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Impact of Rotten Cocoa Pod on Soil Microorganisms from Ikeji-Arakeji Metropolis, Osun State, Nigeria

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Abstract— *Soil is a mixture of broken rocks and mineral which contain living organisms, in which microorganisms can transfer either by erosion or wind dispersal to other living things when come in close contact which can be as a source of contact to pathogenic microbes and can ponderous to health hazard of the community. This study investigates the microbial analysis and also to check the influence of soil with fallen rotten cocoa against soil without rotten cocoa on soil microbes in Ikeji-Arakeji, Osun state, Nigeria. Total bacterial and fungal counts were determined using pour plating method. Total bacterial count was higher from soil with fallen rotten cocoa pod 92 $\times 10^5$ cfu/ml to soil without fallen rotten cocoa which is 86 $\times 10^5$ cfu/ml and Total fungal count ranges was higher from soil with fallen rotten cocoa pod is 44 $\times 10^5$ cfu/ml to soil without rotten cocoa pod which is 33 $\times 10^5$ cfu/ml. The isolation and enumeration of microbial population was carried out using standard culture-based methods. Bacteria isolates such as Proteus vulgaris, Escherichia coli, Pseudomonas sp, Enterobacter aerogene., Bacillus spp, Staphylococcus spp, Micrococcus sp, Erysipelothrix spp and the fungi isolate include Aspergillus niger, Aspergillus flavus and Rhizopus stolonifer. All the microbes were present for the two samples except Micrococcus sp and Enterobacter aerogenes. The bacteria count were more alpine and higher in bacterial count than the fungal count. The presence of these microorganisms indicates a suitable soil for general plantation by the microbial degradative activities.*

Keywords— Soil, Bacteria, Fungi, Microbial population, pathogenic microbes.

I. INTRODUCTION

Soil is a natural body consisting of layers (soil horizons) that are primarily composed of minerals which differ from their parent materials in their texture, structure, consistency, colour, chemical and biological processes that include weathering associated with erosion. These soil forming factors continue to affect soils even on "stable" landscapes (2). Materials are deposited on their surface, and materials are blown or washed away from the surface. Additions, removals, and alterations are slow or rapid, depending on climate, landscape position, and biological activity. Few soils weather directly from the underlying rock. These "residual" soils have the same general chemistry as the original rocks. More commonly, soils form in materials that have moved in from elsewhere. Materials may have moved many miles or only a few feet. Windblown "loess" is common in the Midwest. It buries "glacial till" in many areas. Glacial till is material ground up and moved by a glacier. The material in which soils form is called "parent material." In the lower part of the soils, these materials may be relatively unchanged from when they were deposited by moving water, ice, or wind. (9).

Cocoa pod waste can be embedded in soils which we have high content of organic matter, increase in nitrogen and cation exchange capacity. Organic waste can provide nutrients for increased plant growth and such positive effect will likely encourage continued land application of these wastes (3). However, excessive wastes such as rotten cocoa pod in soil may increase heavy metal concentration in the soil and underground water. For example, the contamination of soil with heavy metals, even at low concentration, is known to have potential impact on environment quality as well as posing a long term risk to groundwater and ecosystem. Heavy metals may have harmful effects on soils, crops and human health (17). This is because toxic elements accumulate in organic matter in soil and sediments taken up by growing plants. These metals are not toxic as the condensed free elements but are dangerous in the form of

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cations and when bonded to short chain of carbon atoms (16). The extent of contamination arising from leachates' percolation inside the soil is determined by a number of factors that include physiochemical properties of the leachates and soil together with hydrological condition of the surrounding site (15).

Soils vary, depending on the climate, temperature and moisture amounts cause different patterns of weathering and leaching (22). Wind redistributes sand and other particles especially in arid regions. The amount, intensity, timing, and kind of precipitation influence soil formation. Seasonal and daily changes in temperature affect moisture effectiveness, biological activity, rates of chemical reactions, and kinds of vegetation (23).

Slope and aspect affect the moisture and temperature of soil. Steep slopes facing the sun are warmer, just like the south-facing side of a house. Steep soils may be eroded and lose their topsoil as they form (11). Thus, they may be thinner than the more nearly level soils that receive deposits from areas upslope. Deeper, darker colored soils may be expected on the bottom land.(18).

Microorganisms affect chemical exchanges between roots and soil. Humans can mix the soil so extensively that the soil material is again considered parent material. (10). The health hazards associated with the consumption of rotten cocoa pod which may contain toxigenic moulds and also *Bacillus* species such as *Aspergillus* species is well-known. Some produce fungal toxins called aflatoxin, ochratoxin which are of global health concern.

This study investigates the microbial analysis of soil and also to check the influence of fallen rotten cocoa against soil without rotten cocoa on soil microbes in Ikeji-Arakeji, Osun state, Nigeria.

II. MATERIALS AND METHODS

2.1 Sterilization of Equipment and General Procedures

Glassware including petri dishes, conical flask, test tubes, durham tubes and beaker as well hand trowel were washed with detergent and water, rinsed with clean warm water to remove all traces of residual washing compounds. They were air dried and then sterilized in the hot air oven at 160℃ for 1 hour. The work bench was disinfectant by swabbing with 70% ethanol; media were sterilized by autoclaving at 121℃ for 15 minutes holding time. All work in the laboratory was done in a sterile environment.

2.2 Sampling Area

Soil samples used in this study were collected in Ikeji-Arakeji, a town in Oriade local government Area of Osun state Nigeria, which is 37 km from Akure (Capital of Ondo State)..

2.3 Sample collection

The soil sample were collected from various site aseptically from from soil with fallen rotten cocoa pod and soil without fallen rotten cocoa pod in Ikeji- Arakeji, Osun state, Nigeria. The soil sample were collected with a hand trowel in to sterile polythene bags for physiochemical properties while samples for biological characteristics were collected in to a srerile universal bottle. The sample were taken to the laboratory for analysis. The pour plate method was used. Ten-fold serial dilution of each water sample was prepared aseptically in physiological saline of 10-1 up to 10-4 and 0.1 ml aliquot of each dilution

2.4 Bacteriological Analysis

Nutrient agar, Eosine methylene blue, Shigella Salmonella agar and MacConkey agar were prepared using manufacturers direction. Bacteria isolates were characterized on the basis of the colonial morphology and Gram stain reaction. Biochemical tests such as catalase, Coagulase, Motility, Indole, and Oxidase tests were carried out (22).

2.5 Identification of microorganisms

The cultural and morphological features of isolates from soil and water samples were studied by plates reading and Gram staining while the organism were separated by biochemical test (13).

III. RESULT

The Seven bacterial species were isolated from the two different samples. These include *proteus vulgaris*, *pseudomonas* spp. *Enterobacter aerogenes*, *Bacillus* spp, *staphylococcus* spp, *micrococcus* spp *Erysipelothrix* species. The bacterial counts are attributed to contamination by cocoa pod rot.

Table 1 show the total bacterial and fungal count. Table 2 show the colonial morphology of the bacterial isolates, which is based on shape, elevation, edge, optics, pigmentation, cell shape and cell arrangement. These characteristics will enable in identification of bacteria. Table 3 shows the biochemical characteristics of bacteria, these characteristics is based on gram staining reaction, motility, catalase, oxidase, methyl red, Voges proskauer, citrate, urease, sugar fermentation test (such as Glucose and Lactose) and spore staining. The bacteria identify are *Bacillus species*, *Proteus vulgaris*, *Pseudomonas* species,

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Klebsiella species, *Bacillus* species, *Erysipelothrix* species, *Staphylococcus* species, *Pseudomonas aeruginosa*, *Micrococcus* species. Table 4 show the bacterial isolates and their occurrences among the samples whether they are present or absent.

3.1 Fungal isolates

Isolate A

The isolate/organism appear as whitish colonies which turns black due to formation of black conidia, the hyphae are septate and profusely branched. Conidia are borne in chains at the tip of sterigmata. The isolates was identified as *Aspergillus niger*

Isolate B

The organism are white colony at first becoming hairly speckle by the presence of sporangia and then brownish black after (7 days) colonies spread rapidly by means of stolons fixed at various points to the substrate by rhizoids.Rhizoids and stolons are present and hyaline to dark brown**.** The isolate was identified as *Rhizopus stolonifer.*

Isolate C

Bright to yellow green colonies. Has the same colour of green with a tinge of yellow on the reverse side of the plate. Conidiophores coarsely rough, heads vary in size, loosely radiate, phialides borne directly on the vesicle. Conidia are globose (It was identified as *Aspergillus flavus*).

It appeared as whitish colonies which turned black due to the formation of black conidia, colonies spreading rapidly. Under microscopic investigation, the hyphae were septate and profusely branched. Conidia were bonded in chains at the hips of sterigmata. Conidial heads are globose.

After growth, the colonies appeared as black conidial heads and yellow pigment when observed under the microscope. The conidiophores were long, smooth and hyaline (colourless). The conidiophores were attached to septate by means of foot cells. The conidiophores had vesicles in which a single file of sterigma carrying conidia was arranged. The colonies appeared dark brown and mycelium spread very rapidly on the plates. The conidia are seriated and compact head forming chains (It was identified as *Aspergillus niger*).

Table.2: Morphology and biochemical characteristics of bacterial isolates

ISOLATE CODE	SHAPE	ELEVATION	EDGE	OPTICS	PIGMENTATION	CELL SHAPE	CELL ARRANGEMENT
1	Round	Flat	Even	Opaque	White	Rod	Chains
2	Round	Raised	Even	Opaque	Cream	Rod	Chains
3	Round	Raised	Even	Translucent	White	Rod	Chains
4	Round	Raised	Entire	Opaque	White	Rod	Chains
5	Round	Raised	Entire	Opaque	White	Rod	Chains
6	Round	Raised	Even	Opaque	White	Cocci	Cluster
7	Round	Raised	Entire	Opaque	White	Cocci	Cluster

Table.3:Microscopy examination and Biochemical characteristic of bacteria isolated.

Probable Isolates	Gram stain	Spore stain	Coagulase	Catalase	Motility	Glucose	Lactose	Sucrose	Maltose
Proteus vulgaris	_	_	_	+	+	A	A	A	A
Pseudomonas species	_	_	+	+	_	A	A	A	AG
Klebsiella species	_	_	_	+	_	AG	A	A	A
Bacillus species	+	_	_	+	_	A	A	A	A
Erysipelothrix species	+	_	_	_	_	A	NR	NR	NR
Staphylococcus species	+	_	_	+	_	AG	A	A	A

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Micrococcus species	_	_	_	+	+	A	A	A	AG

KEYS: - =Negative, += Positive, A = Acids production only, AG = Acidand gas production, NR= Noreaction.

Table.3: Shows the microbial count of soil.

Sampling site	**Total Bacteria count 10^5Cfu/ml**	**Total fungi count 10^5cfu/ml**
Sample A	8.6×10^5	3.3×10^5
Sample B	9.2×10^5	4.4×10^5

KEYS Sample A: Soil from without rotten cocoa pod. Sample B: Soil with rotten cocoa pod

Table.4: The bacterial isolates and their occurrences among the samples

Bacterial species	Sample A	Sample B
Proteus spp	+	+
Pseudomonas spp	+	+
Bacillus spp	+	+
Micrococcus spp	+	_
Enterobacter aerogenes	+	_
Erysipelothrix spp	+	+
Escherichial coli	+	+

KEYS:

+…….Present

_.....Absent

Sample A…….. Soil with rotten cocoa pod

Sample B……. Soil without rotten cocoa pod

IV. DISCUSSION

Soils supply plants with nutrients that are held in place by the clay and humus content of that soil. For optimum plant growth, the soil components by volume should be roughly 50% solids (45% mineral and 5% organic matter), and 50% voids of which half is occupied by water and half by gas. The fertility of an agricultural soil is intimately linked to its microbiota and the activities and relationship that exist between the microbial groups involved in nutritional cycles, which are essential to the normal functioning and evolution of the soil. Several species of bacteria were isolated from the soil sample which are *proteus vulgaris, pseudomonas spp, bacillus spp, enterobacter aerogenes, staphylococcus aureus, and micrococcus spp*. The microorganisms found in the soil constitute the microbial composition of any soil which is governed by equilibrium created by the association by the association and interacting of all microbes found in the environment.

This is in line with the works of (11). The presence of *Bacillus* is a well known indigenous and persistent bacteria to soil environment (10). The presence of *Erysipelothrix* species in the contaminated soil may be as a result of large quantity of animal excreta in the effluent containing these organisms being discharged into the soil environment(5). Similar findings were reported by (9). The fungi identified are well known soil-inhabiting microorganisms. Their presence indicates possible pollution and may have an effect on the soil ecological balance (13). *Aspergillus, Fusarium, Mucor, Penicillium Rhizopus* and a variety of yeasts have also been reported to be associated with waste biodegradation (19). The presence of these organisms is a pointer to possible pollution and may have an effect on the soil ecological balance. These findings were in conformity to that of (9, 10). The soil with rotten cocoa pod although in small quantities points to high microbial activities in such soil. It revealed a significant difference between the counts in the soil with rotten cocoa pod to the soil without rotten cocoa pod. Therefore, it is highly recommended that the rotten cocoa pod can add nutrient to the soil, instead of inorganic fertilizer that can have adverse effect on the plant it can be replaced by rotten cocoa pod.

V. CONCLUSION

The outcome of this study has been shown that diverse types of bacteria and fungi are associated to the soil with

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rotten cocoa pod also aid in availability of the microbes present in degradation of organic matter. Despite the positive impacts of the cocoa pod rot on soil microbes on the microbial and organic properties of the analyzed soils, disposal of spoilt cocoa pod should not be encourage so that it can be used as organic fertilizer.

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