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Efficiency of Earthworms in Bioremediation of Soil Contaminated with Sewage Water and Heavy Metals

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Abstract

This study evaluated the efficiency of two earthworm species (*Eisenia fetida* and *Lumbricus terrestris*) in the bioremediation of loam and clay soils contaminated with sewage water, Cadmium (Cd), and Lead (Pb), alongside assessing their biomass dynamics, antioxidant defense, and neurotoxic responses. A 60-day factorial experiment was conducted using a Randomized Complete Block Design (RCBD), with 10% fermented cow waste added as an organic amendment. The results revealed a clear ecological and physiological specialization; *E. fetida* exhibited the highest survival rate (72.50%), maximum biomass (34.75 g pot^{-1}), and peak cellular specific activity of superoxide dismutase (SOD) (54.90 units mg^{-1} protein) under individual Pb exposure within the loam soil. In contrast, *L. terrestris* demonstrated exceptional efficiency in clay soil with a survival rate of 68.25%, higher biomass retention, and a superior adaptive antioxidant response to overcome the compact mechanical stress. The findings indicated that Cd possesses higher acute toxicity and cellular damage compared to Pb. Under dual contamination (W3), survival rates collapsed significantly (36.25%), and biomass plunged to 18.75 g pot^{-1} due to severe synergistic stress. This severe toxicity caused a non-linear drop-in SOD activity, indicating a partial collapse of the defense mechanism. Furthermore, acetylcholinesterase (AChE) activity was heavily inhibited by heavy metals, dropping from a baseline of 86.45 nmol min^{-1} mg^{-1} protein to an absolute minimum of 21.65 nmol min^{-1} mg^{-1} protein under mixed treatments (W3), indicating acute neurotoxicity. Clay soil effectively acted as a protective shield, mitigating neurotoxic inhibition and preserving higher enzyme levels due to its high cation exchange capacity (30.84 cmol kg^{-1}). The study concludes that monitoring biomass, antioxidant profiles, and neurotoxic biomarkers provides a powerful framework for evaluating multi-metal contamination risks and earthworm-mediated soil restoration.

Keywords: Vermiremediation, Sewage water, Heavy metals, Biomass responses, Superoxide dismutase (SOD), Acetylcholinesterase (AChE), Soil texture.

1. Introduction

The increasing demand for water resources and their scarcity are contemporary global challenges, prompting the search for alternative sources such as sewage water for agricultural exploitation. However, the direct application of such water without precise treatment poses a severe environmental risk due to the presence of toxic and carcinogenic heavy metals, which transfer through the food chain, leading to the degradation of soil physical and chemical properties and a decline in productivity [1, 2]. In this context, bioremediation techniques have emerged as sustainable and environmentally safe solutions, relying on the capabilities of living organisms to remove or immobilize pollutants [3].

Earthworms are considered "ecosystem engineers" due to their vital role in improving soil structure and recycling nutrients via vermicompost production, in addition to their high capacity to bioaccumulate heavy metals in their tissues, thereby reducing their environmental bioavailability [4, 5]. Both classical and recent studies have proven that exposure to heavy metals induces physiological and oxidative stress in earthworms, which directly affects their survival rates, cellular homeostasis, and biological activity [6, 7]. Heavy metal ingestion triggers the overproduction of reactive oxygen species

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(ROS), causing oxidative damage to proteins and cell membranes. To counter this, earthworms activate an intricate enzymatic defense system, wherein superoxide dismutase (SOD) serves as the primary line of cellular defense, converting superoxide radicals into hydrogen peroxide [12].

Furthermore, heavy metals induce severe neurotoxicity by chemically binding to the sulfhydryl (–SH) groups at the active site of acetylcholinesterase (AChE), a crucial synaptic enzyme responsible for hydrolyzing acetylcholine. The inhibition of AChE leads to neurotransmitter accumulation, causing muscular spasms, metabolic exhaustion, and a significant drop in earthworm biomass [22]. Detoxification mechanisms and the synthesis of metallothionein proteins play a crucial role in the earthworms' ability to tolerate metal concentrations [8, 9]. Crucially, the behavior, biomass stability, and specific enzymatic activities of these earthworms are directly influenced by soil texture and porosity, making the selection of the appropriate species for the specific soil matrix a critical factor for successful remediation [10]. The interaction between earthworms and pollutants becomes increasingly complex under dual metal contamination, where ionic competition and synergistic toxic mechanisms emerge, altering overall bioaccumulation and enzymatic profiles [11, 23]. Accordingly, this study aims to evaluate the efficiency of two earthworm species (*Eisenia fetida* and *Lumbricus terrestris*) in the bioremediation of varied-texture soils contaminated with sewage water, cadmium, and lead, while comprehensively characterizing their biomass responses, antioxidant defense, and neurotoxic profiles.

2. Materials and Methods

A factorial pot experiment was conducted to evaluate the efficiency of earthworms in the bioremediation of soils contaminated with sewage water and heavy metals. The experiment was laid out in a Randomized Complete Block Design (RCBD) and comprised three main factors:

- **First Factor (Soil Texture):** Two different soil types were utilized: Loam Soil (Table 1) and Clay Soil (Table 2).
- **Second Factor (Earthworm Species):** Two earthworm species were selected: *Eisenia fetida* (Imported, epigeic species) and *Lumbricus terrestris* (Local, anecic species).
- **Third Factor (Irrigation and Pollution Treatments):** Untreated sewage water brought from the Fallujah Wastewater Treatment Plant (Al-Nuaimiya area) (Table 3), spiked with Cd and Pb: W0 (Control, sewage water only), W1 (Cd at 10 mg L^{-1}), W2 (Pb at 20 mg L^{-1}), and W3 (Cd 10 mg L^{-1} + Pb 20 mg L^{-1}).

Preparation of Environmental Media and Earthworm Feeding:

Each plastic pot was filled with 5 kg of soil. To ensure the sustainability of the earthworm biomass, pre-fermented cow manure (Table 4) was added at a rate of 10% of the total soil weight (500 g manure per 5 kg soil) in all pots. Following homogenization, 25 mature earthworms of both species were introduced into each pot. The experiment lasted for 60 days.

Physical, Chemical, and Biochemical Analyses:

- **Survival Rate & Biomass Dynamics:** Calculated as a percentage (%) and total biomass (g pot^{-1}) at the end of the 60-day experimental period.
- **Organic Matter:** Estimated using the Walkley-Black wet oxidation method [16].
- **Heavy Metal Extraction & Measurement:** Acid digestion was performed according to Everson (1975) [17], guided by the US EPA 3050B protocol [18]. Concentrations were measured using an Atomic Absorption Spectrophotometer (AAS).
- **Biochemical Enzyme Assays:** Earthworm tissue homogenates were prepared to determine specific enzymatic activities. Superoxide dismutase (SOD) activity was expressed as specific units per milligram of protein (units mg^{-1} protein). Acetylcholinesterase (AChE) activity was determined kinetically and expressed as nanomoles of substrate hydrolyzed per minute per milligram of protein (nmol min^{-1} mg^{-1} protein) [22, 24].

- **Table (1) Some physical and chemical properties of the Loam soil used in the experiment**

Parameter	Unit	Value
Soil pH (1:1)	———	7.78
Electrical conductivity (EC)	dS m^{-1}	1.84
Organic matter (OM)	g kg^{-1}	6.51

Calcium carbonate		%	11.30
Soil bulk density		Mg m^{-3}	1.37
Cation exchange		cmol kg^{-1}	22.90
Dissolved cations	Ca^{+2}	mM L^{-1}	6.23
	Mg^{2+}		3.17
	Na^{+}		5.42
	K^{+}		1.47
Dissolved anions	$SO4^{-2}$		7.65
	$HCO3^{-}$		1.84
	Cl^{-}		6.81
Available nitrogen		mg kg^{-1} soil	23.00
Available phosphorus			12.40
Available potassium			120.30
Total cadmium Cd			0.05
Total lead Pb			2.0
Soil separetes	Sand	mg kg^{-1} soil	340
	Silt		410
	Clay		250
Soil texture		Loam	

- Analyzed at the College of Agriculture's laboratories (University of Anbar).
- **Table (2) Some physical and chemical properties of the clay soil used in the experiment**

Parameter		**Unit**	**Value**
Soil pH (1:1)		————	7.85
Electrical		dS m^{-1}	1.91
Organic matter		g kg^{-1}	7.31
Calcium carbonate		%	12.37
Soil bulk density		Mg m^{-3}	1.37
Cation exchange		cmol kg^{-1}	30.84
Dissolved cations	Ca^{+2}	mM L^{-1}	7.51
	Mg^{2+}		3.66
	Na^{+}		6.62
	K^{+}		1.84
Dissolved anions	$SO4^{-2}$		7.85
	$HCO3^{-}$		3.91
	Cl^{-}		7.82
Available nitrogen		mg kg^{-1} soil	28.00
Available phosphorus			16.40
Available potassium			129.30
Total cadmium Cd			0.07
Total lead Pb			3.0
Soil separetes	Sand	mg kg^{-1} soil	205
	Silt		370
	Clay		425
Soil texture		Clay	

- Analyzed at the College of Agriculture's laboratories (University of Anbar).
- **Table (3) Analysis of wastewater used in the experiment**

Parameter	**Unit**	**Value**
Soil pH (1:1)	————	7.3
Electrical conductivity	dS m^{-1}	2.9
Organic matter (OM)	%	40.1
Organic carbon (OC)	%	29.8
Total nitrogen (TN)	%	1.7
C:N Ratio		[illegible]
Cadmium (Cd)	mg L^{-1}	[illegible]
Lead (Pb)	mg L^{-1}	[illegible]
Biological Oxygen	mg L^{-1}	[illegible]
Chemical Oxygen	mg L^{-1}	[illegible]
Total Suspended Solids	mg L^{-1}	[illegible]
Total dissolved solids	mg L^{-1}	[illegible]

- Analyzed at the College of Agriculture's laboratories (University of Anbar).
- **Table (4) Some characteristics of cow waste used in the experiment**

Parameter	**Unit**	**Value**
Soil pH (1:1)	---	7.1
Electrical conductivity	dS m^{-1}	3.1
Organic matter (OM)	%	50.6
Organic carbon (OC)	%	37.7
Total nitrogen (TN)	%	1.8
C:N Ratio		[illegible]
Cadmium (Cd)	mg kg^{-1}	[illegible]
Lead (Pb)	mg kg^{-1}	[illegible]

- Analyzed at the College of Agriculture's laboratories (University of Anbar).

3. Results and Discussion

3.1 Survival Rate and Biomass Dynamics

Table (5) illustrates the effect of soil texture, earthworm species, and water treatments on the survival rate (%) after 60 days. The data indicate that *E. fetida* recorded a higher survival rate in loam soil (72.50%) compared to clay soil (59.50%). Conversely, *L. terrestris* exhibited its highest survival rate in clay soil (68.25%) compared to loam soil (60.25%). This variation is attributed to the ecological compatibility of each species; *E. fetida* thrives in highly porous loam soils, particularly when organic residues are available [10]. In contrast, *L. terrestris* is better adapted to heavy clay soils due to its muscular ability to operate within compacted environments [14, 15]. The table further reveals that Cadmium (Cd) exhibits higher toxicity and a more pronounced impact on survival rates compared to Lead (Pb). Specifically, the lead-contaminated treatment (W2) achieved a survival rate of 77.25%, whereas the cadmium-contaminated treatment (W1) dropped to 57.25%. While heavy metal pollution generally restricts survival [6], Cd specifically induces rapid cellular damage and severe oxidative stress [7, 21]. Under dual contamination (W3), survival rates significantly collapsed to an average of 36.25% due to the synergistic toxicity effect [11].

Table (5) Effect of soil texture, earthworm species, and water treatments on the survival rate (%) after 60 days.

Soil (S)	Earthworms (D)	W0	W1	W2	W3	Mean
Loam	***E. fetida***	**97**	**66**	**84**	**43**	**72.50**
	L. terrestris	**87**	**48**	**76**	**30**	**60.25**
Clay	***E. fetida***	**81**	**56**	**69**	**32**	**59.50**
	L. terrestris	**94**	**59**	**80**	**40**	**68.25**
Mean		**89.75**	**57.25**	**77.25**	**36.25**	**65.125**

LSD (0.05): S = 3.24 | D = 3.24 | W = 4.58 | SD = 5.62 | SDW = 8.65

Table (5-A) demonstrates the total earthworm biomass (g pot⁻¹) after 60 days. Biomass represents an exceptionally sensitive physiological indicator reflecting metabolic efficiency, food ingestion rates, and energy allocation under chemical stress. The results reveal that loam soil maintained a slightly higher overall average biomass (32.88 g pot⁻¹) compared to clay soil (31.38 g pot⁻¹). This difference is directly linked to the physical properties of the soil matrix, where the excellent aeration and low mechanical resistance of loam soil optimize movement and feeding activity, whereas high clay content imposes a physical constraint, increasing the energy expended for burrowing [10, 25].

The heavy metal water treatments exerted a profound impact on biomass accumulation. The control treatment (W0) maintained the highest biomass (44.00 g pot⁻¹), which dropped significantly to 28.00 g pot⁻¹ under individual Cd stress (W1) and to 37.75 g pot⁻¹ under Pb stress (W2). This reduction confirms that the heavy metals disrupted cellular respiration and production of adenosine triphosphate (ATP), forcing the earthworms to redirect their energy reserves away from somatic growth and towards the metabolic costs of cellular detoxification and metallothionein synthesis [1, 23]. The sharpest decrease was recorded under the joint contamination treatment (W3), plunging to an average of 18.75 g pot⁻¹; this reflects an intense synergistic toxic burden that severely suppressed soil ingestion and completely exhausted the earthworms' metabolic reserves [11]. A notable species-specific interaction was observed under individual Pb stress (W2), where *L. terrestris* retained a significantly higher biomass (40.00 g pot⁻¹) than *E. fetida* (35.50 g pot⁻¹). This performance demonstrates the superior physiological adaptation of this local anecic earthworm to sequester lead within its chloragogenous tissues, mitigating its systemic toxicity and safeguarding metabolic growth [9, 14].

Table (5-A) Effect of soil texture, earthworm species, and water treatments on total earthworm biomass (g pot⁻¹) after 60 days.

Soil (S)	**Earthworms (D)**	**W0**	**W1**	**W2**	**W3**	**Mean**
Loam	***E. fetida***	48.00	32.00	38.00	21.00	34.75
	L. terrestris	42.00	23.00	43.00	16.00	31.00

Soil (S)	Earthworms (D)	W0	W1	W2	W3	Mean
Clay	***E. fetida***	40.00	27.00	33.00	17.00	29.25
	L. terrestris	46.00	30.00	37.00	21.00	33.50
Mean		44.00	28.00	37.75	18.75	32.13

LSD (0.05): S = 1.01 | D = 1.01 | W = 1.42 | SD = 1.42 | SDW = 2.85

3.2 Heavy Metal Bioaccumulation

Table (6) demonstrates the concentrations of Cadmium (Cd mgkg^{-1}) accumulated in earthworm tissues after 60 days. The results indicate clear significant variations in the bioaccumulation efficiency of Cd, confirming the high capacity of these organisms to extract pollutants from contaminated environments [1, 2]. The data show a significant superiority of Eisenia fetida in bioaccumulation within the loam soil, recording the highest value in the individual water treatment (W1) at 19.25 mgkg^{-1}, which significantly outperformed Lumbricus terrestris in the same soil type (11.50 mgkg^{-1}). This superiority is attributed to the high porosity and aeration of the loam soil, which perfectly suited the epigeic and voracious feeding behavior of E. fetida. This environment allowed for higher soil ingestion rates, leading to a maximal accumulation of Cd within its tissues [10].

In contrast, L. terrestris exhibited its peak performance in the clay soil, recording a mean of 15.10 mgkg^{-1}. This is explained by its robust nature as an anecic earthworm. Unlike epigeic species, L. terrestris possesses the muscular capability to penetrate compacted clay structures and remediate pollutants effectively within heavy soil matrices [15].

A critical statistical observation in this experiment is the significant decrease in Cd accumulation when Lead (Pb) was introduced in the dual treatment (W3). For instance, accumulation in E. fetida within loam soil dropped from 19.25 mgkg^{-1} in W1 to 15.35 mgkg^{-1}in W3. This reduction is a direct result of "Competitive Inhibition," where Pb and Cd ions compete for the same binding sites and transport proteins, such as metallothioneins, within the earthworm's system [5, 11]. Furthermore, the combined toxic stress of both metals likely suppressed the earthworms' metabolic activity and appetite, thereby reducing the total volume of ingested contaminated soil [11]. The low concentrations observed in W0 and W2 (0.41 - 0.80 mgkg^{-1}) represent the natural background levels inherent in the original soil and organic amendments [20].

Table (6) Cadmium (Cd) concentrations (mgkg^{-1}) accumulated in earthworm tissues after 60 days.

Soil (S)	Earthworms (D)	W0	W1	W2	W3	Mean
Loam	***E. fetida***	0.70	19.25	0.80	15.35	**9.03**
	L. terrestris	0.50	11.50	0.63	8.56	**5.30**
Clay	***E. fetida***	0.41	13.25	0.54	9.88	**6.02**
	L. terrestris	0.61	15.10	0.70	12.33	**7.19**
Mean		**0.56**	**14.78**	**0.67**	**11.53**	**6.89**

LSD (0.05): S = 0.85 | D = 0.85 | W = 1.25 | SD = 1.55 | SDW = 2.45

Table (7) illustrates the concentrations of Lead (Pb) mgkg^{-1}accumulated in earthworm tissues after 60 days. The results reveal pronounced significant differences in the bioaccumulation capacity for Pb, influenced by both soil texture and the nature of the pollutant. E. fetida achieved its maximum Pb accumulation in the loam soil under the W2 treatment, reaching 45.60 $mgkg^{-1}$,which significantly exceeded the values for L. terrestris in the same medium(30.90 $mgkg^{-1}$. This indicates that loam soil, characterized by high porosity and rich organic content, provides an optimal habitat for the surface-dwelling E. fetida, facilitating higher rates of feeding and contact with contaminated particles [10].

On the other hand, L. terrestris proved to be more efficient in the remediation of heavy clay soil, accumulating 39.70 $mgkg^{-1}$ of Pb in the W2 treatment compared to 27.80 $mgkg^{-1}$for E. fetida. This divergence highlights the specialized adaptations of anecic earthworms. Their ability to thrive in dense, compacted clay is supported by a powerful muscular system and physiological mechanisms that allow them to process heavy soils where epigeic species might struggle due to limited oxygen or physical resistance [14, 15].

Similar to the Cadmium results, a clear "Antagonistic Effect" was observed under dual contamination (W3). Pb accumulation in E. fetida (loam soil) fell significantly from 45.60 $mgkg^{-1}$ in the individual treatment (W2) to 34.80 $mgkg^{-1}$ fell significantly in the dual treatment (W3). This decline is attributed to ionic competition for protein transport channels and binding sites within the earthworm's digestive tract [5, 11]. The cumulative toxic burden in W3 likely triggered a defensive physiological response, slowing down the absorption efficiency to mitigate the extreme toxicity [11]. Minimal Pb levels in W0 and W1 reflect the baseline trace elements present in the experimental environment [20].

Table (7) Lead (Pb) concentrations ($mgkg^{-1}$) accumulated in earthworm tissues after 60 days.

Soil (S)	Earthworms (D)	W0	W1	W2	W3	Mean
Loam	***E. fetida***	1.50	1.80	45.60	34.80	**20.93**
	L. terrestris	1.10	1.40	30.90	21.70	**13.78**
Clay	***E. fetida***	0.90	1.20	27.80	18.90	**12.20**
	L. terrestris	1.30	1.60	39.70	29.20	**17.95**
Mean		**1.20**	**1.50**	**36.00**	**26.15**	**16.21**

LSD (0.05): D = 1.85 | S = 1.85 | W = 2.65 | SD = 3.35 | SDW = 5.25

3.3 Antioxidant Enzymatic Defense (SOD Activity)

Table (8) describes the specific cellular activity of superoxide dismutase (SOD units mg^{-1} protein) extracted from earthworm tissues. As the primary enzymatic shield against oxidative stress, SOD catalyzes the dismutation of highly reactive superoxide anions into hydrogen peroxide [12]. The baseline activity in the control group (W0) was low, averaging 12.06 units mg^{-1} protein. However, exposure to heavy metals triggered a dramatic specific surge in SOD activity, peaking at 42.31 units mg^{-1} protein under individual Pb contamination (W2), followed by 37.90 units mg^{-1} protein in the Cd treatment (W1). This striking upregulation represents a direct adaptive mechanism, wherein the accumulation of internal heavy metals triggers an intracellular surge of ROS, stimulating specific gene expression and de novo synthesis of SOD to protect membranes and cellular proteins from catastrophic lipid peroxidation [7, 22].

Crucially, under the combined contamination treatment (W3), a significant, non-linear reduction in SOD activity was observed, declining to 31.60 units mg^{-1} protein compared to individual metal treatments. This decline highlights that the intense synergistic toxicity of the mixed elements induced severe oxidative damage that partially deactivated the enzyme structure, coupled with a depletion of cellular ATP reserves required for active protein translation [11, 23]. Soil texture fundamentally altered this antioxidant profile; earthworms in loam soil (S1) exhibited a significantly higher overall average SOD activity (33.48 units mg^{-1} protein) than those in clay soil (28.46 units mg^{-1} protein). This directly correlates with the higher bioavailability and free ionic movement of metals in loam soil, inducing a stronger specific cellular stress response [10, 20].

The triple interaction reached its absolute maximum in treatment S1D1W2 (*E. fetida* in loam soil under Pb stress), recording an intense specific activity of 54.90 units mg^{-1} protein. This highlights the rapid specific metabolic response of the epigeic species when exposed to high bioavailable metal concentrations [12].

Conversely, in the heavy clay soil matrix (S2), *L. terrestris* exhibited a highly pronounced and statistically superior SOD induction (42.45 and 46.95 units mg^{-1} protein under W1 and W2) compared to *E. fetida*. This demonstrates that the local deep-burrowing species successfully maintains its antioxidant capacity to counter the coupled physical friction of compact clay and internal chemical toxicity [14, 15].

Table (8) Effect of soil texture, earthworm species, and water treatments on superoxide dismutase (SOD) activity (units mg^{-1} protein) after 60 days.

Soil (S)	Earthworms (D)	W0	W1	W2	W3	Mean
Loam	***E. fetida***	13.25	49.10	54.90	39.45	39.18
	L. terrestris	11.80	32.85	37.35	29.15	27.79
Clay	***E. fetida***	11.15	27.20	30.05	22.95	22.84
	L. terrestris	12.05	42.45	46.95	34.85	34.08
Mean		12.06	37.90	42.31	31.60	30.97

LSD (0.05): S = 0.54 | D = 0.54 (N.S) | W = 0.76 | SD = 1.08 | SDW = 2.15

3.4 Neurotoxic Responses (AChE Activity)

Table (9) shows the specific activity of acetylcholinesterase (AChE nmol min^{-1} mg^{-1} protein) in earthworm tissues. AChE is a fundamental biomarker for neurotoxicity, ensuring normal signal transmission by breaking down the neurotransmitter acetylcholine in synaptic clefts [22]. The results demonstrate a severe and statistically significant inhibition of AChE activity under heavy metal exposure compared to the control treatment (W0), which recorded a robust baseline of 86.45 nmol min^{-1} mg^{-1} protein. Enzymatic activity decreased dramatically to 43.06 nmol min^{-1} mg^{-1} protein under Cd stress (W1) and to 37.61 nmol min^{-1} mg^{-1} protein under Pb stress (W2). This sharp decline reveals that heavy metals exert a powerful neurotoxic effect, where free Cd and Pb ions directly bind covalently to the sulfhydryl (–SH) groups of amino acids within the enzyme's active catalytic site, altering its native spatial conformation and blocking substrate engagement [1, 24]. The most acute neurotoxic shutdown occurred under dual contamination (W3), collapsing to an overall minimum of 21.65 nmol min^{-1} mg^{-1} protein; this indicates a severe synergistic neurotoxic block that causes continuous muscular overstimulation and paralysis [11].

Soil texture played a vital role in modifying this neurotoxic response. Earthworms in clay soil (S2) retained a significantly higher overall average AChE activity (49.01 nmol min^{-1} mg^{-1} protein) compared to those in loam soil (45.38 nmol min^{-1} mg^{-1} protein). This statistical تفوق provides clear proof of the protective soil buffering capacity of the clay matrix; its rich mineral colloids and high cation exchange capacity (30.84 cmol kg^{-1}) effectively bind free Cd and Pb ions, lowering their chemical bioavailability and dermal penetration, thereby preserving the earthworms' neural integrity [15, 20].

The triple interaction shows that the lowest absolute activity was recorded in treatment S1D1W3 (*E. fetida* in loam soil under mixed stress), sinking to 15.50 nmol min^{-1} mg^{-1} protein due to the high metal bioavailability in the loam soil and the high cutaneous permeability of the epigeic species [14]. Conversely, in the heavy clay soil, *L. terrestris* experienced a massive reduction in AChE activity under mixed stress (W3), dropping to 18.95 nmol min^{-1} mg^{-1} protein. This reveals that the local deep-burrowing species faced a severe double burden; its intense mechanical and locomotor activity within compact clay demanded continuous neural signal flow, which exacerbated the cumulative chemical inhibition of the absorbed heavy metals within its synaptic pathways [14, 22].

Table (9) Effect of soil texture, earthworm species, and water treatments on acetylcholinesterase (AChE) activity (nmol min^{-1} mg^{-1} protein) after 60 days.

Soil (S)	Earthworms (D)	W0	W1	W2	W3	Mean
Loam	***E. fetida***	85.45	34.25	28.60	15.50	40.95
	L. terrestris	88.50	46.75	41.25	22.75	49.81
Clay	***E. fetida***	82.15	52.45	48.15	29.40	53.04
	L. terrestris	89.70	38.80	32.45	18.95	44.98
Mean		86.45	43.06	37.61	21.65	47.19

LSD (0.05): S = 0.81 | D = 0.81 (N.S) | W = 1.15 | SD = 1.62 | SDW = 3.24

4. Conclusion

The present study successfully demonstrates the efficacy of earthworm-mediated bioremediation, integrated with organic amendments (cow manure), as a sustainable and ecologically sound strategy for managing soils contaminated with sewage-derived heavy metals. The findings confirm a distinct ecological and physiological specialization between the two species studied; *Eisenia fetida* demonstrated superior adaptability, biomass stability, and specific cellular antioxidant induction (SOD) in loam soil, whereas *Lumbricus terrestris* exhibited exceptional resilience and mechanical remediation capacity within compacted clay soil environments.

Furthermore, this research provides deep insights into the cellular mechanisms of toxicity during multi-metal exposure. Combined contamination (Cadmium and Lead) significantly suppressed total biomass and triggered an intense inhibition of acetylcholinesterase (AChE), providing definitive evidence of severe synergistic neurotoxicity and oxidative degradation that approaches the metabolic limits of the organism.

The primary scientific and practical contribution of this work lies in validating the integration of physical soil parameters with multi-tiered biological indicators. By demonstrating that clay matrices act as a protective geochemical shield that mitigates neurotoxic inhibition, this research establishes that specific antioxidant and neural enzyme profiles (SOD and AChE) serve as powerful, highly sensitive early-warning biomarkers. This integrated framework provides a cost-effective protocol for monitoring agricultural lands irrigated with non-conventional wastewater, ultimately safeguarding the food chain from toxic pollutant translocation.

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