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Possible Prevention of Environmental Health Hazard of Lead-Based Paints

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Abstract: The aim of this present study was to identify possible lead poisoning prevention strategies. Six fresh and five spoilt paint samples were collected from a reputable paint industry in Lagos, Nigeria. The microbial population count in the fresh paint samples monitored at two week interval in a ten-month study ranged from 1.6×10^{1}-5.5×10^{3} cfu mL^{-1} while that of spoilt paint samples ranged from 1.0×10^{7}-3.4×10^{10} cfu mL^{-1} for bacteria and fungi, respectively. The isolated bacteria were identified as *Bacillus polymyxa*, *B. brevis*, *B. laterosporus*, *Proteus mirabilis*, *Escherichia coli*, *Lactobacillus gasseri* and *L. brevis* based on isolate's phenotypic profiles using the Analytical Profile Index (API) ID 32E test systems. The fungal isolates were *Aspergillus niger*, *A. flavus* and *Penicillium citrinum*. In addition to these organisms, a strain of *Pseudomonas aeruginosa* which was observed to possess plasmids with molecular weights ranging from 0.030 to 0.112 kb was isolated from the spoilt paint samples. Atomic Absorption Spectrophotometric (AAS) analysis revealed that the spoilt paint samples had higher concentrations of heavy metals ranging from 2.0-5.3, 2.5-5.8 and 3.0-5.0 mg kg^{-1} while the fresh paint samples had 2.0-2.5, 1.0-3.0 and 2.0-3.1 mg kg^{-1} for Pb, Cu and Mn, respectively. Subsequent to curing experiment with 0.002% (v/v) Sodium Dodecyl Sulphate (SDS), *Pseudomonas aeruginosa* was observed to have lost the two existing plasmids. The incorporation of SDS (which successfully cured plasmids in paint spoilage organism) in paint formulation, suggests an environmental health risk prevention strategy because it will reduce spoilage. Also, the use of lead in providing durability in paints may be prevented.

Key words: Lead, paint, health hazard, prevention, sodium dodecyl sulphate

INTRODUCTION

Lead pollution is a serious environmental problem that has detrimental effects on human health and the environment. The addition of considerable amounts of lead into paints as pigments was once wide-spread because of its use as pigments, dispersing agent and drying agent but mostly because it provided durability to the paint (Rabin, 1989; Fassin and Naude, 2004). Chronic lead exposure may lead to developmental delay and the effects are usually felt after it has accumulated in the body over a period of time. The symptoms of lead poisoning are anaemia, weakness, constipation, colic, palsy and often a paralysis of the wrist and ankles (Bellinger *et al*., 1992; Schwartz, 1994; Banks *et al*., 1997; Satcher, 2000; Dietrich *et al*., 2001; Fassin and Naude, 2004). Child-lead poisoning gained wider recognition since, the mid-1920s as a common childhood disease resulting from lead paint in the home (Rabin, 1989;

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Marino *et al*., 1990). However, awareness that lead-based paint is a source of lead poisoning in children dates back to the first few years of the twentieth century (Berney, 1993; Fassin and Naude, 2004).

As a result of the wide-range of organic and inorganic molecules present in paint, many different types of microorganisms grow in them provided that favourable environmental conditions (humidity, temperature, light and to a lesser extent pH) are met (Ciferri, 1999). The most commonly isolated bacteria genera in paints include *Bacillus, Pseudomonas, Enterobacter*, *Proteus*, *Aerobacter*, *Escherichia*, *Micrococcus*, Serratia and Aeromonas etc. (Miller, 1973; Jakabowski *et al*., 1983; Opperman and Gull, 1984; Obidi *et al*., 2009). Studies have shown that some fungi are also associated with the deterioration of paints. These fungi include *Rhizopus arrhinus*, *Aspergillus niger*, *A. ustus*, *Penicillium citrinum*, *Chaetomium globosum* and *Alternaria alternata* etc. (Adeleye and Adeleye, 1999; Obidi *et al*., 2009). Allsopp and Seal (1986), Gillatt (1992) and Grant *et al*. (1993) reported different fungal genera such as *Aspergilus*, *Fusarium*, *Geotrichum*, *Penicillium*, *Saccharomyces*, *Scopulariopsis*, *Sporobolomyces* and *Torula* as the most commonly isolated fungi from water-based paints. Pseudomonads have been found to be the most commonly encountered group, comprising of at least 75% of isolates in spoilt emulsion paints (Dey *et al*., 2004). Spoilt emulsion paints have become a source of concern to marketers and consumers and now constitute a major problem bewildering the paint industry. The lead paint industry has claimed that interior lead paints were discontinued altogether by 1940 (Rabin, 1989). However, a further indication that the use of lead paint for improving shelf life for interiors was still substantial in 1940 comes from its detection in high concentrations (2 mg cm^{-2} or more) in about one third of the houses in Pittsburgh painted with such paints (Corn, 1975). Incredibly, as late as 1971, there is evidence that significant amounts of lead paints were being sold for residential interior uses (Rabin, 1989). A similar study concerning application of lead paints to houses in Washington D. C; New Haven, Chicago, Paris, Europe, Australia, Asia and Africa yielded very similar results (Corn, 1975; Fee, 1990; Spurgeon, 2006; Mathee *et al*., 2007).

Elimination of plasmids in paint spoilage organisms may reduce the prevalence of deterioration, thereby, excluding the use of lead to proffer durability, giving the continual toll taken by child lead poisoning and the considerable resources that will be required to deal with the problem. Considering the use of lead in paints and the serious consequences on children's health and educational attainment, there is need for greater vigilance and a more pro-active approach to lead hazard prevention in the public health community. This includes research to identify the full extent of sources and risk factors as well as implementation of the most appropriate lead poisoning prevention mechanisms. The aim of this present study was therefore to identify possible lead poisoning prevention strategies.

MATERIALS AND METHODS

Collection of Samples

Six samples of freshly produced water-based paints and five samples of spoilt paints were aseptically collected from a paint manufacturing industry located at Ikeja, Lagos, Nigeria in 4 L plastic containers. They were monitored for microbial growth at intervals of 2 week for a 10 month study period by adopting the standard plate count technique. The paints were stirred continuously for 3 min inside the cans for proper mixing before samples were taken. Ten gram of each paint sample was weighed into 90 mL of sterile distilled water and shaken vigorously to obtain a homogenous solution. The paint samples dissolved readily in water since, they were water-based. Aliquots (0.1 mL) from both low (10^{-2}, 10^{-4}) and high (10^{-6},

10^{-8}) ten-fold serial dilutions of paint samples were plated on Nutrient Agar (NA), Mac Conkey Agar (MCA) and Potato Dextrose Agar (PDA) plates, respectively in triplicates. The plates were incubated aerobically with NA and MCA plates at 37°C for 1-3 days and PDA plates at room temperature (30±2°C) for 3-6 days. (Nwachukwu and Akpata, 2003). Identification of pure cultures of bacteria isolated from the various samples was carried out using standard cultural, morphological, taxonomic and biochemical characteristics (Buchanan and Gibbons, 1974; Sahin *et al*., 2002) and the analytical Profile Index (API) identification test systems (bioMerieux Vitek, Inc. Hazelwood, MO. USA). Fungal isolates were identified based on morphological and microscopic examination (Smith, 1969).

Determination of Heavy Metals

The presence of Pb, Cu and Mn in fresh and spoilt paint samples was determined using the flame Atomic Absorption Spectrophotometer (AAS) as described by Stafilov and Zendelovska (2002). The stepwise procedure involved the digestion of 5 g paint sample with 10 mL concentrated nitric acid (99% purity), evaporation of the sample on a hot plate in a fume cupboard until the brown fumes disappeared. The remaining solution was transferred to a 100 mL standard flask, made up to mark with distilled water and filtered with a filter paper into a plastic bottle prior to analysis. Stock solutions of 1000 ppm were prepared for Pb, Cu and Mn by weighing 1.6 g $PbNO_3$; 2.5 g $CuSO_4$ and 3.1 g $MnSO_4$ in 1 L distilled water each in the 1000 ppm stock standard. Working standard solutions of 2, 4 and 6 ppm were then prepared from the stock solution and were used in preparing a standard curve for instrument calibration. The absorbance of the working standard was read at 279.48, 324.75 and 279.43 nm for Pb, Cu and Mn, respectively. The concentration of the Pb, Cu and Mn in the digested paint sample was then determined by aspiration of the digested filtrate with the flame AAS (Perkins Elmer-Analyst 200), determining the absorbance value and extrapolating from the standard curve obtained from the working standards.

Detection of Plasmids

The test was carried out to determine if the gene for paint degradation is plasmid-mediated or not and to ascertain whether or not plasmids code for antibiotic resistance and spoilage. Two different protocols were adopted for detection of plasmid DNA from the isolated bacterial strains. The first, which was used for Gram negative isolates, utilized the Alkaline lysis method of Birnboim and Doly (1979). The stepwise protocol involved cell lysis, precipitation of plasmid DNA, agarose gel electrophoresis and viewing of the amplified plasmid DNA bands over UV (ultraviolet) transilluminator. A second method, (TENS Mini-prep procedure) (Leeh and Brent, 1987) was adopted for plasmid DNA isolation for the Gram-positive isolates. The procedure involved the use of TENS solution (TE buffer containing 0.1 N NaOH and 0.5% sodium dodecyl sulphate). Overnight cultures of 15 mL were spinned for 10 sec in a microfuge to pellet cells at 1000 rpm. The supernatant was then gently removed, leaving 50-100 μL together with cell pellet. This was then vortexed at high speed to re-suspend the cells completely. The mixture was then vortexed. Three hundred microliter of TENS was added and the suspension was mixed for 2-3 min until the mixture became sticky. The tubes were constantly stored on ice after each step to prevent them from the degradation of chromosomal DNA which may be precipitated with plasmid DNA. To this mixture, 150 μL of 3.0 M sodium acetate (pH 5.2) was added, vortexed for 2-5 min to mix completely. The mixture was then spinned for 2 min in a microcentrifuge to pellet the cell debris and the chromosomal DNA. The supernatant was transferred to a fresh tube, mixed well with 0.9 mL of 100% (v/v) ethanol which has been pre-cooled to -20°C. The mixture was

spinned for 20 min to pellet the plasmid DNA and white pellets were observed. The supernatant was discarded and the pellet was rinsed twice with 1 mL of 70% (v/v) ethanol and pellets were dried under vacuum for 2-3 min. The pellets were re suspended in 20-40 μL of TE buffer for further use.

Agarose Gel Electrophoresis of DNA

The agarose gels were run in a horizontal gel electrophoretic apparatus using TBE (Tris, Boric acid, EDTA, 0.8% agarose) buffer (Meyers *et al*., 1976). The agarose gel in TBE buffer was boiled intermittently until the solution became clear. The solution was allowed to cool to 45°C and 7 μL of ethidium bromide was added. The function of the ethidium bromide is to intercalate the basis of the DNA so that it fluoresces when viewed under the UV light. The gel was then poured into the gel plate with the comb in place and allowed to solidify. Subsequently, the gel tray and comb was removed and put into the tank containing the gel buffer, making sure that the buffer covered the gel completely. Two microliter of the tracking dye (bromophenol blue) was mixed with 1 μL of the marker and loaded into the first well. Two microliter of the bromophenol blue was then mixed with 20 μL of the samples and also loaded into the designated wells. Following the loading of the wells, the tank was covered, plugged into power and allowed to run from the negative to positive direction making sure it didn't run more than 3/4 of the gel. Finally, the gel was viewed on the UV transilluminator using protective goggles. Amplified plasmid DNA appeared as sharp bands that fluoresced when excited with UV light. The molecular weight of isolated plasmids was determined as described by Ilori (1998). The plasmids isolated from *Pseudomonas aeruginosa* were run in agarose gel alongside the plasmids that were isolated from the bacteriophage lambda (λ) DNA fragmented in a restriction digestion with Hind 111 endonuclease. The digestion reaction resulted in 8 double stranded DNA fragments. The DNA molecular weight marker provided accurate sizing of fragments over a broad range of sizes. Upon electrophoretic separations, 7 bands of known molecular weights (23130, 9416, 6557, 4361, 2322, 2027 and 564 bp, respectively) were visible. The smallest fragment resulting from the Hind 111digestion (125 base pair) could not be detected on the gel due to its small size. All fragments were present in equimolar amounts therefore, the smallest band will only be visible on over-loaded gels when stained with ethidium bromide. The DNA fragment mixture showed the typical pattern with 7 bands in agarose gel electrophoresis. A standard curve was made by plotting the relative mobilities on gel against the logs of their molecular weights. The molecular weights of plasmids isolated from the *Pseudomonas aeruginosa* were then determined by extrapolating from the standard curve.

Curing of Plasmids with Sodium Dodecyl Sulphate

The curing procedure was done as described by Sonstein and Baldwin (1972) to determine whether the plasmids isolated from *Pseudomonas aeruginosa* (OB-6) encoded the spoilage trait or not. To achieve this, *Pseudomonas aeruginosa* grown on yeast extract trypticase soy (YETS) (0.3% yeast extract and 1.5% agar) were harvested from the YETS plates and emulsified in normal saline at a density of about 10^5 cells per ml. This was then inoculated into YETS broth containing 0.002% (v/v) SDS and incubated with constant shaking at 37°C for 18 h. After incubation, the developed colonies were screened for loss of the plasmids.

Selection of Cured Strain

At the end of the incubation, 0.1 mL aliquots of the suspension were plated on NA plates. The plates were incubated aerobically at 37°C for 24 h. Changes in the original

colonial morphology were observed. The parent strains and mutants that had lost the plasmids were plated out on fresh NA plates, incubated aerobically at 37°C for 24 h. Subsequently, they were tested for antibiotic sensitivity.

Antibiotic Susceptibility Tests of Plasmid-Bearing and Plasmid-Cured Strains

The antibiotic sensitivity of the plasmid-bearing *Pseudomonas aeruginosa* (OB-6W) and the plasmid-cured strain (OB-6C) to 8 antibiotics was determined using the Kirby-Bauer disc diffusion technique (Bauer *et al*., 1966). The purpose of this experiment was to determine the agar disc diffusion zone diameters of the plasmid-bearing *Pseudomonas aeruginosa* strain (OB-6W) isolated from spoilt paints to selected antibiotics and to compare these zone diameters with those of the cured prototype. Single discs (Oxoid Ltd., Basingstoke Hampshire, England) were employed to test the sensitivity of the plasmid-bearing and plasmid-cured strains to antibiotics. The zones of inhibition produced by the test organism (plasmid-bearing strain) were compared directly with that of the control strain (plasmid-cured strain). The zones of inhibition observed around the antibiotic discs were taken as indication of sensitivity and were interpreted based on the National Committee for Clinical Laboratory Standards (NCCLS, 1997).

RESULTS

Populations of Microorganisms from Paint Samples

Ten morphologically different microorganisms were isolated. In addition to these, the spoilt samples had 1 more organism designated OB-6. The heterotrophic bacteria isolated in the study were identified to be *Bacillus polymyxa* (OB-1), *Bacillus brevis* (OB-2), *Bacillus laterosporus* (OB-3), *Proteus mirabilis* (OB-4), *Escherichia coli* (OB-5), *Pseudomonas aeruginosa* (OB-6), *Lactobacillus gasseri* (OB-7) and *Lactobacillus brevis* (OB-8). The fungal isolates (OB-9, OB-10 and OB-11 were identified as *Aspergillus niger*, *A. flavus* and *Penicillium citrinum*, respectively based on macroscopic and microscopic characteristics. Isolate OB-6 occurred only in all spoilt paint samples. It was therefore, selected for antibiotic susceptibility tests. The bacterial population in the fresh paint samples (PS-1-PS-6) monitored every two weeks from the day of production ranged from 1.6×10^{1}-4.7×10^{5} cfu mL^{-1}, while the fungal population ranged from 1.0×10^{1}-5.5×10^{3} cfu mL^{-1}, respectively over a period of 10 months. The mean changes in population density of microorganisms in fresh paint samples PS1-PS6 are shown in Fig. 1. The main feature of the growth pattern of the isolated organisms as shown in Fig. 1 is an observable and definite lag period of 5 months. Subsequently, there was steady increase in the microbial population densities till the 10th month. The microbial population densities in the spoilt paint samples ranged from 2.5×10^{10}-3.4×10^{10} cfu mL^{-1}, 1.0×10^{7}-2.9×10^{7} cfu mL^{-1} and 2.2×10^{5}-3.2×10^{5} for bacteria, coliform and fungi, respectively (Table 1). The Atomic Absorption Spectrophotometric (AAS) analysis of the paint samples revealed that the spoilt paint samples had higher concentrations of heavy metals ranging from 2.0-5.3, 2.5-5.8 and 3.0-5.0 mg kg^{-1} while the fresh paint samples had 2.0-2.5, 1.0-3.0 and 2.0-3.1 mg kg^{-1} for Pb, Cu and Mn, respectively (Table 2).

The eight isolated bacterial strains were screened for the presence of plasmid DNA. The plasmid isolation techniques revealed that only *Pseudomonas aeruginosa* (isolated from spoilt paints) haboured 2 plasmids of different molecular weights (Fig. 2, 3). The other organisms did not show the presence of any plasmid DNA. The result of molecular weights of the isolated plasmids determined in a previous study revealed that the molecular weights of the plasmids contained in *Pseudomonas aeruginosa* ranged from 0.030 to 0.112 kb.

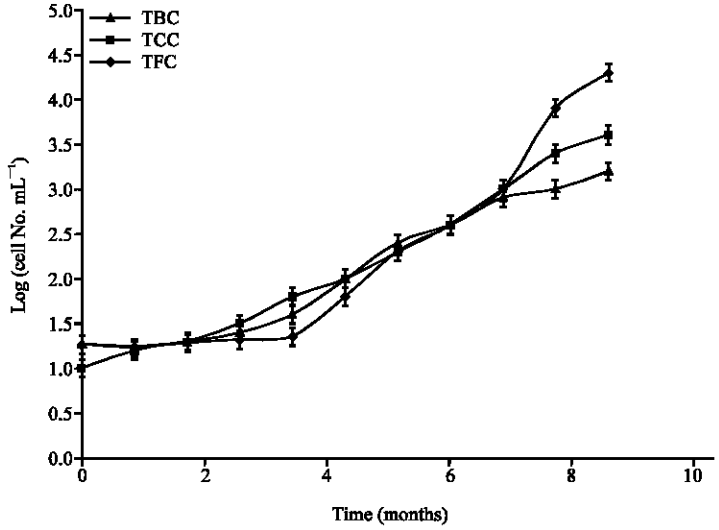

Fig. 1: Mean changes in microbial population density in fresh paint samples PS1-PS6. TBC: Total bacterial count; TCC: Total coliform count; TFC: Total fungal count. Data represent the averages of triplicate determinations

Table 1: Microbial population densities in spoilt paint samples

Paint sample	Total bacterial counts ($\times 10^{10}$ cfu mL^{-1})	Total coliform counts ($\times 10^{7}$ cfu mL^{-1})	Total fungal counts ($\times 10^{5}$ cfu mL^{-1})	Fungal isolates	Bacterial isolates
PSA	2.9	1.1	2.5	OB-9	OB-2, OB-3, OB-4, OB-6, OB-7
PSB	3.4	1.1	3.2	OB-9,	OB-1 OB-6, OB-11 OB-7, OB-8
PSC	3.0	1.0	2.8	OB-10,	OB-3, OB-4, OB-11 OB-6, OB-7
PSD	2.5	2.9	2.5	OB-10	OB-2, OB-4, OB-6
PSE	3.1	1.1	2.2	OB-11	OB-1, OB-5, OB-6

Values presented are means of triplicate samples

Table 2: Atomic absorption spectrophotometric analysis of heavy metals in various paint samples

Paint sample	Status	Mn	Cu	Pb
		(mg kg^{-1})		
D/E chocolate	SS	3.0	5.8	3.0
D/E summer blue	SS	5.0	3.7	2.0
Shell	SS	5.0	4.3	2.0
Estrucian red	SS	4.5	2.5	5.3
Brilliant white	WS	2.4	1.5	2.6
D/W/S	WS	4.1	3.1	3.0
Summer blue	WS	3.8	3.2	3.3
Magnolia	WS	3.9	3.6	2.0
Shell	FS	3.1	1.0	2.5
Summer blue	FS	3.0	1.8	2.0
Magnolia	FS	2.0	3.0	2.4
Brilliant white	FS	2.6	2.0	2.1

SS: Spoilt paint samples; WS: Warehouse samples in storage; FS: Fresh samples. Values are means of triplicate determinations

Prior to the curing experiments, it was observed that *Pseudomonas aeruginosa* (OB-6) had 2 plasmids of molecular weight ranging from 0.030-0.112 kb. However, after the curing experiments, *Pseudomonas aeruginosa* was found to have lost the 2 plasmids that it

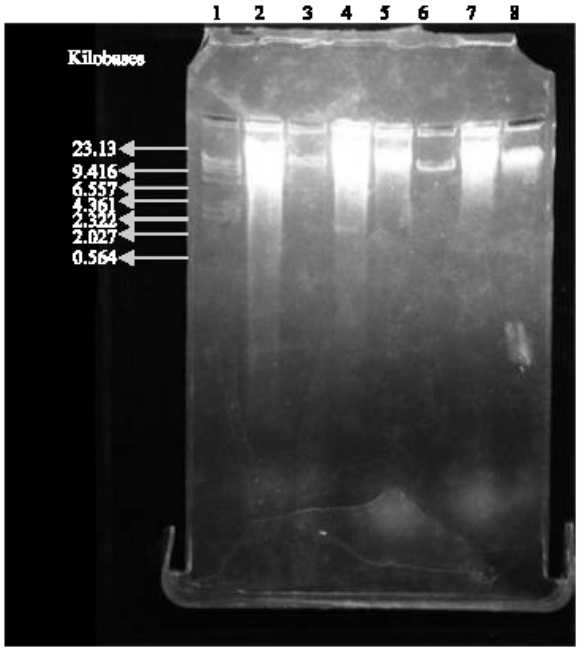

Fig. 2: Agarose gel electrophoresis plate showing no detectable plasmid from bacterial strains. Organisms were screened following the TENS-Mini Prep. Protocol of Leeh and Brent (1987). Lanes: 1, λ DNA. Hind 111 digested (marker); 2, OB-1; 3, OB-1; 4, OB-2; 5, OB-2; 6, OB-3; 7, OB-7; 8, OB-8

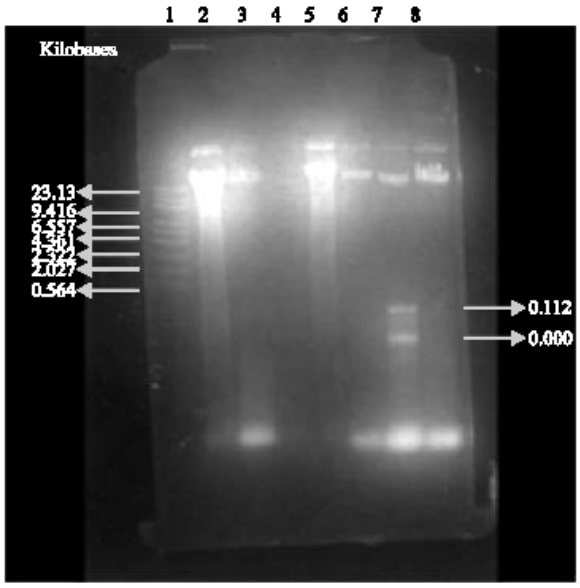

Fig. 3: Agarose gel electrophoresis plate showing plasmid DNA isolated from OB-6 using the protocol of Birnboim and Doly (1979). Lanes: 1, λ DNA Hind 111 digested (marker); 2, OB-4; 3, OB-4; 4, OB-5; 5, OB-5; 6, OB-5; 7, 0B-6; 8, OB-4

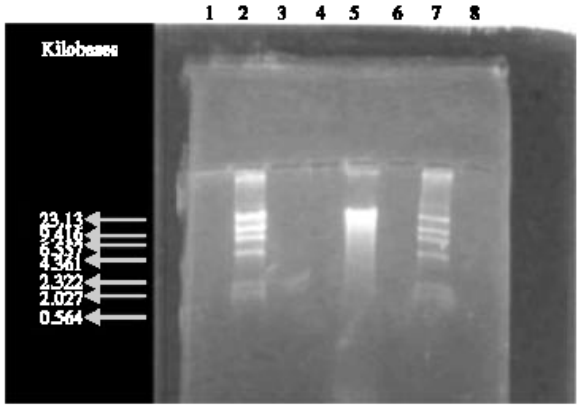

Fig. 4: Agarose gel electrophoresis plate of cured plasmids of *Pseudomonas aeruginosa* OB-6 (C) following the protocol of Birnboim and Doly (1979). Lanes: 2, DNA Hind 111 digested (marker); 5, OB-6(C); 7, λ DNA Hind 111 digested (marker)

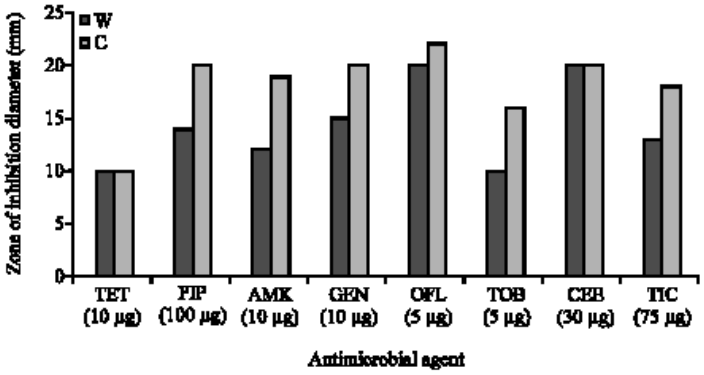

Fig. 5: Antimicrobial sensitivity patterns of plasmid-bearing and plasmid-cured strains of *Pseudomonas aeruginosa*. TET: Tetracycline; PIP: Piperacillin; AMK: Amikacin; GEN: Gentamycin; OFL: Ofloxacin; TOB: Tobramycin; CEF: Ceftazidime; TIC: Ticarcillin; W: Plasmid-bearing; C: Plasmid-cured strain. The disc potency is indicated in parentheses

possessed earlier on (Fig. 4). Therefore, growth in YETS broth containing 0.002% v/v SDS resulted in the complete elimination of the plasmids. This result suggests that SDS is an effective curing agent.

Antibiotic Sensitivity Pattern of Plasmid-Bearing and Plasmid-Cured Strains of *Pseudomonas aeruginosa*

The sensitivity and resistance patterns of the plasmid-bearing *Pseudomonas aeruginosa* and the cured strain to selected antibiotics are presented in Fig. 5. The susceptibility classification of the strains was based on the zone-size interpretative chart for disc diffusion susceptibility testing of the National Committee for Clinical Laboratory Standards (NCCLS). The plasmid-bearing strain OB-6 (W) was resistant to ceftazidime, amikacin, tobramycin,

gentamycin and tetracycline. However, after the curing experiments, it was observed to be sensitive to tobramycin, gentamycin and amikacin.

DISCUSSION

The results obtained in this study demonstrate that microorganisms utilize paints as a source of nutrients since, the constituents of paints are conducive to increased cell multiplication and population build-ups. Similar observation was also made by Adeleye and Adeleye (1999). Therefore, a can of water-based paint is highly susceptible to deterioration. Work done by Da Silva (2003) also proved that the various organic constituents of paints such as pigments, additives, binders etc. which act as nutrients for microorganisms, help to stimulate microbial growth. It is noteworthy to observe that *Ps. aeruginosa* (OB-6) occurred only in the spoilt paint samples. This is most likely possible because the pseudomonads can degrade an exceptionally wide variety of organic molecules. Thus, they are very important in the mineralization process. This may account for their persistence in spoilt paints. Interestingly too, the molecular studies of the isolated organisms showed that of all the microorganisms isolated, *Ps. aeruginosa* (OB-6) was the only organism that harboured plasmids. It is not unlikely therefore, that paint degradation was effected by *Ps. aeruginosa* (OB-6). The mean changes in the microbial population densities in the fresh paint samples (PS1-PS6) during a study period of 10 months (Fig. 1.) revealed a protracted lag phase of 4 to 5 months before exponential growth of the organisms. The observed protracted lag phases are indicative of the gradual reduction in the impact of the biocides incorporated in paints during production (Russell and Mc Donelli, 2000; Gilbert and McBain, 2001; Stickler, 2002; Russell, 2003; Petersen *et al*., 2004). In addition, the observed consistent increase in the microbial population counts in the fresh paint samples after the lag phase from the initial 1.6×10^{1} cfu mL^{-1} at day zero to the levels of 4.7×10^{5} cfu mL^{-1} at the 10th month (Fig. 1) suggests the exhaustion and limitation of the incorporated biocides or resistance developed against them by the indigenous contaminants. The microbial increases also revealed the role of microorganisms in the deterioration of water-based paints and reduction of their shelf lives. The isolation of *Pseudomonas aeruginosa* (OB-6) repeatedly only in the spoilt paint samples is consistent with earlier publications that *Pseudomonas aeruginosa* constitutes 75% of isolates in spoilt paints (Dey *et al*., 2004). Spoilt paints were found to contain higher amounts of lead. The results of concentration of heavy metals in the fresh and spoilt paint samples indicated that higher concentrations of these heavy metals were observed in the spoilt samples. This ranged from 2.0-5.3, 2.5-5.8 and 3.0-5.0 mg kg^{-1} for Pb, Cu and Mn, respectively. The use of Pb concentrations as high as 2.6-10.8% rather than the acceptable limit of 0.06% to improve the durability and shelf life of paints has however been previously reported (Rabin, 1989). This, however, has been found to be hazardous and leads to high blood lead levels (Marino *et al*., 1990). The persistence of *Ps. aeruginosa* observed in the spoilt paints could be linked to its ability to resist the high concentrations of heavy metals observed in the spoilt paints and its possession of plasmids. This finding also reflects the observation of Silver and Misra (1988) that the development of resistance to heavy metals by microorganisms is plasmid-mediated. The results obtained from the antibiotic resistance patterns revealed that the antibiotic resistant *Pseudomonas aeruginosa* (OB-6) was also resistant to biocide ZN489 to a large extent in a previous study (Obidi *et al*., 2010). This organism poses particular problems in spoilt paints and has been found to possess plasmids. To establish a link between the ability to cause spoilage and the presence of the plasmid in the wild strain, the cured and the wild strains of *Ps. aeruginosa* (OB-6) were tested for

degradative ability on the physico-chemical properties of fresh, sterile paints and subsequently, subjected to antibiotic sensitivity (Obidi *et al*., 2010). Agarose gel electrophoresis of the plasmid extract from the cured strain of *Ps. aeruginosa* OB-6(C) showed the absence of the plasmids found in the wild strains OB-6(W). It was observed that the cured strains of *Ps. aeruginosa* had lost the two plasmids it earlier had (Fig. 4), it had also lost its degradative ability. Further proof of this was indicated by the results of the comparative evaluation of the spoilage potentials of the wild and cured strains of *Ps. aeruginosa* exhibited in the physico-chemical parameters of fresh and sterile paint samples. The wild strain showed higher degradative ability than the cured strain. It also had higher resistance to antibiotics than the cured strain as previously described (Obidi *et al*., 2010). The cured strain was found to have lost the initial resistance to tobramycin, gentamycin and amikacin. This further confirmed that the gene for degradation of paint may be plasmid-borne. Sodium dodecyl sulphate, probably affected the genes for degradation and spoilage. Since, SDS is known to cause disruption of biological materials (Sonstein and Baldwin, 1972), the possibility exists that SDS as a curing agent cures the organism of its plasmid by disrupting the membrane sites of plasmid attachment. The present sensitivity of the cured strain to tobramycin, gentamycin and amikacin was thought to be due to the loss of these plasmids. The incorporation of SDS in paint formulations will therefore, not only prevent spoilage but will result in a reduction of lead (which is used for durability) in paint manufacture. *Pseudomonas aeruginosa*, suspected to be the specific spoilage organism (SSO) (Koutsoumanis, 2001), occurred only in the spoilt paint samples regularly in highest frequencies. The spoilt paints also had higher concentration of lead. There seems to be a connection between the presence of lead in spoilt paints and the gene for degradation which may be plasmid-borne. Therefore, the incorporation of SDS in paints which resulted in the successful curing of plasmids in the spoilage organism and hence, reduction in spoilage consequently provides a possible means of reduction in lead concentration in paints. The incorporation of SDS also will reduce the use of lead in providing durability in paints and therefore, suggests an environmental health risk prevention.

REFERENCES

Adeleye, I.A. and O.A. Adeleye, 1999. Isolation and identification of microbes associated with paints and weathered painted walls. J. Sci. Res. Dev., 4: 71-76.

Allsopp, D. and K.J. Seal, 1986. Introduction to biodeterioration. Proceedings of the 13th International Biodeterioration and Biodegradation Symposium, (IBBS'86), Edward Arnold, London, pp: 13-16.

Banks, E.C., C.E. Ferretti and D.W. Shucard, 1997. Effects of low level lead exposure on cognitive function in children: A review of behavioural, neuropsy-chological and biological evidence. Neurotoxicology, 18: 237-281.

Bauer, A.W., W.M. Kirby, J.C. Sherris and M. Turck, 1966. Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol., 45: 493-496.

Bellinger, D.C., K.M. Stiles and H.L. Needleman, 1992. Low level lead exposure, intelligence and academic achievement: A long term follow-up study. Pediatrics, 90: 855-861.

Berney, B., 1993. Round and round it goes: The epidemiology of childhood lead poisoning. Milbank Q., 71: 3-39.

Birnboim, H.C. and J. Doly, 1979. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acids Res., 7: 1513-1523.

Buchanan, R.E. and N.E. Gibbons, 1974. Bergey's Manual of Determinative Bacteriology. 8th Edn., Williams and Wilkinson Co., Baltimore, UK., pp: 1246-1249.

Ciferri, O., 1999. Microbial degradation of paintings. Applied Environ. Microbiol., 65: 879-885.

Corn, J.K., 1975. Historical perspective to a current controversy on the clinical spectrum of plumbism. Mibank Mem. Fund. Q., 53: 93-114.

Da Silva, V.Q., 2003. Microbial deterioration of paints. Microbiologist, 4: 43-43.

Dey, B.K., M.A. Hashim, S. Hassan and B.S. Gupta, 2004. Microfilteration of water-based paint effluents. Adv. Environ. Res., 8: 455-466.

Dietrich, K.N., M.D. Ris, P.A. Succop, O.G. Berger and R.L. Bornschein, 2001. Early exposure to lead and juvenile delinquency. Neurotoxicol. Teratol., 23: 511-518.

Fassin, D. and A.J. Naude, 2004. Plumbism reinvented, childhood lead poisoning in France, 1985-1990. Public health then and now. Am. J. Public Health, 94: 1854-1863.

Fee, E., 1990. Public health in practice: An early confrontation with the 'silent epidemic' of childhood lead paint poisoning. J. History Med. Allied Sci., 45: 570-606.

Gilbert, P. and A.J. McBain, 2001. Biofilms: Their impact upon health and their recalcitrance towards biocides. Am. J. Infect. Control, 29: 252-255.

Gillatt, A.C., 1992. Bacterial and fungal spoilage of water-borne formulations. Additives, 10: 387-393.

Grant, C., I.C. Wright, W.R. Springle and M. Greenhalgh, 1993. Collaborative investigation of laboratory test methods for evaluation of the growth of pink yeasts on paint films. Int. Biodeteriorat. Biodegradat., 32: 279-288.

Ilori, M.O., 1998. Determination of molecular weights of plasmids. Ph.D. Thesis, Department of Botany and Microbiology, University of Lagos, Lagos, Akoka.

Jakabowski, J.A., J. Gyuris and S.L. Simpson, 1983. Microbiology of modern coating system. J. Coat. Technol., 58: 49-57.

Koutsoumanis, K., 2001. Predictive modeling of the shelf life of fish under non-isothermal conditions. J. Applied Environ. Microbiol., 67: 1821-1829.

Leeh, K. and R. Brent, 1987. Minipreps of Plasmid DNA. In: Current Protocols in Molecular Biology, Ausubel, F.M., R. Brent, R.E. Kingston, D.D. Moore and J. Seidman *et al.* (Eds.). John Wiley and Sons, New York, pp: 161-164.

Marino, P.E., P.J. Landrigan, J. Graef, A. Nussbaum, G. Bayan and S. Boch, 1990. A case report of lead paint poisoning during renovation of a victorian farmhouse. Am. J. Public Health, 80: 1183-1185.

Mathee, A., H. Rollin, J. Levin and I. Naik, 2007. Lead in paints: Three decades later and still a hazard for African children. Environ. Health Perspect., 151: 321-322.

Meyers, J.A., D. Sanchez and S. Falkow, 1976. Simple agarose gel electrophoresis method for the identification and characterization of plasmid deoxyribonuclic acid. J. Bacteriol., 127: 1529-1537.

Miller, W.G., 1973. Incidence of microbial contamination of emulsion paints during the manufacturing process. J. Oil Colour Chem. Assoc., 56: 307-310.

NCCLS, 1997. Methods for Dilution in Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically. NCCLS, Wayne, PA, pp: 226-294.

Nwachukwu, S.C.U. and T.V.I. Akpata, 2003. Isolation of Microorganisms by Spread Plate Technique. University of Lagos Press, Lagos, pp: 3-6.

Obidi, O.F., O.O. Aboaba, M.S. Makanjuola and S.C.U. Nwachukwu, 2009. Microbial evaluation and deterioration of paints and paint-products. J. Environ. Biol., 30: 835-840.

Obidi, O.F., S.C.U. Nwachukwu and O.O. Aboaba, 2010. Investigation on the biodegradative potential on Water-based paints. Researcher, 2: 57-67.

Opperman, A.A. and M. Gull, 1984. Presence and effects of anaerobic bacteria in water-based paints. J. Coat. Technol., 56: 51-57.

Petersen, G.D., J. Dahlllof and L.P. Nielsen, 2004. Effects of zinc pyrithione and copper pyrithione on microbial community function and structure in sediments. Environ. Toxicol. Chem., 23: 921-928.

Rabin, R., 1989. Warnings unheeded: A history of child lead poisoning. Am. J. Public Health, 79: 1668-1674.

Russell, A.D. and G. Mc Donelli, 2000. Concentration: A major factor in studying biocidal action. J. Hosp. Infect., 44: 1-3.

Russell, A.D., 2003. Similarities and differences in the response of microorganisms to biocides. J. Antimicrob. Chemother., 52: 750-763.

Sahin, N., I. Gokler and U.T. Abdurrahman, 2002. Isolation, characterization and numerical taxonomy of novel oxalate-oxidizing bacteria. J. Microbiol., 40: 109-118.

Satcher, D.S., 2000. The surgeon general on the continuing tragedy of childhood lead poisoning. Public Health Rep., 115: 579-580.

Schwartz, J., 1994. Low-level lead exposure and children's IQ: A meta-analysis and search for a threshold. Environ. Res., 65: 42-55.

Silver, S. and T.K. Misra, 1988. Plasmid-mediated heavy metal resistance. Annu. Rev. Microbiol., 42: 717-743.

Smith, G., 1969. Classification and Identification of Fungi. 6th Edn., Edward Arnold, London, pp: 56-64.

Sonstein, S.A. and J.A. Baldwin, 1972. Loss of the penicillinase plasmid after treatment of *Staphylococcus aureus* with sodium dodecyl sulphate. J. Bacteriol., 109: 262-265.

Spurgeon, A., 2006. Watching paints dry: Organic solvent syndrome in late twentieth-century Britain. Med. Hist., 50: 167-188.

Stafilov, T. and D. Zendelovska, 2002. Determination of trace elements in iron minerals by atomic absorption spectrophotometry. Turk. J. Chem., 26: 271-280.

Stickler, D.J., 2002. Susceptibility of antibiotic-resistant Gram-negative bacteria to biocides: A perspective from the study of catheter biofilms. J. Applied Microbiol., 92: 163S-170S.