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ANALYSIS ON RESISTANCE DEVELOPMENT OF MICROORGANISMS IN SOIL SAMPLE ISOLATED FROM DIFFERENT AGRICULTURAL FIELD SAMPLE

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ABSTRACT

The current study aims to assess the physicochemical properties and prevalence of microbial communities in soils samples collected from different locations of Nilgiris district, India. Following morphological, biochemical, and 16S rDNA sequence screening of the bacterial populations in the soil, Bacillus megaterium, Bacillus cereus, Pseudomonas aeruginosa, and Chromobacterium pseudoviolaceum were detected. Agar dilution was used to determine the lowest inhibitory concentration of isolated bacteria against Cd, Pb, Fe, and Cu since high concentrations of hazardous metals have a deleterious effect on bacterial development. The majority of the metal-tolerant isolates were shown to be resistant to both penicillin and methicillin, according to co -resistance of antibiotic analysis. But P. aeruginosa has demonstrated resistance to other antibiotics, including Vancomycin, Cefdinir, Ampicillin, and Kanamycin.

Keywords: Soil, Tolerant, Resistance, Antibiotic Analysis, Bacterial development.

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INTRODUCTION

1. Soil

Soil, the biologically active, porous medium that has developed in the uppermost layer of Earth's crust. Soil is one of the principal substrata of life on Earth, serving as a reservoir of water and nutrients, as a medium for the filtration and breakdown of injurious wastes, and as a participant in the cycling of carbon and other elements through the global ecosystem. It has evolved through weathering processes driven by biological, climatic, geologic, and topographicinfluences Nilgiris ,the District is situated at the Northwestern part of the State and Western Ghats and surrounded by the Coimbatore and Erode District, Kerala State and Karnataka State on Eastern, Western and Northern side, respectively .The overall soil reaction (pH) of The Nilgiris district ranged from 4.10 to 7.90 with a mean of 5.68, indicating that the soils are acidic to neutral in nature. Most of the samples were reported to contain low salt status with a mean EC of 0.31 dSm-1, rich in organicmatter (>0.75%) and non-calcareous in nature

.It is a horticultural district which occupies a special significance in hilly regions of Tamil Nadu. The Geographical area of the district is 2545 square Kilometer consists of 6 taluks and unique feature is that 56% of the total area is under natural vegetation. The entire district is situated between 900-2636 meters above MSL.

(Elena radu et.al, (2020))

2. Components of Soil

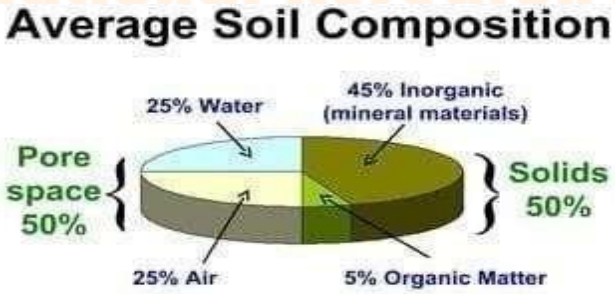

Figure 1.1: Soil Composition (Source: https://plantlet.org/composition-of-soil/)

3. Soil Biodiversity

Soil provides a vital habitat, primarily for microbes (including bacteria and fungi), but also for microfauna (such as protozoa and nematodes), mesofauna (such as microarthropods and enchytraeids), and macrofauna (such as earthworms, termites, and millipedes). The primary role of soil biota is to recycle organic matter that is derived from the "above-ground plant-based food web". (**Kieran Osbiston et.al, (2021))**

4. Role of soil in agriculture field

Soil is essential for life on Earth. It acts as a water filter, a growing medium, and a home for billions of organisms. Soil also helps to fight disease by supplying most of the antibiotics used to treat infections .Soil functions are essential for our survival. They include air quality and composition, temperature regulation, carbon and nutrient cycling, water cycling and quality, natural "waste" (decomposition) treatment and recycling, and habitat for most living things and their food. We rely on these functions for our very survival, and we could not live without them. (**Heike Schmitt et.al. (2006))**

5. Herbicide

Herbicides are used widely in row-crop farming, where they are either applied before or after planting. Spraying herbicides prevents or minimizes other vegetation, maximizes crop production, and improves harvesting. When used in wild lands, herbicides can increase the diversity of native species. (**Jayaraj et.al, (2023))**

Contact herbicides – They are applied by spraying on the plant and mainly act only on the tissues that have been touched by the herbicide. Their efficiency is not influenced by weather conditions. The action of these herbicides can be observed quite quickly,
usually within 24 hours. However, this type of herbicide is not suitable for weeds.

Systemic herbicides – They are designed to act on the entire plant. The efficiency of these herbicides is good, but the effect is slower. It should also be noted that this type of active substance needs favorable weather conditions such as periods of precipitation shortly after application

Residual herbicides – They persist in the soil for a longer time and can protect crops from the next wave of unwanted weeds. The effectiveness of these herbicides depends on the spectrum of weeds, how well the land has been worked, and the amount of precipitation. These types of herbicides are more effective in adequate moisture conditions.

Glyphosate (IUPAC name: ***N*-(**phosphonomethyl) glycine) is a broad- spectrum systemic herbicide and crop desiccant. It is an organophosphorus compound, specifically a phosphonate, which acts by inhibiting the plant enzyme 5- enolpyruvylshikimate-3- phosphatesynthase(EPSP).

Specifically a phosphonate, which acts by inhibiting the plant enzyme 5- enolpyruvylshikimate- 3-phosphate synthase (EPSP). It is used to kill weeds, especially annual broadleaf weeds and grasses that compete with crops. In particular types of soil or weather conditions, however, glyphosate can leach out and pose a potential pollution threat to water courses. Impact on soil micro-organisms that are vital to soil health: Glyphosate has been shown to negatively affect the abundance of the culturable bacterial community, and the total bacterial composition.

1.6 Resistance development (Julija Armalyte et.al, (2019))

There are three common methods of developing resistance in the host.

i) Selection
ii) Hybridization
iii) Mutation

- Selection is an old practice of developing resistant varieties. When a large number of individuals grow under disease favorable environment, some individuals show some resistance to the disease which might be selected and tested again before recommendation as a resistant variety.
- Hybridization involves the crossing of two individuals (parents) with good commercial qualities lacking resistance to specific pathogens and another, a source of resistance lacking desired commercial traits.
- The source of resistance can be obtained by selection from variety or species much prevalent in the area.

- If such variety is not available in the area under cultivation in cultivated varieties or species, the desired individual can be obtained from some other species or related wild plants.
- Successful crossing of wild Lycopersicon pimpinellifolium with cultivated tomato Lycoperiscon esculentum has produced material for the development of varietiesresistance to Fusarium wilt.

1.7 Sequencing

Instead of having to culture a specific microorganism, extract its DNA, and then sequence the DNA, metagenomic sequencing allows scientists to directly extract and sequence DNA from their environmental sample. Scientists typically use a method called "shotgun sequencing" in order to sequence the metagenome of the human microbiome.

REVIEW OF LITERATURE

Heike Schmitt et.al, (2006) conducted research on tetracycline and resistance in agricultural soils has intensified, focusing on microcosm and field studies. Improved waste management and promoting soil health, are proposed to reduce tetracycline's environmental impact. We used their methods for better result.

Soumitra Nath et.al,(2019) proposed a project to limit the spread of Antimicrobial Resistanceacross environment by using techniques like Agar dilution to find inhibition of bacteria to metals. We used Agar dilution method.

Mila D. Kaleva et.al, (2023) evaluate the presence, virulence, antibiotic resistance and biofilmformation of E. coli. They incorporated various techniques like PCR, MALDI- TOF-MS, disk diffusion methods. We used PCR method.

S. Silambarasan et.al, (2010) find antibiotic resistance and metal tolerant bacteria. They done gram staining, Muller Hinton agar by disc diffusion method, well diffusion method, Atomic Absorption Spectrophotometer. We used gram staining and disc diffusion method.

Baye Sitotaw et.al, (2022) they investigated antibiotic production potential of bacteria isolated from MSWDS. Here they used nutrient Agar, serial dilution, streak plating, McFarland standard. We used serial dilution for better result.

Irene M. Unger et.al, (2012) proposed that using VFS to reduce VA loss fromagro ecosystems won't diminish the primary functions associated with VFS use in agriculture.

Davis Gislin et.al, (2018) identified similarity in the bacterial species using BLAST. To isolate bacteria they used serial dilution and gram stained, PCR. We used serial dilution and gram staining to get good result.

Jessica K. Vaughan (2018) proposed that possible pathogenic enteric bacteria were identified .Here they used serial dilution, tryptic soy broth, gram staining. We used serial dilutionfor better result.

Elena radu et.al, (2020) they affirmate that high prevalence and abundance of naturally occurring ARGs, considered as background concentration, can be found in forest soil.

Julija Armalyte et.al, (2019) Analyzed of antimicrobial resistance in soils demonstrates microorganisms not acquire genetic determinants.

Jayaraj et.al, (2023) they study that bacteria from agriculture soil system that have been polluted with pesticides were isolated, identified and their ability to tolerate pesticides were examined

Abdullah Ibn Mafiz et.al, (2018) they study was to investigate antibiotic resistance bacteria and antibiotic resistance genes in urban agriculture soil using phenotypic and metagenomic tool. A total of 207 soil bacteria were recovered from 41 Soil samples collected from an urban agriculture garden in Detroit, USA.

Joseph Jackson Adu-Gyamfi et.al, (2019) Antibiotics kill bacteria, healing infections that cause diseases and death. Unfortunately, bacteria have learned how to quickly evolve and resist the effect of antibiotics antibiotic effect.

Nakuleshwar Dut Jasuja et.al, (2013) they work deals with the isolation and characterization of microorganisms from polyhouse agriculture soil of Jharna village (Rajasthan). Several bacteria and fungi were isolated from polyhouse soil, using serial dilution method. These bacterial isolates were Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia, Enterobacter aerogenes, Shigella sp., Proteus mirabilis, Bacillus anthracis, Bacillus subtilis, Staphylococcus aureus and Staphylococcus epidermidis species which were further identified on the basis of colony morphology.

Kieran Osbiston et.al, (2021) Agricultural intensification like, many anthropogenic activities, impacts negatively on the environment. Examples of these impacts include deforestation, soil degradation and pollution of water and soil. The preventative and therapeutic use of antibiotics in animal farming has been shown to contribute to an increase in AMR, via manure storage and the use of manure solids or wastewater as soil fertilizer.

Lars Bogø Jensen et.al, (2001) Temporary effects on resistance levels among the bacterial populations resulting from spread of animal waste (both manure and slurry) were found for both Pseudomonas spp. and the B. cereus group. This conclusion was reached since the resistant isolates were mostly recovered from soil after spread of manure or slurry. Since the samples taken before spread of animal waste represent last years' spread of animal waste and spread of animal waste is done yearly, no permanent effect was observed.

MATERIALS AND METHODOLOGY

SAMPLE PREPARATIOIN

Collecting three different Soil from three different field from Nilgiris District. (**Julija Armalyte et.al, (2019))**

Figure 3.1: Tea Cultivation Land

Figure 3.2: Agricultural Land (Potato Field)

Figure 3.3: Eucalyptus Land

MEDIA PREPARATION

The preparation of Nutrient Agar culture Medium step by steps- Suspend the 7 grams of Nutrient Agar powder in 250 ml of Purified/distilled water. Heat to boiling to dissolve the ingredients completely. (**Nakuleshwar Dut Jasuja et.al., (2013))**

STERILIZATION

Process that removes, kills, or deactivates all forms of life (particularly microorganisms such as fungi, bacteria, spores, and unicellular eukaryotic organisms) and other biologicalagents (such as prions or viruses) present in or on a specific surface, object, or fluid.

Autoclave is run at a temperature of 121° C for at least 45 minutes by using saturatedsteamunder at least 15 psi of pressure. Things to be sterilized

- Prepared Agar media
- Test tubes Filled with 9ml of distilled water
- Petri dishes
- Tips

SERIAL DILUTION

Serial dilution in analytical chemistry is the stepwise dilution of a highly concentrated solution to obtain a solution with a reduced concentration. A serial dilution definition is a stepwise series of dilutions that are performed to reduce the concentration of a substance in a solution to a more usable concentration. (**Jessica K. Vaughan (2018))**

PLATING

- Plating is done with the 10-5 in the plated agar medium
- Spread plate method is done using sterilized Lrod.
- Incubate the petriplates for 24hours at 37 ºC.

CULTURING

It allows the multiplication of bacterial cells in or on a culture medium under controlled laboratory conditions. The exact conditions required for optimal replication will depend on the target bacterial species. (**Lars Bogø Jensen et.al.,(2001))**

SEQUENCING

For the identification of the type of bacteria the sequencing is done.

RESULT AND DISCUSSION

Serial dilution made for the collected soil samples. Platting done for the dilution 10^{-6} and 10^{-7} by spread plate method. The growth of colonies in 10^{-6} and 10^{-7} dilutions was observed. The taken culture is given for the sequencing.

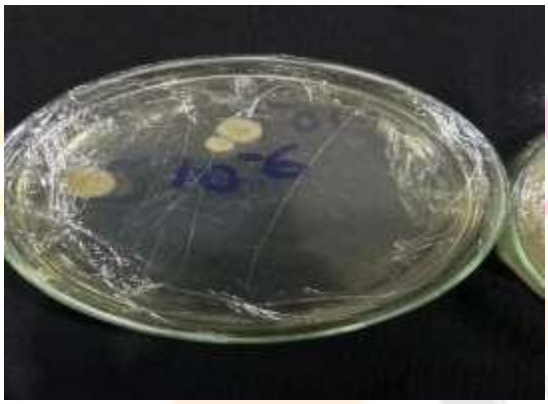

Fig.4.1. Colonies growth in 10-6

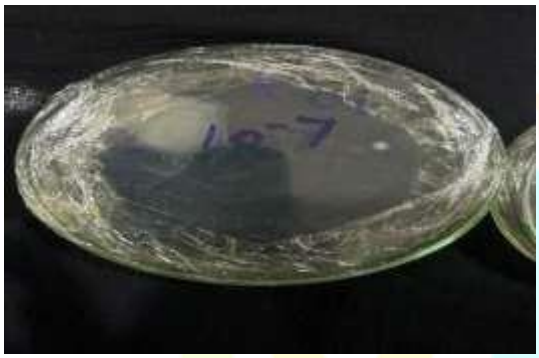

Fig.4.2. Colonies growth in 10^{-7}

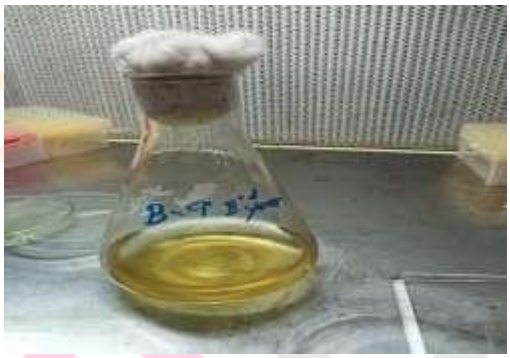

Fig.4.3. Culturing

CONCLUSION AND FUTURE WORKS

In this work the microorganism isolated were sent for sequencing. The colonies sent for sequencing need to be screened with the available online database. Molecular Analysis can be performed in future to know the characteristics of the isolated organisms from soil. If it is novel, need to do molecular characterization for the organism to know the molecular property against the pesticide used in that particular field.

REFERENCES:

Schmitt, H., Stoob, K., Hamscher, G., Smit, E., & Seinen, W. (2006). Tetracyclines and tetracycline resistance in agricultural soils: microcosm and field studies. *Microbial ecology*, *51*, 267-276.

Nath, S., Paul, P., Roy, R., Bhattacharjee, S., & Deb, B. (2019). Isolation and identification of metal-tolerant and antibiotic-resistant bacteria from soil samples of Cachar district of Assam, India. *SN Applied Sciences*, *1*, 1-9.

Kaleva, M. D., Ilieva, Y., Zaharieva, M. M., Dimitrova, L., Kim, T. C., Tsvetkova, I., & Najdenski, H. (2023). Antimicrobial Resistance and Biofilm Formation of Escherichia coli Isolated from Pig Farms and Surroundings in Bulgaria. *Microorganisms*, *11*(8), 1909.

Silambarasan, S., & Jayanthi, A. (2010). A Study on Antibiotic Resistance and Metal Tolerance of Bacteria Isolated from Industrial Site. *Nature, Environment and Pollution Technology*, *9*(2), 261-266.

Sitotaw, B., Ayalew, F., Girma, A., Mekonnen, A. B., Bin Jardan, Y. A., Nafidi, H. A., & Bourhia, M. (2022). Isolation and identification of promising antibiotic-producing bacteria. Open Chemistry, 20(1), 1283-1291.

Unger, I. M., Goyne, K. W., Kennedy, A. C., Kremer, R. J., McLain, J. E., & Williams, C.F. (2013). Antibiotic effects on microbial community characteristics in soils under conservation management practices. *Soil Science Society of America Journal*, *77*(1), 100- 112.

Gislin, D., Sudarsanam, D., Raj, G. A., & Baskar, K. (2018). Antibacterial activity of soil bacteria isolated from Kochi, India and their molecular identification. *Journal of genetic engineering and biotechnology*,

16(2), 287-294.

Vaughan, J. (2018). Antibiotic Resistance of Bacteria Isolated from Soils.

Radu, E., Woegerbauer, M., Rab, G., Oismüller, M., Strauss, P., Hufnagl, P., & Kreuzinger, N. (2021). Resilience of agricultural soils to antibiotic resistance genes introduced by agricultural management practices. *Science of The Total Environment*, *756*,143699.

Armalytė, J., Skerniškytė, J., Bakienė, E., Krasauskas, R., Šiugždinienė, R., Kareivienė, V., ... & Ružauskas, M. (2019). Microbial diversity and antimicrobial resistance profile in microbiota from soils of conventional and organic farming systems. *Frontiers in microbiology*, *10*, 892.

Jayaraj, J., Shibila, S., Ramaiah, M., Alahmadi, T. A., Alharbi, S. A., Mideen, P. K., & Sivagnanam, A. (2023). Isolation and Identification of bacteria from the agricultural soil samples to tolerate pesticides dimethoate, thiamethoxam and Imidacloprid. *Environmental Research Communications*, *5*(7), 075011.

Mafiz, A. I., Perera, L. N., He, Y., Zhang, W., Xiao, S., Hao, W., & Zhang, Y. (2018). Case study on the soil antibiotic resistome in an urban community garden. *International journal of antimicrobial agents*, *52*(2), 241-250.

Adu-Gyamfi, J. J., Heng, L. K., Eklund, G., & Souza, M. D. (2019). Antimicrobial movement from agricultural areas to the environment: the missing link. a role for nuclear techniques. *FAO Land and Water Discussion Paper*, (13).

Jasuja, N. D., Saxena, R., Chandra, S., & Joshi, S. C. (2013). Isolation and identification of microorganism from polyhouse agriculture soil of Rajasthan. *African Journal of Microbiology Research*, *7*(41), 4886-4891.

Osbiston, K., Oxbrough, A., & Fernández-Martínez, L. T. (2021). Antibiotic resistance levels in soils from urban and rural land uses in Great Britain. *Access Microbiology*, *3*(1).

Jensen, L. B., Baloda, S., Boye, M., & Aarestrup, F. M. (2001). Antimicrobial resistance among Pseudomonas spp. and the Bacillus cereus group isolated from Danish agricultural soil. *Environment international*, *26*(7-8), 581-587.