

# Biochemical and Microbial Responses to Trace Element Contamination in Coal Waste–Impacted Soils: Implications for Sustainable Agriculture

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## Abstract

Coal mining, coal combustion residues and waste from several industrial activities are an increasing source of soil degradation, soil health decline, heavy-metal contamination, declining agricultural land fertility and productivity, and overall human health in many regions across Africa, including Nigeria. They further disrupt microbial communities, nutrient cycles and metabolic dynamics and agricultural sustainability, posing serious risk to the entire ecosystem. This study sought the ecological impacts of coal waste contamination on the physicochemical parameter, trace element concentrations, pollution indices and microbial community and also used principal component analysis and multivariate analysis to assess variables to stall biases in the interpretation of results for soil samples collected from coal waste contaminated soil and the control sites in Enugu State, Nigeria. Physicochemical parameters, trace element concentrations, and microbial profiles were assessed using standard analytical protocols. Atomic absorption spectrophotometry and the Kjeldahl method were used for elemental quantification. We assessed the pollution indices, including Contamination Factor (CF), Geo-accumulation Index (Igeo), Enrichment Factor (EF), and Pollution Load Index (PLI). The qualitative microbial assessments and biochemical characterisation were performed using microscopy and culture-based techniques. Statistical analysis was done using R. The test included ANOVA and Principal Component Analysis (PCA). Coal-contaminated soils indicated significant reductions in nitrogen (↓45.5%), organic matter (↓22.6%), calcium (↓24.7%), and effective cation exchange capacity (↓24.4%), with slight acidification (pH 6.35 → 6.25) compared to the control soil samples. The coal-waste contaminated and control soil samples showed that trace element levels, like molybdenum (Mo), were above WHO limits. The pollution indices showed moderate Cu contamination (CF = 1.40, Igeo = 0.48, EF = 1.40), a strong Fe depletion (CF = 0.08, Igeo = -2.64), and a high pollution (PLI = 11.7) in the contaminated soil. The morphological and biochemical test//assays for the microbial identification suggested the presence of stress tolerance and remediation organisms, *Bacillus* and *Pseudomonas* species, indicative of how effective they are at bio-decontamination processes. Coal waste contamination causes ecological strains such as nutrient disruptors and microbial instability. Hence, the incorporation of remediation strategies like bio-organic enhancement, phytoremediation, microorganism-assisted remediation, and native microbial inoculants is necessary to revitalise soil health and support sustainable agriculture aligned with the Sustainable Development Goals.

**Keywords:** Soil contamination; Trace element pollution; Indigenous microbial inoculants; Sustainable land restoration; Agroecological risk assessment

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## Introduction

Coal mining, its uses and associated processes have long been recognized for their significant economic contributions, providing a substantial portion of the world's energy needs (Gopinathan et al., 2023). The environmental footprint of coal-waste from coal powered industries continues to pose as it affects soil health, biodiversity and the entire microbial community, and agricultural activities are also greatly affected. This is peculiar in areas where there is a combination of agricultural and industrial activities (Gopinathan et al., 2023).

In the southeast of Nigeria, the use of coal power by manufacturing industries has led to the deposition of coal waste directly onto adjacent agricultural farmlands. Unlike other traditional coal mining residues, this form of contamination is widespread and frequently ignored but has a huge impact on trace elements in the soil and microbial communities.

Although these trace elements are only required in small quantities, they play a great role in the growth processes of plants and animals such that when there is an interruption due to coal waste contamination, harsh ecological imbalances occur and plant health suffers. Heavy metals, polycyclic aromatic hydrocarbons (PAHs), and residual carbon compounds make up the mixture in coal waste, and they are capable of changing the physical and even chemical properties of soil, which include pH, bulk density, and the organic carbon content (Wong, 2003). These changes would then go on to interrupt the flow of nutrients and reduce the availability of these essential nutrients to plants for their growth, eventually leading to long-term soil infertility and low crop productivity. In addition, microbial communities which support soil health are very sensitive to these disturbances, and studies have shown that a variety of microorganisms are seen in coal-contaminated soils, and certain taxa such

as *Anaerolineae*, *Streptosporangiales*, and *Chryseolinea* do well in them due to their ability to adapt metabolically and break down carbohydrates (Zhang et al., 2025b).

These changes can lead to plant poisoning, delay in plant growth, and a serious reduction in crop yields. The variety of plant and animal life is also affected by coal contamination, which includes the introduction of harmful substances that can destroy dwelling places of organism, lead to species loss, and make ecological communities fall apart. When plants cannot survive in polluted conditions, it reduces the variety of plants. This makes soil more likely to wash away, losing its quality, and hurting the environment.

The latest study in areas of coal contamination in China showed that the communities of microbes were enhanced as a form of survival in response to contaminants. These microbial changes have significant implications for agriculture, but they are often overlooked in Africa, particularly in Nigeria, where coal waste is indiscriminately deposited into farmlands near industrial areas (Su et al., 2023; Chen et al., 2024).

The impacts of the activities of coal-powered industries on soil trace elements, microbial communities, biochemical characterisation and agriculture in Enugu are the focus of this research. This study sought to explain the mechanism by which pollutants from coal waste influence soil structure, microbial diversities and availability of nutrients using pollution indices, multivariate assessments, correlation matrix and heat map. The result from this study will contribute to creating a well-detailed understanding of the extent of coal waste pollution of our study area and also provide scientific advice for policymakers for sustainable land management in coal-laden agricultural zones.

## Materials and Methods

### *Study Area*

The research was carried out in the Obinagu-Ulo community in Ezeagu Local Government Area of Enugu State, Nigeria. This community is a few kilometres away from the popular Oji River and is near a company which powers its operations using coal. The community experiences a yearly temperature from 24°C to 30°C and an average rainfall between 1,200 and 1,500 mm, mostly in the wet season.

### *Sampling Design and Soil Preparation*

Nine coal waste samples were collected in an outspread manner 15 metres apart in the contaminated site of study (N06°29.073', E007°59.132') in June 2023. Samples used for control were collected from Ameti-Ugwunani-Aku, Igbo-Etiti LGA (N06°04.0', E007°02.0'), which does not have an industry near it. The collected samples were prepared as composites, dried using an oven and sieved with a 2mm sieve. Storage was done in sterile polyethylene bags until laboratory analysis.

### *Physicochemical Analysis*

Parameters analysed included particle size distribution, pH, electrical conductivity, biochemical oxygen demand (BOD), colour, turbidity, and phosphate (PO<sub>4</sub>). Particle size was determined using hydrometer readings after Calgon dispersion. pH was measured using a digital pH meter after 30-minute equilibration in distilled water. Exchangeable acidity and cations (Ca<sup>2+</sup>, Mg<sup>2+</sup>) were quantified via titration with sodium hydroxide (NaOH) and ethylenediaminetetraacetic acid (EDTA), respectively. Phosphorus was extracted using Bray II reagent and quantified spectrophotometrically.

### *Determination of Heavy Metals and Nitrogen*

Trace metal concentrations were determined in soil samples using Atomic Absorption Spectrophotometry (AAS) ContrAA 300, Analytik Jena, Germany, except for nitrogen content, which required the Kjeldahl method for its assessment.

The soil was checked for metals such as Cu (324.8 nm), Fe (248.3 nm), Zn (213.9 nm), Mo (313.3 nm), and B (249.7 nm) at particular wavelengths shown in bracket using AAS. Acetylene and air (70%) as carrier gases with the wideness of slit at 0.7nm and hollow cathode lamps of analytical grade (HCL) were used. Blank samples and standard reference materials were also done to ensure quality of results. Data was checked and machines were checked to be properly calibrated. Each test and measurement was run three times.

As mentioned earlier, the Kjeldahl method was used to check for nitrogen to get the total nitrogen content, this helps to figure out soil fertility and how much nitrogen are available to the plants.

### *Microbial Enumeration and Isolation*

We counted bacteria using the pour plate technique. We diluted the samples in an isotonic solution of sodium chloride and distilled water by following a 10-fold serial dilution, plated them on nutrient agar and counted colonies (Cowan and Steel, 1990). If counts were  $\geq 10^2$  CFU/mL it meant contamination.

### *Isolation and Identification of Bacteria*

Samples were swabbed directly on nutrient agar and MacConkey agar to grow bacteria. Dominant colonies were picked and purified. Several tests which include gram staining, movement tests in Motility–Indole–Urease medium (MIU medium), haemolytic activity, biochemical tests like catalase, oxidase, indole, citrate, sulphate reduction, methyl red (MR), Voges-Proskauer (VP), triple sugar iron (TSI), and urease tests were carried out to identify the purified bacteria (Merchant and Packer, 1967; Cheesbrough, 1985, 2006; McFadden, 2000).

### *Morphological and Biochemical Characterisation*

Bacteria were stained using special dyes through the Gram Staining method so they could be visible under the microscope. Cells were observed at  $\times 1000$  magnification using special oil to aid the viewing of bacteria (oil immersion). Tests were also carried out to see how the bacteria reacted

to certain chemicals like checking if they produced enzymes which broke down substrates. Changes in color, production of gas bubbles, pattern of growth were recorded as results.

### *Stock Culture Maintenance*

Separated bacteria grew in liquid food (nutrient broth) then was spun using a centrifuge. Salt water mix (PBS) was used to wash them up. Samples were stored in unique tubes (cryovials) with a sugar solution (glycerol) to keep them safe in the freezer at 20°C (Aziz et al., 2024).

### *Pollution Indices*

Contamination Factor (CF), Geo-accumulation Index (Igeo), Enrichment Factor (EF), and Pollution Load Index (PLI) were used to determine the extent of soil contamination and its ecological impact.

1. Contamination Factor (CF) was used to measure how much metal has built up in the contaminated soil compared to the control soil.

2. The Geo-accumulation Index (Igeo) checks the level of metal pollution in soil using a logarithmic scale, specifically log base 2 of [Concentration in contaminated soil  $\div$  (1.5  $\times$  Concentration in control soil)].

When  $I_{geo} \leq 0$ , it indicates soil is unpolluted; when  $I_{geo}$  is 1–2, it shows there is a case of moderate pollution; and when  $I_{geo} > 5$ , it points out that soil is extremely polluted.

3. Enrichment Factor (EF) is used to compare the proportion of a metal to a reference element (usually iron due to its stability) in both contaminated and control soils.

(Metal  $\div$  Reference element in control soil)

4. The Pollution Load Index (PLI) gives a total measure of pollution across multiple metals.

( $CF_1 \times CF_2 \times CF_3 \times \dots \times CF_n$ ) raised to the power of  $1/n$

The Pollution Load Index (PLI) is interpreted as follows:  $PLI = 1$  indicates a baseline level,  $PLI > 1$  signifies the presence of pollution, and  $PLI > 10$  indicates severe pollution.

### **Statistical Analysis**

All data, physicochemical and microbial, were analysed using R in version 4.3.1. For each soil type measurement, samples were taken in five replicates and presented as mean  $\pm$  standard error (SE). Contaminated and control samples were compared using one-way ANOVA with significance set at  $p < 0.05$ .

### **Results**

#### *Soil Physicochemical Properties*

The physical and chemical properties of the soil were changed as can be seen in Table 1. Comparison of the result from analysis carried out on control and contaminated soil revealed that sand content decreased marginally from  $88.4 \pm 0.35\%$  to  $87.6 \pm 0.23\%$  ( $p=0.12$ ), silt content increased from  $5.4 \pm 0.12\%$  to  $6.9 \pm 0.12\%$  ( $p=0.01^{**}$ ) which is likely due to coal deposition, clay content decreased from  $6.2 \pm 0.12\%$  to  $5.5 \pm 0.12\%$  ( $p=0.08$ ). Generally, soil texture remained mostly sandy. Soil was seen to be slightly more acidic as shown in the pH value, from  $6.35 \pm 0.03$  to  $6.25 \pm 0.03$  ( $p = 0.14$ ) indicating the harmful impact on useful soil microorganisms and their activities. There is a decrease in nitrogen (down to 45.5%) and organic manure content further showing the loss of soil fertility and reduced microbe activity.

Calcium levels dropped significantly from  $8.5 \pm 0.17$  cmol/kg to  $6.4 \pm 0.17$  cmol/kg ( $p = 0.01^{**}$ ), likely due to nutrient leaching or coal-induced immobilisation. Sodium increased slightly from  $0.11 \pm 0.01$  cmol/kg to  $0.12 \pm 0.01$  cmol/kg ( $p = 0.09$ ), though this change wasn't statistically meaningful. Potassium stayed the same at  $0.17 \pm 0.01$  cmol/kg ( $p = 1.00$ ), showing its ability to not be affected by coal contamination.

Soak nutrient holding capacity is reduced as seen in decreased Effective cation exchange capacity

(ECEC) from  $17.84 \pm 0.15$  cmol/kg to  $13.49 \pm 0.12$  cmol/kg ( $p = 0.01^{**}$ ) showing that nutrients could wash away easily. Base saturation decreased slightly from  $87.9 \pm 0.25\%$  to  $85.1 \pm 0.35\%$  ( $p = 0.07$ ), while exchange acidity

dropped from  $2.16 \pm 0.02$  to  $2.00 \pm 0.03$  cmol/kg ( $p = 0.11$ ). Even though the changes seem small now, they show that coal pollution is slowly destroying the soil's chemical makeup.

**Table 1:** Physical and Chemical Properties of Soil Samples from Collected from Control and Contaminated Sites

| Parameter             | Control<br>(Mean $\pm$ SE) | Contaminated<br>(Mean $\pm$ SE) | Change    | ANOVA p-value | Interpretation                                |
|-----------------------|----------------------------|---------------------------------|-----------|---------------|-----------------------------------------------|
| Sand (%)              | $88.4 \pm 0.35$            | $87.6 \pm 0.23$                 | ↓ 0.9%    | 0.12          | Texture remains sandy; minimal change         |
| Silt (%)              | $5.4 \pm 0.12$             | $6.9 \pm 0.12^{**}$             | ↑ 27.8%   | 0.01          | Coal ash may increase fine particles          |
| Clay (%)              | $6.2 \pm 0.12$             | $5.5 \pm 0.12$                  | ↓ 11.3%   | 0.08          | Slight reduction; may affect water retention  |
| pH (H <sub>2</sub> O) | $6.35 \pm 0.03$            | $6.25 \pm 0.03$                 | ↓ 1.6%    | 0.14          | Mild acidification due to coal residues       |
| Nitrogen (%N)         | $0.11 \pm 0.01$            | $0.06 \pm 0.01^{***}$           | ↓ 45.5%   | < 0.001       | Major fertility loss; microbial stress likely |
| Organic Manure (%)    | $2.21 \pm 0.05$            | $1.71 \pm 0.07^{**}$            | ↓ 22.6%   | 0.01          | Reduced organic input; microbial decline      |
| Calcium (cmol/kg)     | $8.5 \pm 0.17$             | $6.4 \pm 0.17^{**}$             | ↓ 24.7%   | 0.01          | Nutrient leaching or binding by coal waste    |
| Sodium (cmol/kg)      | $0.11 \pm 0.01$            | $0.12 \pm 0.01$                 | ↑ 9.1%    | 0.09          | Slight increase; may affect soil structure    |
| Exchange Acidity (EA) | $2.16 \pm 0.02$            | $2.00 \pm 0.03$                 | ↓ 7.4%    | 0.11          | Minor shift; not ecologically critical        |
| ECEC (cmol/kg)        | $17.84 \pm 0.15$           | $13.49 \pm 0.12^{**}$           | ↓ 24.4%   | 0.01          | Reduced nutrient-holding capacity             |
| Base Saturation (%)   | $87.9 \pm 0.25$            | $85.1 \pm 0.35$                 | ↓ 3.2%    | 0.07          | Slight drop; cation availability affected     |
| Potassium (cmol/kg)   | $0.17 \pm 0.01$            | $0.17 \pm 0.01$                 | No change | 1.00          | Stable; unaffected by contamination           |

All values shown are averages based on three soil samples from each location (contaminated and control sites). The  $\pm$  numbers after each value show the standard error, which gives you an idea of how consistent the measurements were across the three samples. The table also contains the following: \*\*\* means that  $p < 0.001$  (Highly Significant), \*\* means that  $p < 0.01$  (Significant, No asterisk means that  $p \geq 0.05$  (Not Significant), SE means standard error, Exchange Acidity ECEC means Effective Cation Exchange Capacity cmol/kg means centimoles of charge per kilogram of soil and %N means Percentage Nitrogen pH (H<sub>2</sub>O)

Figure 1 shows a bar chart that compares the two soil samples. The blue bars represent contaminated soil, while the red bars show normal, unpolluted soil (the control).

Each pair of bars shows a different soil property like pH level, nutrient content, or acidity. By placing them side by side, a visual observation of how much pollution has affected each property is given. There are observable reductions in nitrogen (%N), organic manure, calcium (Ca), and effective cation exchange capacity (ECEC),

hinting at nutrient loss and low soil fertility in contaminated soil. Silt content increased while clay content reduced. Reduction in pH is also seen with minor changes in exchangeable acidity (EA). Potassium (K) and sodium (Na) levels are maintained. Cation availability is seen due to a slight decrease in basic saturation (BS). In all, the above chart supports that soil contamination due to coal waste can reduce the quality of the soil, as the soil structure and dynamics are distorted as well as ecological stress reducing agricultural output and activities.

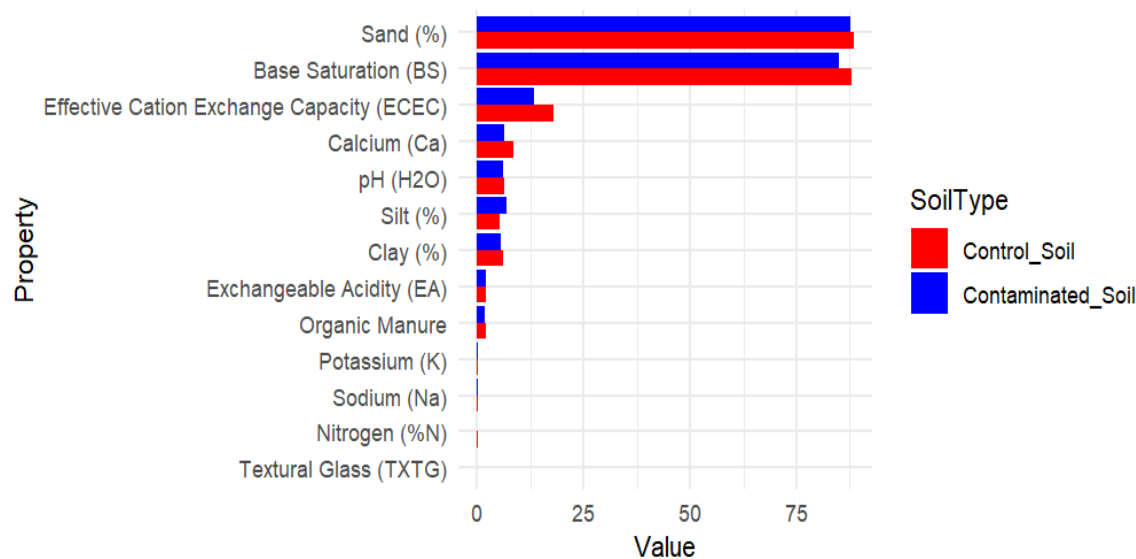


Fig. 1. Comparison of soil properties between the control and contaminated soil

#### *Trace Elements and Pollution Indices*

The summary of trace elements (mean  $\pm$  SE) and pollution indices in this study is indicated in Table 2. The mean  $\pm$  SE as summarized shows the changes in the concentrations of trace elements between contaminated soils and control soils. Copper (Cu), iron (Fe), nitrogen (N), and zinc (Zn) showed differences, while boron (B) and molybdenum (Mo) did not change. The value for copper (Cu) increased ( $0.10 \pm 0.00$  to  $0.14 \pm 0.00$ ) but remained below the limit of 36 mg/kg, indicating the early stage of pollution from coal ash. Iron (Fe) reduced from  $0.25 \pm 0.01$  in the control to  $0.02 \pm 0.00$  in the contaminated, suggesting leaching of nutrients. Nitrogen levels dropped in the polluted soil from  $0.11 \pm 0.00$  to

$0.06 \pm 0.00$ , this means less food for plants and fewer microbes that make sure nutrients are circulated. Zinc also decreased from  $0.05 \pm 0.01$  to  $0.02 \pm 0.00$  but stayed within safe limits according to WHO standards (under 300 mg/kg). Molybdenum went above WHO safety limits in both clean and polluted soil, which is not unusual as this often happens naturally because of the rocks and minerals in the area. Boron stayed about the same in both types of soil. These findings match what other researchers (Antoniadis et al., 2020) found out as they stated that the level of trace elements can change in different parts of the world and molybdenum levels in soil often go above WHO safety limits in different parts of the world.

**Table 2.** Levels of Trace Element in Soil Contaminated by Coal and Control Soils Compared to Global Soil Thresholds (Mean  $\pm$  SE)

| Element | Contaminated<br>(Mean $\pm$ SE) | Control<br>(Mean $\pm$ SE) | WHO<br>Limit<br>(2006) | Antoniadis<br>et al.<br>(2023) | EU<br>(Germany) | China | F-value   | p-value | Exceeds<br>WHO<br>limits |
|---------|---------------------------------|----------------------------|------------------------|--------------------------------|-----------------|-------|-----------|---------|--------------------------|
| B       | 0.12 $\pm$ 0.00                 | 0.12 $\pm$ 0.01            | 0.50                   | 0.5–5.0                        | 1.0             | 2.0   | 0.00      | 1.000   | No                       |
| Cu      | 0.14 $\pm$ 0.00*                | 0.10 $\pm$ 0.00            | 36.0                   | 20–100                         | 60.0            | 100   | 50.0<br>0 | < 0.001 | No                       |
| Fe      | 0.02 $\pm$ 0.00*                | 0.25 $\pm$ 0.01            | 0.30*                  | Not<br>regulated               | -               | -     | 25.0<br>0 | < 0.001 | No                       |
| Mo      | 1.61 $\pm$ 0.08                 | 1.77 $\pm$ 0.05            | 0.60                   | 0.5–5.0                        | 1.0             | 2.0   | 1.74      | 0.161   | Yes                      |
| N       | 0.06 $\pm$ 0.00*                | 0.11 $\pm$ 0.00            | -                      | Not<br>regulated               | -               | -     | 5.00      | < 0.001 |                          |
| Zn      | 0.02 $\pm$ 0.00                 | 0.05 $\pm$ 0.01            | 300.0                  | 100–300                        | 200.<br>0       | 300   | 3.00      | 0.0065  | No                       |

In the table above, values with \*\*\* means  $p < 0.001$  (Highly Significant), values with \*\* means  $p < 0.01$  (Significant) and values no asterisk means  $p \geq 0.05$

In Table 3, three different scoring systems (CF, Igeo, and EF—basically different ways to measure contamination) to figure out how bad the pollution really is

In Table 3, three different scoring systems, Contamination Factor (CF), Geo-accumulation Index (Igeo), and Enrichment Factor (EF) were used to measure coal contamination, its effect on the environment as well as how it affects trace elements in soils used for agriculture. Copper had the highest contamination scores, especially when compared with the other elements, with a CF of 1.40, an Igeo of 0.48, and an EF of 1.40 however its contamination is considered moderate. Iron is disappearing from the soil fast given its CF of 0.08 and an Igeo of -2.64,

probably because the soil's acidity changed. Molybdenum is above WHO limits, but has a CF of 0.91, Igeo of -0.06, and EF of 0.91, showing that it is not a major problem. This may reflect natural geogenic abundance rather than coal-derived input.

Along with Iron (Fe), Zinc (Zn) and Nitrogen (N) were seen to be lost but not much contaminated, as they have CF values below 1 and negative Igeo scores with low EF values (0.40 and 0.55, respectively).

Boron (B) remained stable in all soil types, with a CF and EF of 1.00 and an Igeo of -0.32, indicating no contamination.

**Table 3.** Pollution Indices for Trace Elements in Control and Contaminated Soils

| Element | Contamination Factor (CF) | Geo-accumulation Index (Igeo) | Enrichment Factor (EF) | Interpretation                      |
|---------|---------------------------|-------------------------------|------------------------|-------------------------------------|
| B       | 1.00                      | - 0.32                        | 1.00                   | No contamination                    |
| Cu      | 1.40                      | 0.48                          | 1.40                   | Moderate contamination              |
| Fe      | 0.08                      | - 2.64                        | 1.00                   | Strong depletion                    |
| Mo      | 0.91                      | - 0.06                        | 0.91                   | Exceeds WHO limit, no contamination |
| N       | 0.55                      | - 0.29                        | 0.55                   | Depleted, not significant           |
| Zn      | 0.40                      | - 0.32                        | 0.40                   | Depleted, not significant           |

Contamination Factor (CF), Geo-accumulation Index (Igeo), and Enrichment Factor (EF); CF < 1 = low contamination, CF = 1–3 indicates moderate levels of contamination, and CF > 3 = high contamination. Igeo ≤ 0 = unpolluted conditions and Igeo ≥ 0 = escalating pollution. EF separates natural from anthropogenic origins, with values < 1 implying natural background levels, 1–3 denoting minor enrichment, and values > 5 reflecting substantial human influence.

### *Heat Map*

The heatmap as shown in Fig. 2 uses a blue–grey–red gradient to present the mean concentrations of zinc (Zn), nitrogen (N), molybdenum (Mo), iron (Fe), copper (Cu), and boron (B) across both control and contaminated soils. The concentration of these trace elements in control and contaminated soil is shown on the heatmap, with the red colours indicating the highest concentration, blue showing the lowest and grey showing middle values. Molybdenum (Mo) is very high compared to the other trace elements, as shown with a deep red in both soils despite its low contamination. Iron (Fe) shows a change as it appears as grey in control but gradually moves to blue in contaminated soil, pointing out its reduction due to contamination. The rest of the elements, Zn, N, Cu, and B,

appear light blue in both samples, showing low and stable levels. The heatmap (a color-coded chart) makes it easy to see what is happening: iron (Fe) is decreasing while molybdenum (Mo) stays high, and other elements did not show much change. This clearly shows coal mining and coal waste is negatively affecting with the soil.

The dendrogram (a tree-like diagram) puts contaminated and control soil samples into completely separate groups showing they are chemically different. Statistical tests (using ANOVA ( $p < 0.001$ )) confirmed that iron and copper are the biggest markers of contamination. Iron especially stands out because it drops so much in contaminated soil.

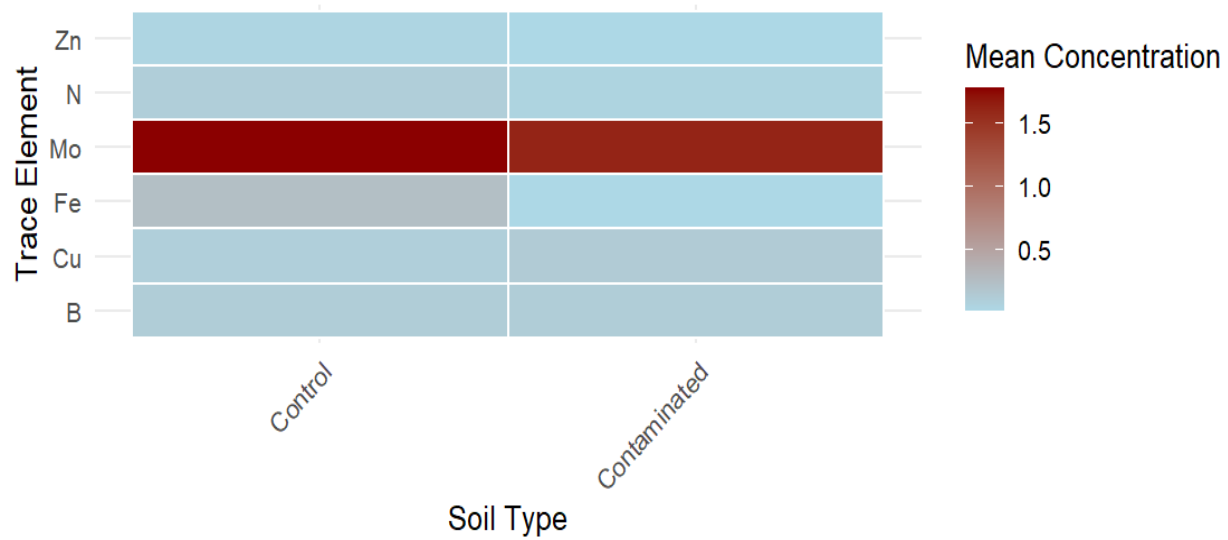


Fig. 2: Heat Map of Trace Element Concentrations

#### *Correlation Matrix*

The correlation matrix (a chart showing relationships) revealed that the physical and chemical properties of contaminated and control soils have very strong connections between their properties as the numbers (correlation coefficients) were close to +1.0, meaning they move together in predictable ways. This tells us that the integrity of the soil remains consistent despite contamination by coal waste. However, such behaviour tends to hide the gradual changes in nutrients available and changes in microbial

community. For example, even though sand content and potassium levels remain stable, main fertility indicators such as total nitrogen (%N), organic manure, calcium, and effective cation exchange capacity (CEC) show a noticeable decrease in contaminated soils, which affects the retention of soil nutrients, affects the activity of microorganisms, and causes a big reduction in plant production (Wong, 2003; Chaubey and Singh, 2012).

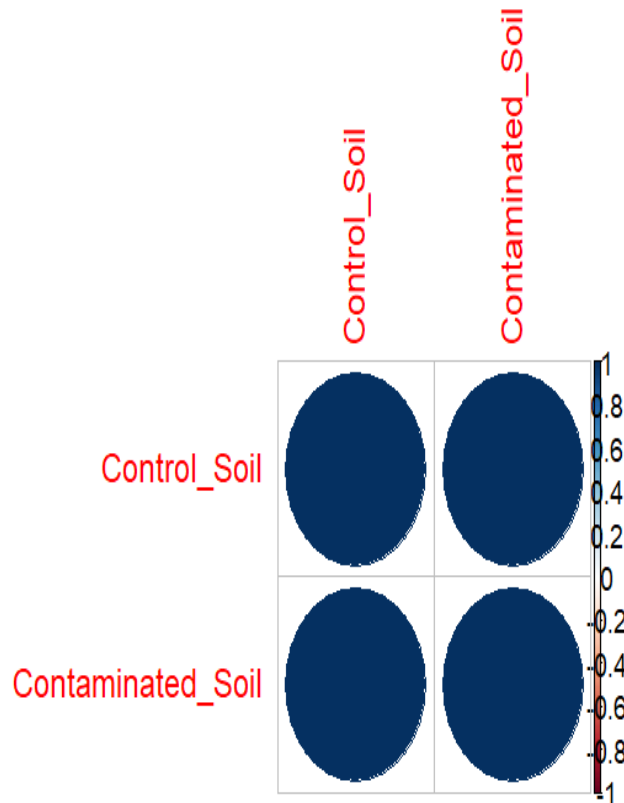


Fig 3: Correlation matrix (circular heatmap) comparing control and coal-contaminated soils

### *Multivariate Analysis*

The Principal Component Analysis (PCA) is shown in Fig. 4. This Principal Component Analysis (PCA) was done to examine the multivariate structure of soil samples and to identify the variables most responsible for distinguishing between contaminated and control sites. The first principal component (PC1) accounted for 64.9% of the total variance, while the second component (PC2) explained an additional 28.0%, resulting in a cumulative variance of 92.9%.

The noticeable difference shows that the plot below gives a well understood and trustworthy representation of the set of data. The biplot showed a clear separation between the contaminated soil and control soil as samples which were contaminated combined separately from control samples pointing out that the main

factor responsible for the difference between both soils is the level of contamination. Contaminated soil showed different levels of pollutant buildup as seen in the broad spread of contaminated samples while control soil has a uniform chemical makeup, seen in the tight grouping. The lines pointing in different directions in the above diagram show that Copper (Cu), Zinc (Zn), Iron (Fe) play a recognized role in identifying polluted soils as seen in their increase in the contaminated sample, Molybdenum (Mo) and Boron (B) were moderately influenced, maybe from pollution or things added to amend the soil. Nitrogen levels show natural differences in fertility of soil. Overall, the analysis highlights how pollution changes soil composition and raises the need for more study on the impacts of coal waste pollution.

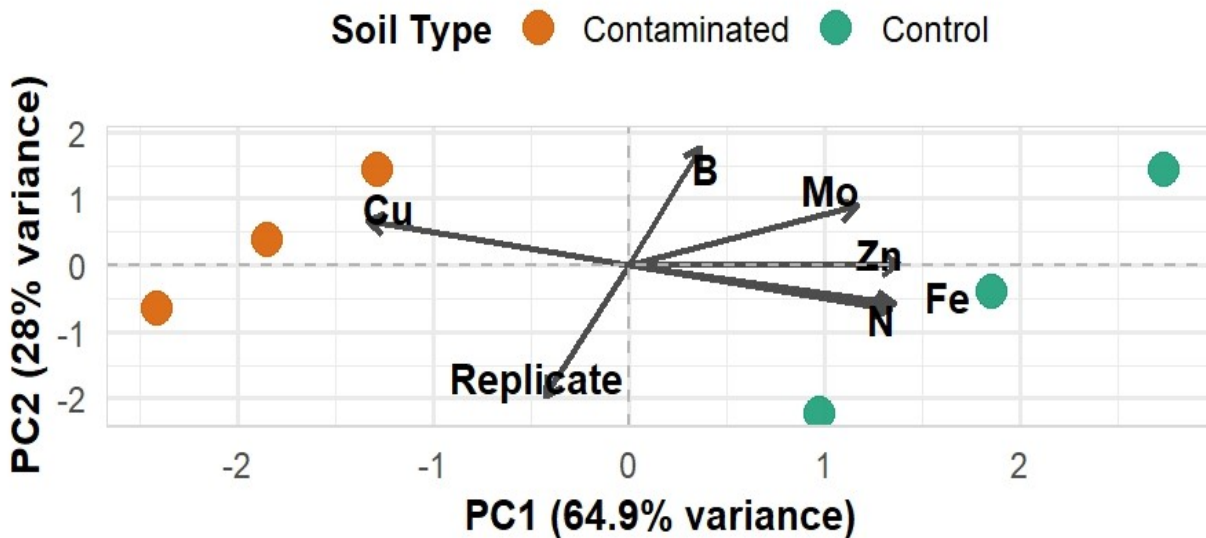


Fig 4: Principal Component Analysis (PCA) plot of trace element concentrations in control and coal-contaminated soils.

#### Microbial Community Structure

Table 4 shows the biochemical and morphological test results for nine different types of bacteria identified from coal-contaminated soil samples in this study. A column exists for each species and a row for each test or attribute. *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Pseudomonas fluorescens*, *Bacillus alvei*, *Micrococcus luteus*, *Micrococcus lylae*, *Bacillus megaterium*, *Bacillus licheniformis*, and *Bacillus cereus*. This figure tried to explain some important biochemical and physical characteristics. Motility, which indicates movement typically facilitated by flagella, indicates the movement capacity, whereas cell morphology shows the classification of the organisms as spherical (cocci) or rod-shaped (bacilli). The Gram stain indicated differences between gram-positive and gram-negative bacteria according to the composition of their various cell walls. Amino acid was also shown using ornithine decarboxylation reactions to indicate amino acid metabolism, while catalase, an antioxidant activity, was measured by the

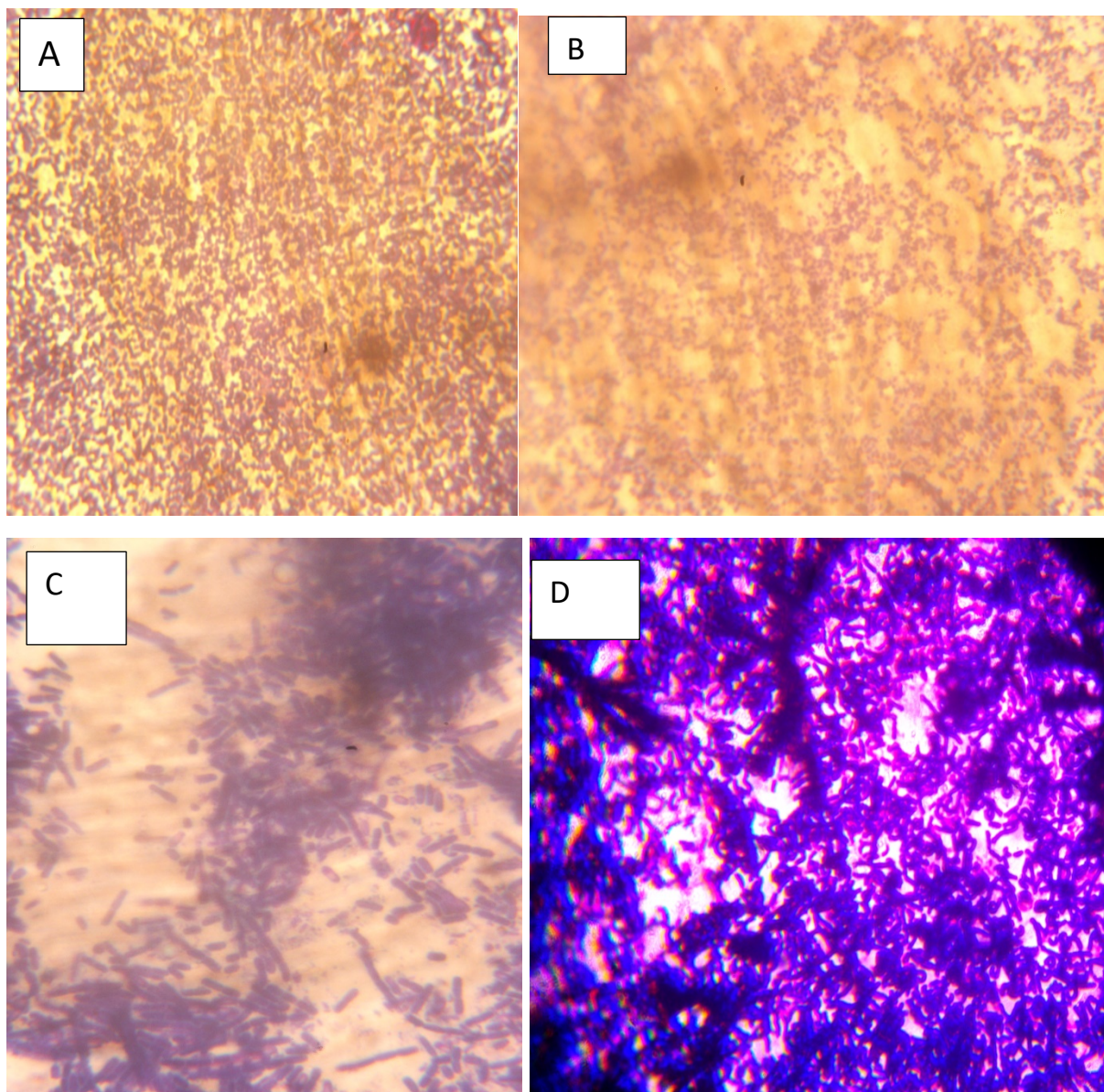
degradation of hydrogen peroxide; bubble formation signified a successful result. The presence of cytochrome c oxidase confirmation was done using oxidase testing. This test plays a significant role in the electron transport chain. The conversion of tryptophan to indole was done to assess indole production. We also assessed the ability of the organisms to utilise citrate as their only carbon source, which was evaluated by citrate utilisation tests. The hydrolysis of urea to ammonia was also confirmed to show an alkaline shift to enable the estimation of urease activity, while the fermentation of glucose and lactose fermentation was also confirmed to show glucose/carbohydrate metabolism. The methyl red test shows mixed acid fermentation by the low pH shift, butanediol fermentation was also identified by the Voges–Proskauer test to detect the presence of acetoin, and hydrogen sulphide production. This is confirmed by the visual appearance of a black precipitate.

| TESTS             | <i>Pseudomonas aeruginosa</i> | <i>Staphylococcus Aureus</i> | <i>Pseudomonas Fluorescens</i> | <i>Bacillus Alvei</i> | <i>Micrococcus Luteus</i> | <i>Micrococcus Lylae</i> | <i>Bacillus Megaterium</i> | <i>Bacillus licheniformis</i> | <i>Bacillus cereus</i> |
|-------------------|-------------------------------|------------------------------|--------------------------------|-----------------------|---------------------------|--------------------------|----------------------------|-------------------------------|------------------------|
| CELL SHAPE        | Rod                           | Cocci                        | Rod                            | Rod                   | Cocci                     | Cocci                    | Rod                        | Rod                           | Rod                    |
| MOTILITY          | +                             | –                            | –                              | –                     | –                         | –                        | –                          | –                             | –                      |
| GRAM STAIN        | –                             | +                            | +                              | +                     | +                         | +                        | +                          | +                             | +                      |
| ORNITHINE         | –                             | -                            | -                              | +                     | NA                        | NA                       | -                          | –                             | –                      |
| CATALASE          | +                             | +                            | +                              | +                     | +                         | +                        | +                          | +                             | –                      |
| OXIDASE           | +                             | +                            | +                              | +                     | +                         | +                        | +                          | +                             | +                      |
| INDOLE            | –                             | NA                           | -                              | +                     | NA                        | NA                       | -                          | -                             | NA                     |
| CITRATE           | –                             | NA                           | +                              | -                     | NA                        | NA                       | +                          | -                             | NA                     |
| GLUCOSE           | –                             | –                            | +                              | +                     | NA                        | NA                       | NA                         | NA                            | –                      |
| LACTOSE           | –                             | NA                           | -                              | +                     | NA                        | NA                       | NA                         | NA                            | NA                     |
| UREASE            | +                             | –                            | -                              | -                     | -                         | -                        | –                          | –                             | –                      |
| HYDROGEN SULPHIDE | –                             | NA                           | -                              | NA                    | NA                        | NA                       | NA                         | NA                            | NA                     |
| METHYL RED        | –                             | NA                           | +                              | +                     | NA                        | NA                       | -                          | -                             | NA                     |
| VOGES-PROSKAUER   | –                             | NA                           | +                              | +                     | NA                        | NA                       | -                          | -                             | NA                     |
| COAGULASE         | NA                            | +                            | NA                             | NA                    | -                         | –                        | NA                         | NA                            | –                      |
| MANNITOL          | NA                            | +                            | +                              | +                     | NA                        | NA                       | +                          | –                             | –                      |

“+” = Positive result (test was successful or trait is present); “–” = Negative result (test failed or trait is absent); “NA” = Not applicable

The photomicrographs presented in Fig. 5 present microscopic visuals of six different bacterial species, indicating their morphological diversity and staining characteristics. *Bacillus cereus* and *Bacillus megaterium* exhibit rod-shaped structures typical of bacilli, often appearing in chains or scattered arrangements. *Pseudomonas fluorescens* and *Pseudomonas aeruginosa* also display rod-shaped morphology

but with slender profiles and more distributed cell-like structure. However, *Bacillus alvei* shows more clusters of bacilli with uniform staining. *Micrococcus luteus*, the only spherical organism in the set, is characterised by cells arranged in tetrads or clusters, with intense colouration and compact density. These visible differences aid in preliminary identification and support biochemical profiling for accurate classification.



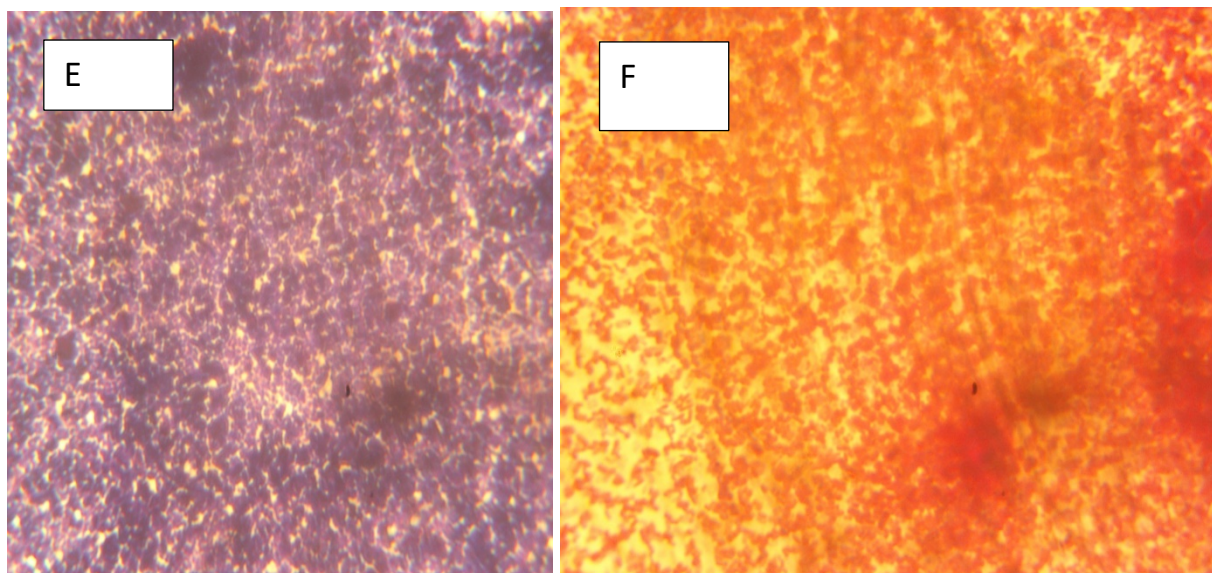


Fig. 5: Photomicrographs of coal contaminated soil revealed rod-shaped *Bacillus* spp and cocci-shaped *Micrococcus* spp., aligned with Gram-positive morphology. The panel shows different bacterial species as follows: (A) *Bacillus cereus*, (B) *Bacillus megaterium*, (C) *Pseudomonas fluorescens*, (D) *Pseudomonas aeruginosa*, (E) *Bacillus alvei*, and (F) *Micrococcus luteus*.

## Discussion

The recent industrial activities of coal-powered industries for activities like quarrying, tile production, construction, and waste and effluent production have led to the deep decline in the quality of our environment. This is because the natural ecosystems are distorted, affecting biodiversity, soil structure and quality, and this is not sustainable despite the foreseen economic gains and modernisation benefits. In our study areas, pollution of these areas is visibly observed and perceived, and health impact is worsening due to the ongoing dumping of waste from various sources from the industries. This comes from the mining of various industrial raw materials and uncontrolled waste disposal, which is further complicated due to its constant release and seepage into agricultural farmlands. The real implication is the exposure to several toxic chemicals, heavy metals, organic waste and trace elements. For example, arsenic (As), mercury (Hg), chromium (Cr) and lead (Pb); polycyclic aromatic hydrocarbons and other persistent contaminants. These contaminants persist in the environment, seeping into soil and water systems and creating long-term threats to ecosystems and

human health (Onyedikachi et al., 2018; Onyedikachi et al., 2021).

The distortion of soil physicochemical characteristics due to coal waste and other quarry-related operations in our study and those of others that are widely sourced and documented in scientific literature has constantly grown on a daily basis despite the effort made by researchers, remediation policies and activities of many remediation projects, indicating the buildup and bioaccumulation, seepage and persistence of these contaminants, which disturbs the nutrient cycles and metabolic pathway dynamics of the natural microbiome and soil and ecosystem communities (Rouhani, Skouse, 2023). In this present study, the physicochemical characteristics generally show the gradual and worsening case of soil health and discrepancy and shift in the physicochemical character of a healthy soil affected by the constant loading of coal waste due to the daily activities of the industries. The disruption of the nutrient cycles and dynamics, particularly nitrogen and calcium, and the reduction of ECEC and organic matter tell a story about the nutrient deficiency of the coal waste-laden soil compared to those from the control soils, indicating weak soils. This occurrence not

only undermines agricultural activities and outputs, but it also affects the economic prowess of the locals who depend on agriculture as their source of livelihood. In addition to this unfavourable condition, the problem of point sources of contamination due to the constant seepage and movement of these metals by percolation, flooding, rainfall, etc. intensifies the transportation of these invasive harmful metals, endangering ecological communities and human health, especially for those with unstable health, children, the aged, and pregnant women, intensifying ecological and human health dangers to the vulnerable population, especially concerning the effect of extractive industries on soil texture, fertility, and elemental makeup. For example, Omang et al. (2023) noted that soils in and around mining areas, as well as industrial waste pits, are, in essence, holding compartments for potential toxic elements, which are later moved in various ways, including leaching, runoff, and uptake by plant roots. Their analysis of soil composition also reveals high amounts of Zn, Fe, Mn, and Cu, which are essential components of this study and demonstrate their dual nature as "essential nutrients, whereas their excess can be toxic, especially in aquatic ecosystems." Children are most vulnerable to adverse health effects of these metals due to their lighter body mass and higher susceptibility, especially if they consume vegetables that were cultivated from polluted areas. This ability to be affected further underscores that our study requires distinct health-risk analysis for farming areas where pollution has occurred (Onyedikachi et al., 2021).

Likewise, Onyedikachi et al., in 2018, performed a health risk screening of soils and plants around quarries in Ebonyi State, Nigeria, and reported that Fe, Mn, Zn, Pb, Cu, and Cd were identified as major pollutants. Their study indicated that these metals built up a lot in the edible parts of plants, and cassava plus leafy vegetables were soaking up far too much of them. What this study tells us is that quarry dust and coal ash are adding trace elements to the soil, and this messes with microbial activity and how well the soil holds onto nutrients. Other studies conducted in Nigeria have linked both cancer-causing and non-cancer

health risks to heavy metal exposure from industrial waste, quarry locations, and areas influenced by coal. For example, Adetola et al. (2024) discovered high levels of 17 heavy metals in soils around the Ratcon quarry in Ibadan. In the same way, Onyedikachi et al. 2019 revealed that mixed waste from tile factories, which is often found near coal-burning waste, caused metals to build up in edible crops. Due to these incidents, concerns about food safety and how people get exposed to these metals have been raised. Thus, coal waste becomes a major source for heavy metal distribution and disruption of nutrients; hence, we require a combined cleanup effort as well as stricter government policies. Another study by Nganje et al. (2021) discussed that trace elements like Zn, Mn, and Cu are often needed for plants to grow; however, when they exceed the permissible limits, it becomes a negative effect on the plant and human health. For instance, they can actually become toxic to plants when there is too much in the soil; this is especially true in soils that have a load of abundant organic matter or good cation exchange capacity. These studies further reiterate that emissions from their industrial activities alter and lower soil fertility, making the crops' growth distorted and their output decline, as observed in this study. Acidic conditions and low cation exchange capacity are aligned to over-increase the contamination levels. This worsening case leads to the wide and fast spread across agricultural areas. Recently, even other metals like the rare earth metals (REEs), as described by Ribeiro et al. (2024), have also indicated that it is becoming a concern, indicating the mechanisms and the concerns of soil contamination are becoming aggressive and environmentally disruptive, corresponding to the pattern as was observed with the studied trace elements in this study and as reported by other literature. This study's results further confirm and emphasise that industrial activities and constant exposures to their effluents are an example of a sweet-bitter cola experience, as they alter soil chemistry and structure in major ways through dust settling on the ground, leaching, and physical disturbance. When trace elements build up, they interfere with nutrient cycling, reduce the variety of microbes in

the soil, their habitat, the symbioses and interaction, and chemical dynamics. These communities form adaptation qualities, forming extracellular polymer substances that are a covering/protection against stress (Nur et al., 2025). Some elements like Zn and Cu might stay within safe limits for farming, but others like Pb and Cd can cause sustained problems for the environment and human health. The cancer and non-cancer health risk has been assessed in our previous studies, further strengthening the fact that human health is at risk due to bioaccumulation of these toxic substances over a period of a lifetime (Onyedikachi et al., 2021).

The assessments of trace elements in this study were done to ascertain the heavy metal burden in the coal-contaminated soils and control soil. The comparative analysis found different patterns in the levels and abundance of the trace metals. This gives us an insight into the elemental behaviour as explained by the physiochemical test analysis, which reflects the effect of coal waste on the environment. The various metals are as follows:

For instance, boron (B) levels were constantly at the same levels and also were in concentrations below the different permissible limits we got from some globally accepted standards. This indicates that B is immobilised in the soil, perhaps adsorbed in various polymers found in the microbiome. Some living microorganisms or their dead biomasses adsorb some metals on their functional groups or even the biofilms produced by them. This makes the metals immobile and unable to reach the plants for absorption into their stems, and other edible part of the plants through the plant roots or even percolate deeply as much in exposed soils. This makes sense, as boron is known to have low solubility and is only taken up selectively in soil systems, especially in alkaline and acidic environments (Wong, 2003). Boron helps to stabilise the cell walls (cross-linking RG-II polysaccharides).

Generally, the uptake pattern and the way B is distributed are dependent on two transporter families that work well together. These are Nodulin-26-like Intrinsic Proteins (NIPs) and Boron Transporters (BORs), which act by influxing boric acid, the form of B in the soil, into

cellular compartments. For example, e.g., AtNIP5;1 in Arabidopsis and OsNIP3;1 in rice, and Boron Transporters (BORs) act as efflux proteins that can export borate ions and even load them into the xylem. For example, AtBOR1 is available for deficiency tolerance and toxicity protection while maintaining homeostasis. Together, NIPs and BORs maintain boron homeostasis, balancing uptake and efflux to prevent both deficiency and toxicity. Nodulin-26-like Intrinsic Proteins (NIPs): Influx channels that import boric acid into cells (e.g., AtNIP5;1, OsNIP3;1).

Boron Transporters (BORs): Efflux transporters that export borate ions or load them into the xylem (e.g., AtBOR1 for deficiency tolerance, AtBOR4 for toxicity protection). Boron is indispensable yet tricky to manage because it is immobile in phloem and tightly regulated in soils. Plants rely on NIPs and BORs to balance uptake and efflux, and genetic manipulation of these transporters is a promising path for sustainable agriculture (Zhou et al., 2025).

Copper (Cu) levels were higher in contaminated soils, but they were still within the safe limits set globally (Antoniadis et al., 2020). Coal combustion residues known as coal ash often contain trace metals like Cu which can leach out, binding to soil particles; hence, frequent deposits of this ash mean the Cu abundance increases, leading to a Cu spike in the soil. Swain (2024) suggested the Cu could be used as an agent for biomonitoring and could be used to indicate industrial pollution, so over time the continuous dumping of this coal ash could get to very dangerous levels, which will lead to degraded ecological systems. The effect often occurs in ripples, as not just the soils are destroyed but entire ecosystems.

On the contrary, iron (Fe) levels went down noticeably in contaminated soils, which, from a layman's point of view, should be elevated just like other metals; however, iron could be leached/washed or solubilised deep down into the soil or may be immobilised in acidic soils as insoluble iron oxide, making it unavailable to plants. This dual behaviour explains why acidic soils are usually rich in iron (Wang et al., 2025). Fe is needed in many microbial processes and nutrient cycling, so when it is rendered

unavailable, it could represent a huge problem, as soil function and biodiversity activities are not optimal. This is particularly a problem as Fe is important in nitrogen fixation, DNA synthesis and repair (ribonucleotide reductase is the enzyme that requires iron (Fe) that converts ribonucleotides into building blocks of DNA) and in respiration and energy production. Musa and Ahmed (2025) observed similar drops in Fe in other coal-affected soils, which tells us that we need to understand the geochemical background to find out the real dynamics and biochemical process of Fe in diversity. Molybdenum (Mo), on the other hand, had concentrations above WHO guidelines in both contaminated and control soil; however, there was no statistical difference between the two, indicating that the inheritance may be sourced naturally based on the geogenic character of the soils instead of from coal. Antoniadis et al. (2020) discussed that Mo concentrations vary in different soils, and based on globally accepted permissible limits, if Mo permitted limits are exceeded, it heightens the need for caution. For example, the fact that they are exceeded the WHO stipulated threshold/permissible limits tells us that there is urgent need to take precaution or implement policies that could help in the application of remediation techniques or even preventive measures.

The pollution index assessment in the Coal-waste-contaminated cultivated Soils showed consistently that the pollution indices calculated such as CF, Igeo, EF, and PLI are very good tools that could help determine ecological risk and trace contamination sources across different states and geopolitical zones in Nigeria and should be recommended especially through policy advice to assist in understanding ecological health. For instance, Afonne et al. (2022), had identified cadmium as the dominant contaminant in Ihiala soils, with Cu and Pb showing moderate enrichment. Also, Musa and Ahmed (2025) have also reported moderate Fe but low Cu enrichment in Taraba, these highlights regional differences shaped by industrial intensity and soil buffering capacity and could boost the understanding of the uniqueness in ecological endowment, problems and solutions for different regions and culture.

In this study, the EF values affirms that Cu has anthropogenic origins, since EF above 1 ( $> 1$  indicates anthropogenic origin) (Swain, 2024) on the converse Zn and N ( $EF < 1$ ) reflect natural sources, this shows that distinct selective accumulation type and history not only suggests the source of pollution but also affects microbial activities and communities, plant uptake, and food safety. This hypothesis was confirmed by a study by Odiete and Chinemelu (2024) that presented the results of severe coal-related pollution in Effurun and Enugu soils and their PLI values exceeding 57 and Igeo values above 5 for several assessed metals (trace elements). These findings insinuate that industrial activities and coal waste residues are a major contributor to trace metal buildup and that applying several techniques and multiple indices provides us with a more comprehensive/robust insight into contamination patterns, and with evidence guiding targeted remediation and -based results that could be applied for sustainable land-use, and planning in industrial regions and arable lands that are exposed or impacted overtime.

Looking at the pollution indices, most trace elements stayed within acceptable safety levels, but the change in dynamics is ecologically relevant and of importance to understand the effect of the various changes and interactions, especially as the microbiome is implicated. The high Igeo values for Cu as seen in this study put them in the "strongly to extremely polluted" category, which is expected as Cu loads are observed in soil due to urban runoffs, industrialisation and mining activities (Onyedikachi et al., 2018). This corresponds to other studies, like in Taraba State, Nigeria (Musa and Ahmed, 2025); Odiete and Chinemelu (2024) also reported extreme PLI values in auto-mechanic soils in the Niger Delta, which reinforces how severe industrial contamination is in this region. The fact that B and Mo levels stayed relatively unchanged reflects that the elements, the metals, move and seep in soil as water percolates aside from the mechanisms for biosorption into soil matrices and other nuances and dynamics in the soil, which were not fully explained in this study. These patterns may affect and influence biodiversity, microbial communities

and their ecosystem interactions, nutrient cycles, and plant uptake, which shapes the broader ecological space.

The Nitrogen (N) levels dropped substantially in contaminated soils as expected, this further shows that the nutrient cycle has been distorted leading loss of diversity, microbial shift, crop stress because the microorganism is exposed to poor nutrition leading to their low yield. Zhang et al. (2025) found that nitrogen loss in coal contaminated soils just like the coal-waste-contaminated soils in our study may lead to the loss of organic matter and the oxidative stress and toxicity effect on their indigenous microbes. There are no international standards for N levels, which makes it hard to gauge the risk, but it's obviously fundamental for the ecosystem.

Zinc (Zn) concentrations were lower in contaminated soils too, perhaps due to less availability or immobility or due to competition with other metals are competing with it. However, Swain (2024) explained that Zn forms stable compounds in polluted areas, so the depletion we observe could explain the poor nutrient availability that harm microbes and plant health due to Zinc depletion activated by pollution.

These differences tell us that coal waste influences trace elements differently and most elements get immobilized or some remain unchanged while some are spike up due to soil chemistry and coal waste residues.

The heatmap analysis (Fig. 2) reveals discrepancies in heavy metal concentrations between the selected study area (coal-contaminated soil) and control. A blue–grey–red gradient is used in the figure to show the average concentrations of trace elements like zinc (Zn), nitrogen (N), molybdenum (Mo), iron (Fe), copper (Cu), and boron (B) in both control and contaminated soils. The red colour indicates the highest concentrations, the grey comes as the middle/medium and the lowest is depicted with blue.

Mo showed in a repeated manner the highest enrichment in both soil types, this suggested that it is naturally abundant/ or its resistant to

contamination effects. These observations confirms that there is substantial enrichment of the soil from constant exposure from coal waste even previous study supports this claim, this is due heavy metal buildup in soil (Zhang et al., 2025).

This heatmap shows how Fe concentration drops especially as the colour changed from grey to lighter blue in contaminated soils. This aligns with recent study that demonstrated that contamination can reduce how available Fe is by changing soil chemistry (Ijaz et al., 2025). Zinc, nitrogen, copper, and boron showed no obvious change in colour, as they were within the zone of pale blue colours that indicated low and safe concentration(s). This includes boron (B), which again represented a natural geogenic source rather than an anthropogenic source. This is confirmed by a similar pattern in a non-industrial agricultural soil too (Musa & Ahmed, 2025). Other studies report that Zn levels vary depending on local pollution sources (Antoniadis et al., 2020). These elemental distribution patterns further reiterate how environmental disturbance shapes soil chemistry. However, as revealed by this study, the buildup of Mo and the converse reduction of Fe in this study collectively show that contamination and constant exposures to trace elements constantly influence trace element profiles unevenly (Dhaliwal et al., 2019, Boothman, Coiro and Moran, 2022).

Soil contamination by Fe and Cu is often used to check fundamental pollution indicators because Fe concentrations in the soil reduce due to contamination by trace elements, while Cu often builds up to toxic levels, which interferes with microbial communities and enzyme functions, and plant and soil health is disrupted as well. The following studies have also confirmed this; for instance, high Cu levels near pollution hotspots have documented ecological consequences (Angon et al., 2024). Industrial activities increase Cu and mercury buildup in soil, lowering enzyme activities and biological functions (Száková et al., 2025). The manner and style in which soil mineralogy and pH change alter metal retention, concentration and mobility makes metal behaviour in soils even more complicated (Popoola et al., 2024). This complexities/behaviour indicates that there is need to consider the chemical, biological, and

physical properties of the soil to in order to manage soil contamination effectively to ensure positive outcome. This is because the trace elements express and interact differently individually and collectively in the soil. Also, their site-specific characteristics determine the method and strategy to be used for targeted cleanup and a sustained land use planning.

The correlation matrix explains positive correlation between the physicochemical properties of control and contaminated soils, this approach explains further the heatmap which sought to explain the comparative contamination pattern of the study areas. The coefficients close to/nearing +1.0 shows that the structural integrity of the soil matrix aligned and despite the changes seen in the values and the coal-waste contamination of the Soil. This shows that soil structure and physicochemical qualities mirrors a structured relationship despite the coal-waste-contamination. However, this needs further confirmation because ecological shifts and various dynamics is needed to make this evidence-based conclusion. Also, this study showed other essential fertility indicators/parameters like total nitrogen (%N), organic manure, calcium (Ca), and effective cation exchange capacity (ECEC) already having a marked reduction in contaminated soil. This reduction symbolizes that despite the positive correlation there could be severe harm due to the coal waste-contamination that affects microbial viability, nutrient retention, and plant productivity (Chaubey and Singh, 2012; Wong, 2003b). In addition, the sand content and potassium (K) levels remain unchanged across both samples i.e. the coal contaminated soil and the control suggesting that soil qualities could be influenced by adaptation meaning that soils may develop resilience over time and grow resistance to coal-derived pollutants, even amidst degradation of soil fertility parameters that reflects the sustained ecological risks from industrial contamination.

The Principal Component Analysis (PCA) biplot showed a separation between the soil samples (coal waste contaminated soil and the control). This indicates variance between the two soil samples. With PC1 64.9% and PC2 accounting for 28% of the total variance. The PCA showed that the two-dimensional structure captures 92.9% of the dataset's variability. This confirms that the

elemental/trace metal composition of the soils is due to contamination. It also explains how soil samples are distributed along PC1, representing copper (Cu), boron (B), molybdenum (Mo), and zinc (Zn), which are somewhat elevated in contaminated soils, while iron (Fe) and nitrogen (N) are more associated with control samples. These observations agree with Zhang et al. (2025); whose study identified heavy metals like Hg, Cd, and Pb and suggested they are responsible for ecological risk in coal-impacted agricultural soils and are also the dominant contributors to this risk using a PCA-PMF (Principal Component Analysis-Positive Matrix Factorisation) approach.

The heat map aligns with the PCA results, which revealed elevated concentrations of Mo across both soil types, with other elements like Cu, Zn, and Fe displaying lower levels than Mo, although with noticeable gradients. The prevalence of Mo in both control and contaminated soils still suggests a natural geological origin or a widespread agricultural use. This is not unique to only Mo, as Mu et al. (2025) also found Hg and Cu to be enriched in top soils of their study samples. The correlation plot further affirms this evidence by the positive associations between soil types, which connotes shared geological characteristics amidst different chemical profiles driven by contamination.

Also, the PCA results for Cu and B indicated a strong contamination, implying enrichment from human activity (anthropogenic source), perhaps from industrial or mining operations or agricultural applications while Fe and N remain unchanged suggesting these elements are less influenced by pollution and geogenic stability. This result corresponds with Wasiu et al. (2025), who observed that while heavy metals like Pb and Cd were elevated near waste dumpsites, nutrients like N and Fe remained relatively unaffected, reflecting their natural abundance and ecological resilience even to the constant coal waste exposure.

The PCA, heat map, and correlation plot in this study demonstrate that coal contamination is a dominant ecological driver that reshapes soil chemistry of our study samples. This has serious potential in changing microbial dynamics. These simulations help to make meaningful assertions

in environmental assessments, confirming if the differences observed are because of contamination and not sampling error. Therefore, there is need for targeted cleanup strategies, especially in regions like Enugu State where agriculture coexists with industrial coal combustion, to ensure agricultural and environmental sustainability.

The micrographs showed microscopic and morphological identification of bacterial species including *Bacillus cereus*, *Bacillus megaterium*, *Bacillus alvei*, *Pseudomonas fluorescens*, *Pseudomonas aeruginosa*, and *Micrococcus luteus*. These organisms have diverse cell shapes, rod-shaped bacilli and spherical cocci, along with characteristics that shows their unique presence and appearance-based pigmentation and staining that explains how they have adapted physiologically to coal-contaminated environments.

*Bacillus* species, particularly *B. cereus* and *B. megaterium* were identified as a spore-forming, gram-positive bacteria specie that can survive in nutrient-poor and metal-rich conditions. This may suggest their presence in the coal-waste sample. These bacteria are known for their roles in biosorption, bioleaching, and degradation of heavy metals enzymatically (Usmani et al., 2019). These microbes have the ability to release biofilms into the soil matrix, adsorbing and attracting trace elements to their functional group on their cell walls. Findings by Li et al. (2022), confirms their presence in coal-waste-laden soil due to their enhanced microbial diversity and metabolic resilience in soils impacted by contaminants like coal waste.

*Pseudomonas fluorescens* and *Pseudomonas aeruginosa* are Gram-negative and oxidase-positive, and are known for their motility and ability to break down toxic metals, polycyclic aromatic hydrocarbons (PAHs), and other toxic pollutants in coal-waste contaminated soil. These observations may indicate that coal-waste environments may support the growth of special microbes with biodegrading characteristics and their ability to survive pollution-laden environments, maybe due to adaptable metabolisms (Wong, 2003b).

*Micrococcus luteus*, a pigmented, catalase-positive coccus, that shows surface colonisation and tolerance to oxidative stress. This organism has been linked to metal resistance and is often found in industrially polluted soils, where it contributes to early-stage ecological stabilisation (Li et al., 2022).

The micrographs are the most glaring microscopy-based evidence of microbial adaptation to coal-derived stressors like the waste from coal-powered industries. The morphological diversity and biochemical traits of these isolates show a functional shift toward survival, detoxification, and potential cleanup traits that could be further enhanced through the bioengineering of these potent microorganisms. The mechanism for these amazing potentials by the microorganisms demonstrates their resilience, microbiome interactions especially the native microbes in restoring soil health and reducing the sustained effects of coal-waste contamination.

The biochemical assessments of microbial isolates from coal waste samples showed different characteristics of metabolically active and stress-tolerant bacteria. These include *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Staphylococcus aureus*, *Micrococcus luteus*, *Micrococcus lylae*, and several *Bacillus* species (*B. alvei*, *B. megaterium*, *B. licheniformis*, and *B. cereus*). Each expressing its enzymatic and metabolic qualities affirming their roles in the ecological profiling in coal-waste-contaminated environments.

Rod-shaped *Bacillus* and *Pseudomonas* species grew more compared to other cultured microorganisms in this study, just as expected, bearing in mind that they are known for being resilient in metal-rich and nutrient-depleted soils. These bacteria were all positive for catalase and oxidase reflecting their ability to release antioxidants, a defence system that enable their survival in toxic environments, like our coal waste-laden soil samples. This protective mechanism is signalled during release of reactive oxygen species induced by toxicity of metals (Usmani et al., 2019). The urease activity observed for *P. aeruginosa* and *B. cereus* implies potential roles in nitrogen cycling and pH

adjustment, particularly under acidic conditions caused by coal residues (Mols and Abee, 2008).

*Pseudomonas fluorescens* and *Bacillus alvei* reacted positively to glucose and lactose fermentation, methyl red, and Voges-Proskauer tests, as glucose metabolisers. Their mixed acid and butanediol fermentation pathways explain their adaptability in environments where carbon sources spike and reduce. These metabolic flexibilities can contribute to breaking down organic pollutants and strengthening their role in carbon metabolism and cleanup (Wong, 2003b).

The round-shaped *Micrococcus* and *Staphylococcus* species were positive catalase and oxidase test. *S. aureus* uniquely expresses its active involvement in coagulase and mannitol fermentation. These qualities are linked to opportunistic colonisation and biofilm formation. Besides, they are often surface-adherent and can tolerate drying out and metal stress, which makes them good indicators of early-stage ecological recovery (Götz et al., 2006).

Most of the bacteria isolates did not indicate motility except *P. aeruginosa*, which showed it to withstand and conserve energy under stress. The expression of hydrogen sulphide and indole production was limited across all isolates, showing reduced anaerobic activity. This is as expected, as the aerobic nature of coal waste-exposed soils could have limited this reaction.

The biochemical traits observed in this study confirm that coal waste contamination drives the selection of metabolically versatile, stress-adapted microbial communities. The predominance of catalase, Gram-positive, oxidase-positive-reactive bacteria shows how they play their ecological roles in detoxification, nutrient cycling, and soil stabilisation, which are important chemical dynamics in biodiversity. This is to prove the need for integrating microbial sequencing to better understand how chemical shifts translate into microbial and elemental responses, and also to optimise the sustenance of ecosystem resilience. The identification of these stress-tolerant bacteria reflects a broader trend of adaptive restructuring and consistency (Zhang et al., 2025). These microbes likely contribute through multiple mechanisms like bioleaching or the removal metals out of

contaminated soils, biosorption to enable toxic elements binding and immobilisation, enzymatic processes to degrade organic pollutants, and also biofilm formation with extracellular polymeric substance (EPS) production stabilises soil structure.

## Conclusion

The evidence from this study indicates that coal-waste contamination substantially alters soil physicochemical properties, trace-element behavior, and microbial community structure in arable lands of Enugu State. The declines in key fertility indicators, including nitrogen, calcium, organic matter, and effective cation exchange capacity, suggest a reduced capacity of these soils to sustain agricultural productivity. Although, molybdenum concentrations exceeded WHO guideline values, there is potential risk of transfer into the food chain indicating serious public health implications. Multivariate analyses like principal component analysis, correlation matrices, heat maps, and pollution indices consistently identified coal waste as the primary environmental gradient accounting for the observed divergence between contaminated and reference soils. Microbial characterisation further shows a dominance of stress-tolerant genera such as *Bacillus* and *Pseudomonas* species, a pattern that reflects adaptive persistence under pollutant pressure and signals their functional importance in contaminated soil systems. These observed shifts may extend beyond soil degradation and therefore signals the urgent remediation strategies.

Recent advances provide insights indicating that the prevalence of resilient microbial taxa observed in this study suggests that microbe-mediated processes may be harnessed or integrated with other remediation frameworks, particular phytoremediation, phycoremediation, and bio-organic soil amendments to solve pollution problems. These approaches are scalable, eco-friendly and sustainable while supporting soil functional recovery. Also, the sustained monitoring of trace elements, coupled with improved organic matter management, may be critical for maintaining soil resilience in coal-waste contaminated regions. These measures align with responsive land management objectives and provide an empirical basis for

policy instruments aimed at reducing long-term ecological and public-health burdens associated with coal-derived pollutants.

Furthermore, the interpretation of findings like microbial analyses were based on culture-dependent techniques, which likely underestimate total community diversity and may exclude unculturable taxa that contribute to ecosystem stability and contaminant transformation. Also, the spatial coverage was confined to selected sites and the sampling occurred within a single season, limiting inference across broader geographic scales and temporal dynamics. These methodological constraints suggest that future investigations would benefit from incorporating DNA-based approaches, such as 16S rRNA sequencing, alongside expanded spatial and seasonal sampling. In this light, direct evaluation of trace-metal bioaccumulation in crops and associated exposure pathways in animals and humans would further refine risk assessment. Addressing these methodological gaps may enhance the resolution of detection frameworks and strengthen the evidence base required for effective management of coal-related soil contamination.

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### Competing Interests

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### Ethics Approval

Not applicable. This study did not involve human participants or animals.

### Consent to Participate

Not applicable.

### Consent for Publication

Not applicable.

### Data Availability

All data generated or analyzed during this study are included in this published article. Additional datasets are available from the corresponding author on reasonable request.

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