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The Physico-chemical Properties and Microbial Isolates of Cross River State Estuary Soils

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Abstract

This study attempted to determine the physico-chemical properties and microbial isolates of estuary Soils in Cross River State. Four soil samples were collected from the estuaries area (Ikang, Atimbo Bridge, Unical Creek) of southern part of Cross River State as the study area. A composite sample was collected from each of the topsoil surface (0-20cm) using soil Auger. Some physico-chemical properties viz., particle size, pH, organic carbon, N, P, Ca, Mg, K, Na, H, Al, ECEC, BS and microbial flora population were determined using standard physico-chemical and biological methods. Results showed that there was a high sand content in Ikang and Atimbo than Unical Creek. Results also showed that most of the chemical properties were low to medium but Mg contents were high in all the samples. The highest microbial population of bacteria and fungi was 79 x 10^6 and 22 x 10^3 respectively. The bacteria microbes identified were *Cyanobacterium spp, Nocardia spp* and *Nostoc spumigerna*, while that of fungi were *penicillium spp*, *Aspergillus flavus*, *fusarium spp* and *Rhizopus spp*. Results depicted that the microbial populations in estuary of Cross River State were not very high.

Key words: Physico-chemical, Estuaries, Microbial isolates, Microbial population, Bacteria and fungi.

Introduction

Estuaries contain salt water and fresh water in different proportions over the length of the estuary and over the course of the day, with more salt water during high tides and less at low tides. Since they are shallow, sunlight penetrates the water, allowing micro-organisms to grow. The rivers that feed the estuaries deposit sediments rich in nutrients, which settle on the sand and mud of the estuary floor. These conditions create unique habitats for micro-organisms and provide an environment for biological diversity in species (fish, shrimp, crabs, and oysters) that are able to adapt to the blackish conditions Frankenberg (1997).

According to Charles, Edward and Brain (2000), estuaries are subjected to both marine influences, such as tides, wave and the flux of saline water, and riverine influences, such as flow of fresh water and sediment. As a result, they may contain many biological niches within a small area and so are associated with high biological diversity.

In estuarine, plants of different groups are present including phytoplankton, benthic diatoms, bacteria and fungi and larger macrophytes. Though estuaries are rich in their nutrients, phytoplantonic organisms are not abundant. This is due to the reduction in light penetration as a result of turbidity (Jannasch, Goldman and Backus, 1974).

Wetland is defined by Andriesse (1986) as a land subjected to excessive wetness, to the extent that the wet condition influences the possible land use. Brinkman and Blokhius

(1980) stated that wetland has free water at or on the surface for two months or more of growing season of perennial crops, grassland, forest or other vegetation. This definition refers to wetland as a natural ecological environment. They concluded that wetland as defined has at least one wet growing season and that wetland soils may therefore alternately support wetland and dry land crops and in fact normally do when cultivated. Jannasch et al., (1974) regards wetland not just as "land" but as "soils" and defined them as soils that are ponded or water saturated during most of the time that they are, or could be used for food crop production.

Cultivated agricultural soils, which used to be fertile, have become severely degraded by slash-and-burn agriculture, which is now being practiced with shortened fallow period because of high population growth rate. Farmers in Nigeria are increasingly interested in wetland soils, which are still fertile (Asawalan et. al. 2001)

According to Asawalan et al., (2001), there is an urgent need for a sustainable utilization of the agricultural opportunities of Akwa Ibom wetland soils for increased food production. Efficient and effective management of wetland soils offer an acceptable alternative for sustainable food production.

Biodiversity refers to all the components of biological life, its diversity and interactions. It includes plants, animals, fungi, bacteria and other micro-organisms as well as the ecosystem and processes of which they are a part (Frankenberg 1997). The study of the microbial isolates and biodiversity vis-à-vis the fertility of the wetland soils would afford us the opportunity of good management of the soils for sustainable agricultural practice especially in Niger Delta of Nigeria.

Attention is being paid for monitoring and assessing the microbiological quality of water resources all over the world (Khalafalla et. al. 1993; Lindskog & Lindskog, 1988). According to (Rangaswami and Bagyaraj, 1993), most of the estuarine bacteria are gram negative. When the source of water pollution is considered, industrial wastewater represents the main source in different parts of the world, e.g. Egypt (Igwe, 2001; Sabae, 2006; Sabae, 2008), Poland (Niewolak, 2000), Nigeria (Ekhaise & Anyasi, 2005) and Brazil (Akaninwor et. al. 2007).

A monitoring program is needed to provide reliable information about the current water quality (Frankenberg, 1997). Therefore, this study was conducted to assess the physico-chemical properties and the microbial isolate of Cross River State Estuary Soils.

Materials and Methods

Location of the Study Area: The research was conducted at Ikang village at Bakassi Local Government Area, Atimbo at Calabar and Unical Creek at Calabar, Cross River State of Nigeria, where there is estuary. Cross River State is located within the latitude 40^0N and 45^0N and longitude $74^0$5'E and $9^0$48'E in the southern part of Nigeria.

Climate: The annual temperature and relative humidity values in Cross River State range from 21^0C-29^0C and 70-85% respectively, while the rainfall values range between 2000mm and 3500mm. The rainy season is generally between March and October.

Sample Collection: A composite soil sample was collected from four different locations of Ikang estuary where the freshwater joins and mixes with salt water, Atimbo, two from Unical

Creek estuaries located behind the Oil-palm plantation. The samples were collected to a depth of 0-20cm using soil auger. The samples were properly labeled and prepared for microbial and physiochemical analysis in the Laboratory. The one for physiochemical analysis were air dried, crushed with mortar and sieved through a 2mm-sized sieve to get fine fraction.

Procedures for Soil Analyses

Soil Reactions (pH): The values were determined by the use of pH meter glass electron employing the 1:2:5 soil water solution ratio (20g of soil to 50ml distilled water). The pH meter was calibrated with buffer solution at pH 4 and pH 7.

Organic Carbon: This was determined by Walkley-Black and wet oxidation method using 20ml concentrated sulphuric acid (H_2SO_4), 10ml Potassium dichromate ($K_2Cr_2O_7$) and finally titrated with standing ferrous sulphide solution ($FeSO_4.7H_2O$) [15].

Available Phosphorus: Available phosphorus was determined by the Bray p-1 method. 0.03N NHF and 0.025H HCl was used as an extract using Spectrophotometer (Thermo Scientific SPECTRONIC 20D+ Spectrophotometers). The phosphorus concentration in the extract was determined by the blue calorimetric method of Murphy and Riley (1982)..

Exchangeable Bases: Ca^{2+}, Mg^{2+}, K^+ and Na^+ were leached from the soil with NH^4.OAC (pH 7) (Ammonia acetate extraction). In this case Ca^{2+} and Mg^{2+} were determined by using EDTA complexometric titration as well as by atomic absorption, whereas K^+ and Na^+ were determined by flame photometer (Jenway Models PFP7).

Exchangeable Acidity: Exchangeable acidity was determined using KCl extraction. In this case KCl solution was added drop by drop to leach the soil sample in a funnel inside a volumetric flask. The leachate was later titrated with 0.01 molar solution of NaOH using phenolphthalein (0.1%) indicator.

Effective Cation Exchangeable Capacity: The determination of effective cation exchange capacity (ECEC) was taken or calculated as the sum of exchangeable acidity and that of exchangeable bases.

Base Saturation: This was obtained as a percentage of total exchangeable bases by the effective cation exchange capacity.

Soil Particle Size Analysis: Soil particle size analysis was determined using hydrometric method in which cation was used as a dispersing agent in a measuring cylinder of 100ml for the particle to settle for reading (Khalafalla, 1993).

Media Preparation and Microbial Analysis Method

Soil Extract Agar: One thousand grams of organic soil was weighed into a conical flask mixed in 1 litre (1000ml) in distilled water and stirred thoroughly using stirring rod and was filtered, the leachate (extract) was used to prepare the agar.

Fifteen grams (15g) of powdered media were suspended in soil extract and boiled to dissolve; it was sterilized by autoclaving at temperature of 121^0C and 15 lbs pressure for 15 minutes. The prepared media was kept in the oven to prevent it form gelling.

Malt Extract Agar: Malt extract agar was used as medium for fungi. 26g of agar was suspended in 1000ml of distilled and 20ml of malt extract was added. The mixture was mixed properly and sterilized by autoclaving at a temperature of 121^0C in 1b/sq inch for 15 minutes.

Serial Dilution Method: 9ml of distilled water was pipette into a clean oven dried test-tubes according to the number samples and sterilized using the autoclave. 10g of the soil samples was suspended in 95ml of sterile distilled water in a beaker. 70% alcohol was used to clean the stirrer rod and the mixture was stirred between 30-60 seconds and allowed to stand for 30 minutes.

1ml of the mixture was introduced into the test tube that contained 9ml of sterilized water using 10ml sterilize pipette starting from the first according to their label in the test tube rack from 10^{-1} to 10^{-5} and was mixed properly. Another clean sterile pipette was used to transfer 1ml from test tube (10^{-1}) to the second test tube (10^{-2}). The same procedure was repeated for remaining test tubes.

1ml of diluted solution was transferred from the 6^{th} (10^{-6}) test tube into a stirred Petri-dish using a sterile pipette. 15ml of soil extract agar was carefully poured into the Petri-dish and stirred carefully to ensure homogenous mixture in order to have discrete colony. The plate was allowed to gel and then inverted, sealed using masking tape to prevent the vapor falling back to contaminate the culture before incubating at 28-37^0C for 24 hours. The same procedure was carried in other samples. The colonies were counted using colony counter (IUL Plate Handler model of 90 mm diameter in plastic).

Direct Inoculation: In the preparation of pure culture from subculture another molten agar was poured into five Petri dishes and allowed to form a gel. A wire loop was sterilized in heat which was used in picking the colony and was streaked on the soil media, by doing so the colonies in the previous culture was transferred into the new culture, sealed with cellotape and kept in room temperature at 28-37^0C for 24 hours.

Gram Staining: Gram stain reaction was used in the preliminary identification of the bacteria. The principle behind this lies in the strength of microorganisms to retain the basic dye methyl noted after discoloration with alcohol. Smears of 24 hours culture of bacteria isolated were spread on a clean grease-free slide. The slides were flamed dried over Bunsen burner until they were fixed. The smears were flooded with methyl (crystal) violet for 20 seconds, and then rinsed off with distilled water. 70% alcohol was used as decolorizer for 10-20 seconds and it was rinsed with water for 2 seconds. Counter stain safranin was added for 20 seconds, and it was washed of with water and finally, the slide was dried by placing it slant position for it to drain off the water. The slide was then observed under X40 magnification (objective microscope lens).

Characteristic and Identification of Isolates: Various biochemical tests were carried out for the identification of isolates. These include gram stain, catalase, oxidase and fermentation tests.

Biochemical Tests for Characterization

Oxidase Test: This test was carried out to determine the presence of oxidase (an enzyme) in the isolates with the ability to catalyze the transport of electron between a redox dye 1% tetramethyl par-a-phenylene diamine hydrochloric acid and electron in the bacteria.

Catalase Test: This was carried out to show if bacteria isolate has peroxidase enzyme which can breakdown hydrogen peroxide into water and oxygen. The reagent used was hydrogen peroxide solution (3%). A little quantity of the organism isolated was taken with a sterile wire loop and placed on a clean glass slide to carry out the test. A drop of hydrogen peroxide was added to the isolate, the observation of bubbles indicated a positive result while a negative result indicated no bubbles H_2O-H_2O.

Sugar Fermentation: The peptone, water, 1% of volume of phenol red indicator was added. This was portioned with 250ml conical flask and 15ml of the different sugar glucose, sucrose, lactose, and mannitol added into different conical flasks. This was then dispersed into test tubes and sterilized at 110^0C for 10 minutes. It was then allowed to cool and inoculated with the test organism and incubated at 37^0C for 48 hours.

Acid production of gas in the upper part of inverted Durham tube indicated positive result and a negative fermentation was shown by no gas production and no color change.

Results and Discussion

Soil Physical Properties: The results obtained from the soil physical analyses are given in Table 1 which shows the physical characteristics of the soil. The texture of the soils varied from sandy loam to loam with generally high silt content and low in clay. That is, the values of clay ranged from 1.0-19.0%, silt ranged between 8.7-37.7% and sand ranged between 52.3-85.3%. The value of clay in Ikang estuary was 1.0%, Atimbo contained 6.0%, Unical Creek contained 17% clay. Ikang, Atimbo, Unical Creek A & B contain 37.7, 8.7, 28.7 and 29.7% silt respectively, and the percentage of sand from Ikang, Atimbo, Unical Creek A and B were 61.3, 85.3, 52.3 and 53.3% respectively. The textural classes of each estuary soils sandy loam, loamy sand, loam and loam respectively. This according to Odunuga et al., (1996) shows that the soils were hydromorphic gley, indicating that the soils were highly sticky and plastic when wet and hard and cracking when dry.

Table 2 shows that chemical properties of the soil. The pH values of the samples varied from 3.8-5.6, with a mean value of 4.9. The pH values of Ikang, Atimbo, Unical Creeks A and B were 3.8, 4.7, 5.5 and 5.6 respectively. According to soil pH classification table by Landon (1990), the soil pH varied from extremely acid to strongly acid. Landon (1991) observed that, the low pH values of the samples indicates that the soils were under reduced condition in the field and that such soil contained pyretic materials (FeS_2) which undergo oxidation from Iron III and sulphuric acid (H_2SO_4) which caused the extreme acidity.

In Table 2, the values of organic carbon content varied between 2.51-3.59g/kg. Organic carbon in Ikang, Atimbo Unical Creeks A and B estuaries were 3.59g/kg, 2.5g/kg, 3.49g/kg and 2.79g/kg respectively. This might be attributed to slow decomposition rate of litter fall, fibrous mangrove rootlets and probably slow rate of silting under water-logging condition of the soil (Brinkman & Blokhius, 1980) and probably because of the ability of clay fraction to hold and retain humus.

Table 2 also shows the total nitrogen content of the soil sample. The values of the total nitrogen varied from 0.2-0.3g/kg with the variation within the samples indicated by CV of 14.5%. Ikang estuary soil had total nitrogen of 0.31g/kg, Atimbo estuary soil contained total nitrogen of 0.22g/kg, Unical Creek A had 0.30g/kg total nitrogen and Unical Creek B 0.24g/kg total nitrogen. According to the rating by Landon (1991), the total N of the soil

studied is medium. Standard rating is shown below:

% of the soil wt	**Rating**
>10	very high
0.5-1.0	high
0.2-0.5	medium
0.1-0.2	low
<0.1	very low

The medium level of total nitrogen content may occur as a result of nitrogen-fixing bacteria not being available to increase the total nitrogen content of the soil and this will affect fertility level of the soil. Ecologically, useful nitrogen comes from the fixation of atmospheric nitrogen gas (N_2) by small group of plants and microorganisms that can convert it into organic form.

Wetlands featured prominently in returning a part of this excess nitrogen to the atmosphere via denitrification. Denitrification requires the prevalent conditions of aerobic and anaerobic (reducing) environment, such as the surface of a marsh as well as a source of organic carbon, usually abundant in most wetlands Akpan-Idiok and Esu, 2001).

In Table 2, it also shows values of available phosphorus which ranged from 23.1-64.8mg/kg with mean value 37.2mg/kg. Available Phosphorus of Ikang estuary soil was 24.50mg/kg, Atimbo estuary had 64.750mg/kg available Phosphorus, Unical Creek A contained 23.125mg/kg available Phosphorus and the available Phosphorus of Unical Creek B was 33.500mg/kg. According to the rating of available phosphorus by Landon (1990), it indicates that the studied soils were low in Phosphorus exception of the soil sample gotten from Atimbo estuary which is medium. The standard rating for available Phosphorus is as follows:

34mg/kg	low
34-68mg/kg	medium
>68mg/kg	high

This result supports Freney, (1967) that available Phosphorus is generally low in wetland soils and neutral waters.

Table 3 shows the exchangeable cations of the soil. This included Calcium, Magnesium, Potassium, Sodium, Aluminium and Hydrogen ions. The values of Calcium ranged from 3.0-9.4cmol/kg with a mean value of 6.95cmol/kg. Standard rating of Calcium is shown below:

>10cmol/kg	high
<4cmol/kg	low

From the rating above, Ca available in the soil was low.

The values of Exchangeable Magnesium ranged from 5.8-23.6cmol/kg. This indicated that the exchangeable Magnesium is high. Standard rating is as follows:

Low	<0.03cmol/kg
Medium	0.03-1.0cmol/kg
High	>11.0cmol/kg

Table 3, also shows the values of sodium (Na) which ranged from 0.10-0.13cmol/kg. Ikang, Atimbo, Unical Creek A and Unical Creek B had 0.13cmol/kg, 0.11cmol/kg, 0.10cmol/kg and 0.12cmol/kg sodium respectively. The rating of exchangeable Na is as follows:

Low	<0.1cmol/kg
Medium	0.1-0.3cmol/kg
High	>0.3cmol/kg

This indicates that the values of exchangeable Na varied from low and medium.

The exchangeable potassium (K) values were also shown in Table 3. The values ranged from 0.14-0.29cmol/kg with a mean value of 0.21cmol/kg. Ikang, Atimbo, Unical Creek A and Unical Creek B contained 0.29cmol/kg, 0.14cmol/kg, 0.19cmol/kg and 0.21cmol/kg K respectively. This indicates that exchangeable K content of the studied soil varied from low to medium in rating using (Singh, 1997) indicator:

Low	0.5cmol/kg
Medium	0.15-0.30cmol/kg
High	>0.30cmol/kg

Since most of the exchangeable bases are low and the studied soils were waterlogged, this indicates that, the acidity of the soil was contributed by these exchangeable bases through loses by leaching the bases. This also indicated that the soil will not be fertile enough for crop production unless these are being replaced.

Table 3 also shows the effective cation exchange capacity (ECEC). The values ranged from 11.69-38.38cmol/kg with a mean value of 24.90cmol/kg. ECEC suggest there was sufficient cation exchange capacity to prevent serious leaching. ECEC is rated as follows:

Very low	<5cmol/kg
Low	5-15cmol/kg
Medium	5-25cmol/kg
High	25-40cmol/kg
Very high	>40cmol/kg

From the above rating, the studied soil varied from low to high ECEC. This is an important reaction in soil fertility, in correcting soil acidity or basicity, in changes altering soil physical properties, and as a mechanism for purifying or altering percolating waters.

Base saturation percentage values were between 77.4-94.7% with a mean value rated as follows:

<20%	low
20-60%	medium
>60%	high

From the rating above, the base saturation percentage of the samples was high.

Results presented in Table 4, shows the microbial count for bacteria. In estuary (1-3), the mean total of the bacterial count for each estuary was as follows: Estuary site 1 (Ikang), 10 x 10^6 CFU/g, Estuary site 2 (Atimbo) 33 x 10^6 CFU/g and Estuary site 3 (Unical Creek), 79 x 10^6 CFU/g. In Estuary site 4 (Unical Creek), no organism was identified during the bacterial count.

Soil microorganism are influenced by various factors, which include: (1) fertility level, (2) moisture content, (3) soil air, (4) temperature, (5) organic matter, (6) H-ion concentration and (7) cultural factors. The availability of N, P and K for plant growth determines the fertility level of soil, and the same elements are also required by the soil

microorganisms. This study outcome suggests the N, P and K levels in the soils were not high; this influenced and explained the low microbes' population in the studied soils. This result is consistent with Rangaswami and Bagyaraj (1993).

Table 5, shows the morphological and biochemical characteristics and identification of bacterial isolates from the estuaries site samples. Three different isolates were isolated from these mixtures. The colonial morphology of various isolates and their corresponding staining reactions, in addition to the biochemical test carried out revealed the identity of the organisms. One bacterium was isolated from each estuary. One gram negative and two gram positive bacteria were isolated. The gram negative bacteria isolated were *Cyanobacterium spp*, while the gram positive bacteria isolated includes *Nocardia spp* and *Noctoc spumigena*. This study outcome did not support Rangaswami and Bagyaraj, (1993) that observed that most of the estuarine bacteria are gram negative as this study found only one gram negative bacteria isolated.

Table 6, shows the microbial count for different fungi. Estuary site 3 has the highest microbial count of 22 x 10^3. In this study the population of bacteria is higher than that of fungi. This contrast the finding of Rangaswami and Bagyaraj (1993) that fungi thrive better than bacteria in acid soils.

Table 7, shows the morphological characteristics and identification of fungal isolates from Estuaries. The probable organisms identified through morphological characteristics were as follow: *Penicillum spp*, *Aspergillus flavus, Fusarium spp* and *Rhizopus spp*.

The effect of *Penicillium* on the estuary was that, it acted as a degrader and the *Aspergillus* acted as a parasite. It was observed that estuarine fungi were involved in decay of wood and leafy material and also caused diseases of plants and animals.

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Table 1: Physical characteristics of soil in Calabar estuaries.

Samples	Clay (%)	Silt (%)	Sand (%)	Textural Class
Ikang (1)	1.0	37.7	61.3	Sandy loam
Atimbo (2)	6.0	8.7	85.3	Loamy sand
Uncial Creek A (3)	19.0	28.7	52.3	Loam
Unical Creek B (4)	17.0	29.7	53.3	Loam
Mean	10.75	26.20	63.05	
Range	1.0-19.0	8.7-37.7	52.5-85.3	
SD	8.65	12.34	27.04	
CV	80.47%	47.10%	42.89%	

Table 2: Chemical properties of the soil.

Samples Soil	Organic reaction (pH)	carbon (g/kg)	Total Available Nitrogen (g/kg)	phosphorus (mg/kg)
Ikang (1)	3.8	3.59	0.31	27.500
Atimbo (2)	4.7	2.51	0.22	64.750
Unical Creek A (3)	5.5	3.49	0.30	23.125
Uncial Creek B (4)	5.6	2.79	0.24	33.500
Mean	4.9	3.10	0.27	37.22
Range	3.8-5.6	2.51-3.59	0.22-0.31	23.125-64.750
SD	1.45	0.53	0.04	3.263
CV	29.59	17.10%	14.81%	87.67%

Table 3: Exchangeable cations.

Samples (%)	Ca^{2+}	Mg^{2+}	K^{+}	Na^{+}	H^{+}	Al^{3+}	ECEC	BS
	Mg/kg	mgl/kg	mg/kg	mgl/kg				
1	9.0	23.0	0.29	0.13	3.44	1.92	38.38	86.0
2	3.0	5.8	0.14	0.11	1.20	1.44	11.69	77.4
3	6.4	12.2	0.19	0.10	1.48	1.16	21.53	87.7
4	9.4	16.8	0.21	0.12	1.20	0.28	28.01	97.7
Mean	6.95	14.60	0.21	0.14	1.83	1.20	24.90	86.45
Range	3.0-9.4	5.8-23.6	0.14-0.29	0.10-0.13	1.20-3.44	0.28-1.92	11.69-38.38	77.4-94.7
SD	2.95	7.51	0.06	0.03	1.08	0.69	11.21	7.11
CV	42.44	51.43	28.57	21.42	59.02	57.5	45.02	8.22

Table 4: Microbial court of estuaries.

Samples	Bacterial count (dilution factor) (CFU/g)	Mean total
Estuary site 1	13×10^6, 7×10^6	10×10^6
Estuary site 2	49×10^6, 17×10^6	33×10^6
Estuary site 3	102×10^6, 56×10^6	79×10^6

Table 5: Characteristics and Identification of Bacterial Isolates from Estuaries

Isolates	Colonial morphology	Shape	Biochemical Test			Sugar Fermentation				Probable Organisms
			Gram reaction	Catalase	oxidase	Glu	Lac	Malt	Suc	
E_1	Bluish colonies	Cocci in clusters	-ve	+ve	+ve	A	A	A	A	*Cyanobacterium Spp*
E_2	Reddish colonies	Cocci	+ve	+ve	-ve	A	A	A	A	*Nocardia Spp*
E_3	Round blue colonies	Cocci	+ve	+ve	+ve	A	-	A	A	*Nostoc spumigena*

Keys: +ve—Positive; -ve—Negative; A—Acid; E1-E3—Estuary sites 1-3; Suc—Sucrose; Man—Mannitol and Lac—Lactose

Table 6: Microbial Count Estuaries

Samples	Fungi count/Dilution factors (cfu/g)
E_1	$17x10^3$
E_2	$8x10^3$
E_3	$22x10^3$
E_4	$19x10^3$

Table 7: Morphological Characteristics and Identification of Fungi Isolates from Estuaries

Isolates	Colonial morphology	Nature of hyphae	Colour of hyphae	Appearance of sporangiospores or conidiophores	Arrangement	Spores	Probable organism
E_1	Small round green colour with smooth appearance	Septate	Green	Single long conidiophore	Single	Tiny spherical spores scattered around	*Penicillium spp*
E_2	Yellowish green colonies with smooth appearance	Septate	Yellow	Elongate conidiophore	Intertwine arrangement	Small round spores crowded	*Aspergillus flavus*
E_3	Pink round colour with smooth appearance	Non-septate	White	---	Single	Small oval spores	*Fusarium spp*
E_4	Wavy white colony with round shape	Non septate	White	Group of long sporangiophore	Single	Oval scattered around	*Rhizopus spp*