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Microbial Ecological Dynamics, Load and Diversity of Sculptured Artworks in Cross River University of Technology Outdoor Gallary

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A B S T R A C T

The microbial ecology of an outdoor artwork gallery was evaluated using basic microbiological standardize techniques. The sculptures made up of wood, cement and cast metal had a mean heterotrophic bacterial count of; wood (3.2×10^4), cement (4.3×10^4) and metal (2.5×10^4) while the fungal count were wood (4.6×10^4), cement (2.7×10^4) and metal (1.8×10^4) colonies forming unit per milliliter of the scrapped solutions. The eight (8) bacterial genera and their frequency of occurrence obtained were; *Bacillus* sp (75%), *Pseudomonas* sp (74.3%), *Enterobacter* sp (71.4%), *Micrococcus* sp (67.1%), *Flavobacterium sp* (54.3%), *Acinobacter* sp (51.1%), *Sarcina* sp (50%) and *Cyanobacterium sp* (32.9%). The eight fungal genera obtained were: *Aspergillus* sp (97.1%), *Penicillium* sp (87.1%), *Fusarium* sp (78.6%), *Cryptococcus* sp (68.6%), *Rhizopus* sp (62.9%), *Alternaria* sp (58.6%), *Mold* sp (52.9%) and yeast (45.7%). Statistically, there was no significant ($p>0.05$) difference between the bacterial and fungal occurrence. However, a significant ($p>0.05$) difference was observed among the sculptured materials: for bacteria; cement > wood > metal while for fungi; wood > cement > metal. The enhanced colonization rate and growth of the microbial isolates correlated ($r=0.01$) positively to the sampled materials as substrates, physicochemical properties of the study site and the presence of the cations and anions as means of electron transport. The presence of these species of bacteria and fungi promote serious deterioration problems to the aesthetic values of these sculptures. Therefore, empirical study targeting the control and preservation of these artworks with natural and environmentally friendly materials is suggested.

Introduction

Art in form of sculpture are primarily developed for aesthetic beautification of environment and documentation of history (Urzi, 2001). The constant exposure of artwork in urban public outdoor galleries is a risk to environmental hazardous element. This includes climatic pollution and biological agent with a reluctant role in the deterioration cultural heritage aesthetic values (*Ameotral*, 2012).

The biodeterioration of these sculptures and other artifacts is the result of interaction between living organisms especially microorganisms, materials and environmental condition (*Capadicasa et al.,* 2010). Therefore the growth of microorganisms on artworks involved into main factors, the chemical composition of the object and the environmental factors (*Lopez-miras et al.,* 2013).

Fungi involved in biodeterioration represent the group of chemoheterotrophs characterized by the presence unicellular or multicellular hyphae and are metabolically versatile (Pinar *et al.,* 2013). The most diverse species of fungi associated with deterioration of artwork are: *Alteruaria, Clasdosporium, Sarcromyces, Peuicillium, ASpergillus, Acremorium, Oidiodendron, Gloeophyllum and Phialophora.* The fungi versatility allows them to colonize on a wide range of substrates such as wood, limestone and metal enhanced by extreme environmental condition and the mutualistic association with cyanobacterium and algae (Pinor *et al.,* 2011; Dakal and Cametro, 2012).

The heterotrophic bacterial species that colonize environmentally exposed artworks nutritionally belongs to three groups; photoautotroph, chemolithoautotrophs and chemorganistroph. Among the phototrophs and chemorganistrophs are the cyanobacterial sulphoxidizing and nitrifying bacteria (Dakal and Camotro, 2012). These bacteria genera include; *Alcalegenes, Bacillus, Pseudomonas, Flavobacterium, Micrococcus, Sarcina, Mycobacterium Streptomyces, Staphylococus, Arsthrobacter, and Paenibacillus* (*Jurado et al.,* 2012, *Ertenauer et al.,* 2013). These groups of bacteria known for their simple nutritional and ecological needs developed, proliferate and colonized monument faster than other groups. They are responsible for the irreversible damage to major artwork, sculpture and monuments. Among them cyanobacterium have the ability to survive under the condition of repeated drying and rehydration occurring on exposed artwork surfaces and to protect themselves from harmful ultraviolet (UV) radiation by synthesizing protective pigments (*Rispim et al.,* 2003).

Environmental physiochemical factors that enhanced colorization of microorganism on artwork and monument includes; Temperature, composition of the material, pollutants, humility, light and wind (Nuhoglulet *et al.,* 2006; *Lan et al.,* 2010). High relative humility in the air and high temperature provides moisture needed to encourage deteriorating chemical reaction and microbial growth. Light accelerates both cumulative and irreversible deterioration of artwork and monument (Grossi *et al., 2006*). Pollutants in the air contribute significantly to the deterioration of archival artwork. The atmosphere contains lots of pollutants of different origin. Persistent air pollutants of industrial cities like oxides, sulphur, nitrogen, and some carbonaceous particles, ash and other particulate matter upon setting on the surface of the artwork, destroy their aesthetic and artistic beauty (Nuhuglu *et al.,* 2006). This research work, therefore aimed at investigating and establishing the microbial dynamics of exposed outdoor artwork in Cross River State University of Technology and to suggest empirical measure to control their menace.

Materials and Methods

Sample selection

Sculptured status of three years old, made out of wood, cement stone and metal were selected for the microbial investigation. The materials chosen had been exposed to both physical and bio-weathering conditions within this period. The study was conducted between the transitional period of the year between the setting of the wet months and the onset of the dry months. This period of the year is characterized by minimum rainfall, ambient temperature, low moisture, adequate sunshine and wind.

Sample collection and microbial isolation

The method described by Cheesbrough (2000) and earlier adopted by () was used. The various objects selected were swapped with sterile swap sticks and spatula. The swapped materials and particles collected from respected objects were aseptically put in 50mLtryptophane broth supplemented with physiological saline (0.8%w/v) solution in a 100mL capacity sterile flasks. Then transported in an ice-packed cooler to the laboratory for analysis and microbial assay.

Determination of the physicochemical properties of the sculptured materials

The physical and qualitative chemical properties of the gallery were determined as described by Anne (2019).

The pH of the swapped solution was measured with a pH meter (SANXON PHB-3 pH meter, China and Jenway)

Mean temperature of the air circulation around the gallery side was taken by hanging the thermometer in strategic position within the site for morning, afternoon and evening.

The amount of moisture in the air was equally determined with a moisture strip () placed around the objects.

The qualitative presence of the cations and anions in the sampled object were determined as described by APHA (1998).

Procedures for each of the ions were adopted accordingly by Royal society of chemistry; www.rse.org (downloaded) and wired chemist: www.wiredchemist.com > chemistry. Traces of the ions per sample were considered as being present and or not present after treatment with:

Fe^{3+} precipitated (ppt) in 0.1M H_2s solution at pH 9.

Ca^{2+}, k^+, Na^+ ppt in 0.2M $(NH4)_2$ CO_3 at pH 10 while other ions were soluble

NO^-_3, PO_4^{3-}, CO^-_3, SO_4^{2-} ppt in 0.1M H_2S at pH 5.

Bacterial isolation, heterotrophic load and identification

The sample for bacterial isolation was cultured on nutrient agar (brand) supplemented with a combination of a broad spectrum antibiotic to inhibit the growth of fungal contaminants as described by ().

A ten-fold serial dilution of the sample was made and 1.0mL of 10^{-4}diluents was spread plated in triplicate. The plates were incubated at room temperature (28±2^{o}C) for 18h. The heterotrophic colonies were counted and recorded as CFUmL^{-1} of the representative samples. Discrete colonies were picked and subcultured on MaConkey, sorbitol, etc for growth. Then bacterial isolates obtained were identified based on their; Cultural and morphological growth characteristics, Gram reaction, biochemical and sugar fermentation properties as described by Cooke (1987).

Fungal isolation, heterotrophic load and identification

The method described by () was adopted. The samples were respectively cultured on potato dextrose agar (brand name) fortified with antibacterial agent (chloramphenicol/ streptomycin) to inhibit the growth of bacterial contaminants. A ten-fold serial dilution of the sample was made and 1.0mL of 10-4diluents was aseptically spread plated in triplicates. Then the plates were incubated at room temperature (28±2^{0}C) for 72h. The mixed colonies developed were counted and recorded as colony forming units (CFUmL^{-1}) per millilitre of the representative samples.

Discrete colonies were picked and subcultured on Sabouraud agar (brand name) and Rose Bengal agar for confirmed growth and characterization. The obtained isolates were identified on the basis of their cultural, microscopic and morphological examination and characterization of colony colour, pigments, types of hyphae, aerial hyphae, substrate hyphae, mycelium, asexual and sexual spores conidiospores and sporangiospores as described by Barnett and Hunter (1998).

Statistical analysis

Data collected were analyzed using IBM SPSS statistic version 20 (IBM corporation, 2011). Simple means, percentages and frequencies were computed. Also means were computed using Analysis of variance (ANOVA) to determined associations.

Results and Discussion

Table 1 shows the qualitative ecological bio-physicochemical parameters of the three samples. The temperature was seen to fluctuate between 28^{o}C to 30^{o}C. The atmospheric moisture and pH of the objects were considered low, moderate and high with no tangent difference. The cations (Na^+, K^+, Cu^{2+}, and Ca2$^+$) and anions (NO_3^-, CO_3^-, PO_4^{3-}, and SO_4^{2-}) were qualitatively considered as being absent (Nil) or present. The metal objects were of cast materials that were made of little or no quantified cations and anions composition. However cement based sculptures contained more of the cations and anions than metal and wood.

Table 2 shows the total heterotrophic bacterial and fungal contaminants count per sample material. The wood sample had the highest fungal load (4.6 x10^4) while the cement base sample had the highest bacterial load (4.3 x 10^4).

The total bacterial count from all the samples was 10.0 x 10^4 CfumL^{-1} more than fungal load of 9.1 x 10^4 CfumL$^{-1.}$ Similarly, the metal sample had the lowest bacterial count (2.5x104 CfumL^{-1}) and fungal count (1.8x10^4 CfumL^{-1}) with no significant difference in the load of both bacterial and fungal counts.

Table 3 indicates the bacterial contaminant isolated and their frequency of occurrence from each sample. Eight (8) bacterial genera were isolated and *Bacillus* sp (75.7%) was the most isolated contaminant followed by *Pseudomonas* sp (74.3%) while *Cyanobacterium* sp was the least isolated with a frequency of 32.9%. However, *Enterobacter* sp was not isolated from cement, *Flavobacterium sp* from wood and *Sarcina* sp from metal (Table 4).

Table 5 shows the distribution and frequency of bacterial isolates from the three samples. High prevalence of *Bacillus* sp (50%), *Pseudomonas* sp (38.2%) and *Micrococcus* sp (19.3%) were observed with a significant ($p<0.5$) difference to *Enterobacter* sp (4.9%), *Acinetobacte r*sp (6%) and *Sarcina* sp (6%). *Cyanobacterium* sp (8%), *Flavobacterium* sp (4.5%). Table 6 shows the distribution and frequency of fungal isolates in the three sampled materials. *Aspergillu s*sp (37.8%), *Penicillium sp* (30.2%) and *Fusarium sp* (30.7%) were observed with a prevalence significantly ($p>0.5$) difference to *Alternaria* sp (17.6%), *Rhizopus* sp (14.3%), *Mold* sp (11.1%), Yeast (4.9%) and *Cryptococcus sp* (3.8%).To be continued

The biophysicochemical nature of the gallery is considered as a conducive condition for enhance microbial growth. The presence of adequate moisture (humidity), temperature and pH promote the high ecological microbial diversity and heterotrophic number of the isolates. In a similar study Etim and Antai (2014) demonstrated that adequate pH and temperature encourage the growth and colonization of bacteria and fungi in painted art objects. Therefore, the high microbial load and diversity is considered a consequence of these environmental conditions as different cells exhibit different response to changes in temperature and pH.

The presence of the cations and anions as part of the sample composition in contact with the moderate moisture enhances the growth, diversity and subsequent colonization of the sculptures. The sources of these ions are considered a function of both anthropogenic and bio-weathering activities. Haavisto (2002) and Luciana *et al.*, (2004) has opined that metals and heavy metals had increased rapidly as a result of metal contamination in soil, waste treatment plant and wood impregnation plants. Secondly, these ions (K^+, Na^+, Fe^{3+}, Ca^{2+}, Cu^{2+}, NO_3^-, SO_4^{2-}, CO_3^-) in the environment play an integral role in the life processes of these microbial isolates. Bruins *et al.*, (2000) had suggested that these ions serve as essential micronutrients to microorganisms. The author submitted that ions are used for redox processes, molecules stability through electrostatic interactions, as components of various enzymes and for the regulation of osmotic pressure. This result therefore in concert with the above information, shows that the high bacterial and fungal heterotrophic load, speciation and frequencies of occurrences to a larger extend had been a factor of the ecological parameters of the exposed gallery and the composition of the sculpture materials.

The high numbers of heterotrophic bacterial and fungal count is considered the effect of substrate composition. The wood with a high cellulolistic organic matter had high of fungi (4.6×10^4 $CfumL^{-1}$) than bacteria (3.2×10^4 mL^{-1}) the bacterial load appeared higher in the cement based sculptures than fungal load of ($2.7\times10^4 CfumL^{-1}$). Similarly, the metal works had more bacterial load ($2.5\times10^4 CfumL^{-1}$) than fungi ($1.8\times10^4 CfumL^{-1}$).

Table.1 shows some biophysicochemical properties of the samples.

Parameters	**Wood**	**Cement**	**Metal**
Temperature	28±3°C	29±1°C	30±2°C
Moisture	Moderate	High	Moderate
pH	High	High	Low
Na^+	Nil	Present	Nil
K^+	Nil	Low	Low
NO_3^-	Present	Present	Present
PO_4^{3-}	Present	Present	Present
CO_3^-	Nil	Present	Nil
Ca^{2+}	Nil	Present	Nil
SO_4^{2-}	Present	Present	Nil
Cu^{2+}	Nil	Nil	Present

Table.2 Mean total heterotrophic count for bacteria and fungi per sample material.

Sample material	Sample size (n=70)	Bacterial isolates (cfumL^{-1})	Fungal isolates (cfumL^{-1})
Wood	25	3.2 X 10^4	4.6 X 10^4
Cement base	30	4.3 X 10^4	2.7 X 10^4
Metal	15	2.5 X 10^4	1.8 X 10^4
Total	70	-	-

Table.3 Frequency of bacterial isolates in wood, cement and metal samples.

Bacterial isolates	No. of sample (N=70)	Frequency of occurrence (%)
***Pseudomonas* sp**	52	74.3
***Enterobacter* sp**	50	71.4
***Acinetobacter* sp**	40	51.1
***Bacillus* sp**	53	75.7
***Micrococcus* sp**	47	67.1
***Flavobacterium* sp**	38	54.3
***Cyanobacterium* sp**	23	32.9
***Sarcina* sp**	35	50.0

Table.4 Frequency of fungal isolates in wood, cement and metal samples

Fungal isolates	No. of sample (N=70)	Frequency of occurrence (%)
***Aspergillus* sp**	68	97.1
***Penicillium* sp**	61	87.1
***Fusarium* sp**	55	78.6
***Cryptococcus* sp**	48	68.6
***Rhizopus* sp**	44	62.9
***Alternaria* sp**	41	58.6
***Mold* sp**	37	52.9
***Yeast* sp**	32	45.7

Table.5 Distribution and frequency of bacterial species from different sample materials

Bacterial species	Collection site/sample			
	Wood (n=25)	Cement(n=30)	Metal(n=15)	Total(n=70)
Bacillus sp	15(60)	13(43.3)	7(46.7)	45(50)
Pseudomonas sp	12(48)	8(26.7)	6(40)	26(38.2)
Enterobacter sp	2(8.0)	0(0.0)	1(6.7)	3(4.9)
Acinetobacter sp	2(8.0)	1(3.3)	1(6.7)	4(6.0)
Micrococcus sp	7(28)	5(16.7)	2(13.3)	14(19.3)
Flavobacterium sp	0(0.0)	2(6.7)	1(6.7)	3(4.5)
Sarcina sp	2(8.0)	3(10)	0(0.0)	5(6.0)
Cyanobacterium sp	1(4.0)	4(13.3)	1(6.7)	6(8.0)

Table.6 Distribution and frequency of fungal species from different sample materials

Fungal species	Collection site/ sample material			
	Wood (n=25)	Cement(n=30)	Metal(n=15)	Total(n=70)
Aspergillussp	10(40)	12(40)	5(33.3)	27(37.8)
Penicillin sp	6(24)	8(26.7)	6(40)	20(30.2)
Fusariumsp	8(32)	6(20)	6(40)	20(30.7)
Cryptococcus sp	2(8.0)	1(3.3)	0(0.0)	3(3.8)
Rhizopussp	5(20)	3(10)	2(13.3)	10(14.3)
Alternariasp	4(16)	5(16.7)	3(20)	12(17.6)
Mold sp	5(20)	2(6.7)	1(6.7)	8(11.1)
Yeast sp	2(8.0)	0(0.0)	1(6.7)	3(4.9)

The bacteria speciation showed that *Bacillus* (75.7%) dominated the artworks, followed closely by *Pseudomonas sp* (74.3%) and *Enterobacter sp* (71.4%). However, their differences were not statistically significant ($p>0.05$) compared to those with a percentage frequencies less than 70%. A report similar to this study had acknowledge that *Pseudomonas* sp, *Bacillus* sp, *Micrococcus* sp and *Enterobacter* sp are soil and organic waste borne bacteria. Their high incidence of occurrence could be attributed to their exposure to human and the inanimate objects considered in this study (Sterflinger and Pinar, 2013), (Duan *et al.*, 2017).

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