



Research Article

Utilising Microflora from Poultry Litter Effluent for Bioremediation of Lead and Nickel Polluted Soil in Maiduguri, Borno State

Musa Saidu Mshelia^{1*}, Ibrahim Shettima Muhammad², Usman Abdullahi Ibrahim², and Usman Yuguda³
¹Works, Physical Planning and Development Department, University of Maiduguri, Maiduguri, Borno State
²Department of Civil and Water Resources Engineering, University of Maiduguri, Maiduguri, Borno State
³Civil Engineering Technology Department, Mai Idris Aloomo Polytechnic Geidam, Yobe State
*Corresponding author's Email: mshmshelia01@unimaid.edu.ng, doi.org/10.55639/607.343332

ARTICLE INFO:

Keywords:

Bioremediation,
Heavy Metals,
Poultry Litter Effluent,
Bacteria,
Soil Pollution

ABSTRACT

The study investigates the cost-effectiveness and environmental compatibility of using poultry litter effluent to remediate Pb and Ni-polluted soil, focusing on its microbial composition and potential for heavy metal remediation. Various bacterial and fungal species were identified, including pathogenic strains such as *E. coli*, *Staphylococcus aureus*, *Salmonella enteritidis*, *Shigella*, *Bacillus sp.*, *Klebsiella sp.*, *Aspergillus fumigatus*, *Fusarium oxysporum*, *Mucor sp.*, *Candida sp.*, *Aspergillus niger*, *Penicillium sp.*, *Trichoderma sp.*, and *Saccharomyces sp.* Microbial counts ranged from 5.36×10^6 to 1.60×10^8 CFU/ml for bacteria and 1.03×10^3 to 8.36×10^4 CFU/ml for fungi. The efficiency of poultry litter effluent in reducing lead (Pb) and nickel (Ni) concentrations in soil samples was assessed. After remediation, Sample A showed a minimal decrease in Pb and Ni concentrations, while Samples B and C showed significant reductions. After treatment with poultry litter effluent, Pb and Ni levels decreased significantly (statistically significant at $P < 0.05$). This shows that *Pseudomonas sp.*, *Saccharomyces sp.*, *Bacillus sp.*, *Aspergillus sp.*, *Penicillium sp.*, and *Candida sp.* can bioaccumulate and absorb Pb and Ni. The sample that was treated with 300 ml of poultry litter effluent showed the highest efficacy in Pb removal, achieving a rate of 92%. On the other hand, the sample that was treated with 200 ml of poultry litter effluent showed an 81% decrease in Ni content. The study can serve as a reference for scholars studying bioremediation in polluted soils, as well as a strategic plan for NESREA and other global environmental protection organisations involved in the bioremediation of contaminated soil.

Corresponding author: Musa Saidu Mshelia, **Email:** mshmshelia01@unimaid.edu.ng
Works, Physical Planning and Development Department, University of Maiduguri, Maiduguri, Borno State

INTRODUCTION

Human activities, which include industrialization, urbanization, technological advancements, and dangerous agricultural methods, have significantly accelerated pollution and environmental degradation (Priya *et al.*, 2023). The ecosystem has been degraded by toxic chemicals and harmful heavy metals, leading to pollution of soil, surface water, and groundwater, posing a significant threat to all living forms on earth. Heavy metals are poisonous and cannot be broken down into innocuous substances by biological, chemical, or physical processes. Unlike organic substances, inorganic substances may persist in the environment for a long time and can only be converted into less harmful forms (Tayang and Songachan, 2021). At lower concentrations, heavy metals like iron, copper, and zinc are necessary for biological systems (proteins and enzymes) (Atigh *et al.*, 2020). However, even at low concentrations, most heavy metals such as lead, mercury, arsenic, chromium, nickel, cadmium, and others are toxic and harmful to humans, plants, and animals (Igiri *et al.*, 2018). Chronic human exposure to these metals can have fatal health implications, as indicated in Table 2.2. Inhalation of polluted air, ingestion of contaminated water, eating food grown on contaminated land, and contact with contaminated soil or industrial waste can all expose humans to heavy metals. The buildup of heavy metals from the surrounding soil and water can contaminate vegetables, cereals, fruits, fish, and shellfish. Heavy metal poisoning of soil is a global issue that requires collaboration between governments and scientific organisations (Bakshi and Banik, 2018). Heavy metals' health effects include cancer, organ damage, nervous system damage, stunted growth and development, and, in severe circumstances, death. Autoimmunity is a condition in which the human immune system assaults its cells as a result of lead and mercury exposure. Heavy metal exposure can cause joint illnesses such as rheumatoid arthritis and renal

disease, as well as circulatory and brain system problems (Godleads *et al.*, 2014; Xu *et al.*, 2019). According to Jalilvand *et al.* (2019), there are approximately 5 million contaminated sites worldwide, encompassing 20 million hectares of polluted land, where heavy metal concentrations are higher than their geo-balance or regulatory norms. They go on to say that these heavy metal-polluted locations have economic costs of around \$20 billion per year.

Remediation of polluted soils and sites is an important step in protecting the environment and living species, and it must be incorporated into the broader interdisciplinary scenario of strategic green transition. Several approaches are used to remediate polluted soil and water. However, these methods (physical, chemical, and thermal) are now regarded as obsolete since their sole remedial goal is to eliminate contaminants without regard for their adverse effects (Wan *et al.*, 2019; Mishra *et al.*, 2021; Dhaliwal *et al.*, 2020). Moreover, these procedures have proven to be exceedingly expensive in both energy and economic terms, as well as highly intrusive, further compromising the already precarious environmental condition (Song *et al.*, 2019; Vocciante *et al.*, 2021). The main goal of soil remediation is to produce an environment that is good for both ecological and human health (Wuana and Okieimen, 2011; Asrari, 2014; Henry *et al.*, 2015). Given all of these consequences, there is a need for a more effective and environmentally friendly technique for remediating contaminated soils and water, and bioremediation may be the best alternative to the existing complex and challenging physical and chemical procedures. This integrated strategy aims to enhance remediation outcomes, maximize social and economic benefits, and minimize environmental harm caused by remediation efforts (Grifoni *et al.*, 2022).

Bioremediation is the process of cleaning a contaminated environment by modifying enzymes while absorbing various harmful metals.

The process is environmentally friendly and novel for ecological restoration by using microorganisms with unique catabolic abilities and their byproducts, like biosurfactants and enzymes, to improve remediation effectiveness. Microorganisms interact with enzymes to break down complex compounds into simpler nutrients, mobilize metal ions, enhance pollution absorption by microorganisms, and facilitate bioremediation (Sharma and Kumar, 2021). Bioremediation is more effective than other methods for removing heavy metals due to its ecologically friendly characteristics, cost-effectiveness, and lack of labour and effort needed (Jeyakumar *et al.*, 2023). The strategies employed in bioremediation include biostimulation, bioaugmentation, bioaccumulation, biosorption, phytoremediation, and rhizoremediation (Mshelia *et al.*, 2023). Metals such as cobalt, selenium, zinc, cadmium, copper, vanadium, arsenic, chromium, mercury, nickel, iron, and lead are very toxic to humans and other living things. The removal of harmful substances from the environment is necessary to prevent adverse effects and recover valuable resources. Metals such as Pb, Cr, Cd, Hg, and Ni are more hazardous in their mixed or elemental states. Metals released into the environment can rapidly accumulate in human tissue or cells. Previous studies have detected heavy metals in the liver, muscle tissues, and gills of many fish species in contaminated maritime habitats. Metals may accumulate in many human body organs once they enter the food chain. Despite the widespread usage of heavy metals in manufacturing, anyone working in or living near these facilities is at high risk of exposure and contamination. Excessive quantities of heavy metals may have adverse effects on humans, other animals, and the environment (Sharma *et al.*, 2021).

MATERIALS AND METHODS

Materials

The materials used in this study consist of soil samples, poultry litter effluent, and distilled water. The investigation used laboratory-grade

nickel in the form of (NiSO₄, 6H₂O), and lead in the form of (PbO). Some of the equipment used in the study includes the Microwave Digester Model: Master 40 by Sineo Chemistry Technology, China; Atomic Absorption Spectrophotometer Type 210VGP AAS by Buck Scientific, USA; Muffle Furnace Type FM 515 by P-Select, Pizzato, Italy; and a 15-litre autoclave steriliser.

Sample Collection

Poultry Litter

The researchers obtained poultry litter from the University of Maiduguri's poultry research farm, which is located at coordinates 11.802734°N, 13.210522°E. 4 kg of poultry litter was measured and mixed with 20 litres of distilled water obtained from the University of Maiduguri Teaching Hospital Kidney Centre. We degraded the combination in the laboratory for a week to produce effluent. For the experiment, filtering the degraded poultry litter resulted in the collection of liquid waste.

Soil Sample Preparation

Soil samples were obtained at a depth of 7–15 cm behind the Environmental Engineering Laboratory, Faculty of Engineering, University of Maiduguri (11.813049°N, 13.205356°E). In order to get a uniform particle size, the soil sample was dried in the laboratory for 24 hours at room temperature and then sieved through a 2mm mesh. After being artificially contaminated with 50g of laboratory-grade Pb, and Ni salts, the soil sample was air-dried for 24 hours. These salts were obtained from Park Scientific in Northampton, England; BDH Laboratory Supplies in Poole, England; and LobaChemie Pvt. Ltd. in Coloba, Bombay, India. The soil sample was put in a plastic container measuring 200mm in diameter and 150mm in depth. The containers were labelled A, B and C. Prior to introducing the abattoir and poultry litter effluents, a soil sample weighing 60g was obtained from each container for the purpose of determining the concentrations of heavy metals (Pb, and Ni) present in the soil samples.

Bioremediation Process Setup

Sample A (the control sample) was not exposed to poultry litter effluent. Sample B was administered 200 ml of poultry litter effluent, while Sample C was administered 300 ml of

poultry litter effluent. All the samples in the containers were kept in the laboratory at ambient temperature for three weeks. Subsequently, all the samples were exposed to air and watered with a set amount of distilled water, namely 200 ml every 5 days. Throughout the study, soil samples were collected every 7 days to examine the concentration of heavy metals in the samples. All analyses were conducted three times.

Elemental Analysis with Atomic Absorption Spectrophotometer (AAS)

Heavy metal levels in the samples were analysed using an atomic absorption spectrophotometer (AAS 210 VGP, Buck Scientific Company, USA) at the Yobe State University Department of Chemistry Analytical Laboratory, Yobe State. Measuring the absorbance determined the concentration of heavy metals in the soil sample. The equipment identified the heavy metals using absorption/concentration mode and recorded the result for each solution. We used the same analytical method to determine the amounts of heavy metals in both digested blank solutions and spiked samples.

Microbiology Analysis

Microbiological analysis was performed at the Department of Biological Sciences laboratory at the University of Maiduguri in Borno State, Nigeria. In order to isolate bacteria from the effluent sample, a 1 ml portion of the effluent was mixed with 9 ml of distilled water. The mixture was rapidly shaken, and the resulting solution was diluted sequentially in ten-fold increments. 0.1 ml portions of the contents in each of the test tubes (10^{-2} , 10^{-3} , 10^{-5}) were aseptically distributed onto petri dishes containing nutrient agar, eosin methylene blue agar, mannitol salt agar, and MacConkey agar. These agar plates were prepared following the instructions provided by the manufacturers. The plates were then incubated at a temperature of 37°C for a duration of 24 hours. In order to isolate fungi from the effluent sample, the same method as previously explained was

used, using Sabouroud dextrose agar and incubating at a temperature of 30°C for a duration of 3 days. After the incubation period, colonies with distinct characteristics were chosen and sub-cultured on nutrient agar for bacteria and Sabouroud dextrose agar for fungi in order to get pure samples. These samples were then used for the identification of the isolated bacteria and fungi. Additionally, the bacterial isolates were subjected to a series of tests, including catalase, methyl red, indole, oxidase, Voges-Proskauer, citrate, aerobic, and anaerobic tests, in order to identify them. The bacterial isolates were characterised and classified using their biochemical characteristics, using the approach outlined in Bergey's Manual of Determinative Bacteriology (Holt et al., 1994). The fungi were identified using their macroscopic and microscopic features, as well as references such as "Smith's Introduction to Industrial Mycology" (Onions et al., 1981) and "Introduction to Food-Borne Fungi" (Samson and Reenen-Hockstra, 1988).

Determination of Total Bacterial and Fungal Counts

Poultry litter effluent samples were diluted by a factor of ten up to 10^{-5} in sterile distilled water to count the bacteria and fungi present in triplicate. Bacterial plate counts were conducted on nutritional agar, while fungal counts were done on Sabouroud dextrose agar using the pour-plate method. Successive dilutions of 0.1 ml slaughterhouse and poultry litter effluent samples were pipetted onto sterile Petri dishes containing nutritional agar and Sabouroud dextrose agar for bacterial and fungal culture, respectively. The samples were then incubated at 37°C. The total number of bacteria and fungi was calculated using a colony counter and reported as colony-forming units per millilitre (CFU/ml).

$$CFU = \frac{\text{Number of colonies} \times \text{Total Dilution Factor (TDF)}}{\text{Volume of culture plated (ml)}} \dots \dots \dots (1)$$

Bacteria colonies ranging from 30 to 300 were counted, with fewer than 30 considered too few to count (TFTC) and more than 300 considered too many to be counted (TMTC), according to Kurna *et al.* (2016). Fungal colonies between 10 and 150 were counted, with fewer than 10 and more than 150 being disregarded, according to ESA (2012).

Data Analysis

Data collected from the study were subjected to a two-way repeated measures analysis of variance

(ANOVA) using GraphPad Prism version 9.0 to compare the reduction in heavy metal levels in each sample examined, setting a significance threshold at 5%.

RESULTS AND DISCUSSION

Bacterial and Fungal Isolates

Table 1 of this study lists the identified bacteria from poultry litter effluent sample. The bacteria were identified based on their colony form and biochemical tests, whereas the fungal isolates were identified based on their cultural, microscopic, and macroscopic properties.

Table 1: Morphological and biochemical observations of different bacteria isolated from poultry litter effluent sample.

Form	Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular
Surface	Smooth	Smooth	Smooth	Smooth	Smooth	Shiny	Smooth	Smooth
Colour	Yellow	White	Yellowish	Colorless	Greenish	White	Cream	Whitish
Margin	Entire	Entire	Entire	Entire	Entire	Entire	Undulate	Entire
Elevation	Flat	Convex	Raised	Convex	Convex	Convex	Raised	Convex
Opacity	Transparent	Transparent	Opaque	Transparent	Rough	Moist	Opaque	Translucent
Shape	rod	rod	cocci	rod	rod	rod	rod	rod
Gram Reaction	-	-	+	-	-	-	+	-
Catalase	+	+	+	+	+	+	+	+
Oxidase	-	-	-	-	+	-	-	-
Citrate	+	-	-	-	+	+	+	+
MR	+	+	+	+	+	-	+	-
VP	-	-	-	-	-	+	-	+
Indole	+	+	-	+	-	-	+	-
Ae/An	F	F	F	F	A	F	A	F
Bacteria Identified	<i>Salmonella enteritidis</i>	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>	<i>Shigella</i> sp.	<i>Pseudomonas aeruginosa</i>	<i>Enterobacter</i> sp.	<i>Bacillus</i> sp.	<i>Klebsiella</i> sp.
Sample	PLE	PLE	PLE	PLE	PLE	PLE	PLE	PLE

*MR- Methyl Red, VP- Vogues Proskauer, PLE- Poultry Litter Effluent.

Pseudomonas aeruginosa, *Enterobacter* sp., *Staphylococcus aureus*, *Salmonella enteritidis*, *Shigella* sp., *Bacillus* sp., *Klebsiella* sp., and *E. coli* were identified in the poultry litter effluent. While *Aspergillus fumigatus*, *Fusarium oxysporum*, *Mucor* sp., *Candida* sp., *Aspergillus niger*, *Penicillium* sp., *Trichoderma* sp., and *Saccharomyces* sp. were the fungi identified in the poultry litter effluent. Microbes, including *E. coli*, *Staphylococcus* sp., *Enterobacter* sp., *Salmonella* sp., *Aspergillus niger*, and *Penicillium* sp., are pathogenic. Several research investigations have identified pathogenic bacteria with comparable traits in poultry litter (Mshelia *et al.*, 2023).

Enumeration of Bacteria and Fungi in Poultry Litter Effluent

Table 2 shows the microbial counts in poultry litter effluent. The result shows that the bacterial count varied from 5.36×10^6 - 1.60×10^8 CFU/ml, whereas the fungal count ranged from 1.03×10^3 - 8.36×10^4 CFU/ml. Furthermore, *E-coli* has the

highest count of 3.35×10^8 CFU/ml, while *Staphylococcus* sp. has the least count of 2.14×10^7 CFU/ml. *Aspergillus niger* has the highest fungal count of 8.36×10^4 CFU/ml, while *Candida* sp has the lowest fungal count of 1.03×10^3 CFU/ml.

Table 2: Bacterial and Fungal count for poultry litter effluent.

S/No.	BACTERIA	CFU/ml	FUNGI	CFU/ml
1	<i>Salmonella enteritidis</i>	2.61×10^8	<i>Aspergillus niger</i>	8.36×10^4
2	<i>Bacillus</i> sp.	5.36×10^6	<i>Fusarium oxysporum</i>	7.09×10^3
3	<i>Escherichia-coli</i> .	3.35×10^8	<i>Aspergillus fumigatus</i>	6.45×10^5
4	<i>Staphylococcus aureus</i>	2.14×10^7	<i>Mucor</i> sp.	4.82×10^3
5	<i>Klebsiella</i> sp.	3.14×10^8	<i>Penicillium</i> sp.	4.73×10^3
6	<i>Shigella</i> sp.	4.41×10^7	<i>Trichoderma</i> sp.	8.0×10^4
7	<i>Pseudomonas aeruginosa</i>	1.60×10^8	<i>Candida</i> sp.	1.03×10^3
8	<i>Enterobacter</i> sp.	2.75×10^8	<i>Saccharomyces</i> sp.	6.91×10^3

*CFU/ml; Colony Forming Unit/Milliliter.

Lead (Pb) Polluted Soil Remediation

The initial Pb content in sample A was 38.28 ± 0.04 mg/kg; however, it was slightly

reduced to 35.24 ± 0.07 mg/kg in week 1, 32.15 ± 0.07 mg/kg in week 2, and 29.13 ± 0.07 mg/kg in week 3 (Table 3).

Table 3: The mean standard deviation concentration for Lead in soil remediated with poultry litter effluent.

Sample Id	Initial Conc.	Week 1	Week 2	Week 3
	$\bar{X} \pm SD$	$\bar{X} \pm SD$	$\bar{X} \pm SD$	$\bar{X} \pm SD$
A - Control	38.28 ± 0.04	35.20 ± 0.07	32.15 ± 0.07	29.13 ± 0.07
B – 200ml PLE	38.75 ± 0.17^a	28.11 ± 0.21^b	18.78 ± 0.34^c	9.35 ± 0.11^d
C – 300ml PLE	39.05 ± 0.85^a	28.54 ± 0.67^b	19.29 ± 0.66^c	$3.09 \pm 5.35^{c,d}$

* PLE Poultry Litter Effluent

*Values with different superscripts within a row are significantly different ($P < 0.05$).

Before remediation, the Pb concentration in sample B was 38.75 ± 0.17 mg/kg, and after remediation, the concentration was significantly reduced to 28.11 ± 0.21 mg/kg in week 1, 18.78 ± 0.34 mg/kg in week 2, and 9.35 ± 0.11 mg/kg in week 3. The reduction in Pb concentration in the sample might be related to research by Farhan & Khadom (2015) and Alori *et al.* (2018) that indicated *Pseudomonas* sp. and *Saccharomyces* sp. can be used to remediate Pb-polluted soil due to the bacteria's Pb accumulation potentials. In sample C, a

substantial reduction was seen in the first, second, and third weeks following remediation with poultry litter effluent compared to the Pb initial concentration (39.05 ± 0.85 mg/kg, 28.54 ± 0.67 mg/kg, 19.29 ± 0.66 mg/kg, and 3.09 ± 5.35 mg/kg). The decrease in Pb concentration in the sample was consistent with the research of Ramasamy *et al.* (2011) and Njoku *et al.* (2020), which found *Bacillus* sp. and *Aspergillus fumigatus* to be viable biosorbents for removing Pb from contaminated soil. *Staphylococcus* sp. can uptake Pb through biosorption by 88.33% of $1000 \mu\text{g/ml}$

to 1600 μ g/ml, as stated by Aslam *et al.* (2020); *Enterobacter* sp. can remove up to 85% of the 50ppm concentration of Pb in 96 hours, according to Aisami *et al.* (2020); and Espinoza-C *et al.* (2021) reported a 69.33% to 90.23% removal of Pb by *Saccharomyces* sp. Luna *et al.* (2016) also observed that *Candida* sp. produces biosurfactant with Pb removal efficiencies of 79%. According to Godleads *et al.* (2014), bacteria that can survive in the presence of high concentrations of lead use a number of different strategies to avoid being poisoned by the metal. These strategies include the use of an efflux

mechanism, extracellular sequestration, biosorption, precipitation, morphological changes to the cells, increased production of siderophores, and intracellular lead bioaccumulation.

There was a small decrease in the concentration of Pb within the control sample (A) from 8% at week 1, 16% at week 2, and 24% at week 3. While looking at the rate decrease of Pb in samples B and C spiked with poultry litter effluent from week 1 to week 3 after remediation, the decrease is bigger, going from 27% to 92% (Fig. 1).

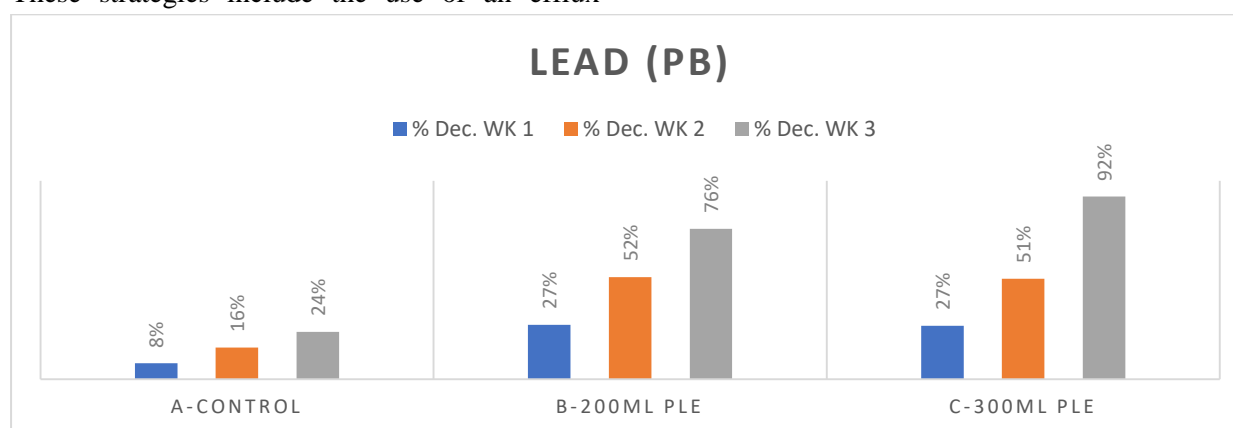


Fig. 1: Percentage decrease in lead concentration at different weeks.

At week 1, samples B and C had a Pb concentration reduction of 27% each. At week 2, sample B has a reduction of 52% in Pb levels, while sample C has a reduction of 51%. At week 3, after remediation with the effluent, samples B show a 76% drop in lead levels, while sample C has the highest lead content reduction of 92%.

Nickel (Ni) Polluted Soil Remediation

One of the heavy elements utilised to pollute the soil samples was Ni. The initial mean \pm SD Ni content in sample A was 41.80 \pm 0.20 mg/kg, 39.55 \pm 0.23 mg/kg at week 1, 37.44 \pm 0.19 mg/kg at week 2, and 35.29 \pm 0.04 mg/kg at week 3 (Table 4).

Table 4: The mean standard deviation concentration for Nickel in soil remediated with poultry litter effluent.

Sample Id	Initial Conc.	Week 1	Week 2	Week 3
	$\bar{X}\pm$ SD	$\bar{X}\pm$ SD	$\bar{X}\pm$ SD	$\bar{X}\pm$ SD
A - Control	41.80 \pm 0.20	39.55 \pm 0.23	37.44 \pm 0.19	35.29 \pm 0.04
B – 200ml PLE	44.33 \pm 0.90 ^a	28.23 \pm 0.92 ^b	22.74 \pm 1.01 ^c	8.45 \pm 7.32 ^{b,c,d}
C – 300ml PLE	45.95 \pm 4.78 ^a	29.67 \pm 3.93 ^b	23.98 \pm 3.46 ^c	12.63 \pm 1.63 ^{c,d}

* PLE Poultry Litter Effluent

*Values with different superscripts within a row are significantly different (P < 0.05).

The Ni concentration in sample C was 44.33 ± 0.90 mg/kg before remediation; following remediation, the concentration dropped significantly ($P < 0.05$) to 28.23 ± 0.92 mg/kg, 22.74 ± 1.01 mg/kg, and 8.45 ± 7.32 mg/kg in weeks 1, 2, and 3, respectively. *Aspergillus* sp. can bioaccumulate and biosorb Ni, as stated in two separate studies by Tastan *et al.* (2010) and Pundir *et al.* (2018). *Aspergillus* sp. may have contributed to the reduction in Ni concentration in the soil sample after remediation by being present in the poultry litter effluent. The initial concentration of Ni in sample E was 45.95 ± 4.78 mg/kg. After remediation, the concentration significantly dropped in weeks 1, 2, and 3, with mean \pm SD values of 29.67 ± 3.93 mg/kg, 23.98 ± 3.46 mg/kg, and 12.63 ± 1.63 mg/kg, respectively. The bioremediation potentials and presence in the effluent of microorganisms *Bacillus* sp., *Penicillium* sp., and *Candida* sp. in the bioaccumulation and biosorption of Ni, as

mentioned by Anahid *et al.* (2011) and Ghosh *et al.* (2022), can be attributed to the reduction in concentration of Ni in the soil sample remediated with the poultry litter effluent. The ability of microorganisms like *Bacillus* sp., *Aspergillus niger*, *Penicillium* sp., and *Candida* sp. to bioaccumulate and biosorb Ni has been studied by Tastan *et al.* (2010), Anahid *et al.* (2011), Pundir *et al.* (2018), and Ghosh *et al.* (2022). This can be explained by the fact that the concentration of Ni in the soil samples that were cleaned up with the effluents went down. *Bacillus* sp. can uptake Ni up to $50 \mu\text{g/ml}$ to $150 \mu\text{g/ml}$ intracellularly (bioaccumulation) and by passive phenomenon (biosorption), as mentioned by Goyal *et al.* (2019), while *Penicillium* sp. has the capability to uptake $7.5 \mu\text{g/l}$ of Ni in 140 minutes, according to Sundararaju *et al.* (2020).

The percentage reduction in Ni concentration in soil samples after three weeks of remediation with poultry litter effluent is shown in Fig. 2.

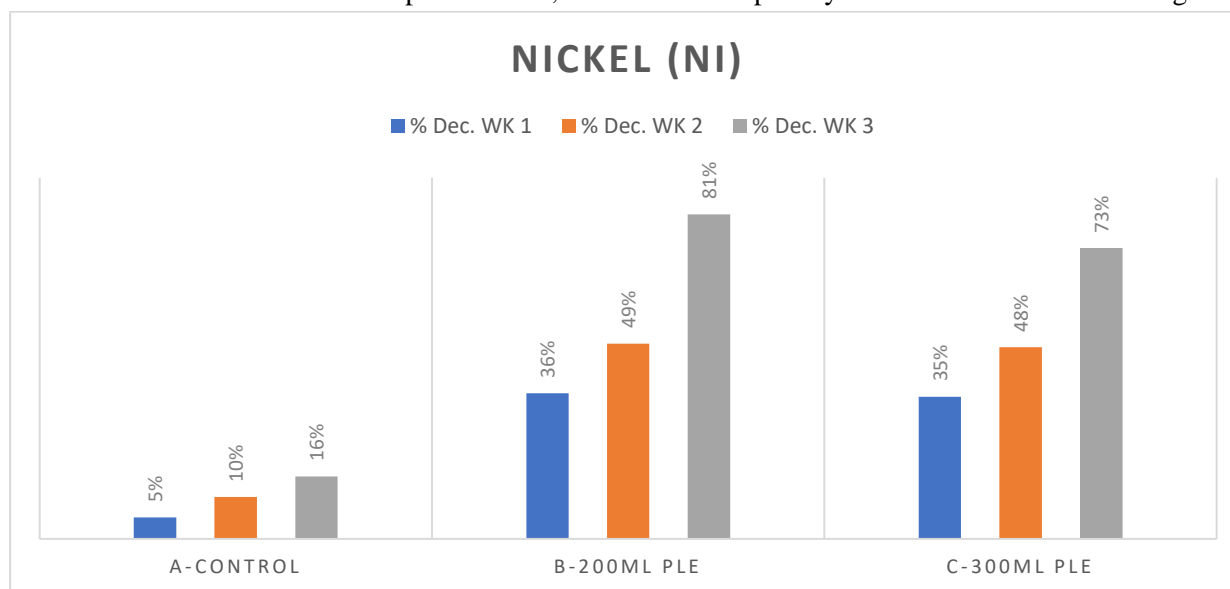


Fig. 2: Percentage decrease in nickel concentration at different week.

The reduction of Ni in samples B and C spiked with poultry litter effluent is significant, ranging from 35 to 81 percent from week one to week three after remediation. At week 1, sample C shows the smallest percentage loss (35%), whereas sample A has the highest percentage

decline (36%). At week 2, Sample C has the lowest percentage decline (48%), while Sample A has the highest percentage decline (49%). Sample C had a 73 percent reduction in Ni after remediation with the effluents, whereas sample A had an 81 percent reduction after week 3.

Efficacy of Poultry Litter Effluent in Bioremediation

To assess the effectiveness of poultry litter effluent in the bioremediation of polluted soil, the concentration of heavy metals in the soil was initially noted. Before treatment, the concentrations of lead and nickel in the soil ranged from 38.75 ± 0.17 mg/kg to 39.05 ± 0.85 mg/kg for lead and 44.33 ± 0.90 mg/kg to 45.95 ± 4.78 mg/kg for nickel, respectively. After it was remediated with the effluent, the concentration dropped to 3.09 ± 5.35 mg/kg and 8.45 ± 7.32 mg/kg. The samples remediated with poultry litter effluent showed a 92% and 81% reduction in lead and nickel concentrations. From the study, it was observed that poultry litter effluent was effective in the bioremediation of lead and nickel-polluted soil, and it was noted that this efficacy was increasing over time. However, further increasing the remediation time will also enhance the effectiveness of the bioremediation process. The lower amount of lead and nickel in the soil samples that were remediated with poultry litter effluent is due to the bioaccumulation and biosorption abilities of the microorganisms found in the effluent, including *Saccharomyces* sp., *Pseudomonas aeruginosa*, *Rhizopus* sp., *Bacillus* sp., *Enterobacter* sp., *Aspergillus fumigatus*, *Staphylococcus aureus*, *Aspergillus niger*, *Penicillium* sp., *Candida*, and *Salmonella enteritidis*. The findings of this study are in agreement with the research findings of Tastan *et al.* (2010), Anahid *et al.* (2011), Ahemad and Malik (2012), Farhan and Khadom (2015), Moukhtari *et al.* (2018), and Ho *et al.* (2018). Pundir *et al.* (2018), Ghosh *et al.* (2022), Hu *et al.* (2023) and Wróbel *et al.* (2023) found that these microorganisms have the potential to be useful sources of and effective tools for bioremediation of heavy metal-polluted soil.

CONCLUSION

This study aimed to evaluate the effectiveness of poultry litter effluent in bioremediating polluted

soil. The soil had high concentrations of lead and nickel before treatment, which dropped after being remediated with the effluent. The results showed a 92% and 81% reduction in lead and nickel concentrations, respectively. This was due to the bioaccumulation and biosorption abilities of microorganisms found in the effluent, including *Saccharomyces* sp., *Pseudomonas aeruginosa*, *Rhizopus* sp., *Bacillus* sp., *Enterobacter* sp., *Aspergillus fumigatus*, *Staphylococcus aureus*, *Aspergillus niger*, *Penicillium* sp., *Candida*, and *Salmonella enteritidis*. The bacterial and fungal species identified are both beneficial and pathogenic strains, with varying population densities. Significant reductions in lead and nickel concentrations over a three-week period demonstrated the remediation potential of the poultry litter effluent. The highest efficacy in lead removal was achieved with 300 ml of effluent, resulting in a 92% reduction. The study provides novel insights into the potential use of microflora derived from poultry litter effluent for soil bioremediation in heavy metal contamination cases. The study can serve as a reference for scholars studying bioremediation in polluted soils and a strategic plan for NESREA and other global environmental protection organisations involved in the bioremediation of contaminated soil.

Limitation and Future Prospects

Although there have been many well-established research papers, the use of poultry litter effluent and microbes to remove heavy metals is mostly limited to laboratory settings. The main impediment and disadvantage of this technology is its restricted capability for large-scale production and commercialization. In order to enhance productivity and accomplish sustainable development objectives, it is essential to advance the integration of technology in bioremediation. It is crucial to carry out progressive and exploratory research in this setting, which entails comprehensive investigations on molecular-level remediation processes and models. Also, there is

a need to study the long-term effects of poultry litter effluent on soil health and the microbial diversity of the effluent.

REFERENCES

- Aisami, A., Mahdi, Z. A., Chindo, H. A., James, I. J., and Garba, L. (2020). Evaluation of Lead Resistance by Newly Identified Enterobacter Cloacae Strain Ahz 01 Isolated from Contaminated Soil. *Greener Journal of Biological Sciences*, 10(2), 42-47.
- Alori, E. T., Joseph, A., Adebisi, O. T. V., Aluko, A. P., and Onyekankeya, C. (2018). Bioremediation Potentials of Sunflower and Pseudomonas Species in Soil Contaminated with Lead and Zinc. *African Journal of Biotechnology*, 17(44), 1324- 1330. doi:10.5897/AJB2018.16523.
- Anahid, S., Yaghmaei, S., and Ghobadinejad, Z. (2011). Heavy Metal Tolerance of Fungi. *Scientia Iranica, Transactions C: Chemistry and Chemical Engineering*, 18(2011), 502-508. doi:10.1016/j.scient.2011.05.015.
- Aslam, F., Yasmin, A., and Sohail, S. (2020). Bioaccumulation of Lead, Chromium, and Nickel by Bacteria from Three Different Genera Isolated from Industrial Effluent. *International Microbiology*, 23(2), 253-261. doi:10.1007/s10123-019-00098-w.
- Asrari, E. (Ed.). (2014). Heavy Metal Contamination of Water and Soil: Analysis, Assessment, and Remediation Strategies (1st ed.). Apple Academic Press. <https://doi.org/10.1201/b16566>.
- Atighi, Z. B. Q., Heidari, A., Sepehr, A., Bahreini, M., and Mahbub, K. R. (2020). Bioremediation of Heavy Metal Contaminated Soils Originated from Iron Ore Mine by Bio-Augmentation with Native Cyanobacteria. *Iranian (Iranica) Journal of Energy and Environment*, 11(2), 89-96. doi:10.5829/ijee.2020.11.02.01.
- Bakshi, S., and Banik, C. (2018). The Impact of Heavy Metal Contamination on Soil Health (Vol. 2). Cambridge, UK: *Burleigh Dodds Science Publishing Limited*.
- Dhaliwal, S. S., Singh, J., Taneja, P. K., and Mandal, A. (2020). Remediation Techniques for Removal of Heavy Metals from the Soil Contaminated through Different Sources: A Review. *Environmental Science and Pollution Research*, 27(2), 1319-1333. doi:10.1007/s11356-019-06967-1
- ESA. (2012). Milk and milk products: Enumeration of colonyforming units of yeasts and/or moulds. (ES ISO 6611:2012). Ethiopia: *Ethiopian Standard Agency*
- Espinoza-C, M., Gómez, A. V. S., Valladares, M. R., Flores, E., and Castañeda, M. T. (2021). Bioremediation of Lead in Water by Two Pure Microorganisms and Bacteria-Yeast Consortium. *BioTecnología*, 25(2), 33-46.
- Farhan, S. N., and Khadom, A. A. (2015). Biosorption of Heavy Metals from Aqueous Solutions by *Saccharomyces Cerevisiae*. *International Journal of Industrial Chemistry*, 6(2), 119- 130. doi:10.1007/s40090-015-0038-8.
- Ghosh, S., Bhattacharya, J., Nitnavare, R., and Webster, T. J. (2022). *Heavy Metal Removal by Bacillus for Sustainable Agriculture*. (M. T. Islam, M. Rahman, and P. Pandey Eds.): Springer, Cham.
- Godleads, A., Prekeyi, T.-F., Ehinomen, I., and Odukonya, E. (2014). Assessment of Heavy Metals Bioremediation Potential of Microbial Consortia from Poultry Litter and Spent Oil Contaminated Site. *International Journal of Environmental Bioremediation & Biodegradation*, 2(2), 84-92. doi:10.12691/ijebb-2-2-6.
- Goyal, P., Belapurkar, P., and Kar, A. (2019). A Review on in Vitro and in Vivo

Conflict of Interest

The authors declare no conflict of interest

- Bioremediation Potential of Environmental and Probiotic Species of Bacillus and Other Probiotic Microorganisms for Two Heavy Metals, Cadmium and Nickel. *Biosciences, Biotechnology Research Asia*, 16(1), 0113. doi:10.13005/bbra/2714.
- Grifoni, M., Franchi, E., Fusini, D., Vocciant, M., Barbafieri, M., Pedron, F., . . . Petruzzelli, G. (2022). Soil Remediation: Towards a Resilient and Adaptive Approach to Deal with the Ever-Changing Environmental Challenges. *Environments*, 9(2). doi:10.3390/environments9020018.
- Henry, H., Naujokas, M. F., Attanayake, C., Basta, N. T., Cheng, Z., Hettiarachchi, G. M., . . . Scheckel, K. G. (2015). Bioavailability-Based in Situ Remediation to Meet Future Lead (Pb) Standards in Urban Soils and Gardens. *Environmental Science & Technology*, 49(15), 8948-8958. doi:10.1021/acs.est.5b01693.
- Hu, F., Wang, P., Li, Y., Ling, J., Ruan, Y., Yu, J., and Zhang, L. (2023). Bioremediation of Environmental Organic Pollutants by Pseudomonas Aeruginosa: Mechanisms, Methods and Challenges. *Environmental Research*, 239, 117211. doi:https://doi.org/10.1016/j.envres.2023.117211.
- Igiri, B. E., Okoduwa, S. I. R., Idoko, G. O., Akabuogu, E. P., Adeyi, A. O., and Ejiogu, I. K. (2018). Toxicity and Bioremediation of Heavy Metals Contaminated Ecosystem from Tannery Wastewater: A Review. *Journal of Toxicology*, 2018, 16. doi:10.1155/2018/2568038.
- Jalilvand, N., Akhgar, A., Alikhani, H. A., Rahmani, H. A., and Rejali, F. (2019). Removal of Heavy Metals Zinc, Lead, and Cadmium by Biomineralization of Urease-Producing Bacteria Isolated from Iranian Mine Calcereous Soils. *Journal of Soil Science and Plant Nutrition*. doi:10.1007/s42729-019-00121-z.
- Jeyakumar, P., Debnath, C., Vijayaraghavan, R., and Muthuraj, M. (2023). Trends in Bioremediation of Heavy Metal Contaminations. *Environmental Engineering Research* 28(4).
- Kurna, B. B., Yadima, S. G., & Usman, B. (2016). Bacteriological Quality Assessment of Irrigation Water and Irrigated vegetables in Maiduguri, Borno State, Nigeria. *Civil and Environmental Research*, 8(11).
- Luna, J. M., Rufino, R. D., and Sarubbo, L. A. (2016). Biosurfactant from Candida Sphaerica Ucp0995 Exhibiting Heavy Metal Remediation Properties. *Process Safety and Environmental Protection*, 102, 558-566. doi:https://doi.org/10.1016/j.psep.2016.05.010.#
- Mishra, S., Lin, Z., Pang, S., Zhang, Y., Bhatt, P., and Chen, S. (2021). Biosurfactant Is a Powerful Tool for the Bioremediation of Heavy Metals from Contaminated Soils. *Journal of Hazardous Materials*, 418, 126253. doi:https://doi.org/10.1016/j.jhazmat.2021.126253
- Mshelia, M. S., Jones, A. N., and Yadima, S. G. (2023). Bioremediation of Soil Polluted with Cadmium and Zinc Using Abattoir Effluent in Maiduguri, Borno State, Nigeria. *Nigerian Journal of Engineering*, 29(3), 28-28.
- Njoku, K. L., Akinyede, O. R., and Obidi, O. F. (2020). Microbial Remediation of Heavy Metals Contaminated Media by Bacillus Megaterium and Rhizopus Stolonifer. *Scientific African*, 10, 1-8. doi:10.1016/j.sciaf.2020.e00545.
- Pundir, R., Chary, G. H. V. C., and Dastidar, M. G. (2018). Application of Taguchi Method for Optimizing the Process Parameters for the Removal of Copper and Nickel by Growing Aspergillus Sp. *Water Resources and Industry*, 20, 83-92. doi:10.1016/j.wri.2016.05.001.
- Priya, A. K., Muruganandam, M., Ali, S. S., and Kornaros, M. (2023). Clean-up of Heavy Metals from Contaminated Soil by Phytoremediation: A Multidisciplinary and Eco-Friendly Approach. *Toxics*, 11(5). doi:10.3390/toxics11050422.

- Ramasamy, R. k., Congeevaram, S., and Thamaraiselvi, K. (2011). Evaluation of Isolated Fungal Strain from E-Waste Recycling Facility for Effective Sorption of Toxic Heavy Metal Pb (II) Ions and Fungal Protein Molecular Characterization- a Mycoremediation Approach. *Asian J. Exp. Biol. Sci.*, 2(2), 342-347.
- Sharma, P., and Kumar, S. (2021). Bioremediation of Heavy Metals from Industrial Effluents by Endophytes and Their Metabolic Activity: Recent Advances. *Bioresource Technology*, 339, 125589. doi:https://doi.org/10.1016/j.biortech.2021.125589
- Sharma, P., Pandey, A. K., Kim, S.-H., Singh, S. P., Chaturvedi, P., and Varjani, S. (2021). Critical Review on Microbial Community During in-Situ Bioremediation of Heavy Metals from Industrial Wastewater. *Environmental Technology & Innovation*, 24, 101826. doi:https://doi.org/10.1016/j.eti.2021.101826
- Song, Y., Kirkwood, N., Maksimović, Č., Zheng, X., O'Connor, D., Jin, Y., and Hou, D. (2019). Nature Based Solutions for Contaminated Land Remediation and Brownfield Redevelopment in Cities: A Review. *Science of The Total Environment*, 663, 568-579. doi:https://doi.org/10.1016/j.scitotenv.2019.01.347.
- Sundararaju, S., Manjula, A., Kumaravel, V., Muneeswaran, T., and Vennila, T. (2020). Biosorption of Nickel Ions Using Fungal Biomass *Penicillium* Sp. Mrf1 for the Treatment of Nickel Electroplating Industrial Effluent. *Biomass Conversion and Biorefinery*. doi:10.1007/s13399-020-00679-0.
- Tastan, B. E., Ertug̃rul, S., and Dönmez, G. (2010). Effective Bioremoval of Reactive Dye and Heavy Metals by *Aspergillus Versicolor*. *Bioresource Technology* 1, 11, 870–876. doi:10.1016/j.biortech.2009.08.099.
- Tayang, A., and Songachan, L. (2021). Microbial Bioremediation of Heavy Metals. *Current Science*, 120(6), 00113891.
- Vocciante, M., Dovì, V. G., and Ferro, S. (2021). Sustainability in Electrokinetic Remediation Processes: A Critical Analysis. 13(2), 770.
- Wan, X., Lei, M., and Chen, T. (2019). Review on Remediation Technologies for Arsenic- Contaminated Soil. *Frontiers of Environmental Science & Engineering*, 14(2), 24. doi:10.1007/s11783-019-1203-7.
- Wróbel, M., Śliwakowski, W., Kowalczyk, P., Kramkowski, K., and Dobrzyński, J. (2023). Bioremediation of Heavy Metals by the Genus *Bacillus*. *International Journal of Environmental Research and Public Health*, 20(6), 4964.
- Wuana, R. A., and Okieimen, F. E. (2011). Heavy metals in Contaminated Soils: A review Of sources, Chemistry, Risks and Best Available Strategies for Remediation. *International Scholarly Research Network ISRN Ecology*, 2011(402647), 1-20. doi:10.5402/2011/402647
- Xu, J., Liu, C., Hsu, P.-C., Zhao, J., Wu, T., Tang, J., . . . Cui, Y. (2019). Remediation of Heavy Metal Contaminated Soil by Asymmetrical Alternating Current Electrochemistry. *Nature Communications*, 10(2440), 1-8. doi:10.1038/s41467-019-10472-x.