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The Role of Microbe/Metal Interactions In The Degradation of Emulsion Paintings of Buildings In Port Harcourt, Nigeria

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Abstract

In Nigeria, it is common to notice "infected patches" on emulsion paintings, mostly on the walls of buildings and fences. These emulsion paintings contain a wide range of constituents and therefore provide ecological niches that may be exploited by a large variety of microbial species. Samples of these paintings were collected from three different locations in the University of Port Harcourt, Nigeria. In order to determine the microbes in both degraded and undegraded walls, some of the samples were cultured and incubated for 24hours at 37^{o}C. The colonies that developed were subjected to different tests: motility, methyl-red, voges proskauer, oxidase, indole, citrate utilization and catalase tests.Algae, bacteria and fungi were present in this environment but no algae was found in the undegraded wall The pH range of the degraded wall was also determined and found to be 6.59-7.04. While that of the undegraded wall was found to be 6.78-7.79. The heavy metal content of these samples was determined using Atomic Absorption Spectrometry. It was found that Fe, Na^{+}, Cr, Ni, and Zn dorminated these areas followed by Ca^{+}, Ti, and Cd.

Key words: Microbes, Bacteria, Fungi, Algae, Heavy Metals, pH, Emulsion Paintings

Introduction

Beveridge and Doyle (1989), Ehrlich and Brierley (1990), Gadd (1993), Macaskie (1987), and so many others carried out extensive work on the interactions of microbes and metals and made several reports. The uptake of trace metals and their subsequent utilization in enzyme activation occur in all microbes (Wackett *et al*, 1989). Since ferric iron in the environment at around neutral pH exists mainly in a water-insoluble form, its uptake under aerobic conditions requires the microbial formation of ligands called siderophores, to render the ferric iron soluble (Neilands 1974). A number of

microbes are able to use some metals as electron donors or acceptors in energy metabolism (Ehrlich, 1996). Depending on the element, the metal species may be in the simple ionic form or in the form of oxyanions. As energy sources, oxidizable metals may satisfy the entire energy demand of an organism (Chemolithotrophs). For example, the eubacteria *Thiobacillus ferrooxidans*, and the *archaea Acidianus brierleyi*, and *sulfolobus acidocaldarius* are able to obtain all their energy for growth from the oxidation Fe(11) to Fe(111)

(Ehrlich,1996); *stibiobacter senarmontii* from the oxidation of Sb_2O_3 to Sb_2O_5 (Lyalikova *et al*,1976), and *Pseudomonas asenitoxidans* from the oxidation of AsO^{2-} to AsO_4^{3-} (IIyaletdinov and Abrashitova,1981).

In the original concept, as formulated by von Wolzogen Kuhr and van der Vlugt (1934), sulphate reducing bacteria promote biocorrosion of cast-iron metal surfaces anaerobically through cathodic depolarization. In this model, an iron surface exposed to aqueous moisture undergoes the spontaneous reation:

$$Fe^0 + 2H_2O \text{ ----} \rightarrow Fe^{2+} + 2OH^{--} + H_2 \text{ ---------} \quad (1)$$

with the reaction

$$Fe^0 \text{ ----}\rightarrow\text{----------} Fe^{2+} + \text{……} 2e \text{ ----------} \quad (2)$$

at anodic regions, and the reaction

$$2H_2O + 2e \text{ -----}\rightarrow 2OH^- + H_2\text{----} \quad (3)$$

at cathodic regions.

The H_2 generated in a cathodic region was thought to accumulate at the iron surface where it was generated: its build-up causing passivation (polarization) of the surface, i.e., its build-up having stopped further corrosion.

Sulfate-reducing bacteria, when using this hydrogen in their reduction of sulfate, as illustrated by this reaction:

$$4H_2 + SO_4^{2-} + H^+ \rightarrow HS^- + 4H_2O\text{---------} \quad (4)$$

were thought to depolarize the surface, thereby promoting a continuation of the corrosion process.

The sulfide they generate could react with Fe^{2+} produced at anodic area which would also help to promote corrosion if iron sulfide did not precipitate on the surface as a uniform film that would passivate the iron surface as long as the film was undisturbed. Although some past experiments seemed to lend support to this model, the general view now is that anaerobic biocorrosion is the result of several different microbiological reactions.

The growth of microorganisms on paintings may cause aesthetic and structural damage. With aesthetic damage, one must consider pigment discoloration, stains and the formation of a biofilm on the painted surface, whereas with structural damage, one must consider the cracking and disintegration of paint layers, the formation of paint blisters, and the degradation of support polymers or of glues and binders resulting in the detachment of the paint layer from the support (Ross,1963).Of course, the two types of damage are strongly linked, and in the long run, structural damage greatly affects the aesthetic quality of a painting. In fungal colonization of mural paintings,

Saiz-Jimenez and Samson (1991) have shown that at the beginning, the growth of fungi on the surface of a mural caused only aesthetic damage since there was little or no alteration of the painted surface. Later on, fungal growth in depth occurred. Hyphae penetrated the painted layer, degrading some of its components (especially glues and binders), which resulted in a decrease in the cohesion of the painted layers, thus giving rise to exfoliations, cracking and loss of the paint. Similarly, cyanobacteria and algae growing on paintings exposed to light may cause considerable damage. Besides the aesthetic damage caused by a green, black, brown, or yellow algae patina covering the painted portions, these organisms may cause weathering of the surface layers, accelerating the detachment of portions of the painted layer as well as the underlying plaster (Klens and Lang,1956).

Gettens and coworkers were among the first to point out in1941 that paintings could be defaced or destroyed by the growth of those small, parasitical plants, commonly called ”mould”or“mildew”. They reported that these organisms could cause changes to the painted surfaces through staining, discoloration or formation of patinas and efflorescence. Besides, they showed that many of such organisms, especially the fungi, could grow between the paint layers and the wall, causing a swelling of the paint film which could lead to the detachment of portions of the painted layer and their disaggregation from the wall.

Experimental Procedure

Sample site description

The sample sites are located in Choba Park, Delta Park and Abuja Park of the University of Port Harcourt, Nigeria (see fig 3 showing the map of University of Port Harcourt). In Choba Park, the engineering workshop building was used as site 1, while in Delta Park, a hostel building was used as site 2, but in Abuja Park, a complex building (housing the College of Natural and Applied Sciences) was used as site 3. These buildings were clearly marked as Research Sites for the period of work.

Sampling Procedure

Both degraded and undegraded emulsion paintings were scraped from the walls of these research sites in the University of Port Harcourt, Nigeria, using a plasticc spatula, into clearly labelled plastic bags. They were later transferred into preconditioned glass containers and stored in a refrigerator.

Sample Treatment and Analysis

With the aid of a spatula, the sample was transferred into a sterile agar plate. Using a sterile pipette, a 0.1ml of physiological saline solution was transferred into the plate. The inocula were inoculated by spread plate technique in triplicates using a bent glass rod. The cultured plates were all inoculated for 24 hrs at 37^0C. After the incubation period, the colonies that developed were counted and the average mean value calculated and used for the calculation of colony forming units per gram (Cfu/g).

Enumeration of Total Fungi

With a spatula the sample was transferred into a potato dextrose agar plate. The medium was aseptically dispensed into sterile petri dishes and allowed to solidify at room temperature. The solidified dry medium was then inoculated after performing ten fold serial dilutions. The cultured plates were all incubated for 72 hours at room temperature. After incubation, growth was observed and colonies counted in the plates. The colony forming units per gram were calculated from the mean value of the plates.

Figure 1: Plate 10 (A freshly degraded emulsion painting (infected wall))

Figure 2: Plate 7 (An undegraded emulsion painting (good wall))

Purification of Bacteria Isolates

Once the total heterotrophic bacteria were counted, culture plates from each sample were selected for purification. Based on cultural characteristics, the colonies were picked, labeled and transferred to fresh sterile nutrient agar plates and incubated for 24 hours at room temperature. After incubation, the isolates were all gram stained and viewed under the oil immersion objective (x 100) of a compound microscope. Mixed cultures were further sub-cultured to obtain a pure culture.

Gram staining

To confirm the purity of the isolates, the gram staining procedure was performed as outlined, herewith using clean labeled and grease-free slides. A smear of each isolate was prepared, fixed and flooded with 0.5% crystal violet (name of a stain) and allowed to stain for 60 seconds. It was rinsed under slow running tap water and gram iodine was introduced into the smears for 30 seconds. It was rinsed off the same way and immediately decolorized using 95% ethanol within the period of 10-15 seconds, keeping the slides in a slanting position. The slides were rinsed under slow running water. The decolorized smears were all stained for 60 seconds with 1% safarine and finally blotted dry for microscopy using the immersion objective lens of a microscope. The gram reaction of the isolates was observed and noted.

Spore staining

The technique was performed by using Malachite green as the initial stain, while safarine was used for counter staining. A smear of each isolate was made to the grease–free labeled clean slide. It was allowed to fix by air drying. The fixed smear was placed on a beaker of water that was previously heated. The slide was flooded with 5% malachite green and boiled using a bunsen flame for 2 minutes, not allowing the smear to dry. The steaming allows heat to permeate the spore coat in order to ensure full penetration of the dye into the spore. Upon washing out the initial stain, 1% safarine was used to counter stain. The smear was rinsed under slow running tap water and air-dried. The slide was viewed under a microscope (x 100 objective lens). Vegetable cells took up the counter stain, thus appearing red, while the endospores retained green (Malachite Test).

Motility Test

The test was performed to determine the presence of flagella in the bacterial isolates with the use of a semi solid agar medium. The medium is commonly referred to as motility medium. It was prepared by weighing its components, dissolving them in deionised water and boiling the solution to dissolve the agar completely. The medium was dispensed in 10ml aliquots into test tubes plugged with cotton wool and finally sterilized by autoclaving for 15 minutes at a $1.2kg/cm^{2-}$ pressure and a temperature of 121^0C. It was then allowed to cool and solidify in an upright position. The incolua was labeled and inoculated aseptically using a straight wire. The process was carried out for 72 hours at 37^0C.

Motility was observed as hazy growth that spreads throughout the stab line rendering the medium slightly opaque. Non motile ones grew only along the stab line.

Biochemical Test for identification of bacteria isolates

All the tests were carried out in duplicates and controls were set up alongside the tests. They were, generally, not inoculated unless otherwise stated. Each isolate was examined for its ability to ferment some sugar (Carbohydrate), and then tested for the production of indole, the production of hydrogen sulphide from triple sugar iron agar, citrate utilization, catalase production, starch hydrolysis, methyl red and vogu vogues-proskaur (MRVP) and oxidase test.

Determination of heavy metals in degraded and undegraded emulsion paintings by the APHA 3111B AAS method (American Public Health Association)

Sample treatment and analysis (APHA 3111D (V))

The sample was perfectly washed in deionised water by shaking it for 10 minutes. Then it was air dried for 15 hours. After it was oven dried to a constant weight at 55^0C, 0.1g of the sample was dissolved by using HNO_3/$HCLO_4$. The residue was filtered and diluted to desired volume with distilled water. The blank (distilled water) was first aspirated, then the quality control standard solution, and finally the sample solution according to the guildelines in table 8 below:

Table 1: Summary of operating conditions for AAS machine (APHA 3111B)

Metal	Lamp current (mA)	Wavelength (nm)	Slit width (nm)	Silt Height	Flame type	Fuel Flow(L/MIN)	Air Flow(L/min)
Cd	8.00	228.80	0.50	Normal	Air - acetylene	1.20	10.0
Co	15.00	240.70	0.20	Normal	Air - acetylene	1.20	10.0
Cr	15.00	357.90	0.30	Normal	Air - acetylene	1.60	10.0
Cu	15.00	324.7	0.50	Normal	Air - acetylene	1.00	10.0
Pb	8.00	217.0	1.00	Normal	Air - acetylene	1.10	10.0
Zn	10.00	213.9	1.00	Normal	Air - acetylene	1.10	10.0
Ni	15.00	232.0	0.20	Normal	Air - acetylene	1.00	10.0
Fe	15.00	248.30	0.20	Normal	Air - acetylene	1.20	10.0
Mn	15.00	279.50	0.20	Normal	Air - acetylene	1.00	10.0
V	18.00	310.30	0.20	Normal	N_2O- acetylene	6.00	10.0
Ba	15.00	55.36	0.50	Normal	N_2O- acetylene	6.00	10.0

Results and Discussion

Microbial Analysis

Results

Degraded Wall

Table 2: Microbial Density of Samples in Degraded Wall

S/N	THBC	THFC	ALGAE
Site 1	2.6×10^5cfu/g	$1.8\times \mathbf{10}^3$cfu/g	$1.4\times \mathbf{10}^2$cfu/g
Site 2	$3.12\times \mathbf{10}^5$cfu/g	$2.4\times \mathbf{10}^3$cfu/g	Nil
Site 3	$2.14\times \mathbf{10}^5$cfu/g	$1.3\times \mathbf{10}^3$cfu/g	$2.26\times \mathbf{10}^2$cfu/g

Sample Code:
THBC→Total heterotrophic Bacteria Count
THFC→Total heterotrophic fungi count
CFU/G→Colony forming units per gram of sample

Table 3: Identification of Bacteria in Degraded Wall

Isolate lab no	Gram reactions	Cell Morphology	Catalase test	Oxidase test	Moltility	Spore test	Citrate	Starch hydrolysis	MR	VP	SUGAR FERMENTATION					Probable Generae
											Indole	Glucose	Sucrose	Maltose	Lactose	
1A	+	Rods	+	-	+	+	+	+	+	-	-	A	-	A	A	Bacillus Sp
1B	-	Rods	+	-	-	-	+	-	-	+	-	A	A	-	-	Flavobacterium Sp
2A	+	Rods	+	-	+	-	-	+	-	-	-	A/G	A/G	A	A/G	Arthrobacter Sp
2B	+	Cocci	+	-	-	-	+	-	-	-	-	A/G	A	A	A	Microcolus Sp
2C		Rods	+	-	-	-										
2D	+	Rods	+	-	+	+	+	+	+	-	-	A	-	A	A	Bacillus Sp.
A	+	Rods	+	-	+	+	+	+	-	+	-	A	A	A	A	Bacillus Sp.
3B	-	Rods	+	-		-					-					
3C/	+	Rods	+	-	+	+	+	-	-	+	- A	A	-	A	A	Bacillus Sp.

Sample Code:

+	:	Positive
-	:	Negative
A	:	Acid production
G	:	Gas production
A/G	:	Both acid and gas production

MR : Methyl red test
VP : vogues Proskauer test.

Table 4: Identification of Fungi on Degraded Wall

Sample Number	Name
1A	Rhizopus SP
2A	Aspergillus SP. (niger)
2B	Aspergillus Flavus
2C	Fusarium sp
3A	Aspergillus sp

Undegraded Wall

Table 5: Microbial Density of Samples in Undegraded Wall

S/N	THBC	THFC	ALGAE
Site 1	$1.65x\ 10^3$cfu/g	$1.7\ x10^3$cfu/g	Nil
Site 2	$1.03x\ 10^4$cfu/g	$3.6x\ 10^2$cfu/g	Nil
Site 3	$5.4x\ 10^3$cfu/g	$8.0x10^2$cfu/g	Nil

Sample Code:
THBC→Total heterotrophic bacteria Count
THFC→Total heterotrophic fungi count
CFU/G→Colony forming units per gram of sample

Table 6: Identification of Bacteria in Undegraded Wall

Isolate lab no	Gram reactions	Cell Morphology	Catalase test	Oxidase test	Moltility	Spore test	Citrate	Starch hydrolysis	MR	VP	SUGAR FERMENTATION					Probable Generae
											Indole	Glucose	Sucrose	Maltose	Lactose	
1A	+	Rods	+	-	+	+	+	+	-	+	-	A / G	A	A	-	Bacillus Sp
1B	+	Rods	+	+	-	+	+	+	-	+	-	A / G	A	-	-	Bacillus Sp
2A	+	Rods	+	-	+	+	+	+	-	+	-	A / G	-	A	-	Bacillus Sp
2B	+	Rods	+	-	+	+	+	+	-	+	-	A / G	-	A	-	Bacillus Sp

2C		Rods	+	+	-	+		+	-	+		A / G	A		-	Bacillus Sp
2D	+	Rods	+	-	+	+	+	-	-	+	-	A / G	A	A	A	Bacillus Sp.
A	+	Rods	+	-	-	+	-	-	-	+	-	A / G	-	A	-	Bacillus Sp.
3B	+	Rods	+	+		+					-	A / G	-		-	Bacillus Sp
3C /	+	Rods	+	-	+	+	-	-	-	+	-	A / G	-	A	A	Bacillus Sp.

Sample Code:

⇒ + : Positive
- : Negative
A : Acid production
G : Gas production
A/G : Both acid and gas production
MR : Methyl red test
VP : Voges proskauer test.

Table 7: Identification of Bacteria in Undegraded Wall

Sample Number	**Name**
1A	Aspergillus sp
2A	Rhizopus sp
2B	Aspergillus sp
2C	Aspergillus sp
3A	Aspergillus sp

From the results, it is obvious that at site 2, bacteria and fungi were most abundant, hence causing much aesthetic and structural damage, so much so that there were no algae growing on it. Furthermore, at site 1, the population of bacteria and fungi was such that it allowed the growth of some algae with some damage on the painted wall. At site 3, it was observed that the bacteria and fungi were least abundant, hence causing little damage on the painted wall. It therefore follows that an increase in the population of the bacteria and fungi causes more damage to the painted wall and decreases the population of the algae. In all the samples, the bacteria were most abundant, especially the bacillus species. In the case of the fungi colony, no yeast was identified but moulds were present. The most abundant in the moulds were the

aspergillus species, followed by the rhizopus and fusarium species. The least of the microbes identified were the algae, but at site 2, no algae were present. However, this observation is supported by the work of Gettens and coworkers in 1941 in which they showed that microbes, especially the fungi, could grow between the paint layers and the wall, causing a swelling of the paint film which could lead to the detachment of portions of the painted layer and their disaggregation from the wall. Besides, work by Agrawal and others in 1988 has also shown that paintings contain a wide range of organic and inorganic constituents, that they provide different ecological niches that may be exploited by a large variety of microbial species, and that many of the components of painting and additives are biodegradable. Thus, microbial activities contribute greatly to the degradation of emulsion paintings.

Analysis of Heavy Metals in Degraded and Undegraded Emulsion Paintings.

Results:

Table 8: Mean values of concentrations of heavy metals in degraded sites with their standard deviations (mg/kg)

HEAVY METALS	SITE 1	SITE 2	SITE 3
Titanium	0.97±0.02	0.97±0.02	0.94±0.02
Nickel	10.75±0.16	10.75±0.16	10.56±0.16
Iron	78.35±0.17	78.35±0.17	77.46±0.06
Zinc	4.47±0.20	4.47±0.20	4.48±0.17
Chromium	11.34±0.09	11.34±0.09	11.30±0.02
Calcium	2.70±0.24	2.70±0.24	2.59±0.01
Sodium	35.26±0.25	35.26±0.25	34.97±0.06
Cadmiom	0.05±0.02	0.05±0.02	0.02±0.01

Table 9: Correlation of Degraded Sites

	SITE 1	*SITE 2*	*SITE 3*
SITE 1	1		
SITE 2	1	1	
SITE 3	1	1	1

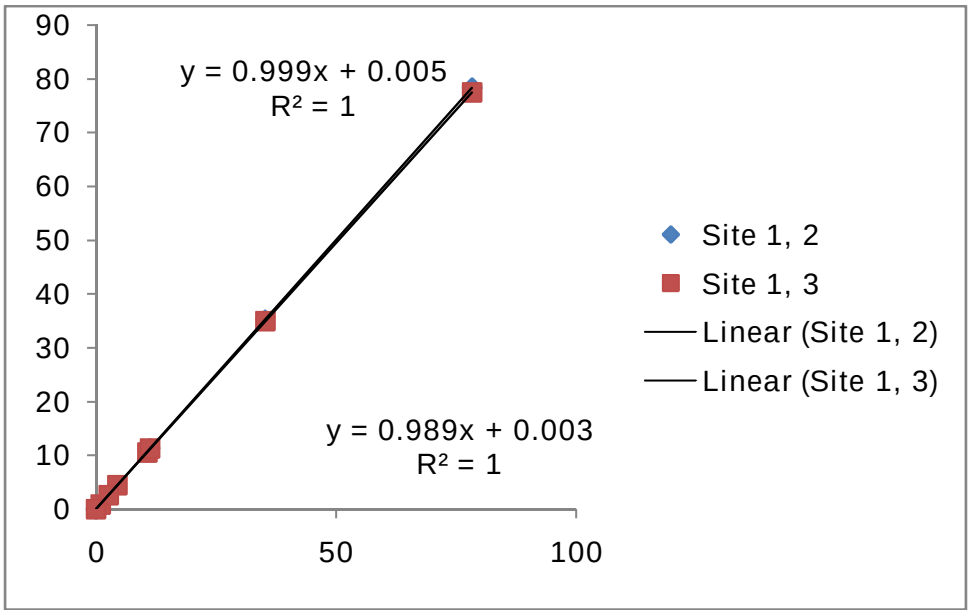

Figure 3: Graph of Correlation between Heavy Metal Concentrations in Degraded sites

Table 10: Mean values of concentrations of heavy metals in undegraded sites with their standard deviations (mg/kg).

HEAVY METALS	SITE 1	SITE 2	SITE 3
TITANIUM	0.65±0.04	0.65±0.05	2.71±2.85
NICKEL	6.91±0.61	7.45±0.13	7.41±0.35
IRON	50.34±0.76	54.49±0.41	52.91±0.23
ZINC	2.50±0.44	2.85±0.10	2.42±0.14
CHROMIUM	6.62±0.24	7.45±0.27	6.74±0.23
CALCIUM	1.44±0.21	1.57±0.26	1.43±0.02
SODIUM	34.12±0.51	32.39±0.44	31.59±0.44
CADMIUM	0.02±0.00	0.01±0.00	0.02±0.01

Table 11: Correlation of Undegraded Sites

	SITE 1	*SITE 2*	*SITE 3*
SITE 1	1		
SITE 2	0.997309074	1	
SITE 3	0.996718624	0.999136327	1

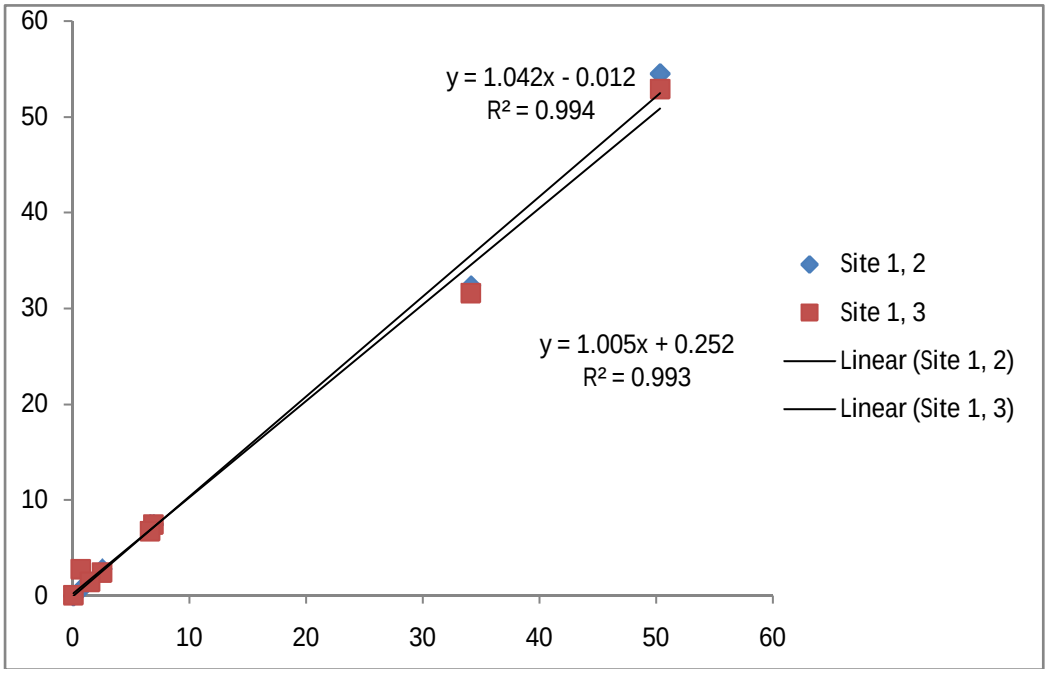

Figure 4: Graph of Correlation between Heavy Metal Concentrations in Undegraded sites

Discussion

The results in tables 15 and 16 indicate the concentrations of heavy metals in both degraded and undegraded sites. It is observed that there is high deposit of iron, sodium, chromium and nickel in both sites, although this is higher in the degraded sites. Perhaps the only reason is that there is no microbe/metal interaction yet in the undegraded sites. But in table 15, there is a microbe/metal interaction and other microbial activities giving rise to degradation. The city of Port Harcourt is home to many oil and gas companies and other production companies. Hence, it experiences a high level of industrial activities. A comparison of results in tables 14,15, and 16 reveals a higher amount of metal content in the last two tables than in the first one. This is clearly illustrated as shown in figure one. This difference in amount of metal content can only be explained by the fact that there is a gradual build up of these heavy metals from the environment. Work by Battarbee, Niragu and others in 1988 and 1989 showed that these metals are released into the environment by both natural and anthropogenic sources, especially industrial activities and automobile exhausts. Gadd, Ehrlich, Macaskie, Beveridge, Brierley and so many others carried out extensive work on the interactions of microbes and metals and made several reports. The uptake of trace metals and their subsequent utilization in enzyme activation occur in all microbes (Wackett et al 1989). Enzymatic microbial detoxification of harmful metals is another type of microbe/metal interaction. In this process, a toxic metal species may be converted to a less toxic or non-toxic entity by enzymatic oxidation or reduction. The bacterial oxidation of AsO_2 to AsO_4^{3-} by a strain of *Alcaligenes faecalis*, and the reduction of CrO_4^{2-} to $Cr(OH)_3$ by *P. fluorescens* are examoles of

such redox reduction (Ehrlich, 1996; Wang and Shen, 1995). Besides, prockaryotic and eukaryotic microbes are capable of accumulating metals by binding them as cations to the cell surface in a passive process (Beverridge and Doyle, 1989, Gadd 1993). These results support our observations that there is usually a gradual build-up of metals from the environment on the painted surface and that they contribute extensively to the degradation of this surface.

The results here, R=1 and R^2=1, for each correlation, show that there is a perfect linear correlation of the concentrations of heavy metals for the three sites in the degraded area. This means that as the concentrations of heavy metals increase in site1 there is a perfectly corresponding increase of the concentrations of heavy metals in site 2 as well as in site 3. An interesting factor also is that the total variation in the values of the concentrations in all the three sites is predictable by the regression line for both site 1 and site 2 and between site 2 and site 3 as well as between sites 1 and 3. Just as it is in the degraded sites, there is a perfect correlation of metallic concentration in the undegraded sites as well. In this case, 99% of the total fluctuation is also predictable by the regression lines as indicated by the value R^2=0.99.

Conclusion

It is obvious from all the results that algae, bacteria, fungi and heavy metals play a major role in the degradation of emulsion paintings on the walls of buildings and fences in Port Harcourt, Nigeria. This degradation in the emulsion paintings is primarily caused by the growth of fungi between the painting and the wall and the various microbe/metal interactions.. All these activities weaken the paint structure and the adhesion of the paint to the wall, causing it to break away from the wall eventually.

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