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Microbial evaluation and deterioration of paints and paint-products

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Abstract: *The microbial quality of materials and final products of a reputable paint industry in Lagos area were analysed. The bacterial contaminants isolated in the paint-products included Bacillus brevis, B. polymyxa, B. laterosporus, Lactobacillus gasseri, L. brevis, Esherichia coli and Proteus mirabilis. The fungal contaminants detected in the paints were mainly Aspergillus niger, A. flavus and Penicillium citrinum. The microbial populations in the raw materials ranged from 1.0 x 10^6 – 9.5 x 10^6 cfu g^{-1} for bacteria and between 1.25 x 10^4 and 6.8 x 10^4 cfu g^{-1} for fungi while those present in packaging materials ranged from 3.45 x 10^6 – 7.65 x 10^6 cfu g^{-1} for bacteria and 2.4 x10^3 – 2.8 x 10^3 cfu g^{-1} for fungi respectively. The bacterial populations in the fresh paint samples monitored every two weeks from the time of production ranged from 1.6 x 10^1 – 4.7 x 10^5 cfu ml^{-1} while the fungal populations ranged from 1.0 x 10^1 – 5.5 x 10^3 cfu ml^{-1} over a ten-month study period. The optical density at 600 nm increased while transmittance, pH, specific gravity and viscosity of the paint samples decreased over the period suggesting gradual deterioration of the aesthetic qualities of the paint-products with time as indicated by the measured parameters.*

Key words: *Paints, Contaminants, Deterioration, Aesthetic qualities*
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Introduction

Paints are uniformly dispersed mixtures having a viscosity ranging from a thin liquid to a semi-solid paste, consisting of a pigment suspended in a liquid vehicle such as oil or water. With a brush or roller or spray gun, paint is applied in a thin coat to various surfaces such as wood, metal, or stone. Although, their primary purpose is to protect the surface to which they are applied from corrosion, oxidation, environmental weathering or other types of deterioration, paints also provide decorative finish (Briggs, 1980; Adeleye and Adeleye, 1999). The components of paints include vehicle, pigment, additive and solvent (Briggs, 1980). The various organic materials of paints represent a carbon source for practically all species of microorganisms and act as nutrients to stimulate microbial growth both in-can and on the dry paint film. This seriously compromises the adhesion and durability of the paint as well as its decorative function (Da Silva, 2003). Unfortunately, microbial contamination of paints can be from a number of sources such as raw materials, manufacturing plant process units and packaging materials (Gillatt, 1992).

The major groups of microorganisms involved in paint deterioration are bacteria and fungi, which can grow on applied paint films and solvent and water-based coatings (Gaylarde and Gaylarde, 2005). Most commonly isolated bacterial species in paints include *Bacillus, Pseudomonas, Enterobacter, Proteus, Escherichia, Micrococcus, Serratia, Aeromonas* and so on (Jakabowski *et al*., 1983; Opperman and Gull, 1984). A wide range of anaerobic bacteria including *Bacteroides*, *Clostridium, Desulphovibrio* and *Bifidobacterium* have also been isolated in paints (Opperman and Gull, 1984). Grant *et al*. (1993) also reported *Rhizopus arrhinus, Aspergillus niger, A. ustus, Penicillium citrinum, Chaetomium globosum, Alternaria altanata* fungi associated with the deterioration of paints. The biodegradability, structure and microbial aetiology of biodeteriorated monuments and painted surfaces have been investigated (Altenburger *et al*., 1996; Ciferri, 1999; Ogbulie and Obiajuru, 2004; Theron and Cloete, 2004; Gonzalez and Saiz- Jimenez, 2005; Imperi *et al*., 2007). Factors that influence deterioration of paints and paint-products include among others: the anaerobic environment in the paint can, the organic nature of the paint components, the microbial quality of the packaging materials and the hygiene level of the manufacturing plant processing units. The consequences of this microbial deterioration such as foul smell, viscosity loss, discolouration and visible surface growth have serious economic implications on the paint industry. The aim of this study was, therefore, to investigate the microbial quality of paints with a view to improving the shelf life of paint and paint products.

Materials and Methods

Collection of samples: Solid raw materials, packaging materials and freshly produced liquid paint samples (FS-1 and FS-2) were collected from a reputable paint industry located in Lagos area, Nigeria into sterile Mc Cartney bottles. All samples were transferred to the laboratory for analysis.

Isolation of microorganisms: Ten gram (10 g) from a solid raw material sample or 10 ml from a liquid paint sample were transferred aseptically to a test tube containing 90 ml sterile distilled water to give 10^{-1} dilution from which higher dilutions(10^{-2}-10^{-8}) were made. The packaging materials were dipped and immersed in 150 l sterile distilled water for 5 min after which they were removed. Ten ml of

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this water sample was transferred aseptically to a universal bottle containing 90 ml sterile distilled water to give a 10^{-1} dilution from which higher dilutions were made. Aliquots (0.1 ml) from each dilution was plated out on nutrient agar (NA) for bacteria, Mac Conkey agar (MCA) for coliforms and potato dextrose agar (PDA) for fungi respectively in three replicates (Nwachukwu and Akpata, 2003). Incubation was carried out at 37°C for 24 hr (for bacteria) and at room temperature for 72 hr for isolation of fungi. Pure cultures were prepared for the different types. The isolation procedure was carried out on the fresh paint samples FS1 and FS2 for ten months at two weeks interval. Comparisons of means was done by the analysis of variance (ANOVA) using the computer software EPIINFO version 6.04 (Center for Disease Control, Atlanta). Analysis of variance was used to determine if there was any variation in the microbial growth of the fresh paint samples at 0 and at the 10th month.

Identification of isolates: The pure cultures of bacteria isolated from the various paints samples were identified by conventional bacteriological test methods and by reference to the keys outlined by Buchanan and Gibbons (1984) and phenotypically by the API 20 E and ID 32 E test systems (bio Merieux Vitek, Inc, Hazelwood, MO, USA). The fungal isolates were identified based on their morphological characteristics as described by previous workers (Smith, 1969; Dutta, 1970).

Determination of physico-chemical parameters -

Specific gravity: The specific gravity determinations of paint samples were carried out by pycnometry as described by Ohwoavworhua and Adelakun (2005). A pycnometer of approximately 50 ml capacity was washed, dried in the oven and placed in the desiccator to cool to room temperature before removal. It was weighed and the weight recorded as M1 (g). Paint sample (50 g) was transferred into the pycnometer. The pycnometer with its content was weighed and the weight recorded as M2 (g). The pycnometer containing the sample was filled with distilled water and shaken many times to allow all trapped air within the pycnometer to be expelled. Then, the pycnometer and its content were weighed and the weight recorded as M3 (g). The contents of the pycnometer were removed and the pycnometer was washed, cleaned and then refilled with distilled water. The outer surface of the pycnometer was dried, using a tissue paper, it was carefully weighed and the weight recorded as M4 (g). The specific gravity of the paint sample was calculated as follows :

$$\text{Specific Gravity (SG)} = \frac{M2-M1}{(M4-M1)\,(M3-M2)}$$

Optical density (OD) and transmittance: A rapid simple method of estimating growth with fine optical instrument was adopted as described by Rieck *et al*. (1993). The photoelectric colorimeter (Model: AE- 11C Tokyo Erma Optical works, Ltd Japan) used in the study was standardized by adjusting it to read 100% light transmittance with 5 ml of distilled water in a 1 cm glass cuvette placed in it at 600 nm. Five milliliters each of serially diluted paint samples obtained from 10^{-3}, 10^{-5} and 10^{-7} dilutions were poured into the cuvette and placed in the colorimeter which had two scales. The bottom scale displayed the absorbance and the top scale, % transmittance. The test was carried out at two weeks intervals for ten months and the displayed results were recorded.

pH and viscosity: The pH of the paint samples was determined with the use of a digital pH meter (Model: Jenway M50/Rev CE 350 EU) in 1: 200 solution of the paint samples in distilled water. The pH meter was calibrated using phthalate buffer (pH, 4.0) and phosphate buffer solutions (pH, 7.0). The viscosity of the paint samples was measured according to the procedure of Rammohan and Yassen (2003) using a glass capillary viscometer (Model : Capirograph Toyoseikh Seisaku-Sho Ltd.). The paint sample was allowed to flow through an outlet tube (measuring tube which is narrowed into a capillary tube above the outlet). Two annular reference marks on the measuring tube were used. The time it took for the sample meniscus to drop from the upper to the lower reference mark was measured manually with a stop watch (seconds). The centistokes (CST) of the paint sample was calculated by multiplying the measured time by the viscometer calibration factor at room temperature (30 ± 2°C). The pH and CST measurements were done at two weeks intervals for the ten-month study period.

Results and Discussion

The types and population densities of microorganisms detected in the raw materials and packaging materials are shown in Tables 1 and 2. From these tables, the raw and packaging materials for paint production were heavily contaminated with a variety of bacteria ranging from 1.0×10^6-9.5×10^6 cfu g^{-1} and from 1.25×10^4-6.8×10^4 cfu g^{-1} for fungi in the raw materials. The total bacterial population detected in the packaging materials ranged from 3.45×10^6-7.65×10^6 cfu g^{-1} while the fungal population was between 2.4×10^3 and 2.8×10^3 cfu g^{-1}. Fresh paint samples, FSI and FS2, monitored from day 0 to the 10th month supported the growth of *Bacillus brevis, B. laterosporus, B. polymyxa, Lactobacillus gasseri, L. brevis, Proteus mirabilis, Escherichia coli, Aspergillus niger, A. flavus*, and *Penicillium citrinum*. The fresh paint samples also showed increasing optical density and decreasing specific gravity, pH, transmittance and viscosity (Fig. 3, 4 and 5). The mean changes in the population density of microorganisms detected in sample FS1 and FS2 are shown in Fig. 1,2. As the microbial population and optical density increased, the pH, transmittance, specific gravity and viscosity decreased with time. In the two paint samples, there was a lag period of about 5 months after which a steady exponential growth was observed. Bacteria and fungi are heterotrophic microorganisms and depend on preformed organic matter as principal sources of energy and cellular carbon (Prescott *et al*., 2002). Their occurrence therefore in the raw materials (Table 1) in large numbers is an indication that the raw materials were particularly rich in organic nutrients utilizable for microbial growth. The high counts of bacteria in the paint samples arising from various sources including raw materials and packaging materials and utensils suggest that the shelf life of the paints could be adversely affected.

Table - 1: Microbial types and population density in the raw materials used in paint production

Raw materials codes	Total bacterial counts (x 10^6 cfu g^{-1})	Total coliform counts (x 10^6 cfu g^{-1})	Total fungal counts (x 10^4 cfu g^{-1})	Fungal types	Bacterial types
HSD H_20	4.40	6.50	1.25*	*Aspergillus flavus* *Penicillium citrinum*	*Bacillus polymyxa* *Proteus mirabilis* *Escherichia coli*
CD H_20	2.70	4.50	1.50	*Penicillium citrinum*	*Bacillus polymyxa* *Proteus mirabilis* *Escherichia coli*
MU H_20	6.45	7.05*	1.50	*Aspergillus niger*	*Bacillus brevis* *Proteus mirabilis*
MN 288	2.35	2.20	0.00	ND	*Bacullus laterosporus* *Proteus mirabilis* *Lactobacillus gasseri*
MN 239	2.05	2.50	3.50	*Aspergillus flavus* *Penicillium citrinum*	*Bacillus polymyxa* *Bacullus laterosporus*
MN 277	7.00	3.50	2.25	*Aspergillus flavus*	*Escherichia coli* *Lactobacillus brevis*
MN 409	7.50	3.90	3.59	*Aspergillus flavus* *Penicillium citrinum*	*Bacillus polymyxa* *Proteus mirabilis*
MN 280	7.80	1.50	2.35	*Penicillium citrinum*	*Bacillus brevis* *Lactobacillus gasseri*
MN 231	3.75	5.60	0.00	ND	*Proteus mirabilis* *Lactobacillus gasseri*
MN 236	2.00	2.50	1.30	*Aspergillus niger*	*Bacillus laterosporus*
Z4 726	5.40	3.00	0.00	ND	*Bacillus laterosporus* *Lactobacillus gasseri* *Lactobacillus brevis*
MN 236X	2.05	2.10	0.00	ND	*Bacillus brevis* *Proteus mirabilis*
Z4441	6.10	5.60	0.00	ND	*Bacillus laterosporus* *Lactobacillus gasseri*
MN 252	3.00	7.00	6.80	*Aspergillus niger*	*Bacillus polymyxa* *Proteus mirabilis*
MN 261	3.34	4.50	0.00	ND	*Bacillus laterosporus* *Escherichia coli* *Lactobacillus brevis*
ZN 490	7.60	3.00	0.00	ND	*Bacillus brevis* *Bacillus laterosporus*
RN 300	3.20	3.20	3.00	*Penicillium citrinum*	*Bacillus brevis* *Lactobacillus gasseri*
RN 375	9.50*	3.50	0.00	ND	*Bacillus laterosporus* *Proteus mirabilis*
Z4726	5.30	1.20*	0.00	ND	*Bacillus polymyxa* *Escherichia coli*
Z4740	7.30	2.95	0.00	ND	*Bacillus brevis* *Bacillus laterosporus* *Escherichia coli*
L1140	3.15	3.50	4.20*	*Aspergillus niger* *Penicillium citrinum*	*Bacillus polymyxa* *Bacillus brevis* *Lactobacillus gasseri*

ZN 465	1.00*	2.60	1.50	*Aspergillus niger*	*Proteus mirabilis*
Z4 899	3.50	3.00	0.00	ND	*Bacillus brevis* *Bacillus laterosporus*
ZN 470	5.20	1.40	0.00	ND	*Bacillus polymyxa* *Lactobacillus brevis*
MN 241	2.70	2.40	3.50	*Penicillium citrinum*	*Bacillus brevis* *Bacillus laterosporus*
MN 286	2.40	2.90	0.00	ND	*Bacillus laterosporus* *Escherichia coli* *Lactobacillus gasseri*

ND = None detected, * = Values indicate significant microbial difference p<0.001; 0.0167; 0.0118 for bacteria, coliform and fungi respectively

Table - 2: Microorganisms and their population densities in packaging materials used in paint production

Raw materials codes	Total bacterial counts (x 10^6cfu g^{-1})	Total coliform counts (x 10^6cfu g^{-1})	Total fungal counts (x 10^4 cfu g^{-1})	Fungal types	Bacterial types
C/T 20LTS	3.45*	2.90*	2.40*	*Aspergillus niger*	*Bacillus brevis* *Escherichia coli* *Lactobacillus gasseri*
D/W/S Plastic	4.28	4.70	0.00	ND	*Bacillus polymyxa* *Escherichia coli* *Lactobacillus brevis*
C/20LTS	3.82	3.42	2.80*	*Aspergillus niger*	*Bacillus brevis* *Proteus mirabilis* *Lactobacillus gasseri*
D/W/S/Metal	4.95	4.90*	0.00	ND	*Bacillus polymyxa* *Bacullus laterosporus*
D/E 20LTS	7.65*	4.01	0.00	ND	*Bacillus brevis* *Escherichia coli* *Lactobacillus brevis*
D/E 4LTS	5.92	4.82	0.00	ND	*Bacillus polymyxa* *Proteus mirabilis* *Lactobacillus gasseri*

ND = Not detected, C/T = Caplux textured; D/W/S = Dulux weather shield; C = Caplux; D/E = Dulux emulsion, * = Values show significant difference p<0.001; 0.001; 0.01039 for bacterial, coliform and fungal respectively

Previous work by Gillatt (1992) also showed that microbiological contamination can originate from a number of sources including make-up and wash waters, other raw materials, the manufacturing plant itself and the final container. Seven bacterial spp. and three fungal spp were isolated. *Lactobacillus* spp are facultative anaerobes, and could survive and reproduce in an environment with low oxygen tension, a condition which is obtainable in packaged paints. Facultative anaerobes do not require oxygen for growth but do grow better in its presence (Prescott *et al.*, 2002). The minimal occurrence of fungi in the packaged finished products could be as a result of their highly aerobic nature, a condition which was probably minimally available in the packaged paint products used for this study. Since this condition was probably not obtainable in packaged paints collected for this study, they could not flourish abundantly. However, a few genera such as *Aspergillus* (which was the most predominant) and *Penicillium* were isolated. Studies have shown that fungi associated with deterioration of paints include *Rhizopus arrhinus, Aspergillus niger, A. ustus, A. flavus, Penicillium citrinum, Alternaria altanata etc.* (Allsopp and Seal, 1980). Adeleye and Adeleye (1999) reported that the genus *Aspergillus* is one of the most frequently isolated fungi from biodeteriorated painted walls. A similar observation was made by Jakabowski *et al*. (1983) and Saad (1992). The increasing incidence of bacterial population from the 5th to 10th month as observed during this study suggests the growth and proliferation of dormant microorganisms which utilized the biodegradable components of the paints such as the additives (glues, emulsifiers, organic binders and thickeners). The protracted lag period from month 0 to 5 for FS1 and FS2 also indicated that the biocides incorporated into the products during production were still effective in inhibiting microbial growth till the 5th month after which the effects of the biocides started reducing. The decrease in pH observed during the study is in line with the trend of changes in the microbial growth system involving acid producing strains. During their metabolism, microorganisms could produce intermediates which

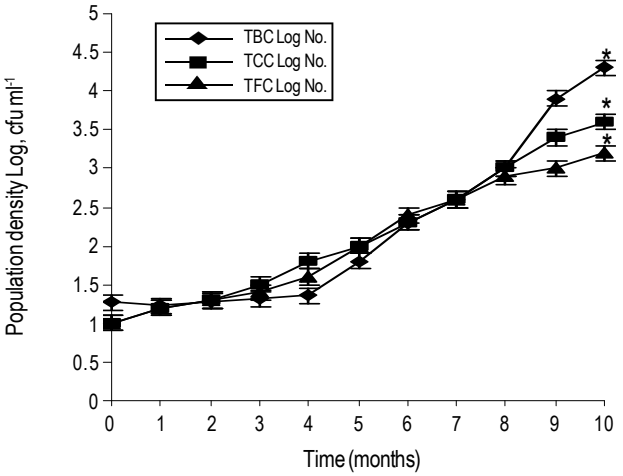

Fig. 1: Mean changes in microbial population density in fresh paint sample FS-1 on 10 months, TBC = Total bacterial count; TCC = Total coliform count; TFC = Total fungal count * = Values of mean in triplicate determination at significance p<0.001

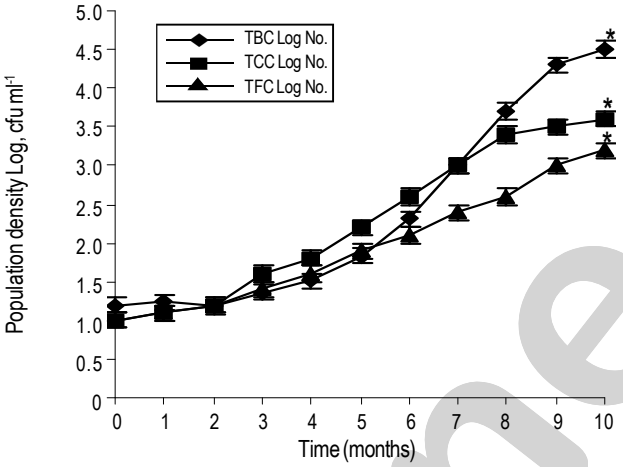

Fig. 2: Mean changes in microbial population density in fresh paint sample FS-2 on 10 months, TBC = Total bacterial count; TCC = Total coliform count; TFC = Total fungal count, * = Values of mean in triplicate determination at significance p<0.001

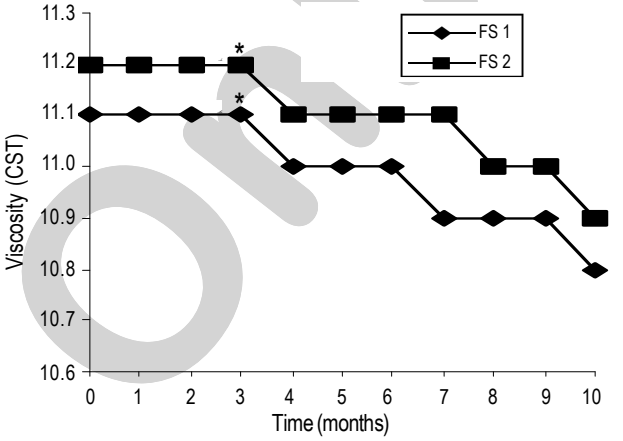

Fig. 5: Mean changes in viscosity of fresh paint samples FS-1-FS-2. * = Values are mean in triplicate determination at significance p<0.026

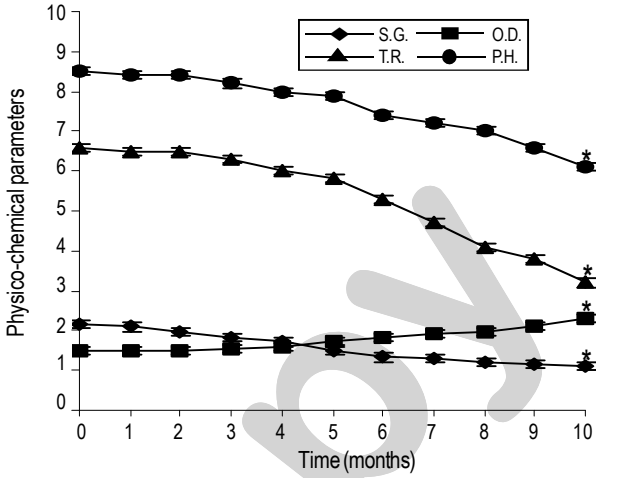

Fig. 3: Mean changes in physico-chemical parameters in fresh paint sample FS-1 at 10 months, SG = Specific gravity; OD = Optical density; TR = Transmittance, * = Values are mean in triplicate determination at significance p<0.001; 0.001; 0.042 and 0.001

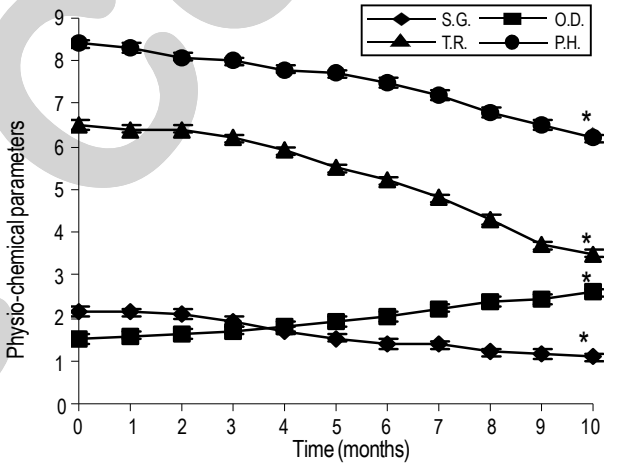

Fig. 4: Mean changes in physico-chemical parameters in fresh paint sample FS-2 at 10 months, SG = Specific gravity; OD = Optical density; TR = Transmittance, * = Values are mean in triplicate determination at significance p<0.001; 0.001; 0.001 and 0.001

could lower the pH values of the medium (Prescott *et al*., 2002). From this study, therefore, the changes in the physico-chemical parameters determined could be attributed to the presence and growth of microbial contaminants such as bacteria, coliform and fungi between the 0 month and the 10th month in the paint samples. Thus, microbial evaluation of paints could be a suitable biological index for the determination of self life of paint products.

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