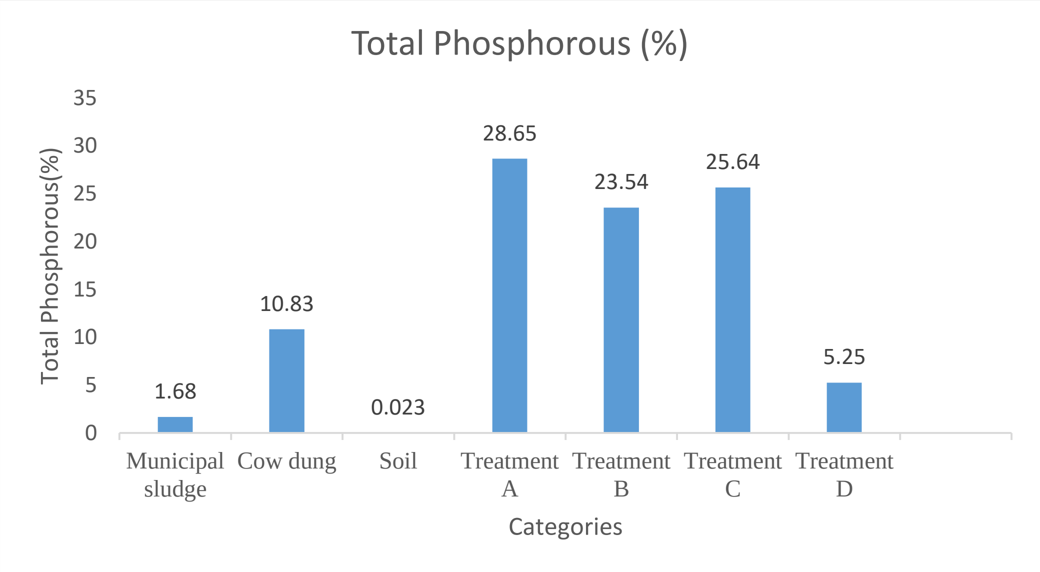

Figure 5: Comparison of Total Phosphorous (TP) before and after vermicompost

C: N ratio:

The carbon-to-nitrogen (C:N) ratio is a widely used indicator of the maturity and stability of organic waste. In the present study, the C:N ratio decreased significantly after vermicomposting, ranging from 79.80 in the initial substrate to 0.645 in the final vermicompost (Table 2).

Vermicomposting enhances the rapid decomposition and mineralization of organic matter, resulting in a reduction of carbon content due to its loss as CO_2, while nitrogen is relatively enriched. This process leads to a decline in the C:N ratio, indicating improved compost maturity. A C:N ratio below 20 is generally considered a sign of well-stabilized and mature compost (Ludibeth *et al*., 2012).

Comparative between before vermicompost and after vermicompost treatments A, B, C had very low C. High nitrogen content shown decrease of C: N ratio. Treatment D had the greatest C:N ratio, implying a higher amount of carbon in the material (Figure 6).

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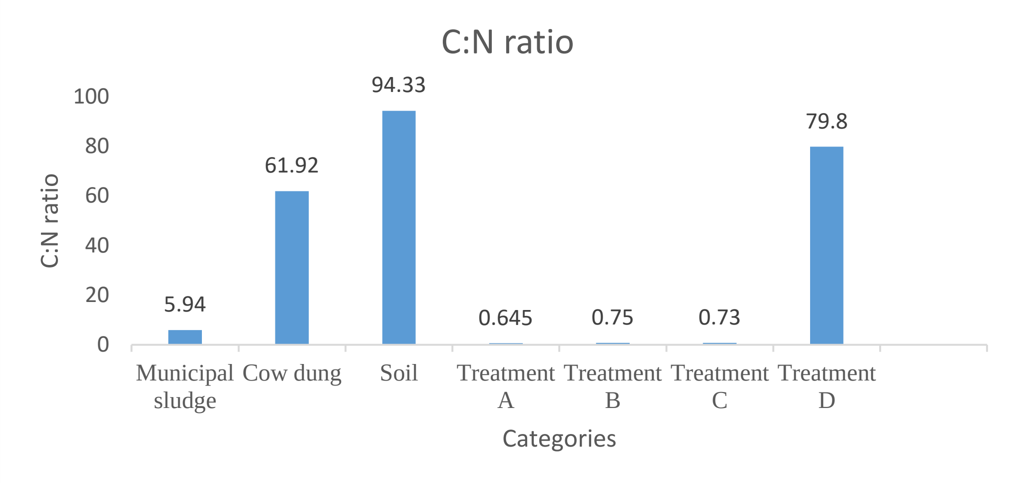

Figure 6: Comparison of C:N ratio before and after vermicompost

Table 2. Physiochemical characteristics of the Municipal sludge, cow dung, soil and vermicompost: (Mean ± SD n=3,)

	Before treatment			**After treatment**			
Physiochemical characteristics	Municipal sludge (MS)	Cow dung	Soil	Group - A [CD (100):MS (100)]	Group – B [CD (150):MS (50)]	Group-C (Positive Control-Cow dung)	Group-D (Negative Control-Soil)
Electrical conductivity(mS /cm)	1.74±0.035	0.62±0.027	1.83±0.02	0.77±0.007	**0.47±0.002**	0.525±0.058	**0.873±0.059**
pH	8.20±0.22	7.55±0.05	7.74±0.19	6.43±0.02	5.14±0.15	**5.07±0.05**	**7.43±0.02**
Total Organic Carbon (%)	21.21±0.21	30.42±0.28	2.67±0.15	**28.38±0.015**	25.24±0.040	26.28±0.020	**1.82±0.020**
Total Nitrogen (%)	3.56±0.05	0.49±0.04	0.03±0.01	**43.94±0.020**	33.42±0.025	35.86±0.025	**0.023±0.002**
Total Phosphorous (%)	1.68±0.14	10.83±0.03	0.023±0.015	**28.65±0.015**	23.54±0.025	25.64±0.020	**5.25±0.032**
C:N ratio	5.94±0.054	61.92±4.59	94.33±46.99	0.64±0.001	0.75±0.00057	**0.73±0.001**	**79.80±6.71**

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Lead Concentration:

Vermicomposting has proven effective in reducing lead concentration in all treatments compared to the initial levels in municipal sludge, soil and cow dung.

Table 3 Lead analysis in municipal sludge before treatment and after treatment (Mean ± SD, n=3)

Parameter	Cow dung (Before treatment)	Soil (Before treatment)	Municipal sludge (Before treatment)	Municipal sludge (After treatment- CD+MS=100:100)	Municipal sludge (After treatment- CD+MS=150:50)	Cow dung (Positive control	Soil (Negative control)
Lead analysis(mg/kg)	2.71±0.098	4.15±0.040	430.56±0.020	**40.17±0.03**	36.07±0.030	**1.45±0.38**	3.92±0.025

Vermicomposting significantly reduced lead (Pb) concentrations across all treatments (A, B, C, and D), demonstrating its effectiveness in heavy metal stabilization (Table 3; Figure 7).

The initial lead concentration in cow dung was relatively low (2.71 mg/kg), and it further decreased after vermicomposting in Treatment C (positive control). Similarly, soil exhibited an initial Pb concentration of 4.15 mg/kg, which declined to 3.92 mg/kg in Treatment D (negative control).

The most substantial reductions were observed in Treatment A (40.17 mg/kg) and Treatment B (36.07 mg/kg), indicating enhanced remediation potential when municipal sludge was combined with organic amendments. The greater reduction in these treatments may be attributed to increased microbial activity and the bioaccumulation and immobilization of heavy metals by Eisenia fetida during vermicomposting.

The decrease in lead concentration can be explained by processes such as adsorption, complexation with organic matter, and incorporation into earthworm tissues, which reduce the

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bioavailability of heavy metals. Overall, the findings highlight the potential of vermicomposting as an effective and eco-friendly approach for the remediation of lead-contaminated substrates.

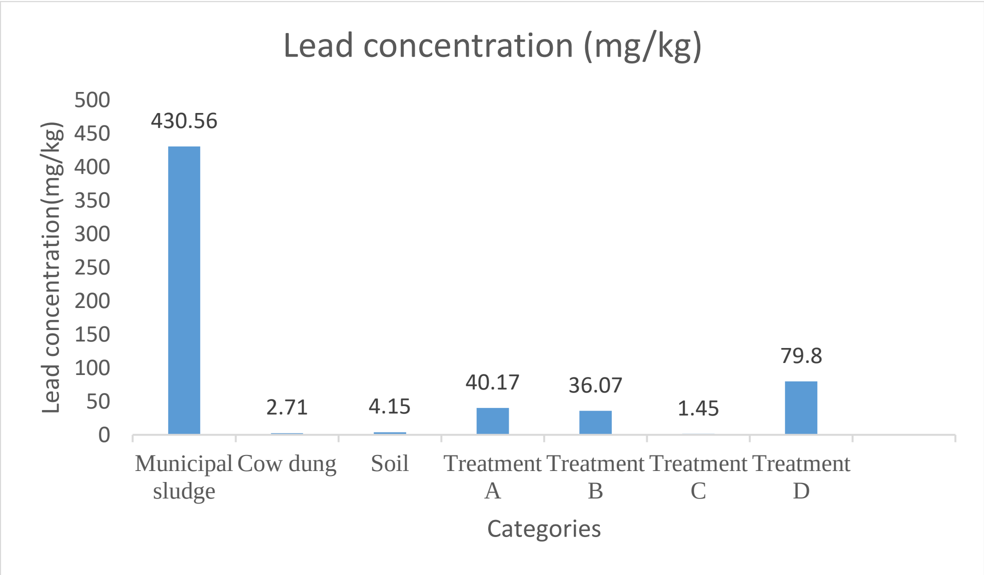

Figure 7: Comparison of Lead concentration before and after vermicompost

IV Conclusion:

Based on the results of the present study, it can be concluded that vermicompost produced using Eisenia fetida with municipal sludge and cow dung exhibited significant improvements in physicochemical and biological properties. Among the different treatments, the mixture of municipal sludge and cow dung (150:50) showed the best overall performance in terms of nutrient enhancement and compost quality.

The presence of earthworms in sludge–cow dung mixtures significantly increased the availability of essential nutrients such as nitrogen and phosphorus, while also improving key parameters including pH, electrical conductivity, total organic carbon, and C:N ratio. The 100:100 treatment demonstrated the highest levels of total nitrogen and phosphorus, indicating effective mineralization and nutrient transformation during vermicomposting.

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Furthermore, the study confirmed the potential of vermicomposting as an eco-friendly approach for the remediation of lead-contaminated sludge. A notable reduction in lead concentration was observed across all treatments, particularly in sludge-amended groups, along with improvements in organic matter stabilization and microbial activity. Similar findings have been reported in recent studies highlighting the efficiency of vermicomposting in heavy metal stabilization and nutrient enhancement (Singh *et al*., 2022; Sharma & Garg, 2023).

The results highlight the multifaceted role of earthworms in enhancing compost quality through mechanisms such as bioaccumulation, transformation of heavy metal speciation, and stimulation of microbial processes. Recent studies also support these mechanisms, emphasizing the role of earthworm–microbe interactions in improving soil health and reducing metal toxicity (Zhang *et al*., 2021; Kumar *et al*., 2024).

Overall, vermicomposting using *E. fetida* can be considered an effective and environmentally sustainable technique for converting municipal sludge into nutrient-rich compost suitable for agricultural applications.

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