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Original Research Article

Analysis of Bisphenol A Related Compounds and Bacteria Diversities in Soil Samples from Refuse Dump Sites within Awka Municipal

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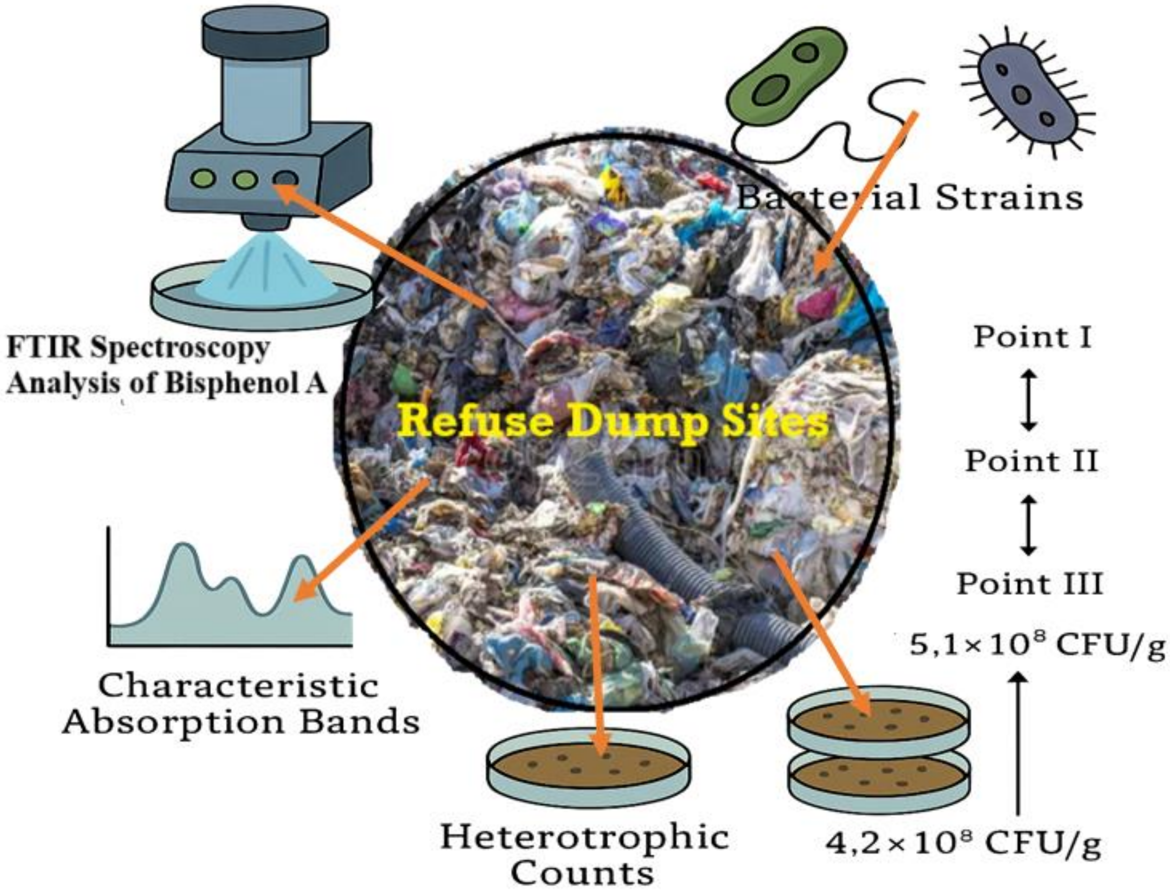
ABSTRACT

Bisphenol A (BPA) is an endocrine-disrupting compound characterized by its aromatic alcohol-based functional groups. This study investigated the presence of BPA and bacterial diversity in soil samples collected from refuse dump sites within Awka metropolis, Anambra State. Fourier Transform Infrared (FTIR) spectroscopy analysis revealed characteristic absorption bands in soil from Point I at 3244.6–3842.9 cm^{-1} (O–H stretching) and 2165–2366.9 cm^{-1} (C–H stretching). In Point II samples, absorption bands were observed at 1654 cm^{-1} and 1718.3 cm^{-1} (Amide I), 1436.9 cm^{-1} (Amide III), and 1407.1 cm^{-1} (–NH_2 bending). Soil from Point III exhibited distinct bands at 1069.7 cm^{-1} (antisymmetric stretching of the C–O–C bridge) and 902.1–687.7 cm^{-1} (skeletal vibrations involving C–O stretching), indicative of saccharide structures. Bacterial heterotrophic counts were assessed using standard microbiological and biochemical techniques. Strains of *Pseudomonas* and *Klebsiella* spp. were the most prevalent across all sites. Peak colony-forming unit (CFU/g) counts were recorded on day 7 of incubation: 5.1×10^8 CFU/g at Point I and 3.9×10^8 CFU/g at Point III. By day 14, counts declined to 4.2×10^6 and 2.9×10^7 CFU/g, respectively. This study underscores the significance of continuous monitoring of soil contaminants and microbial activity at waste disposal sites. The findings provide valuable guidance for environmental monitoring agencies to enhance waste management practices and implement stricter recycling policies.

Keywords: Endocrine-disrupting compounds; Fourier Transform Infrared Spectroscopy (FTIR); Heterotrophic bacterial count; Soil contamination; Environmental microbiology.

Graphical Abstract



1.0 Introduction

Municipal waste management represents one of the most pressing environmental challenges of our time, with waste streams originating from diverse sources including residential areas, administrative buildings, recreational facilities, and public installations [1]. These waste composites exist in solid, liquid, and gaseous forms, each presenting unique environmental persistence challenges [2]. From a broader perspective, waste can be defined as any residual material derived from production processes, consumption activities, or discarded personal belongings that have reached the end of their functional utility [3].

Among municipal waste streams, solid waste demands particular attention due to its persistent nature and significant partition coefficient within biosystems, especially aquatic ecosystems [1]. The environmental impact of solid waste is mediated through complex biochemodynamic interactions that alter ecosystem functionality, with bioavailability being a key determinant of its ecological consequences [4]. Of particular concern are trace metal contaminants - including arsenic, cadmium, chromium, lead, and mercury - which pose substantial risks due to their bioaccumulation potential and participation in biogeochemical cycles [5,6]. Industrial activities exacerbate this problem through the release of effluents containing complexed phenols, surfactants, sulfides, and various organic compounds that persist in soil matrices and disrupt fertility by interfering with nutrient cycling processes [4,7].

The ecological assessment of waste impacts requires careful examination of soil quality indicators, particularly microbial communities that serve as sensitive biomarkers of environmental stress [8]. Soil microbiota play crucial roles in mineralization processes, with population dynamics reflecting the chemical milieu of their habitat [4]. Recent studies have demonstrated that microbial community shifts can serve as reliable indicators of waste-derived contamination [9].

A particularly concerning component of modern waste streams is Bisphenol-A (BPA), an endocrine-disrupting compound first introduced commercially in 1957 [10]. This high-production-volume chemical permeates

daily life through its use in polycarbonate plastics, epoxy resins, and numerous consumer products ranging from food packaging to medical devices [11]. The environmental persistence of BPA and its demonstrated estrogenic activity have raised significant public health concerns, particularly regarding its potential to leach from waste materials into surrounding ecosystems [12].
This study investigates the ecological impacts of municipal waste disposal in Awka Metropolis through two parallel approaches: (1) comprehensive analysis of soil microbial communities at refuse dump sites, and (2) spectroscopic quantification of BPA contamination levels. By employing standardized microbiological enumeration techniques coupled with Fourier-transform infrared spectroscopy, we aim to establish concrete relationships between waste disposal practices and measurable ecological parameters. Our findings will contribute to the growing body of knowledge on waste management strategies while providing specific data to inform local environmental policy decisions.

2.0 MATERIALS AND METHODS

2.1 Materials

All chemicals and reagents utilized in this investigation were of analytical grade purity, procured from certified suppliers including BDH Chemicals Ltd., May & Baker Ltd., and Sigma-Aldrich Chemie GmbH. Critical instrumentation comprised a Fourier-transform infrared spectrometer (PerkinElmer Spectrum Two), digital pH meter (Hanna Instruments HI2210), autoclave (ALP CLA-32), laminar flow hood (Baker Company MSC-12), and precision incubators (Memmert GmbH). Each instrument underwent rigorous calibration according to manufacturer specifications prior to experimental runs, with comprehensive calibration records maintained throughout the study duration to ensure data integrity.

2.2 Methods

2.2.1 Sample Collection and Preparation

Soil sampling was conducted at three active refuse disposal sites within Awka Metropolis, Anambra State, Nigeria (06°28.12'N, 07°32.1'E, elevation 488 ft) following established geospatial sampling protocols. Site selection prioritized locations with minimum five-year operational history, high waste deposition volumes, and absence of formal waste management infrastructure. Composite samples were collected from the plow layer (0-15 cm depth) using sterile stainless steel augers, employing a systematic W-pattern across 10×10 m grids to ensure representative sampling. Immediately following collection, samples were transferred to UV-sterilized polyethylene containers, maintained at 4°C during transport, and processed within six hours to preserve sample integrity. Control samples were obtained from undisturbed adjacent areas located 500 meters from disposal sites.

2.2.2 Microbial Isolation and Enumeration

Microbiological analyses were performed under strict aseptic conditions following modified standard procedures. Total heterotrophic counts were determined through serial decimal dilutions in phosphate-buffered saline followed by spread plating on nutrient agar, with incubation at 30±2°C for 48-72 hours. Coliform analysis employed a membrane filtration technique using 0.45 μm filters cultured on m-Endo agar medium, incubated at 37±1°C for 24 hours. Microbial identification incorporated Gram staining, biochemical profiling using API 20E systems, and molecular confirmation of selected isolates via 16S rRNA sequencing to ensure taxonomic accuracy.

2.2.3 Bisphenol A Quantification

BPA analysis was conducted using optimized Fourier-transform infrared spectroscopy methodology. Sample preparation involved freeze-drying, homogenization through 200 μm sieves, and Soxhlet extraction with methanol:dichloromethane solvent system. Extracts were concentrated under nitrogen stream and prepared as KBr pellets (1:100 sample ratio) for spectroscopic analysis. FTIR scanning was performed across 4000-400 cm^{-1} range at 4 cm^{-1} resolution, with 32 scans per sample and background correction. Spectral interpretation utilized reference libraries with rigorous quality control measures including method blanks, matrix spikes at 50-200 μg/kg concentrations, and daily instrument performance verification.

2.2.4 Statistical Analysis

All experimental measurements were conducted in triplicate to ensure methodological reproducibility. Data analysis incorporated descriptive statistics (mean ± standard deviation), one-way ANOVA with Tukey's post-hoc test (significance threshold $p<0.05$), and principal component analysis for spectral data interpretation. Statistical computations were performed using SPSS version 26 and OriginPro 2021 software packages, with all analytical workflows documented to permit experimental replication. The comprehensive statistical approach ensured robust interpretation of both microbial and chemical analytical results.

3.0 Results and Discussion

3.1 Microbial Heterotrophic Counts in Soil Samples

The heterotrophic bacterial counts from the soil samples collected at various refuse dump sites within Awka metropolis are presented in Tables 1 and 2. Results indicate that microbial populations were significantly higher in all refuse site samples compared to the control soil. The peak microbial activity was observed on day 7 of incubation, followed by a notable decline by day 14, suggesting a reduction in metabolic activity or organismal viability with time.

Table 1: Heterotrophic Counts of Organisms after 7 Days of Incubation

Total Heterotrophic Counts (CFU/ml)	**Point I**	**Point II**	**Point III**
Total Viable Counts (10^{-2})	5.1×10^8	3.3×10^7	3.9×10^8
Total Coliform Counts (10^{-2})	7.02×10^4	8.2×10^3	3.5×10^4
Control	TVC: 3.4×10^5	TCC: 2.6×10^3	

Table 2: Heterotrophic Counts of Organisms after 14 Days of Incubation

Heterotrophic Counts (CFU/ml)	**Point I**	**Point II**	**Point III**
Total Viable Counts (10^{-2})	4.2×10^6	5.6×10^6	2.9×10^7
Total Coliform Counts (10^{-2})	2.30×10^4	2.39×10^4	3.4×10^4
Control	TVC: 4.2×10^3	TCC: 1.2×10^2	

Analyses of the soil microbiota revealed a consistent presence of *Pseudomonas* and *Klebsiella* species across all sampled refuse dump sites, indicating their resilience and ecological dominance in such contaminated environments. These bacterial strains are known for their metabolic versatility and ability to survive in harsh environmental conditions, including polluted soils with complex organic and inorganic contaminants. The microbial enumeration data demonstrated that peak heterotrophic bacterial counts (CFU/g) in all soil samples occurred on day 7 of incubation. Specifically, total viable heterotrophic counts at Point I and Point III were 5.1×10^8 CFU/g and 3.9×10^8 CFU/g respectively. However, a notable decline in viable microbial populations was observed by day 14, with Point I and Point III recording counts of 4.2×10^6 CFU/g and 2.9×10^7 CFU/g, respectively. This reduction in microbial load over time is indicative of nutrient depletion or increasing toxicity due to persistent organic pollutants like bisphenol A (BPA).

It is important to recognize that soil microorganisms function as critical agents of biogeochemical cycling. As living entities, they harbor enzymatic proteins that facilitate the degradation of complex compounds, mineralization of nutrients, and maintenance of soil health [10]. Their population dynamics serve as effective bioindicators of ecological balance and anthropogenic disturbance. The marked decline in microbial activity over time in this study, particularly at Point II, reflects either a low availability of organic substrates required for microbial proliferation or the inhibitory effect of environmental toxicants present at higher concentrations.

Supporting this interpretation, Valero [16] has emphasized that microbial growth in environmental matrices is significantly influenced by the presence of catabolite activators—organic compounds that serve as metabolic cues promoting heterotrophic expansion. In their absence, or under conditions of toxicant accumulation, microbial communities undergo attenuation, leading to reduced diversity and biomass. The findings of this study align with those reported by Ezenwelu et al. [11], who documented similar patterns of declining bacterial diversity and emulsification index in microbial isolates obtained from the Bonny Jetty site. In their study, *Pseudomonas* and *Bacillus* species exhibited hydrocarbon-degrading capabilities and were dominant in polluted environments, further highlighting their ecological relevance in contaminated sites.

3.2 Bacterial Morphological Distribution

Table 3: Bacterial Morphotypes Detected in Respective Soil Samples Across Incubation Days

Source	**Control**	**Point I (Day 0)**	**Point I (Day 7)**	**Point I (Day 14)**	**Point I (Day 21)**	**Point II (Day 0)**	**Point II (Day 7)**	**Point II (Day 14)**	**Point II (Day 21)**	**Point III (Day 0)**	**Point III (Day 7)**	**Point III (Day 14)**	**Point III (Day 21)**
Bacilli	✓	✓			✓				✓				
Spiral		✓											
Cocci		✓											
Cocci in pairs			✓			✓							
Rods						✓				✓			
Large cocci							✓				✓		
Non-sheath spiral									✓				
Staphylococci										✓			

The morphological distribution of bacterial types further confirms microbial diversity in the refuse-contaminated soils. Point I and III exhibited high abundance and diversity of bacilli, cocci, spiral, and staphylococcal morphotypes, whereas Point II recorded comparatively lower diversity, possibly due to higher contaminant load or reduced organic carbon sources.

These findings are consistent with previous studies where catabolite activators such as hydrocarbons enhance microbial proliferation [16]. Conversely, the decline in organismal diversity and counts over time may be attributed to toxicant accumulation and reduced bioavailable nutrients, in line with the findings of Ezenwelu et al. [11].

3.3 FTIR Spectroscopy Analysis of BPA in Soil

Fourier-transform infrared (FTIR) spectroscopy was employed to determine the presence of bisphenol A (BPA)-related compounds in soil samples collected from three refuse dump sites. The analysis revealed several characteristic absorption bands associated with functional groups of BPA and related organic compounds. In Figure 1, the FTIR spectrum for the soil from Point I showed broad absorption bands in the region of 3244.6–3842.9 cm^{-1}, indicating O–H stretching vibrations, a hallmark of hydroxyl-containing compounds such as phenols. The CH-stretching bands observed between 2165 and 2366.9 cm^{-1} further confirm the presence of aliphatic hydrocarbons.

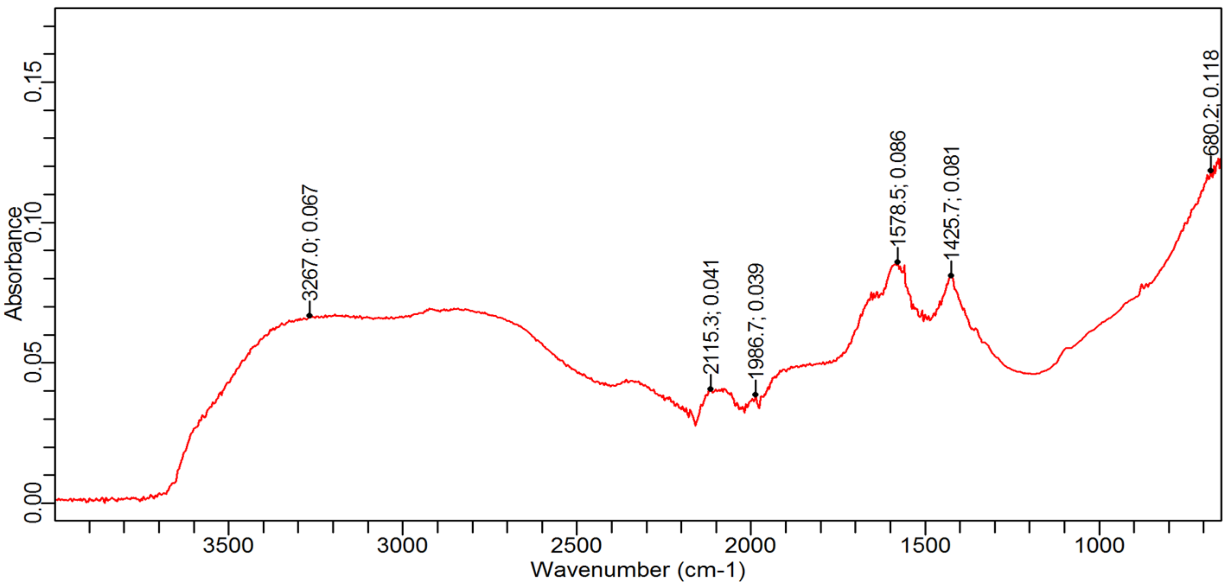


Fig. 1: FTIR spectroscopy analysis of BPA from the point I refuse dump site soil sample.

In Figure 2, representing the Point II soil sample, additional peaks were noted at 1654 and 1718.3 cm^{-1} corresponding to Amide I bands, which typically indicate C=O stretching in protein or peptide-like structures. The presence of bands at 1436.9 cm^{-1} (Amide III) and 1407.1 cm^{-1} ($-NH_2$ bending) further supports the existence of nitrogen-containing compounds, possibly derivatives of BPA degradation or microbial metabolites.

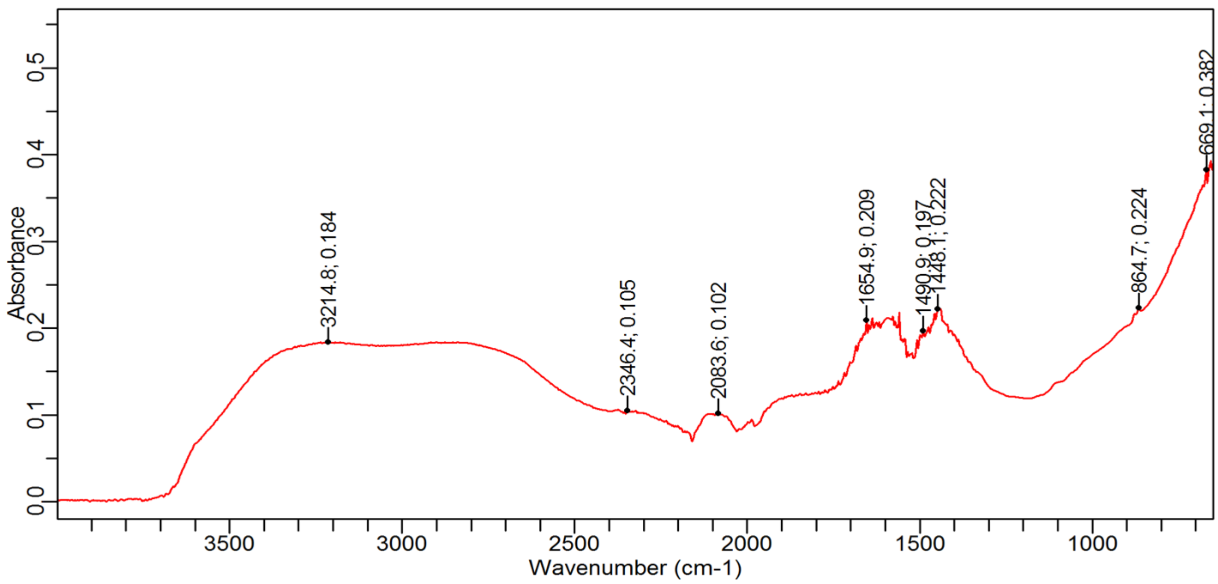


Fig. 2 FTIR spectroscopy analysis of BPA from the point II refuse dump site soil sample.

The FTIR spectrum in Figure 3 from the Point III soil sample displayed prominent absorption peaks at 1069.7 cm^{-1} (anti-symmetric stretching of the C–O–C bridge) and within 902.1–687.7 cm^{-1}, corresponding to skeletal vibrations involving C–O stretching. These features are characteristic of saccharide structures, suggesting microbial polysaccharide or humic interactions in the soil matrix.

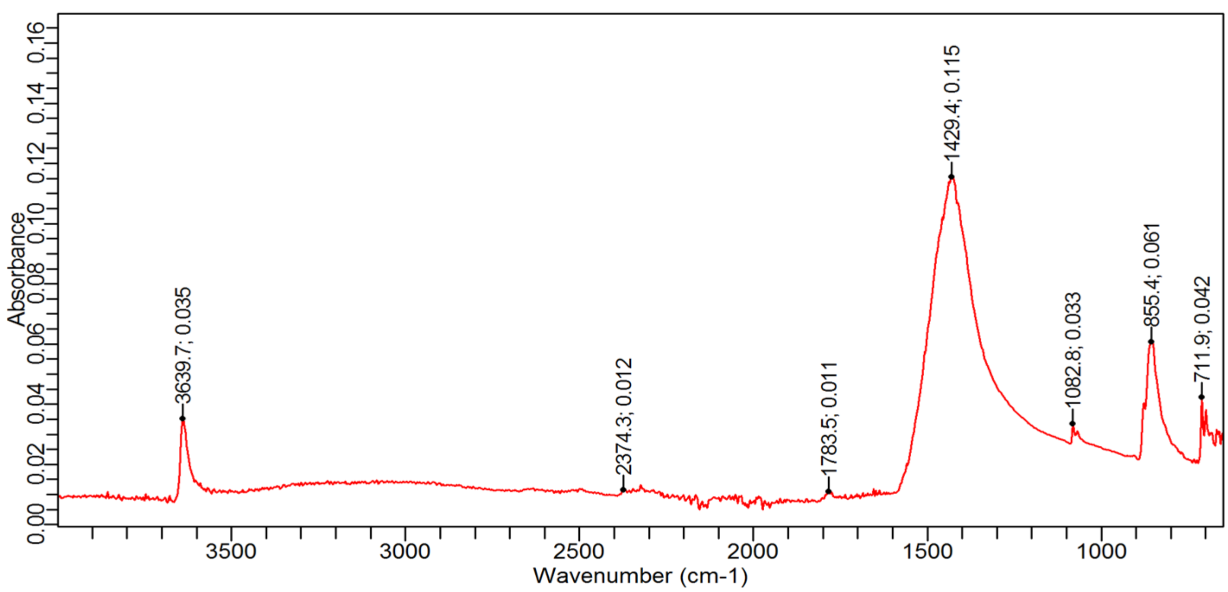


Fig. 3: FTIR spectroscopy analysis of BPA from the point III refuse dump site soil sample.

Comparatively, these findings align with those reported by Antonino et al. [7], who noted similar absorption bands at 3450 cm^{-1} (O–H), 1655 cm^{-1} (Amide I), 1580 cm^{-1} ($-NH_2$ bending), and 1320 cm^{-1} (Amide III) in rubber-packaged water samples. Additionally, Muzzarelli and Rocchetti [15] reported saccharide-related vibrations at 1160 cm^{-1} and between 1032–1082 cm^{-1} in Malaysian bottled water, which further corroborate the structural similarities observed in our samples. The persistence of these bands indicates the infiltration and retention of BPA-related compounds in the sampled soils, likely originating from the prolonged accumulation of plastic and industrial waste at the dump sites.

3.4 Environmental Implications

The spectroscopic and microbiological findings suggest that BPA contamination has a substantial impact on microbial viability and biodiversity in the affected soils. The observed reduction in heterotrophic activity over time reflects a disruption of soil microbial equilibrium, possibly due to BPA's role as an endocrine-disrupting chemical. Since soil microbes are central to nutrient cycling, mineralization, and organic matter decomposition [10], such disruption portends long-term consequences for soil fertility and ecological health.

4.0 Conclusion

This study highlights the environmental and biological implications of uncontrolled refuse dumping in urban centers, with a specific focus on microbial dynamics and BPA contamination in soils. The presence of BPA, as evidenced by FTIR spectroscopy, and the attenuated microbial proliferation in contaminated soils underscore the urgent need for effective regulatory intervention. Bisphenol A, a persistent endocrine disruptor, not only poses direct human health risks but also undermines critical soil microbial functions essential for ecosystem stability.

Therefore, environmental regulatory agencies must strengthen monitoring frameworks, enforce stricter policies on waste disposal, and promote systematic recycling programs to mitigate the growing threat of chemical pollutants in our terrestrial ecosystems.

Conflict of Interest

The authors declared that there is no conflict of interest.

Author's Declaration

The authors affirm that the work presented is original and will accept all liability for any claims about the content.

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