Frontiers in Health Informatics
ISSN-Online: 2676-7104
www.healthinformaticsjournal.com
2024; Vol 13: Issue 8
Open Access

Comparative Analysis of Microbial Profiles in Normal Soil and Soil Contaminated with Chicken Feathers

Philomina A F[1], Dr. Mary Agnes A[2*], Dr. Sindhuja G[3], Yasmin M[4]

[1]Research Scholar, Department of Zoology, Auxilium College (Autonomous), Affiliated to Thiruvalluvar University, Vellore-622006, TN, India.

[2*]Associate Professor, Department of Zoology, Auxilium College (Autonomous), Affiliated to Thiruvalluvar University, Vellore-622006, TN, India. Email id: agnesanthony14@gmail.com

[3]Assistant Professor, Department of Zoology, Islamiah Women's arts and Science College, Affiliated to Thiruvalluvar University, Vaniyambadi, TN, India.

[4]Research Scholar, Department of Zoology, Auxilium College (Autonomous), Affiliated to Thiruvalluvar University, Vellore-622006, TN, India.

Cite this paper as: Philomina A F, Dr. Mary Agnes A, Dr. Sindhuja G, Yasmin M (2024). Comparative Analysis of Microbial Profiles in Normal Soil and Soil Contaminated with Chicken Feathers. *Frontiers in Health Informatics*, *13 (8) 3411-3417*

Abstract:

Soil degradation is a pressing environmental issue exacerbated by factors such as rapid population growth, intensified economic activities, and climate change. Among the many contributors to this degradation, the accumulation of poultry waste, particularly chicken feathers, poses a significant challenge. Globally, approximately 24 billion chickens are slaughtered annually, resulting in the generation of around 8.5 billion tonnes of poultry feathers, with India alone contributing an estimated 350 million tonnes. These feathers, composed primarily of keratin, are highly resistant to natural degradation, leading to their accumulation in the environment.

Microorganisms play a pivotal role in the natural decomposition of keratin-rich materials like feathers, contributing to nutrient recycling and soil health restoration. This study investigates the microbial communities in normal, uncontaminated soil compared to those in soil polluted with poultry feathers. By employing microbial profiling techniques, the study aims to identify the diversity, abundance, and functional roles of these microorganisms in feather degradation. The findings provide insights into the ecological impact of feather pollution on soil microbiota and underscore the potential of microbial communities in developing sustainable waste management strategies. Understanding these microbial dynamics can inform practices to mitigate soil degradation and enhance soil resilience in the face of increasing agricultural and industrial pressures.

Introduction:

Soil is a limited resource essential for food production, carbon sequestration, water and nutrient regulation, contaminant filtration, biodiversity enhancement, heritage preservation, and climate regulation (Arshad and Martin 2002). Soil pollution refers to the introduction into the soil environment, either through human activities or natural processes, at concentrations higher than normal levels. These substances can include chemicals such as heavy metals, pesticides, industrial pollutants and other contaminants (Panagos, P., etal.,2020)

Production of livestock is regarded as a potential source of food for the World's hungry. According to reports, between one-third and half of the entire weight of animals that are slaughtered is left as byproducts of the livestock and poultry

industries that are either partially or completely utilised. But it turns into a significant contaminant of the nation's site and cities when animal faeces are improperly handled, especially when it is dumped into land, river, since this can cause surface and surface water to become contaminated with excess nutrients and intestinal bacteria (Ediene VF.et al 2016). The leftover of the slaughter house, which are frequently divided into solid, liquid and fat categories, could be so organic. Condensed meat, undigested food, bones, hairs, and aborted foetuses make up the solid portion of the wastes. Contrarily, the liquid aspect is made up of water, urine, dissolved solids, blood, and gastrointestinal contents, whilst the fat waste is made up of fats and oil. Primary producers are often destroyed in land and water as result of these slaughter house waste deposition (Saidu, M., & Musa, J. J. (2012).

Feathers, which make up 5-7% of a chicken's total weight, have become significant pollutants because of their resistant nature (Tamreihao, K., et al, 2019). Feathers cover poultry and birds. Poultry feathers constitute 6-9% of a chicken's total weight and are made up of approximately 91% protein, 8% water and 1% lipids. The protein found in feathers is called keratin, fibrous protein containing sulphur. All keratins are notable for their high levels of the sulphur containing di-amino acid cysteine, which serves as a cross-linking point between protein chains. This extensive interchain cross-linking through cystine gives keratins, particularly hard keratin, their notable characteristics of toughness, durability, resistance to degradation, and desirable mechanical properties (Adelina, A., et al 2021).
Microbial genome sequencing has always been limited to the few species that are suitable for pure culture growth. Microbial communities may now be directly sequenced from ambient samples by the advancement of culture-independent techniques over the years. This method is often called "community genomics" or "metagenomics" (Bragg, L., & Tyson, G. W. (2014).

Determining the microbial community structure of the environment by classifying the different microorganisms present and measuring their diversity in terms of species richness or abundance is an important first step in metagenomic analysis. Within the framework of microbial communities, a "species" is a basic and unique taxonomic hierarchy rank. The main factor used to classify organisms into "species" is their general resemblance in morphology and genotype. But the standards that scientists use to classify people into the same species are currently not universal and are typically found to depend on the situation (Doolittle, W. F., & Zhaxybayeva, O. (2009)). One commonly used diversity indicator is species richness, which is the number of unique species that inhabit a certain biological community, habitat, or ecosystem type within a given unit area. On the other hand, species abundance takes into account calculations related to species dominance and/or evenness, which refers to the pattern of the relative abundances of different species in a particular ecosystem. Relative abundance values, in turn, show the quantitative pattern of species commonness and rarity within a sample or population (Levin, S. A., et al 2009).

Materials and Methods:

Two samples, one the normal soil collected from the campus of Auxilium College, Vellore and the other soil sample contaminated with chicken feather are tested for microbes using Microbiome profiling by 16S rRNA gene.

Microbiome profiling by 16S rRNA gene:

Genetic markers with a phylogenetic signal such as 16S rRNA gene, 18S rRNA gene and Internal Transcribed Spacer (ITS) lets microbiologists get a quick and comprehensive overview of microbial diversity in their samples. Such analysis is often used to find patterns in microbial diversity in relation to specific treatments or conditions. Variant level resolution of this analysis lets researchers identify species of interest that are either driving the biological change or are affected by one or more parameters affecting the environment of the microbial community. Using multiple targets one can easily observe multitrophic changes occurring across various kingdoms which is often desirable for environmental DNA (eDNA) based genetic surveys. These methods are often deployed by microbial ecologists who study low diversity environments like human microbiome to complex environments such as soil.

Processing of Samples

Samples were checked for sufficiency of volume for downstream processing. Subsequently, all samples were subjected to a quality check using a spectrophotometer and the A260/280 ratio were recorded followed by a 16S rRNA gene general PCR to check if gene amplification was possible for each sample received. Samples that failed in the PCR were excluded irrespective of them qualifying spectrophotometric QC. This was done to assure success of library prep. The PCR successful samples were quantified with Qubit fluorometer using a dsDNA broad range kit (Thermo Fisher Scientific). The samples were then diluted to 1ng/µL in Low TE and subjected to library preparation workflow for the 16S rRNA gene V3-V4 region using the Illumina demonstrated protocol for 16S Metagenomic sequencing using a phased primer strategy. The protocol uses a phased primer strategy as outlined by Fadrosh et al. (2014). Indexed libraries were purified and pooled at equimolar concentrations to a concentration of 10nM. The pool was further validated using an Agilent Tapestation 4200 high sensitivity D1000 screentape (Agilent) and quantified using the qubit fluorometer high sensitivity ds DNA kit (Thermo Fisher Scientific). The pooled library was sequenced on an Illumina MiSeq instrument using the 2x249 base chemistry of the v2 reagent kit as per manufacturer's instructions. The reads produced were demultiplexed on board the sequencer \and raw fastq files were generated.

The filtered files are processed through the **DADA2** pipeline, which consists of several key steps. After denoising and chimera removal, the final set of sequences is used to construct an Amplicon Sequence Variant (ASV) table. This table contains the abundance of each unique sequence (ASV) across all the samples. The ASVs are assigned to taxonomic classifications (e.g., species, genus) based on reference databases. This allows researchers to identify the microbial communities present in the samples.

Result:

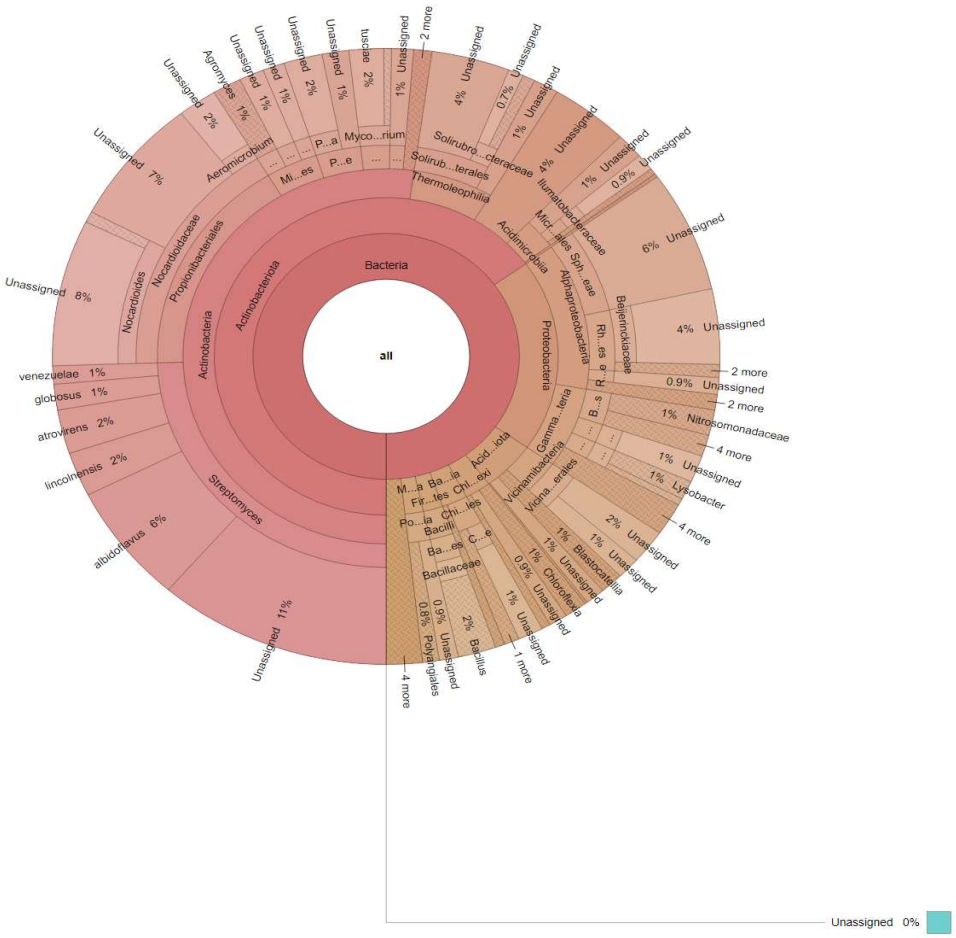
Figure: Species level of Taxonomy of Control A- normal soil

Bacillus niacin
Sreptomyces albidoflavus
Mycobacterium tusciae
Nocardioides albus
Streptomyces atrovirens
Streptomyces lincolnensis
Bacillus aryabhattai
Lysobacter soli
Ensifer meliloti
Streptomyces globosus
Streptomyces venezuelae
Candidatus Entotheonella
Pseudomonas alcaligenes
Agromyces flavus
Bacillus anthracis
Bacillus funiculus

Nocardioides kribbensis

Figure: Level 7- Species level of Taxonomy of Control B - polluted soil

Sreptomyces albidoflavus
Mycobacterium tusciae
Streptomyces atrovirens
Streptomyces lincolnensis
Bacillus aryabhattai
Lysobacter soli
Streptomyces globosus
Bacillus vireti
Streptomyces venezuelae
Staphylococcus nepalensis
Pseudomonas mosselii
Candidatus Glomeribacter;gigasporarum



Krona plot:

Figure: Krona plot of Sample A

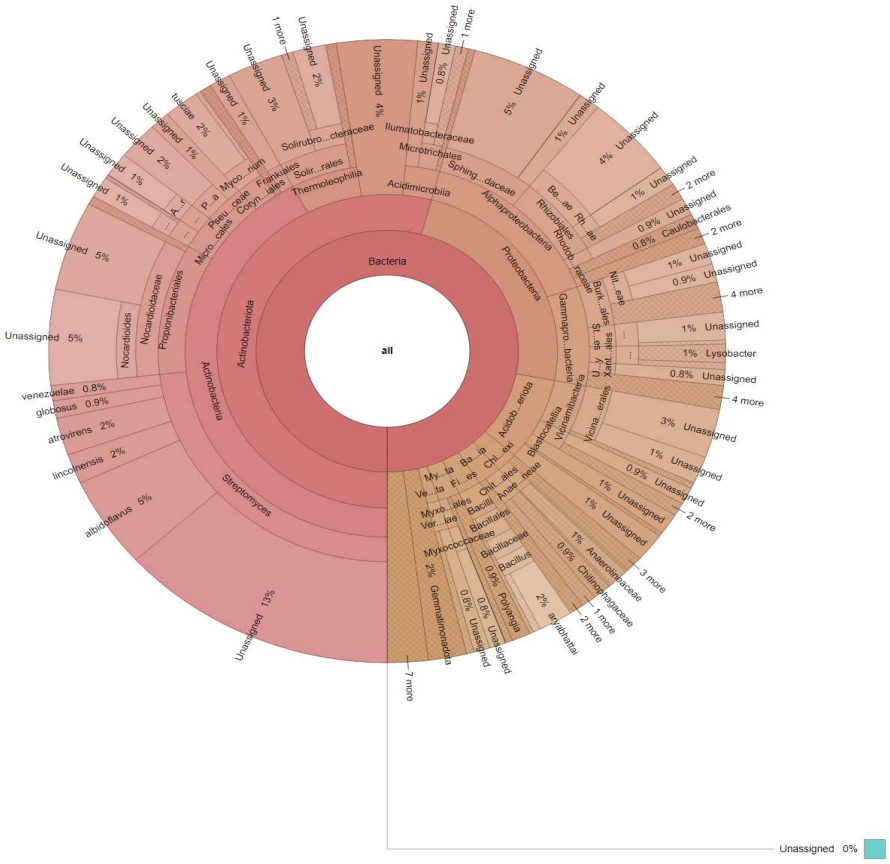


Figure: Krona plot for Sample B

Discussion:

The species that are common in both the samples were *Sreptomyces albidoflavus, Mycobacterium tusciae, Streptomyces atrovirens, Streptomyces lincolnensis, Bacillus aryabhattai, Lysobacter soli, Streptomyces globosus, Streptomyces venezuelae.* The species that are confined to the polluted soil are *Pseudomonas mosselii, Candidatus Glomeribacter;gigasporarum, Bacillus viretti* and *Staphylococcus nepalensis*.

The species that are confined only to the normal soil are *Bacillus niacin*, *Nocardioides albus, Ensifer meliloti , Candidatus Entotheonella , Pseudomonas alcaligenes, Agromyces flavus, Bacillus anthracis, Bacillus funiculus, Nocardioides kribbensis*. The bacteria which are presented only in normal soil and has been destroyed in polluted soil might be the eco friendly soil bacteria.

The Bacillus genus, including species such as *Bacillus subtilis, B. cereus, B. thuringiensis, B. pumilus, and B. megaterium*, is among the most prevalent rhizobacteria known to promote plant growth and development through various mechanisms (Meena, V. S., et al.,2016). Nocardioides, a genus within the Actinomycetes group, is capable of surviving in diverse low-nutrient environments. It utilizes various organic materials, including carbon and nitrogen sources, to degrade pollutants (Ma, Y.,et al.,2023). Bacteria involved in the decomposition of organic matter, such as Candidatus Species and Acidothermus, have shown a positive correlation with soil organic matter content. These microorganisms play a crucial role in breaking down organic materials, thereby contributing to the accumulation and maintenance of soil

organic matter, which is essential for soil fertility and overall ecosystem health (DU, S.,et al, 2017). The Pseudomonas aeruginosa isolates demonstrated several plant growth-promoting traits, such as the production of indole-3-acetic acid, hydrogen cyanide, and ammonia, along with the ability to solubilize insoluble phosphates. Additionally, they exhibited the potential to safeguard plants against fungal pathogens (Ndeddy Aka, R. J., & Babalola, O. O. (2016).

Conclusion:

The study provides a comparative analysis of microbial profiles in normal soil and soil contaminated with chicken feathers, revealing significant differences in microbial diversity and abundance. While certain beneficial microbial species, such as Bacillus and Nocardioides, are prevalent in normal soil, their presence diminishes in contaminated soil, indicating potential disruption of eco-friendly soil bacteria by feather pollution. On the other hand, species unique to polluted soil, such as *Pseudomonas mosselii* and *Staphylococcus nepalensis*, highlight microbial shifts potentially driven by keratin degradation or contamination stress.

These findings underscore the ecological impact of poultry waste on soil health and microbial balance. Importantly, they highlight the role of microbes in keratin decomposition and nutrient cycling, offering insights for sustainable waste management strategies. Harnessing such microbial communities can mitigate the adverse effects of feather contamination, restore soil health, and support agricultural productivity. Further research should explore the functional applications of these microbial taxa in environmental remediation and sustainable agriculture.

Bibliography:

1. Adelina, A., Feliatra, F., Siregar, Y. I., Putra, I., & Suharman, I. (2021). Use of chicken feather meal fermented with Bacillus subtilis in diets to increase the digestive enzymes activity and nutrient digestibility of silver pompano Trachinotus blochii (Lacepede, 1801). *F1000Research*, *10*.
2. Arshad, M. A., & Martin, S. (2002). Identifying critical limits for soil quality indicators in agro-ecosystems. *Agriculture, ecosystems & environment*, *88*(2), 153-160.
3. Bragg, L., & Tyson, G. W. (2014). Metagenomics using next-generation sequencing. *Environmental microbiology: methods and protocols*, 183-201.
4. Doolittle, W. F., & Zhaxybayeva, O. (2009). On the origin of prokaryotic species. *Genome research*, *19*(5), 744-756.
5. DU, S., YU, M., LIU, F., XIAO, L., ZHANG, H., TAO, J., ... & CHEN, X. (2017). Effect of facility management regimes on soil bacterial diversity and community structure. *Chinese Journal of Eco-Agriculture*, *25*(11), 1615-1625.
6. Ediene VF, Iren OB, Idiong MM, 2016, Effects of slaughterhouse effluent on the physicochemical properties of surrounding soils in Calabar Metropolis. Int J Adv Res. 2016; 4:37-41
7. Levin, S. A., Carpenter, S. R., Godfray, H. C. J., Kinzig, A. P., Loreau, M., Losos, J. B., ... & Wilcove, D. S. (Eds.). (2009). *The Princeton guide to ecology*. Princeton University Press.
8. Ma, Y., Wang, J., Liu, Y., Wang, X., Zhang, B., Zhang, W., ... & Cui, X. (2023). Nocardioides:“specialists” for hard-to-degrade pollutants in the environment. *Molecules*, *28*(21), 7433.
9. Meena, V. S., Maurya, B. R., Meena, S. K., Meena, R. K., Kumar, A., Verma, J. P., & Singh, N. P. (2016). Can Bacillus species enhance nutrient availability in agricultural soils?. *Bacilli and agrobiotechnology*, 367-395.
10. Ndeddy Aka, R. J., & Babalola, O. O. (2016). Effect of bacterial inoculation of strains of Pseudomonas aeruginosa, Alcaligenes feacalis and Bacillus subtilis on germination, growth and heavy metal (Cd, Cr, and Ni) uptake of Brassica juncea. *International journal of phytoremediation*, *18*(2), 200-209.

11. Panagos, P., Borrelli, P., & Robinson, D. (2020). FAO calls for actions to reduce global soil erosion. *Mitigation and Adaptation Strategies for Global Change*, *25*, 789-790
12. Saidu, M., & Musa, J. J. (2012). Impact of abattoir effluent on river Landzu, Bida, Nigeria.
13. Tamreihao, K., Mukherjee, S., Khunjamayum, R., Devi, L. J., Asem, R. S., & Ningthoujam, D. S. (2019). Feather degradation by keratinolytic bacteria and biofertilizing potential for sustainable agricultural production. *Journal of basic microbiology*, *59*(1), 4-13.