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MICROBIAL DETERIORATION OF SURFACE PAINT COATINGS.

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ABSTRACT

Bacterial and fungal species associated with the normal and deteriorated painted surface in Owerri, Imo State were isolated and identified. The bacteria genera isolated were *Pseudomonas, Bacillus, Micrococcus, Staphylococcus, Enterobacter* and *Streptomces,* whereas the fungal genera isolated were *Rhizopus, Penicillium, Cladosporium,. Aspergillus, Alternaria, Fusarium,* and *Curvularia.* More bacterial and fungal isolates were obtained from biodeteriorated surfaces than normal painted surfaces. The occurrence of these microbial isolates in normal : deteriorated painted surfaces were found to be *Pseudomonas* (90:100%), *Bacillus* (80:100%), *Rhizopus* (60:100%) and *Aspergillus flavus* (50:90%) *Penicillium, Staphylococcus, Enterobacter, Aspergillus niger, Cladosporium, Alternaria Streptococcus, Fusarium, Curvularia* and *Micrococcus* occurred only between 20 and 70%in the biodeteriorated samples. *Bacillus sp., Pseudomonas sp., Rhizopus sp.* and *Aspergillus niger* were isolated from undeteriorated (normal) painted surfaces. Besides the organisms isolated from normal pain, *Penicillium sp., Cladosporium, Aspergillus flavus, Alternaria sp., Fusarium sp., Micrococcus sp., Staphylococcus aureus Enterobacter sp.,* and *Streptococcus sp.* were isolated in the deteriorated paint. None of the isolates was able to grow on fresh emulsion paint films. This study has established the microbial flora of deteriorated painted surfaces in the tropics.

KEYWORDS:

INTRODUCTION

Microorganisms are ubiquitous in nature and their activities are known to be influenced by factors like moisture content, temperature and pH. Microorganisms have been associated with water activity levels of 0.63 – 0.97 in view of their need for an aqueous medium for growth and proliferation (Tiller, 1982). Hull (1976) defined paints as solutions of wood, metal, oil or other article used either for protection to prevent environmental weathering or to provide a decorative finish. However, these functions may be adversely affected by the paint's susceptibility to microbial growth (Allsopp and Seal, 1986). When microbes colonize painted surfaces, they rely on nutrients provided by a gradual utilization of compounds in the material (Okpokwasili and Ituen, 1996). There is paucity of literature on the microbial deteriogens of painted surfaces in Eastern Nigeria, although it is believed that environmental factors in the area are favourable to microbial growth, Okpokwasili and Ituen (1996).

In Imo State, microbial deterioration of painted surfaces which occur both in the internal and external parts of the building pose more problems at the external part. This could be associated with favourable environmental factors and other conditions that favour such activities. The problems are known to be great in the tropics where fungi and other microbes cause defacement of paint films (Allsopp and Seal, 1986).

Studies by Okpokwasili and Ituen (1996) indicated the presence of fungi and other fouling microflora in Port Harcourt, Nigeria. However, studies by Ross and Hollis (1976) and Miller (1973) revealed that bacterial genera play more role and predominate in in – can deterioration of paints. Several microorganisms have been associated with deteriorated painted surfaces in other parts of the world.

This study was therefore designed to ascertain the microbial deteriogens of painted surfaces in Owerri, Nigeria, a tropical area in Africa.

MATERIALS AND METHOD

SAMPLE COLLECTION

Paint films (scrapings) samples of normal and deteriorated painted surfaces were collected from painted cement wall surface at different locations within Owerri Capital Territory using sterile scapel, blades and containers as described by Okpokwasili and Ituen (1996). The film colour was noted and transported asceptically to the laboratory.

MICROBIAL ISOLATION AND IDENTIFICATION.

After serial dilution with 0.1% sorbitol as described by Ogbulie *et al.* (1998), bacterial isolates were isolated by inoculating 0.1ml of the appropriate dilution into sterile oxoid nutrient media for isolation of the bacterial flora using the pour plate method described by Ogbulie *et al.* (1998). The inoculated plates were incubated at 37^{0}C for 24 hours and the isolates purified for further test. The purified bacterial isolates were examined for colonial morphology as well as their staining reactions. The bacterial isolates were further characterised by subjecting them to standard biochemical tests as described by Cruickshank, *et. al.*, (1975; 1982), Ogbulie *et al.* (1998).

Fungal flora were isolated by inoculating 0.1ml of the appropriate dilution to sterile potato dextrose agar (PDA) containing 100ug/L of chloramphenicol to suppress bacterial growth and incubated at 37^{0}C for 72 hours. A portion of the fungal colony was picked using

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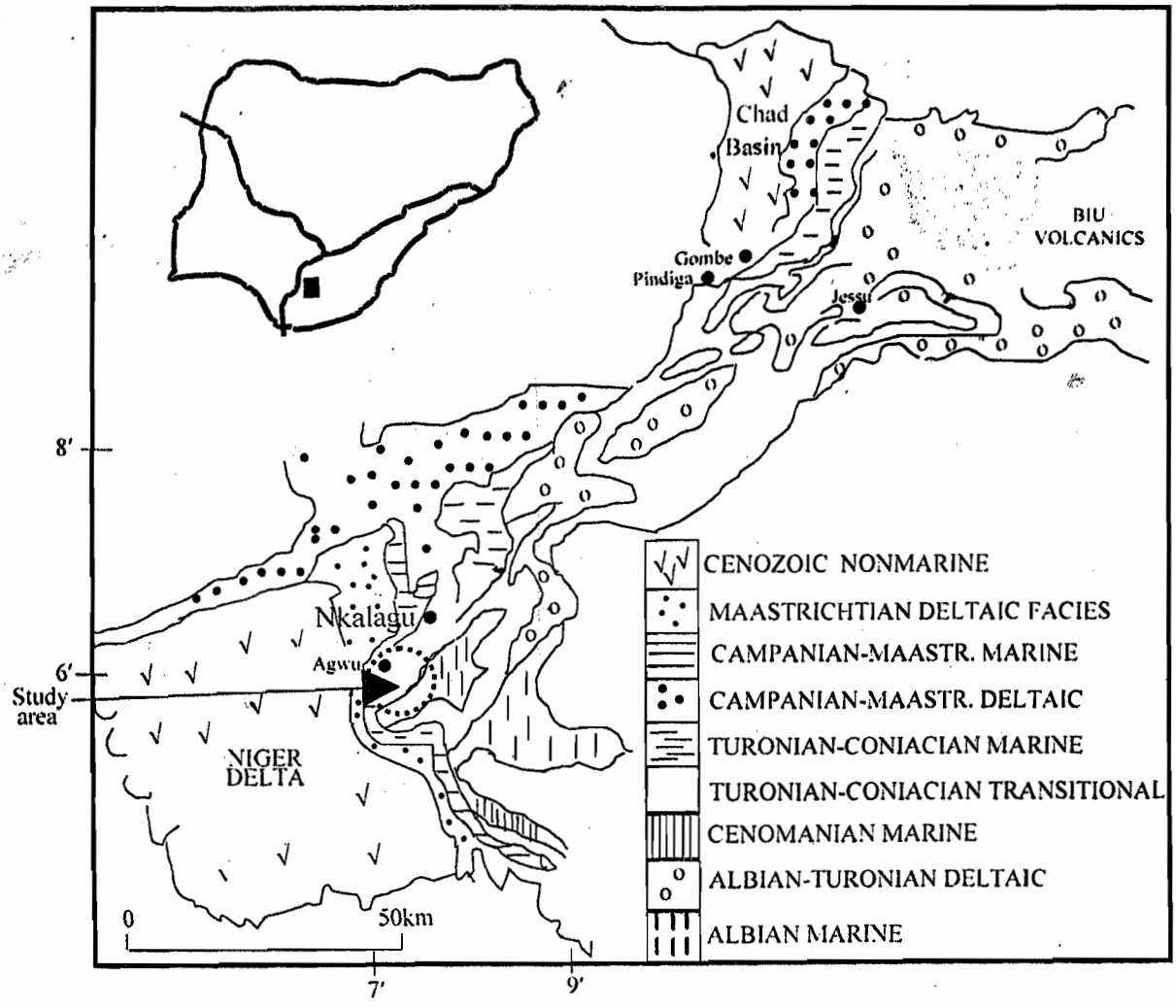

Fig. 1: Geologic map of the Benue Trough showing sample location in Anambra Basin (modified from Petters, 1978)

temperature (40-50°C) and the residue mixed with CdI_2/KI (thoulet) solution (40.0ml; S.G. 1.96). The mixture was ultrasonicated for 30 minutes and then centrifuged for one hour with the floating fractions washed and dried at relatively low temperature (40-50°C). Each sample was weighed, stored in a clean glass sample-bottle, labelled and transferred to a vacuum oven or desiccator.

Infra-red (IR) Spectra

Infra-red spectra of kerogen concentrates were obtained on a Pye Unicam SP3-300IR spectrophotometer using a KBr disc technique (Victorovic *et al.* 1981, Vassalo *et al.* 1983). All samples were carefully and consistently prepared by grinding for 25 seconds to avoid getting different results for different grinding times (Painter *et al*, 1981). For quantitative or semi-quantitative IR spectroscopy, the method of Victorovic *et al* (1981) was adopted where the contents of specific structures absorbing in various regions (band intensities) were expressed in the form of numerical values calculated in the following way:

$$a = \frac{A \times 10^2}{\%T \times \%K}$$

Where a = calculated numerical value

A = area under the band (by integration)

%T = percent transmittance

%K = weight percent kerogen content

RESULTS AND DISCUSSION

Kerogen Content

Table 1 shows the results of the kerogen content from the dry sedimentary organic matter residue. The weight of isolated kerogen concentrate varies from 0.39 to 3.00 gm, that is about 2 to 14wt % (of dry sedimentary organic matter residue). Oil-shales from Lokpanta-Lekwesi area are richer in kerogen concentrates (13.64wt %) than shales from other locations, while oil-shales from Ndeaboh-Lokpanta and Neaboh-Awgu areas contain an average of 6.0 and 8.9wt % of kerogen concentrates respectively. This variation in the content of the kerogen concentrates can be attributed to over-heating or can be explained as the mark of more or less intense oxidation-reduction

Table 2; Identification of fungal isolates.

Isolates	Macroscopic characteristics	Microscopic Characteristics	Organisms
1.	Dense colony mycelia, white, later turn gray or yellowish brown. Reverse is white.	Hyphae have very few septa, stolon run among mycelia connecting unbranched spores and many oval.	*Rhizopus sp.*
2.	Surface at first is white, later powdery bluish green with a white border. Reverse is white.	Septate hyphae with branching conidiophores. Round unbranched chain of conidia borne on sterigmata.	*Penicillium sp.*
3.	Greenish brown surface with grayish velvety nap heaped and slightly folded. Reverse is black	Hyphae septate, conidiophores are dark, branched, vary in length. Conidia from branching free - like chains.	*Alternaria sp.*
4.	Velvety surface with white colour later turning green. Reverse is yellow.	Septate hyphae, unbranched conidiophores. Vesicle completely covered by double sterigmata with chains of round and rough conidia radiating from the vesicle, but vesicle was covered by both double and single radiating sterigmata in all directions.	*Aspergillus flavus*
5.	Wooly white later turned black. Reverse side is yellowish	Septate hyphae, unbranched conidiphores, smooth with variable lenght vesicle and entirely covered by double sterigmata to form radiate head.	*Aspergillus niger*
6.	Grayish white surface and wooly, later turn greenish black with light border.	Myceluim septate and dark conidiophores, short and septate. Conidia are large, brown found singly and club - like in shape.	*Aspergillus niger.*
7.	White and cottony, later violet center with light periphery. Reverse is light in colour.	Septate hyphae, short simple conidiophores bearing small oval ones called conidia, singly.	*Fusarium sp.*
8.	Surface wooly grayish, later brown to black. Reverse is fairly dark.	Septate mycelium, conidiophores simple. Macroconidia large, dark and curved.	*Curvularia sp.*

sterile inoculating needle and subcultured in a fresh PDA plate. The fungal isolates were identified as described by Ogbulie *et al.*, (1998). The isolates were later tested for their ability to grow on freshly opened emulsion paint using the method of Okpokwasili and Ituen (1996).

pH DETERMINATION

The pH analysis was carried out by dissolving 5g of the paint chip samples in 10ml of sterile distilled water adjusted to pH 7 using sterile beaker and glass rod. The pH was thereafter determined using a pH meter. (Model Nº .291 MKS)

RESULT

The findings of this study are summarized in tables 1 to 6. Table 1 shows bacterial isolates and the characteristics used for their identification while table 2 shows the fungal isolates and the macroscopic and microscopic characteristics used for their identification. Amongst the isolates, only *Pseudomonas* and *Bacillus* species were isolated from normal painted surfaces. Table 1 shows that *Pseudomonas sp., Bacillus sp., Staphylococcus aureus, Enterobacter sp., Streptomyces sp.*, and *Micrococcus sp.* were isolated from biodeteriorated painted surfaces. On the other hand Table 2 shows that *Rhizopus sp.*and *Aspergillus niger* were isolated from normal painted surfaces. In addition, *Penicillium sp., Cladosporium sp., Aspergillus flavus, Fusarium sp., Alternaria sp.* and *Curvularia sp.*, were isolated from biodeteriorated painted surfaces.

Further studies on the frequency of occurrence of the isolates in ten replicate samples of painted surfaces with obvious biodeterioration (Tables 3 and 4) revealed that among the bacterial species, *Bacillus sp.*, and *Pseudomonas sp.* occurred in 90% and 70% of samples, while *Staphylococcus aureus* and *Enterobacter sp.* occurred in 30%, and *Streptomyces* and *Micrococcus* occurred in 40% and 20% of the

Table 3: Bacterial Isolates from Normal and Biodeteriorated Painted Surfaces:-

Micro-organisms	NPS	BPS
Pseudomonas sp.	P	P
Bacillus sp.	P	P
Staphylococcus aureus	A	P
Streptomyces sp.	A	P
Micrococcus sp.	A	P
Enterobacter sp.	A	P

Table 4: Fungal Isolates from Normal and Biodeteriorated Painted Surfaces.

Micro-organisms	NPS	BPS
Rhizopus	P	P
Penicillium	A	P
Cladosporium	A	P
Aspergillus flavus	A	P
Aspergillus niger	P	P
Alternaria sp.	A	P
Fusarium sp.	A	P
Curvularia sp.	A	P

KEY: NPS = Normal painted surfaces.
BPS = Biodeteriorated painted surfaces.
A = Absent.
P = Present.

samples, respectively. On the other hand, the frequency in the occurrence of the fungal isolates revealed that *Asergillus niger, Rhizopus sp.*, and *Penicillium sp.* occurred in 60% of the samples. *Cladosporuim sp. and Aspergillus flavus* occurred in 50%, *Altenaria sp.* and *Fusarum, sp.* occurred in 40% and 30% of the samples respectively. The least occurrence of 20% was recorded for *Curvularia* species.

The result of the growth/survival of test isolates in fresh emulsion paint samples revealed that all the isolates were unable to grow or cause any obvious change (deterioration) on the fresh paint samples used.

DISCUSSION

This investigation revealed the association of both bacterial and fungal isolates with biodeterioration of painted surfaces. Six genera of bacteria belonging to the genera *Pseudomonas, Bacillus, Staphylococcus, Streptomyces, Enterobacter,* and *Micrococcus,* and eight species of fungi namely *Rhizopus sp., Penicillin sp., Cladosporium sp., Aspergillus flavus, Aspergillus niger, Alternaria sp., Curvularia sp.*, and *Fusaruim sp.* were isolated from painted surfaces. It was evident in this study that high populations of microbial genera was associated with the deteriorating painted than normal painted surfaces. This particular trend in the diversity of isolates clearly shows that the painted surfaces were undergoing biodeterioration. This is process of microbial growth and proliferation in such an environment might have been enhanced by increased metabolic exudates, which could facilitate the growth of other transient microbial population. It is apparent that the high number of microorganisms associated with deteriorated surfaces might have resulted from the break down of the biocide and/or other antimicrobials in the paint film by the primary colonizers thereby making the subsequent colonization of the surface by other microbes not only easy but rapid. It has been reported elsewhere (Seal and Eggin, 1981; Gilbert and Loveluck, 1975) that apart from the biocide, other ingredients such as resin, oil, emulsifiers, wetting agents, cellulose, plasticizers etc., are subject to microbial attack. This makes the direct attack on the paint possible. Microorganisms isolated from the painted surfaces are similar to those reported by other workers (Drescher, 1958 and Ross, 1980).

The consistent isolation of these groups of organisms indicates that they are the characteristic biodeteriogens of the material in question. This does not mean that all the isolates contribute significantly to the biodeterioration process as some may be mere contaminants. Nevertheless, the high number of bacteria and fungi isolated in the painted surfaces could be as a result of more available nutrient in the material as reported elsewhere (Brierley *et al.,* 1985). Furthermore, the bacteria isolated from the material in question are similar to those listed by Okpokwasili *et al.* (1996) with the exception of *Streptomyces sp.* The resistant spore – forming nature of *Bacillus sp.* could be responsible for its dominance. The fungal species are also similar to those

Table 5: FREQUENCY OF OCCURRENCE OF BACTERIA ISOLATES FROM NORMAL AND BIODETERIORATED PAINTED SURFACE.

Micro-organisms	NNS	NBS	%NS	%BS
Pseudomonas sp.	70	100	7	100
Bacillus sp.	80	100	9	100
Staphylococcus aureus	-	70	5	70
Streptomyces sp.	-	40	4	40
Micrococcus sp.	-	20	2	20
Enterobacter sp.	-	50	5	50

100 normal and biodeteriorated samples were collected for the study

Table 6: FREQUENCY OF OCCURRENCE OF FUNGAL ISOLATES FROM NORMAL AND BIODETERIORATED PAINTED SURFACE.

Micro-organisms	NS	NBS	%NS	%NBS
Rhizopus sp.	60	100	60	60
Penicillium sp.	50	90	50	60
Cladosporuim sp.	-	70	-	50
Aspergillus flavus	-	50	-	50
Aspergillus niger	-	50	-	50
Alternaria sp.	-	40	-	40
Fusarium sp.	-	30	-	30
Curvularia sp.	-	20	-	20

100 Normal and biodeteriorated painted samples were used for the study

KEY: NS = Number of isolates in normal samples.

NBS = Number of isolates in the biodeteriorated

% = Percentage.

P = Present.

listed by Allsopp *et al.*(1986) and Okpokwasili *et al.* (1996) with the exception of *Rhizopus sp.* and *Curvularia sp.*

The presence of *Pseudomonas sp., Bacillus sp., Rhizopus sp.,* and *Aspergillus niger* in the normal painted surfaces compared with the numerous bacteria and fungal genera associated with the biodeteriorated painted surfaces confirms the role of water activity in the biodeterioration process as well as microbial proliferation. It could also be possible that they are the primary colonizers that initiate the entire process when the biocide and physico – chemical quality of the paints have been reduced by the hash tropical climatic conditions brought about by the nature of the paint and the location of the building. Similar suggestion has been reported by Drisko and Crilly (1974). This could also be as a result of increased available substrate for microbial utilization such as additional microbial metabolites or increased moisture content, which have been reported to characterize biodeteriorated painted surfaces (Chua *et al.* 1982). Basically, the biodeteriogens of painted surfaces in Owerri has been documented. Further studies should be directed to ascertain the in – can biodeteriogens of paint and the susceptibility of these isolates to commonly used paint biocides.

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REFERENCES

Allsopp, D and Seal, K. J., 1986. Introduction to Biodeterioration (1st edition). The Chaucer press Bungay Sutlolk 51-53.

Brierley, G. L, Kelly, D. P., Beal, C. J. and Best, D. J., 1985. *Materials and biotechnology principles and Application.* (eds; Hygins, I.J; Best D.J and Jones J.) Blackwell scientific publication Oxford London Pp 208-320.

Clua, N.H., Kinok, S.W., Tan, K.K and Ted, S.P., 1972. Growth on Concrete and other similar surfaces in Singapore. Journal of the Singapore Institute of Architects, 51:13-15.

Cruickshank, R., Dugurd, J.P, Marmoin, B.P and Swain, R.H.A., 1982. The practice of medical microbiology. Churchill Livingstone Edinburgh.

Cruickshank, R., Duguid, J.P., Marmoin B.P and Swain, R.H.A., 1975. Staining methods in Microbiology. Churchill Livingstone New York 34-42.

Drescher, R. F., 1958. Microbiology of paint films in temperate climatic condition. American Paint Journal, 42(27): 800-802.

Drisko, R.W and Crilly, J. B., 1974. Control of algae growth in paint at tropical locations. Journal of Paint technology 46: 48-55

Gilbert, R. J and Loveluick, D. W., 1975. Microbial respects of determination of materials. Academic Press London 205-225.

Hull, E. K., 1976. In: S.R Fin and Taylor G.A (Eds): Introduction to Paint Technology (4th ed.). Wembly, Middlesex.

Miller, W. G., 1973. Incidence of microbial contamination of emulsion paint during manufacturing process. Journal of oil and Colour Chemist Association 56; 307-312.

Ogbulie, J.N., Uwaezuoke, J. C. and Ogiehor, S. I., 1998. Introductory Microbiology practical. Springfield Publishers, Owerri Nigeria.

Okpokwasili, G.C. and Ituen, A., 1996. Fouling microflora of painted surfaces. Materials and Organism 30:155-159.

Ross, R. T., 1980. Effect of moisture in microbial deterioration of paint film. Advances in Applied Microbiology 26: 206.

Ross, R.T and Hollis, G.G., 1976. Microbiological deterioration of pulpwood, paper and Paint In: Miler B.M and Litsky A (Eds): Industrial Microbiology Mc Graw Hill Book Co New York 309-354.

Seal, K. J and Eggin H.O.W., 1981. Biodeterioration of materials. In Essay in Applied Microbiology (eds; Wors, J.R and Richman, M.H) John Wiley and Sans Chuckester, England.

Tiller, A.K., 1982. Proposed Mechanism of Anaerobic Corrosion. Corrosion Process. Darkins, R.W (ed). Applied Science Publishers, London. 155-159.