

Article

Effects of Clay Minerals on Enzyme Activity as a Potential Biosensor of Soil Pollution in Alice Township

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Abstract: Inadequate waste management and illegal trash dumping continue to be the leading causes of severe environmental pollution. Human exposure to harmful heavy metals has emerged as a serious health concern on the continent. Some people in Alice, a small town, grow their food in home gardens. They use animal manure and compost derived from soil obtained from landfills to enhance the fertility of the garden soil. Heavy metal heaps in garbage disposals are constantly present, releasing dangerous amounts of metal into the environment. The harmful effects of heavy metals on plants lead to unsanitary conditions and environmental problems. Animals and people who consume these vegetables may also be at risk for health problems. Assessing the soil's enzyme activity can potentially lessen the negative effects of the accumulated pollutants and improve the soil's overall health and quality. Soil enzymes are biologically active components that have a catalytic impact and are released from root exudates, crop residues, and animal remains. The activity of enzymes serves as an excellent bioindicator of soil cleanliness and quality because they are sensitive to heavy metals. X-ray diffraction (XRD) was used to quantify the mineral elements in soil using 40 kV parallel beam optics, 30 mA, and CuK α radiation. Meanwhile, the activity of the enzyme was assayed in different coupled substrates. Thirteen (13) clay minerals were found, including Talc 2M, Kaolinite 2M, and Chlorite Lawsonite Muscovite 2M1. The detected trace elements have high concentration levels that exceed the World Health Organization's (WHO) allowed levels. The identified elements affected the enzyme activity at different levels. The Mn, Al, Si, V, Ti, and Ca negatively affect soil enzyme activity, specifically invertase (INV). However, the amount of Mg, K, Fe, and Zn showed a slightly positive effect on the same enzyme (INV). According to this view, these elements come from several sources, each with a particular impact on soil contamination and enzyme activity. High levels of heavy metals in this study may be due to improper waste disposal, limited recycling opportunities, lack of public awareness, and inadequate enforcement of waste management regulations. It is essential to employ Fourth Industrial Revolution (4IR) technologies, correct disposal techniques, suitable agricultural methods, preventive regulations, and efficient waste management to mitigate the negative effects of heavy metals on the environment.

Keywords: enzymes; soil pollution; waste disposal; enzyme activity; correlation; trace elements



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1. Introduction

The leading cause of illness and premature death globally is pollution, which has a detrimental impact on the stability of natural resources and their ability to support ecological processes. The Alice region is particularly affected by severe soil contamination, primarily due to the improper disposal and mishandling of various pollutants, both locally and from foreign sources [1]. Situated in a rural area surrounded by agricultural farms specializing in vegetation, livestock farming, and mixed farming, the small town of Alice relies on agricultural activities for income generation, economic growth, and food production.

Workers in the agricultural sector and waste collectors have limited knowledge about heavy metal pollutants in soil, their environmental impact, and their effects on human

health. The public is unaware that some commonly used fertilizers contain hazardous elements that can pose a threat when they accumulate beyond permissible limits in the environment. Fertilizers, insecticides, and organic waste generated during farming operations are the primary sources of sedimentation and soil contamination in agriculture.

Soil enzymes, such as urease (URE), invertase (INV), and phosphatase (PHO), are biologically active components released from root exudates, microbes, crops, and animal remains. These enzymes play a significant role in stabilizing soil structure, decomposing organic matter and waste, converting plant nutrients into accessible forms, and facilitating nutrient cycling [2].

Enzymes are crucial regulators and catalysts of essential soil functions. The accumulation of heavy metals in various ecosystems has significant impacts on ecological equilibrium. It is crucial to develop innovative and tailored remediation technologies to address heavy metal contamination effectively and efficiently in soils [3,4]. Enzymes play a crucial role in the bioremediation process. Identifying and selecting soil enzymes that are highly responsive to toxic elements is essential for monitoring soil pollution and enhancing soil quality during the bioremediation process [5].

Since enzymes are smaller than microbial cells, they can more readily come into contact with pollutants, which speeds up the process of degrading and reducing toxins to a less hazardous or permissible condition. Enzymes are capable of cleaving chemical bonds and facilitating the transfer of electrons from a reduced organic substrate (donor) to another chemical compound (acceptor). They remove several types of inorganic and organic pollutants from the soil, such as lead (Pb), mercury (Hg), polymers, azo dyes, hazardous metals, Polycyclic Aromatic Hydrocarbons (PAHs), and chromium (Cr). These oxidation-reduction processes lead to the pollutants' ultimate oxidation into nontoxic molecules [6].

Currently, there is no universally accepted enzyme as a diagnostic indicator for soil quality based on soil enzymes in diverse environmental conditions. This research aims to identify soil enzymes, assess their activity levels, and evaluate their effectiveness in mitigating harmful pollutants. The study seeks to investigate the impact of hazardous pollutants on the selected biological indicators (URE, CAT, PHO, and INV). This study will provide valuable insights for authorities to consider the use of eco-friendly and cost-effective techniques, such as enzyme activity, which are already present in the soil. It is important to note that many sites still employ outdated waste disposal methods, such as burning, for waste disposal in the area.

2. Methods and Materials

2.1. Sample Sites Description

The study was carried out at two specified collection sites in Alice Town. The first site, designated as the study site, is the Alice landfill site, which is approximately 2 km away from the reference site located within the premises of Fort Hare University's eastern side (as indicated on Figure 1). The landfill site is positioned at latitude $32^{\circ}48'24.88''$ S and longitude $26^{\circ}49'33.37''$ E. Meanwhile, the reference site is located at latitude $32^{\circ}47'07.35''$ S and longitude $26^{\circ}57'26.10''$ E.

The landfill site (denoted as Site 1) is divided into three sections (A, B, and C (Figure 1)), and each section was chosen randomly based on the amount of waste present at each location. The soil at the landfill site is covered with various types of household waste, including hospital waste, broken glass, rusted tins, and metal residues. In contrast, the soil at the reference site (referred to as Site 2 or Section D) is naturally covered with wild plants and small stones.



Figure 1. The study map area [7].

2.2. Elemental Analysis

Soil specimens were collected for the period of four (4) weeks at random depths ranging from 0 to 25 cm. A clean soil auger was used to obtain samples from three sections (A, B, and C) of Site 1 and one section of Site 2 (D). The collected dry soil samples were placed in sterile, marked plastic bags with zippers and delivered to the laboratory for further analysis. In the laboratory, samples were ground with a pestle and mortar and sieved to obtain a fine texture [8].

Diffraction patterns were obtained using a Siemens D5000 powder XRD apparatus. Next, 2.0 g of preserved soil fragments was placed in a 3 mm transparent container after being pulverized, ground, and homogenized to a 10–15 mm powdery size. They were then examined using parallel beam optics at 40 kV, 30 mA, and $\text{CuK}\alpha$ radiation. The scanning settings for the sample were as follows: reflections were scanned for 2 h, the temperature range was set from 0 to 80 °C with a step size of 0.02, and a sum-up interval of 2 s was used in each stage. Each soil sample's elements were detected by comparing them to the 2004 International Center for Diffraction Data (ICDD) database [9].

2.3. Enzyme Activity Analysis

2.3.1. Invertase Activity

Five drops of toluene were introduced into a 100 mL volumetric flask containing 5 mL of distilled water, 15 mL of an 8% sucrose solution, and 5.0 g of soil that had been dissolved in the mixture. The resulting solution was subjected to digestion at 37 °C for a period of 24 h, followed by centrifugation for 5 min at 4000 rpm. Subsequently, a 1.0 mL aliquot was withdrawn and transferred to a 100 mL volumetric flask containing 3 mL of 3,5-dinitro salicylic acid. The mixture was then heated for 5 min and allowed to cool to room temperature. The glucose concentration was determined by measuring the absorbance using a spectrophotometer at 508 nm. The invertase activity was expressed as $\mu\text{g glucose}\cdot\text{g}^{-1}\text{ soil}\cdot\text{h}^{-1}$ [10].

2.3.2. Urease Activity

A combination of 20 mL of distilled water, 5 mL of toluene, 10 mL of a 10% urea solution, and 5.0 g of soil extract was subjected to incubation at 37 °C for 24 h. Following incubation, the mixture underwent centrifugation at 4000 rpm for 5 min. A 1 mL portion was then combined with 4.0 mL of a sodium phenol solution, comprising 100 mL of a 6.6 M phenol solution, 100 mL of a 6.8 M sodium hydroxide (NaOH) solution, and 3 mL of a 0.9% sodium hypochlorite solution. The quantification of released ammonium in the solution was carried out colorimetrically at 578 nm using a spectrophotometer, and the urea concentration was expressed as $\mu\text{gNH}_4\text{N}\cdot\text{g}^{-1}\text{ soil}\cdot\text{h}^{-1}$ [10].

2.3.3. Phosphatase Activity

In this stage, 10 g of soil was infused with 10 mL of disodium phenyl phosphate solution, five drops of toluene, and 10 mL distilled water in a 50 mL polyethylene conical flask. The mixture was incubated at 37 °C for 24 h and centrifuged for 5 min at 4000 rpm. Then, 0.25 mL of ammonia-ammonium chloride buffer was added to make the pH 9.6. A blend of 0.5 mL of 8% potassium ferrocyanide and 0.5 mL of 2% 4-amino antipyrine were added to give a supernatant color. A spectrophotometer was used to determine the phenol concentration colorimetrically at 510 nm. Phosphatase activeness was presented as $\mu\text{gphenol}\cdot\text{g}^{-1}\text{ soil}\cdot\text{h}^{-1}$ [10].

2.3.4. Catalase Activity

A 5.0 g soil sample was dissolved in glassware with 0.5 mL of toluene. The resulting supernatant was refrigerated at 4 °C for 30 min, followed by the addition of 5 mL of 3% hydrogen peroxide (H_2O_2). Subsequently, the solution was further cooled for an hour. The mixture was then subjected to treatment with 2 M sulfuric acid (H_2SO_4), and 0.01 M potassium permanganate (KMnO_4) was introduced to the supernatant until a vivid pink hue developed. The catalase enzyme activities were to be quantified in terms of mL of $\text{KMnO}_4\cdot\text{g}^{-1}\text{ soil}\cdot\text{h}^{-1}$ [10].

2.4. Statistical Analysis

The IBM Statistical Package for Social Science (SPSS) 28, version 28.0, was used to analyze the data (IBM, Armonk, NY, USA). IBM Pearson's Correlation was used to investigate the relationship between soil enzyme activity and trace elements in the soil.

3. Results

3.1. XRD Analysis

The predominant technique employed for the examination of soil minerals is X-ray diffraction. In this study, the powder X-ray diffraction method was utilized to investigate sixteen soil samples obtained from both contaminated and uncontaminated regions.

The data gathered offers a comprehensive understanding of the mineralogical components, as depicted in Table 1, which elucidates the crystalline structure of the minerals. Bulk chemical composition describes the entire mineral and chemical composition of a rock

sample, accounting for all the elements that are present. The visual representation of the findings is illustrated in Figures 2–5.

Table 1. Mineral contents determined by X-ray diffraction analysis (wt%).

Sampling Sites	Lawsonite (%) SrMn ₂ (Si ₂ O ₇)(OH) ₂ ·H ₂ O	Chlorite (%) Mg ₃ Si ₂ O ₅ (OH) ₄	Talc 2M (%) Mg ₃ Si ₄ O ₁₀ (OH) ₂	Muscovite 2M1 (%) KAl ₂ (AlSi ₃ O ₁₀)(OH) ₂	Kaolinite 2M (%) Al ₂ Si ₂ O ₅ (OH) ₄	Quartz high (%) α-SiO ₂	Goethite (%) α-FeOOH	Montroseite (%) V ₈₇₅ Fe ₁₂₅ OOH	Ilmenite (%) FeTiO ₃	Anatase (%) TiO ₂	Pyrolusite (%) β-MnO ₂	Franklinite (%) ZnFe ₂ O ₄	Dolomite (%) CaMg (CO ₃) ₂
1A	12.34	12.21	20.25	7.88	26.48	1.85	5.12	3.31	1.57	6.69	0.31	0.85	1.15
1B	7.12	17.29	25.88	8.88	19.20	1.70	6.65	4.57	2.52	1.10	0.32	2.18	2.58
1C	7.23	17.29	29.04	8.28	12.06	1.38	8.74	5.49	2.20	1.71	0.40	1.09	5.09
1D	18.24	11.25	18.90	8.83	26.52	1.08	4.73	4.30	1.58	1.65	0.16	0.88	1.88
2A	15.54	10.44	23.95	7.17	22.35	1.41	7.12	4.10	1.79	0.86	0.29	1.16	3.80
2B	15.49	13.93	19.70	7.94	25.77	1.41	4.74	3.32	1.85	1.01	0.25	1.26	3.34
2C	15.95	15.19	21.11	8.55	19.81	1.68	5.58	3.45	1.88	1.07	0.26	0.94	4.51
2D	14.19	16.14	23.03	9.38	19.41	1.09	6.57	3.12	2.22	0.79	0.22	0.77	3.08
3A	12.03	13.79	26.59	7.78	14.11	1.05	7.64	5.34	2.18	1.18	0.40	1.68	6.23
3B	15.19	13.99	19.76	8.59	24.60	1.48	4.81	3.53	1.53	1.06	0.26	0.92	4.28
3C	16.19	10.82	21.69	7.59	24.11	1.42	6.26	3.66	1.44	2.17	0.20	0.75	3.70
3D	14.15	14.90	25.31	8.95	14.26	1.98	6.81	4.80	2.44	0.79	0.25	1.14	4.20
4A	13.20	12.77	24.96	7.92	17.50	1.41	6.42	6.05	2.39	0.98	0.27	1.03	5.11
4B	15.81	12.53	21.89	7.34	21.22	1.79	5.28	3.75	2.20	1.74	0.18	1.32	4.95
4C	15.58	12.57	20.39	8.48	23.31	1.75	5.47	4.72	1.71	1.07	0.25	0.90	3.80
4D	12.69	16.20	24.54	7.30	13.51	1.90	6.98	4.77	2.77	0.69	0.43	1.12	7.09

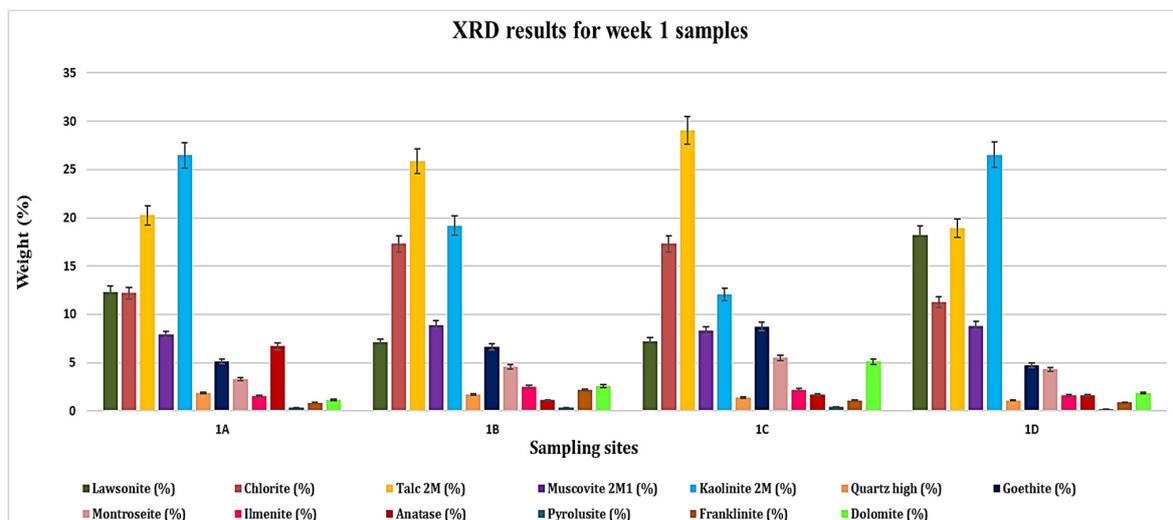


Figure 2. The soil mineralogical composition for week 1 samples.

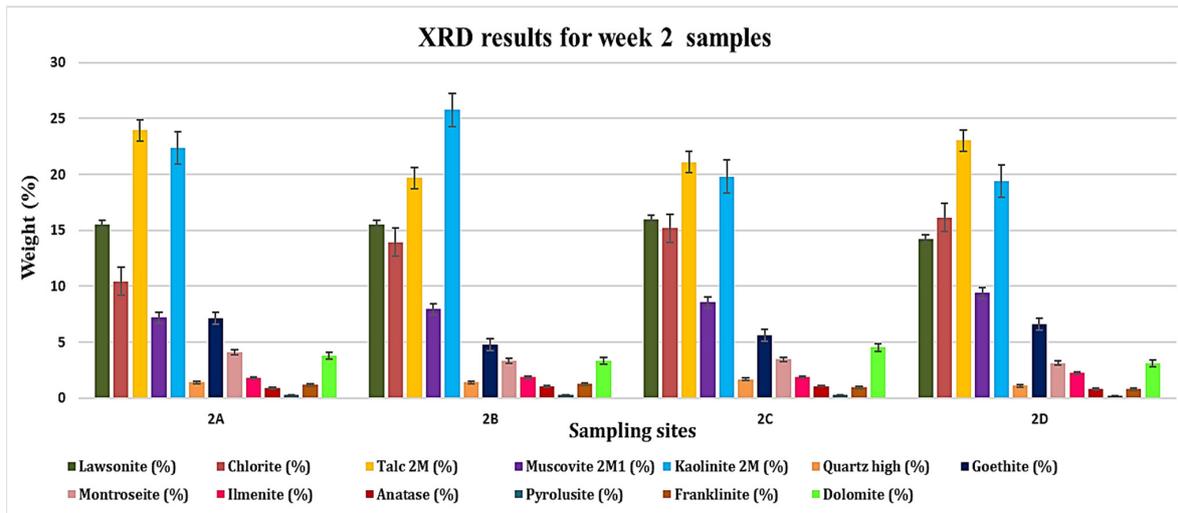


Figure 3. The soil mineralogical composition for week 2 samples.

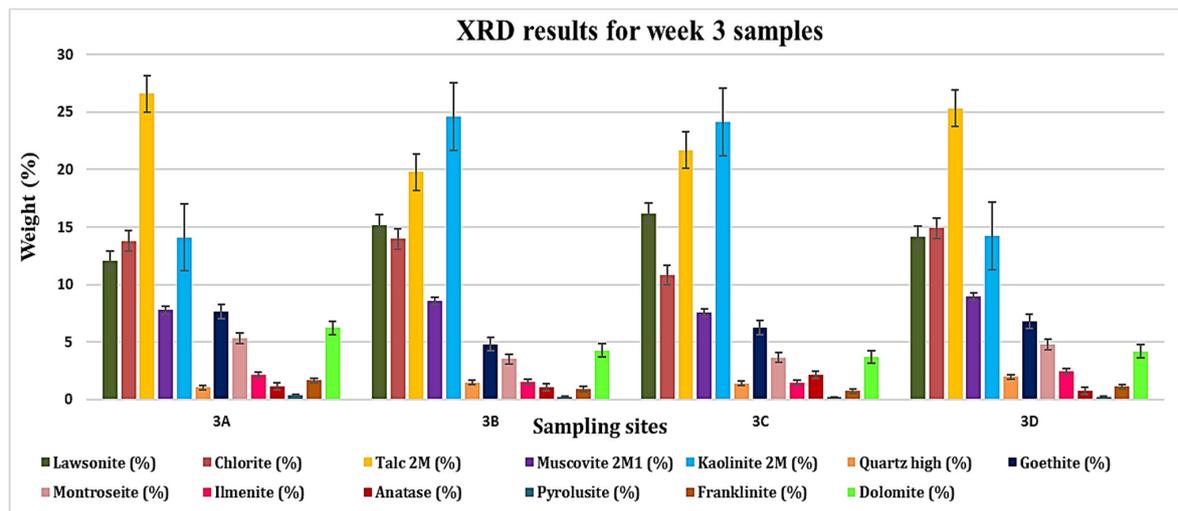


Figure 4. The soil mineralogical composition for week 3 samples.

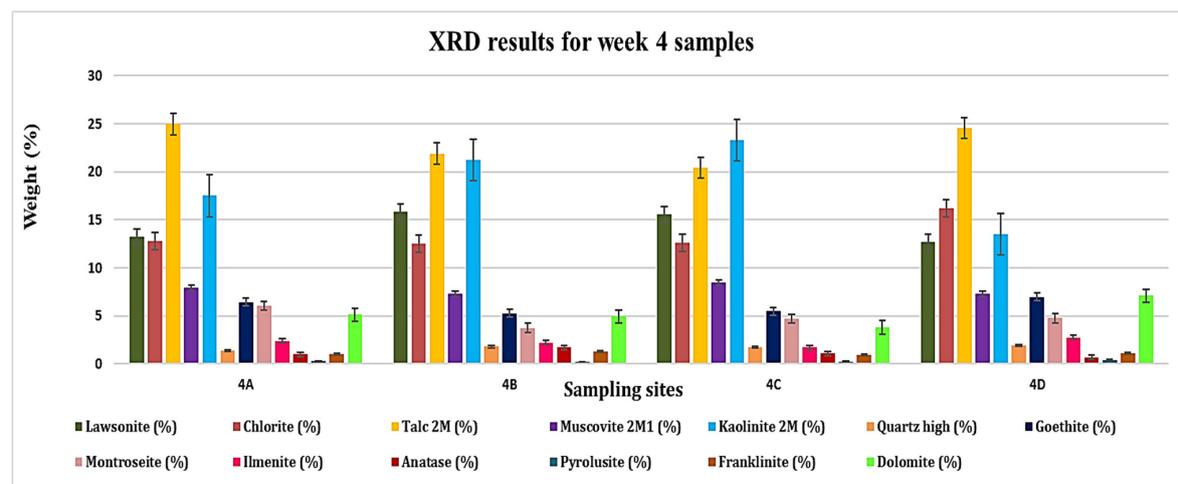


Figure 5. The soil mineralogical composition for week 4 samples.

The X-ray diffraction (XRD) method is utilized for identifying the predominant minerals in soil. The analysis of soil samples from the designated study areas indicates the presence of consistent mineral phases, as detailed in Tables 1 and 2. The chemical composition and mineralogy of the soil samples from both sites exhibit a high degree of similarity. This investigation contributes to the understanding of soil mineral composition and the crystalline structure of these minerals.

Table 2. The observed complexes of major elements by XRD in soil samples.

Major Elements	Observed Element Complexes by XRD in Soil Samples
Mn	$\text{SrMn}_2(\text{Si}_2\text{O}_7)(\text{OH})_2 \cdot \text{H}_2\text{O}$ (lawsonite), MnO_2 (pyrolusite)
Mg	$\text{Mg}_3\text{Si}_4\text{O}_{10}(\text{OH})_2$ (talc 2M), $\text{Mg}_3\text{Si}_2\text{O}_5(\text{OH})_4$ (Chlorite)
Al	$\text{Al}_2\text{Si}_2\text{O}_5(\text{OH})_4$ [kaolinite], $\text{KAl}_2(\text{AlSi}_3\text{O}_{10})(\text{OH})_2$ (muscovite)
K	$\text{KAl}_2(\text{AlSi}_3\text{O}_{10})(\text{OH})_2$ (muscovite)
Fe	FeOOH (goethite)
Ti	TiO_2 (anatase), FeTiO_3 (ilmenite)
V	$\text{V}_{875}\text{Fe}_{125}\text{OOH}$ (Montroseite)
Ca	$\text{CaMg}(\text{CO}_3)_2$ (dolomite)
Zn	ZnFe_2O_4 (franklinite)
Si	SiO_2 (quartz)
Sr	$\text{SrMn}_2(\text{Si}_2\text{O}_7)(\text{OH})_2 \cdot \text{H}_2\text{O}$ (lawsonite)

A total of 13 minerals have been identified in the soil samples, including Lawsonite ($\text{SrMn}_2(\text{Si}_2\text{O}_7)(\text{OH})_2 \cdot \text{H}_2\text{O}$), Talc 2M ($\text{Mg}_3\text{Si}_4\text{O}_{10}(\text{OH})_2$), Chlorite ($\text{Mg}_3\text{Si}_2\text{O}_5(\text{OH})_4$), Kaolinite 2M ($\text{Al}_2\text{Si}_2\text{O}_5(\text{OH})_4$), Goethite ($\alpha\text{-FeOOH}$); Quartz high ($\alpha\text{-SiO}_2$), Montroseite ($\text{V}_{875}\text{Fe}_{125}\text{OOH}$), Franklinite (ZnFe_2O_4), Pyrolusite ($\beta\text{-MnO}_2$), Anatase (TiO_2), Muscovite 2M1 ($\text{KAl}_2(\text{AlSi}_3\text{O}_{10})(\text{OH})_2$), Ilmenite (FeTiO_3), and Dolomite ($\text{CaMg}(\text{CO}_3)_2$).

The identified mineral components are primarily composed of elemental constituents, with their composition represented as a weight percentage. This representation contrasts with the milligrams per kilogram (mg/kg) unit used by the World Health Organization (WHO) in their guidelines for permissible substance limits in the environment. According to the conversion analysis, 1.0 wt% of a substance is equivalent to 10,000 mg/kg. The concentration of all identified mineral elements exceeds the allowable limits recommended by the WHO.

The predominant minerals in the soil samples are Talc, Kaolinite, Chlorite, Lawsonite, and Muscovite, with Talc being the most abundant. Pyrolusite and Franklinite constitute less than 1.5% of the total mineral composition, while other minerals are identified to a moderate extent, with compositional rates ranging between 9.5% and 1.0%. This study also reveals that mineral crystallites belong to different crystal systems, with various minerals exhibiting distinct crystal structures.

The presence of aluminum (Al), silicon (Si), titanium (Ti), and iron (Fe) minerals in the soil is attributed to advanced weathering processes and the type of waste thrown in the landfill site, which contains these metals and released as byproducts in the soil. These mineral elements occur as primary or secondary oxides and have the ability to solubilize cations in their complexes. The presence of aluminosilicates in many minerals is associated with neogenesis, wherein clay particles from complex minerals are mixed with water [11].

During the initial analysis in week 1, it was observed that Kaolinite 2M was the most prevalent mineral at sampling sites 1A (26.48 wt%) and 1D (26.52 wt%). Conversely, talc 2M was the dominant mineral at sites 1B (25.88 wt%) and 1C (29.04 wt%). The mineral percentages for pyrolusite, franklinite, anatase, and dolomite were consistently lower across all sites, ranging from 0.16% to 6.69 wt%.

During the second week of collection, Talc 2M was the primary mineral at three sampling sites: 2A, with a mineral composition percentage of 23.95 wt%; 2C, with a percentage value of 21.11 wt%; and 2D, with a recorded value of 23.03 wt%. Kaolinite was found in high quantities at site 2B, comprising 25.77 wt% of the minerals present.

The samples collected in Week 3 indicated a high presence of Talc 2M at sites 3A (26.56 wt%) and 3D (25.31 wt%), while significant amounts of kaolinite crystallites were observed at sites 3B (24.6 wt%) and 3C (24.11 wt%). Additionally, anatase, pyrolusite, and franklinite were found to have minimal mineral composition percentages across all sampling sites.

In the analysis conducted in the fourth week, abundant quantities of Talc 2M crystallites were found at sampling sites 4A, 4B, and 4D, apart from site 4C, which exhibited a high presence of kaolinite 2M mineral. Anatase, pyrolusite, and franklinite were detected in minimal quantities across all selected sites.

According to the pollution index calculated in Table 3, the concentrations of Mn, Mg, V, Si, K, Zn, Al, Fe, and Ca are toxic to the surrounding environments. This is because their MPI pollution index ranges between 0.39 and 1.85. It ranges from severe contamination to slight pollution, potentially impacting soil, plants, and the overall environment (as presented at Appendix A). Ca and Zn levels in the first week indicated a high pollution index on 1C and 1B. The pollution index levels of titanium (Ti) revealed severe pollution, reaching a maximum level of 2.75. This means that the concentration of Ti in the soil is too high, which poses a risk and potentially harms important aspects of the environment.

Table 3. The metal pollution index (MPI) for detected elements in soil.

Sites	Mn	Mg	Al	Fe	Ti	V	Ca	Zn	Si	K
1A	0.68	1.07	1.00	1.08	1.05	0.77	0.61	0.97	1.71	0.89
1B	0.39	1.37	0.72	1.41	0.67	1.06	1.37	2.48	1.57	1.01
1C	0.40	1.54	0.45	1.85	1.04	1.28	2.71	1.24	1.28	0.94
2A	1.10	1.04	1.15	1.08	1.09	1.31	1.23	1.51	1.29	0.80
2B	1.09	0.86	1.33	0.72	1.28	1.06	1.08	1.64	1.28	0.85
2C	1.12	0.92	1.02	0.85	1.35	1.11	1.46	1.22	1.54	0.91
3A	0.85	1.05	0.99	1.12	1.49	1.11	1.48	1.47	0.53	0.87
3B	1.07	0.89	1.73	0.71	1.34	0.74	1.02	0.81	0.75	0.96
3C	1.14	0.83	1.69	0.92	2.75	0.76	0.88	0.68	0.72	0.85
4A	1.04	1.02	1.30	0.92	1.42	1.27	0.72	0.92	0.74	1.08
4B	1.25	0.89	1.57	0.76	2.52	0.79	0.70	1.18	0.94	1.01
4C	1.23	0.83	1.73	0.92	1.55	0.99	0.54	0.80	0.92	1.16

3.2. Enzyme Activity Analysis in Soil

The determined enzyme activities in the soil are presented graphically in Figure 6 and tabulated in Table 4.

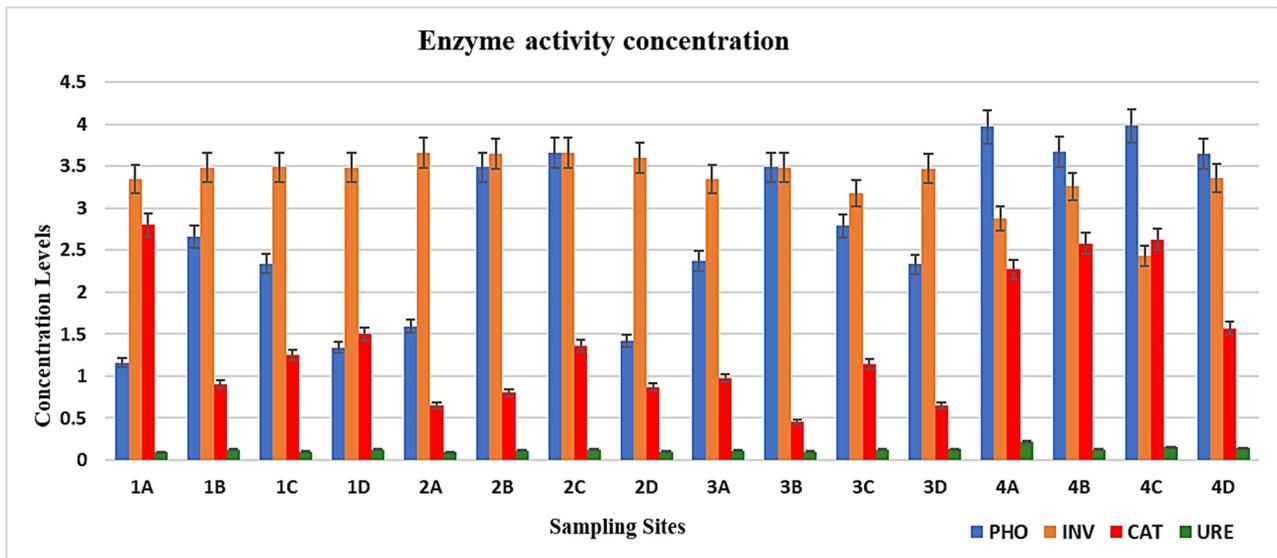


Figure 6. The concentration levels of enzyme activities in soil.

Table 4. The amount of selected enzyme activity in soil fragments.

		Urease ($\mu\text{g NH}_4\text{-N}\cdot\text{g}^{-1}\text{ Soil}\cdot\text{h}^{-1}$)	Invertase ($\mu\text{g Glucose}\cdot\text{g}^{-1}\text{ Soil}\cdot\text{h}^{-1}$)	Catalase ($\text{mL KMnO}_4\cdot\text{g}^{-1}\text{ Soil}\cdot\text{h}^{-1}$)	Phosphatase ($\mu\text{g Phenol}\cdot\text{g}^{-1}\text{ Soil}\cdot\text{h}^{-1}$)
Week 1	Site 1A	0.09 ± 0.05^c	3.35 ± 1.94^d	2.80 ± 1.62^d	1.16 ± 0.67^d
	Site 1B	0.12 ± 0.07^c	3.48 ± 2.01^d	0.90 ± 0.52^{cd}	2.66 ± 1.54^a
	Site 1C	0.10 ± 0.06^c	3.49 ± 2.01^d	1.25 ± 0.72^a	2.34 ± 1.35^c
	Site 2	0.12 ± 0.07^c	3.48 ± 2.01^d	1.50 ± 0.87^a	1.34 ± 0.77^d
Week 2	Site 1A	0.09 ± 0.05^c	3.66 ± 2.11^d	0.65 ± 0.37^b	1.59 ± 0.92^d
	Site 1B	0.11 ± 0.06^c	3.65 ± 2.10^d	0.80 ± 0.46^{cd}	3.49 ± 2.01^{ab}
	Site 1C	0.12 ± 0.07^c	3.66 ± 2.11^d	1.36 ± 0.78^a	3.66 ± 2.11^e
	Site 2	0.10 ± 0.06^c	3.60 ± 2.08^d	0.87 ± 0.50^{cd}	1.42 ± 0.82^d
Week 3	Site 1A	0.11 ± 0.06^c	3.35 ± 1.93^d	0.97 ± 0.56^{cd}	2.37 ± 1.37^c
	Site 1B	0.10 ± 0.05^c	3.48 ± 2.01^d	0.46 ± 0.27^{ab}	3.49 ± 2.01^{ab}
	Site 1C	0.12 ± 0.07^c	3.18 ± 1.83^d	1.14 ± 0.66^a	2.79 ± 1.61^a
	Site 2	0.13 ± 0.07^c	3.47 ± 2.01^d	0.65 ± 0.37^b	2.33 ± 1.34^c
Week 4	Site 1A	0.22 ± 0.13^{cd}	2.88 ± 1.66^c	2.27 ± 1.31^e	3.97 ± 2.29^e
	Site 1B	0.12 ± 0.07^c	3.26 ± 1.88^d	2.58 ± 1.49^d	3.67 ± 2.12^e
	Site 1C	0.15 ± 0.09^c	2.43 ± 1.52^c	2.63 ± 1.52^d	3.98 ± 2.30^e
	Site 2	0.14 ± 0.0^c	3.36 ± 1.94^d	1.57 ± 0.91^a	3.65 ± 2.10^e

Note: Results are presented as mean values (M) \pm standard deviation (SD). At the 95% confidence interval, mean scores with the same alphabetical letters in a column are not statistically different, where $p \leq 0.05$, and those with different letters are statistically different.

Table 4 and Figure 6 reveal the examined enzyme activity concentration levels in the selected soil samples. INV enzyme activity concentrations are significantly high in all collected samples. The lowest values of INV are recorded at site 1A. Meanwhile, the levels of PHO enzyme activity were high, specifically in the samples collected in the 4th week. The PHO values are recorded in the following order of concentration: week 4 > week 3 > week 2 > week 1, indicating that week 1 samples have the lowest levels of PHO activity. The CAT activity

was low between weeks 2 and 3 of the collection period, whereas the highest concentration levels were found in the samples from the fourth week of sampling. The activity levels of the URE enzyme were the lowest among all sampling sites, recorded below $0.50 \mu\text{g NH}_4\text{-N}\cdot\text{g}^{-1} \text{soil}\cdot\text{h}^{-1}$ in all selected collection sites. According to this study, URE and CAT have low activity levels and are, therefore, susceptible to heavy metals. This suggests that the toxicity of heavy metals has a significant impact on soil microbes. Due to their low enzyme potential index, CAT and URE are suitable to be used as soil bioindicators for soil pollution. The order of enzyme potential strength and absorption of these enzymes is as follows: $\text{INV} > \text{PHO} > \text{CAT} > \text{URE}$. These results indicate that the activity of INV and PHO enzymes has great potential to be utilized in the bioremediation of soil pollution due to their high adsorption capacity and activity levels.

3.3. The Correlation Analysis

The impact of trace elements in enzyme activity is detected by Pearson correlation, where the correlation results are presented in Table 5.

The study assayed and analyzed the active elements in soil minerals to assess their impact on enzyme activity using Pearson correlation. The obtained correlation coefficients revealed varying degrees of positive and negative correlations among the detected trace elements in the soil, suggesting diverse sources and differential effects on soil pollution and enzyme activity. For instance, phosphatase (PHO) activity exhibited positive correlations with manganese (Mn), silicon (Si), vanadium (V), and zinc (Zn) concentrations while showing negative correlations with magnesium (Mg), potassium (K), aluminum (Al), iron (Fe), and titanium (Ti) content. Additionally, calcium content demonstrated a significant positive correlation with soil PHO activity. Invertase (INV) activity displayed negative correlations with trace elements such as Mn, Al, Si, V, Ti, and Ca while showing a positive correlation with magnesium, potassium, iron, and zinc.

Conversely, catalase (CAT) activity showed negative correlations with magnesium, iron, potassium, calcium, and zinc levels but was significantly correlated with titanium content and positively correlated with silicon, manganese, and vanadium. Urease (URE) activity was significantly correlated positively with vanadium concentration and positively correlated with magnesium, manganese, silicon, and calcium while displaying negative correlations with available potassium, aluminum, titanium, iron, and zinc concentrations. Furthermore, the correlation analysis revealed significant associations between enzyme activities, with invertase and catalase correlating negatively and phosphatase correlating positively with urease. Additionally, a positive correlation was observed between catalase and urease activities.

Table 5. The correlation analysis between trace elements and enzyme activities.

	Mn	Mg	K	Al	Si	Fe	V	Ti	Zn	Ca	PHO	INV	CAT	URE
Mn	1													
Mg	−0.774 **	1												
K	−0.092	−0.068	1											
Al	0.593 *	−0.893 **	0.003	1										
Si	−0.120	−0.029	−0.160	−0.094	1									
Fe	−0.663 **	0.0948 **	−0.116	−0.844 **	−0.132	1								
V	−0.462	0.700 **	−0.107	−0.672 **	−0.044	0.612 *	1							
Ti	−0.099	−0.263	−0.171	0.397	0.219	−0.251	−0.292	1						
Zn	−0.586 *	0.488	−0.049	−0.334	0.072	0.299	0.322	−0.228	1					
Ca	−0.150	0.517 *	−0.418	−0.731 **	0.071	0.510 *	0.514 *	−0.535 *	0.157	1				
PHO	0.088	−0.072	−0.225	−0.131	0.362	−0.181	0.208	−0.428	0.085	0.564 *	1			
INV	−0.077	0.078	0.119	−0.043	−0.206	0.093	−0.424	−0.045	0.159	−0.128	−0.459	1		
CAT	0.072	−0.229	−0.276	0.181	0.355	−0.279	0.122	0.526 *	−0.221	−0.090	0.212	−0.664 **	1	
URE	0.044	0.122	−0.050	−0.233	0.140	−0.006	0.613 *	−0.296	−0.023	0.332	0.587 *	−0.643 **	0.398	1

Note: * = Correlation is significant at the 0.05 level (2-tailed). ** = Correlation is significant at the 0.01 level (2-tailed).

4. Discussion

Clay-derived elements are widely available in the natural environment and exhibit significant reactivity, often forming during the weathering process of the planet's predominant silicate minerals [12]. The XRD analysis conducted in this investigation revealed the presence of thirteen (13) mineral phases in all soil samples collected from both polluted and unpolluted sites, surpassing the permissible limits recommended by the WHO. Furthermore, the results indicated high concentrations of five (5) minerals, with four belonging to the clayey group. The predominant minerals were found to be Talc 2M, followed by kaolinite 2M, chlorite, lawsonite, and muscovite 2M1. The clayey nature of the tested samples suggests a substantial water retention capacity but limited drainage and aeration capabilities [9].

The high clay content observed in this study may affect the soil's geochemistry due to its porous nature, containing a combination of metal oxides, silicon dioxide (quartz), and other minerals, including aluminosilicates. Because clay has holes between its layers, it is porous, which affects its ability to store water, chemical reactivity, and the availability of nutrients. The presence of the mica group in muscovite 2M1 in all samples indicates the soil's resistance to weathering, while the abundance of aluminum (Al) minerals suggests the weathering of feldspar and felsic rocks.

Clay minerals may contain varying amounts of alkaline earth, alkali metals, and other cations, serving as reservoirs for essential cations (e.g., ammonium ion (NH_4^+) and anions (e.g., phosphate ion (PO_4^{3-} compounds) through reversible binding, which can then be released to microorganisms through electron transfer mechanisms. These clay minerals present in soils offer a range of essential nutrients for bacteria, including potassium (K), sodium (Na), magnesium (Mg), calcium (Ca), iron (Fe), manganese (Mn), cobalt (Co), zinc (Zn), copper (Cu), and molybdenum (Mo) [12].

The identified trace elements contain concentrations that exceed the allowable levels suggested by the WHO, posing a risk to the environment. The excessive presence of essential and non-essential trace elements can lead to physiological and morphological abnormalities, including genetic mutations, such as stunted growth, hemophilia, or cystic fibrosis. Human exposure to heavy metals primarily occurs through the consumption of edible vegetables, accounting for approximately 90% of overall intake, with the remaining 10% attributed to skin contact and the inhalation of polluted dust.

The bioaccumulation of heavy metals in vegetables poses a health risk due to their potential transfer from contaminated land and water into the food chain. Manganese (Mn), as an essential element, plays a role in various physiological functions of the body. Acute exposure to manganese may have potential neuroprotective effects by reducing apoptotic cellular death. Still, exposure to high quantities can lead to harmful conditions such as neurological complications, including Alzheimer's and Parkinson's diseases, resulting in apoptotic cell death and disruption of homeostasis [13].

The present investigation yielded results consistent with those of Xie et al. (2016, as cited in the study), demonstrating a decline in total bioactivity, richness, and microbial diversity as the concentration of heavy metals in soil increased. The presence of heavy metal pollution can lead to detrimental effects on plant growth, soil microbial diversity, and activity and appears to exert a more pronounced influence on genetic structure [14].

Most minerals exist as primary and secondary oxides and have the capacity to dissolve cation contents and complexes. They can modify the characteristics of the soil, including its pH, color, porosity, and inherent chemical composition, thereby influencing the soil quality and potentially polluting the water [13]. These include the aluminum oxides, iron oxides, and titanium dioxides identified in this study. The mineral composition studied by Lu et al., 2018, on the undisturbed soil contaminated by landfill leachate corresponds with this study. They identified the primary minerals in quartz, muscovite, and albite, with a relatively stable content of these minerals [11].

Talc 2M is a type of clay mineral that is electrically neutral and is characterized by van der Waals bonds that link adjacent layers [15]. This magnesium silicate mineral is

highly insoluble and exhibits an affinity for ionic bonding with oxides and hydroxides due to its electronegativity. It particularly favors the formation of pyrolusite in alkaline soils through the higher oxidation of manganese ions (Mn^{4+}) [16]. Kaolinite, a product of feldspar weathering, is a white powdery mineral widely used in the ceramic industry because of its electrical neutrality and high capacity for ion adsorption [14]. The presence of a high amount of kaolinite in this study suggests intense chemical weathering under warm, humid conditions, which could have economic implications for the exploration of kaolin deposits [17].

Olokode and Aiyedun's (2011) study supports these findings, identifying kaolinite as the predominant and primary mineral in all samples. Minor quantities of muscovite, quartz, illite, and zeolites follow it. The chemical composition of clay minerals varies based on the source composition, sedimentary differences, and residual supply input [18]. Additionally, Chen et al. (2019) reported that kaolinite is the predominant clay mineral, comprising 62% to 8.8% of the composition. As leaching progressed, they observed an increase in kaolinite and illite-mica content and a corresponding decrease in quartz content. They also observed a weak correlation between kaolinite, feldspar, and quartz minerals [19].

Umbugadu and Igwe's (2019) study on the characterization of mineral clays and their oxides found that all eight analyzed samples contained three dominant minerals: kaolinite, illite, and muscovite. Kaolinite was present in two samples, while illite was found in three samples and muscovite in four samples. These minerals belong to the silicate group and the phyllosilicate family, with weathering advances attributed to K-feldspar, kaolinite, muscovite, and illite [20]. In contrast, Charles (2016) observed a very low concentration of kaolinite, less than 10%, and a large amount of quartz due to the weathering of quartzite [21].

The study also noted a decrease in enzyme activity, possibly because clay minerals inhibit their enzymatic potential. These findings align with the data from Olagoke et al. (2019), which observed a decline in potential enzyme activity as clay concentrations increased. The authors attributed this effect to the inclusion of montmorillonite, a clay mineral [22]. The greatest decrease in enzyme activity was observed in soil samples with 10% added montmorillonite, indicating a possible link between the reduction in enzyme activity and the increased adsorption of enzymes as the concentration of clay increases. The decrease in enzyme activity caused by clay minerals has been attributed to their adsorption capability [22].

Clay elements can function as effective enzyme blockers due to their ability to interact based on surface charge, exchange cations, and provide precise surface area [23]. The decrease in enzymatic activity observed in soil may be attributed to the presence of clay minerals, which tend to bind with organic compounds. The emergence of enzyme–clay clusters have the potential to modify the entire spectrum of enzymatic activity. The results presented in this study support the previously published findings by Tietjen and Wetzel in 2003, which demonstrated that the adsorption of montmorillonite had an impact on the activity of protease, glucosidase, xylosidase, and alkaline phosphatase. A decrease in each of the enzyme activities was identified [24].

According to this study, exposure to heavy metals significantly decreased the levels of CAT and URE activity. This demonstrates the sensitivity of these enzymes to the toxicity of heavy metals and their negative impact on soil microorganisms. The study also discovered that the region's low temperatures and drought had a detrimental effect on the microbial biomass in the soil. This was due to a decrease in moisture, which consequently reduced enzyme activity derived from residues of microorganisms.

The low enzymatic activity of URE and CAT could be attributed to inadequate substrate levels, decreased enzyme production due to hindered microbial growth, inhibition of the enzyme by trace metals that conceal the active site, denatured proteins, and structural changes affecting enzyme function. When present in larger quantities, all trace elements can be harmful. They can interact with the protein-active moieties of enzymes or enzyme precursor complexes, altering the way bacteria primarily utilize their enzymatic processes.

Consequently, a prolonged buildup of trace minerals in the soil may decrease enzyme activity, microbial biomass, and a range of functions, and it can also alter the genetic makeup of microbial communities over time [25]. The equivalent results were published by Antonious and Turley (2020). They found that high concentrations of Zn in the examined samples caused a reduction in urease enzyme activity. This reduction was attributed to metallic trace interference in the enzyme's active regions [23].

Certain components can damage the structure and operation of membranes by attaching to ligands such as phosphate and protein cysteinyl and histidyl groups. The published results by Yeboah et al. (2021) corroborate this study's findings. They reported a decrease in enzymatic activity, which is associated with a high concentration of metals in the soil. Additionally, the evaluated enzymes in the soil responded differently to increased levels of heavy metals [26].

According to Aponte et al. (2020), the study found that heavy metals have a linearly decreasing effect on the metabolic rate of soil enzymes. The endoenzymes (phosphatases, catalases, and urease) exhibited lower activity. This illustrates how heavy metals have a significantly more harmful effect on living microbes and their endoenzymes. Heavy metals had a more significant impact on the functioning of enzymes involved in the recycling of carbon (C) and sulfur (S) than on those involved in nutrient recycling (nitrogen (N) and phosphorus (P)). Subsequently, exposure to harmful metals has the potential to alter the stoichiometry of S, P, C, and N produced through the enzymatic degradation of organic substances. This, in turn, affects the composition and functioning of microbial populations [27].

The presence of clay minerals has been observed to have a negative impact on the functioning of enzymes, leading to a decrease in both the maximum rate of response and the binding capacity between the enzyme and its target substance [28]. The substrate's interaction with minerals helps protect it from enzymatic deterioration. The ability of elements to adsorb enzymes allows us to predict how minerals will affect enzyme activity. The results show that minerals have a negative effect on soil enzyme activity. The study's results were supported by the observations of Sheng et al. (2022), who claimed that the presence of minerals negatively affected enzyme function. Goethite, hematite, olivine, augite, and quartz concentrations demonstrated a lesser, but still significant, inhibitory effect of up to 30% on β -glucosidase activity [29]. The study by Olagoke et al. (2019) shows that clay-based compounds have a comparable and supportively restrictive impact [22].

In addition, the researchers noticed that numerous dangerous metals, hazardous substances, and harmful gases, such as methane (CH₄) and carbon dioxide (CO₂), are discharged into the surroundings during the bacterial decomposition of waste. This mainly occurs when waste pickers burn garbage within the dumpsite as a result of the accumulation of waste and the absence of waste treatment by authorities. As a result of these activities, people risk contracting infections, colitis, airborne illnesses, and allergies due to the unpleasant odor emanating from the decomposing garbage at the dump site. The results of this study are consistent with the outcomes reported by Njoku et al. (2019). The researchers found that 78% of those polled near the garbage dump reported significant hazardous air pollution caused by unpleasant odors emanating from the landfill. Individuals who lived closer to the dump site experienced diseases such as the influenza virus, eye irritation, and frequent feelings of weakness more often than those who lived farther away. Research findings have indicated that individuals who inhale or ingest sulfur dioxide and nitrogen dioxide often experience symptoms such as pulmonary congestion, difficulty breathing, and respiratory tract infections, especially among those with asthma. Many individuals (56%) living near the dumpsite expressed concern for their safety in the coming years [30].

5. Recommendations and Conclusions

Soil productivity at the examined sites was affected by pollutants caused by trace elements, as indicated by the concentrations and the metal pollution index (MPI) findings.

Soil enzyme activity is a valuable indicator for assessing contamination-related damage to soil functions and monitoring recovery during soil restoration. Since enzyme production in natural environments is typically limited, it can be enhanced under controlled conditions. Enzymes are essential to bioremediation. Therefore, optimizing enzymes from different species, such as fungi and bacteria, is crucial to maximize their stability and efficiency for circumstances or substrates.

This study recommends the purification of PHO and INV enzyme activity to be used as innovative methods for reducing soil pollution and harmful substances in soil. Enzyme purification is necessary to increase the number of required enzymes with high catalytic activity and purity and enhance the binding affinity, polarity, solubility, and size of the enzymes.

These enzymes have a high activity strength and correlate negatively with some identified elements. This means that these enzymes can decontaminate the soil if adequately applied in the environment. People living near polluted areas are consistently exposed to hazardous gases, such as methane, sulfur dioxide, and nitrogen dioxide, which are byproducts of waste reduction processes.

Recommendations include implementing appropriate and effective agricultural methods, efficient waste treatment procedures, preventive regulations, and proper garbage handling to mitigate the adverse impacts of heavy metal pollution. The municipality's environmentalists must implement measures and sanctions to combat soil contamination. This includes implementing more robust regulatory systems, laws, and related measures to regulate the handling, disposal, and penalties for improper management of potentially soil-polluting chemicals and procedures. Site managers must promote public education programs to raise awareness and encourage garbage recycling to protect the environment and people's wellness.

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Appendix A

Interval of contamination/pollution index (MPI) of heavy metals in soil and its significance [14].

MPI	Significance	Remarks
<0.1	Very slight contamination	No negative effect on soil, plant and, environment
0.10–0.25	Slight contamination	No negative effect on soil, plant and, environment

MPI	Significance	Remarks
0.26–0.5	Moderate contamination	No negative effect on soil, plant and, environment
0.51–0.75	Severe contamination	No negative effect on soil, plant and, environment
0.76–1.00	Very severe contamination	No negative effect on soil, plant and, environment
1.1–2.0	Slight pollution	Will pose a negative impact on soil, plants, and environment
2.1–4.0	Moderate pollution	Will pose a negative impact on soil, plants, and environment
4.1–8.0	Severe pollution	Will pose a negative impact on soil, plants, and environment
8.1–16.0	Very severe pollution	Will pose a negative impact on soil, plants, and environment
>16.0	Excessive pollution	Will pose a negative impact on soil, plants, and environment.

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