

Effects of Microbiological Activity on the Conservation of Aboriginal Rock Art

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Abstract

Sampling of pigments and rock surfaces in the West Kimberley region of WA during both wet and dry seasons has provided a useful guide to the processes affecting the deterioration of rock paintings. A comparison of the bacterial counts and the number of yeasts and moulds has shown that significant differences exist between the levels of activity in the two seasons. Measurements of surface pH of the images has shown that the wet season is associated with significant increases in acidity which is due in part to the increased biological activity. The availability of moisture within the shelters is the major mechanism controlling the biological activity. Other factors affecting the deterioration include the presence of excreta associated with a nearby bat colony.

Introduction

In 1988 the Western Australian Museum's Department of Materials Conservation received a grant to study the process of deterioration of Aboriginal rock paintings in the West Kimberley region of Western Australia. The major emphasis of the research, which was carried out in the dry season of June and July 1990, was on microclimatic conditions within shelters. Assessing the overall stability of the images also involved mineralogical, biological and management issues since they impinge on the overall site stability. Following the success of the initial dry season work a subsequent grant was obtained to repeat all the assessments during the wet season in 1992. The wet season pH data from the Napier range was augmented by measurements from an additional site, *Barralumma III*, during the 1992 field work.

The Napier Range is well known for its large number of rock paintings, many of which are predominantly composed of either white or off-white pigments. Some of the images are also executed over a white background applied to the rock or underlying paintings. In a previous study in the area, Clarke (1976) found that the white mineral huntite $[Mg_3Ca(CO_3)_4]$ had been widely used and that its physical properties and method of application predisposed it towards serious deterioration, particularly under the damp or humid conditions which are regularly experienced during the wet season in the tropical north of Australia. During the initial dry season field work, it became apparent that the surface pH of the pigments and the rock substrates varied across individual sites and it differed from the limestone substrates of the Napier Range. Samples of the substrates

adjacent to painted surfaces and of nearby soil were taken for microbiological assay since it was thought that the microflora may be exerting a significant influence on the processes of deterioration. The measurements were repeated in a subsequent wet season which saw significant changes in the values of the surface pH and in the overall level of microbiological activity.

The nature of the deterioration mechanisms on rock painting sites is dependent on the interaction of all the weathering forces on the host rocks and on the pigments themselves. The impact of microflora on the deterioration processes is in part due to the specific interactions of the metabolites of bacteria, fungi, yeasts and moulds with the pigments and the effects of the same on the binding characteristics of the materials. The penetration of hyphae into the images and through the sequential paint layers can cause significant changes in the bonding of the pigments to each other and to the bonding between the substrate and the painted images. The observations in this report were made at the Napier Range sites *Bilyarra*, *Barralumma I*, *II* and *III*, which are all limestone in their basic geology. The implications of biological activity on the conservation management of this unique resource are discussed. Conservation of the paintings has become a major concern since the arrival of the European settlers in the West Kimberley region in the late 19th century. At this time traditional repainting and retouching of the images was halted or significantly reduced as a result of changes in land ownership. Several Aboriginal groups wish to change this situation.

Microbiological Measurements of Pigments and Surface pH

A number of areas were sampled for microbiological activity and the material was placed in sterilised vials containing distilled water and peptone. Because of delays between field and laboratory sampling in the dry season we cannot directly compare our analyses with standards associated with the food industry. However it is useful to note that fresh milk has typical bacterial counts of 30,000 per millilitre. All the bacterial counts are based on a dilution factor of one thousand before the samples were placed on assorted culture media. The data collected for the 1990 dry season at the various sites in the Napier ranges are listed below in Table 1.

The pH values in this Table are those that were obtained on the sites in the Kimberley and do not refer to the acidity readings of the cultures. At a first glance a comparison of the bacterial counts between sites in the same season appears difficult owing to the variability of activity across one area of the same image. However, when the data is grouped into ranges of *sterile*, *moderate* and *high* it is possible to see some trends. Bacterial counts of the order $85,000 \pm 35,000$ can be regarded as *sterile*, counts of the order $480,000 \pm 90,000$ are *moderate*, while counts of $600,000 \pm 100,000$ can be regarded as *high*. The overall level of activity of bacteria, yeasts and

moulds for *Barralumma I* is the greatest, *Bilyarra* is the next most active whilst *Barralumma II* is the least active site. It is important to note that it is a normal situation for counts to vary by more than an order of magnitude across a site. Thus the dust at sample 2 is regarded as sterile, areas such as samples 4-5 and 7-9 are *moderate* and samples 1 and 3 have *high* biological activity. The number of yeasts and moulds that were counted in the *Barralumma I* samples showed a one hundred fold difference in counts. This provides a good indication that the level of activity across a site can vary significantly and so the extent of preservation can similarly be dramatically different.

Since obtaining surface pH measurement on pigments, rock and dust surfaces is a much simpler and less invasive procedure than taking samples of material for microbiological assays, we have used the values of the surface pH as a primary tool in assessing the nature of the surfaces. Since the metabolites of biological activity are often acidic, the surface pH readings on the sites can be used as a guide to differences in the nature of the sites that have a similar geological signature. The pH readings for the three sites in the Napier Ranges are listed for the dry season in Table 2. A comparison of the minimum pH for each of the three sites is listed in Table 2 with the average bacterial counts showing that there is a very strong correlation

Table 1: Microbiological Assays of Sites in the 1990 Dry Season.

Sample number	Site description	Bacterial count	Yeasts & moulds	pH
1	<i>Barralumma I</i> - dust from ledge in large cave orifice	700,000	30	7.38
2	soil below lower dingo	80,000	240	8.44
3	dust above ledge above two white figures on right	850,000	3,000	8.44
4	<i>Barralumma II</i> - dry season	380,000	30	6.30
5	* lower shelf floor	400,000	100	7.4 -8.4
6	* lowest shelf floor	90,000	150	8.4
7	<i>Bilyarra</i> - dry season - lower floor of shelter	650,000	10	7.21
8	upper floor of shelter	500,000	90	8.84
9	beneath Wandjina	510,000	40	7.21

between higher bacterial counts and lower pH or higher acidity. A linear regression on the three data sets gives a correlation coefficient of 0.959 for the relationship between minimum pH and the mean number of bacteria per ml. The equation describing the correlation is,

$$\text{pH}_{\text{minimum}} = 7.51 - 3.07 \times 10^{-6} B$$

where B is the mean number of bacteria on the site. The good correlation coefficient supports the observation that there is a definite correspondence between surface acidity and microbiological activity.

The similarity of the maximum pH values is due to the common nature of the calcareous substrate and the minimum pH values are indicative of the varying levels of microbial activity at the sites. The minimum pH observed at *Barralumma I* was 5.71 and this was associated with a high level of biological activity with a count of 850,000 per millilitre. This location had the highest acidity and highest bacterial count of all the measurements made during the 1990 dry season in the Napier Ranges. This data clearly indicates that the measurement of pH is a good indicator of biological activity.

The 1992 wet season measurements of microbiological activity are listed for the same three sites in Table 3. Between the 1990 dry season and the field work in 1992, fencing of *Barralumma I* to stop cattle rubbing against the images may have resulted in a reduced amount of nutrients from dung deposits being available. The most probable reason why this site has the most acidic mean pH of the three sites in the dry season and in the wet season is the presence of the bat colony in the caves behind the rock paintings. When working at the site the smell of bat urine and faecal material was most marked. A recent report on the effects of bat urine and faeces (Paine: 1993) has noted the devastating

impact of the same on the walls, painted surfaces and brass ornamentation in English churches. Paine noted that the oxidation products of the bat urine, which consists of approximately 63% urea, $\text{CO}(\text{NH}_2)_2$, up to 6% ammonia and 1% uric acid $\text{C}_5\text{H}_4\text{N}_4\text{O}_3$, can react with the pigments and with the substrate. A *sterile* sample of dust from a ledge at *Barralumma I* had a bacterial count one hundred times less than the areas where the soil sample below the dingo painting was taken. The soil in this area had a more moist look and feel than the sample from the ledge. The dry season differences between the *sterile* and *moderate* areas of this site differed in bacterial counts by only one order of magnitude whereas the same areas in the wet season had a 25 fold difference in bacterial counts.

This internal comparison clearly indicates that the increased moisture levels associated with the wet season have a major impact on the overall level of biological activity. Counts of the yeasts and moulds were also generally high in the wet season data. Increases in acidity are to be expected since water is often the main factor controlling the activity of fruiting colonies of fungi and of yeasts and moulds.

The surface pH data for the sites during the wet season in the Napier Ranges are listed in Table 4.

The fall in the mean surface pH of *Barralumma I* from 6.26 ± 0.69 for the dry season to a value of 5.42 ± 0.27 for the wet season is a sign that the increased level of biological activity is the fundamental cause of the shift in the acidity of the site readings. Within the individual readings of activity for a site, there is a good correlation of increased microbiological activity and increases in acidity. The influence of the urine and faecal material from the bat colony at the rear of *Barralumma I* cannot be underestimated

Table 2: 1990 Dry Season pH data For Sites in the Napier Ranges

Site location	Average pH	Maximum pH	Minimum pH
<i>Barralumma I</i>	6.26 ± 0.69	8.44	5.71
<i>Bilyarra</i>	7.37 ± 0.82	8.84	5.95
<i>Barralumma II</i>	7.63 ± 1.00	8.63	6.63

Table 3: Microbiological assay of sites in the 1992 wet season

Sample number	Site description	Bacterial count	Yeasts & moulds	pH
114 M	<i>Barralumma I</i> - wet season dust above dingo	1,700	1,000	7.22
115 M	soil below dingo	177,000	7,500	5.40
116 M	soil above small figures	44,000	2,400	6.40
121 M	<i>Barralumma II</i> - wet season near green algae	220,000	165	6.95
122 M	* blue green algae	1,920,000	1,100	5.05
123 M	* lower shelf soil	27,000	3,700	8.05
SBi 108	<i>Bilyarra</i> - wet season active drip line on calcite straw	>>240,000	145	7.62
SBi 109	Fungal spores	Nil	Nil	6.60

in terms of it supplying a major nutrient source, particularly nitrogen, for microorganisms. Since urea is slightly basic it will not contribute directly to the acidity.

Although no samples of materials were taken for microbiological analyses at *Barralumma III* the pH data obtained at the site during the 1992 wet season showed a bimodal distribution of values. The more alkaline peak was associated with the pH of the soil samples and the more acidic peak was identified with pigment surfaces. The mean surface pH of the substrate and the images was 6.13 ± 0.47 which is 0.34 pH lower than the average for *Barralumma II*. If the average pH is seen to be an indicator of the overall level of microbiological activity then the obvious question is what are the factors that make *Barralumma III* more "active" than *Barralumma II*? The answer lies in the different nature of the sites. *Barralumma III* is some ten metres above the level of the surrounding countryside on a rising incline and there are a large number of trees in the area which provide

some degree of shade for the rock shelter. *Barralumma II* is a very much more sterile rock shelter with little or no vegetation in the immediate vicinity. The pleasant sheltered nature of *Barralumma III* is also appreciated by animals, such as cattle, which have rested in the area. Although not as biologically active as *Barralumma I* with its bat colony, this site is dominated by large amounts of oxalate skin growing over the images which is an apparent manifestation of the presence of metabolites from microflora.

The minimum pH of 5.48 was observed on the surface of a "very old and not so dusty snake" (Haydock:1992). The low pH of this image is probably due to the presence of bacteria since the dust itself has a pH of 6.90 which is very close to the pH of the "very old dusty snake" at pH 7.08. The standard deviation of the mean pH data at *Barralumma III* was 0.47 and this is smaller than the value of ± 0.74 at *Barralumma II*. The smaller range of pH measurements at *Barralumma III* is just a reflection of the more

Table 4: 1992 wet season surface pH for Napier Range sites

Site location	Average pH	Maximum pH	Minimum pH
<i>Barralumma I</i>	5.42 ± 0.27	8.21	5.07
<i>Barralumma II</i>	6.13 ± 0.47	7.08	5.48
<i>Barralumma III</i>	6.47 ± 0.74	8.05	5.05
<i>Bilyarra</i>	7.07 ± 0.64	8.42	5.95

homogeneous nature of the microenvironment of the site which was not repainted in 1987 when both *Barralumma* sites were significantly altered.

All three *Barralumma* sites are still more acidic than the mean value for *Bilyarra* which had a pH of 7.07 ± 0.64 in the wet (see Table 4). Since all four Napier Range sites share a similar geology the most readily discernible factor between the sites in the wet season is the amount of moisture available to the various sites. The other major influence at *Barralumma I* is the bat colony which seems to have a dominating influence on the survival/degradation of the images. Although the data on the mean wet season counts for yeasts and moulds is associated with a large scatter, the mean count for *Barralumma I* was 3633 ± 2793 which is roughly twice the mean value of 1655 at *Barralumma II*. This data further indicates that the bat colony has a major impact on the overall level of biological activity. Since the sampling points at *Barralumma II* had yeast and mould counts that ranged from 165-3700, the standard deviation of the mean is very large at 1495. The influence of water on the overall level of microbiological activity can be gauged from a more detailed discussion of the data in Tables 1 and 3. For the dry season data listed in Table 1, the greatest bacterial count of 850,000 per ml was obtained at sample point 3 at *Barralumma I* which was sheltered from direct sunlight and felt somewhat moist to the touch, compared with the totally desiccated nature of all the other samples. The wet season data in Table 3 had the greatest bacterial count for sample number 122M at *Barralumma II* with 1,920,000 per ml, which was associated with an area of active blue-green algal growth on the ceiling of the shelter. The algal growth was receiving a direct supply of water from the drip line. The impact of water is also clearly seen in the SBi 108 sample from the drip line of a calcite straw at *Bilyarra* where the estimated count was $2 \cdot 2^{1/2}$ million bacteria per millilitre (Tulloch: 1992). Owing to limitations on sample material the results were only reported as being very much greater than 240,000 per millilitre. Although the wet season count of yeasts and moulds at *Bilyarra* was low at 145, this value is three times higher than the dry season data in

1990. This is a clear indication that, under the conditions of a plentiful supply of moisture, it is possible to obtain very high levels of biological activity, even in the absence of major nutrient sources such as the bat colony at *Barralumma I*.

Comparison of Wet and Dry Season Microbiological Data

Owing to differences in sampling methodologies used in the dry and the wet seasons it is not possible to make direct comparisons of the counts. The bacterial counts for the 1990 dry season relate to samples which have been "incubated" in distilled water for a period of six weeks prior to analysis. Because of the gaps of up to six weeks between collecting and analysing the dry season samples, it was decided to collect the wet season samples in dry sterile containers and to incubate the material under controlled conditions in the laboratory. In order to facilitate a more direct comparison of the data from the two seasons, a method of *normalising* the bacterial counts between the 1990 dry season and the 1992 wet season has been developed and is described below.

In trying to gain some semi-quantitative method for comparing the data in the two seasons we need to have some way to *normalise* the data from the Napier Range sites that corrects for the two different methods of sampling. The basis for such a procedure is to standardise the counts for *sterile* areas of the sites in the two seasons. From the data listed in Table 1 two dry season *sterile* areas had bacterial counts of $85,000 \pm 7,000$ and this can be regarded as being typical of an insignificant level of biological activity. In a similar fashion the three samples taken at *Barralumma I* and *II* during the wet season (see Table 3) had mean counts of $24,233 \pm 21,285$ and these too can be regarded as background values. If we *normalise* the background readings to correct for the longer incubation period for the 1990 dry season, the dry season background counts are lowered by a factor of $\{85,000 \div 24,233\}$ or $3^{1/2}$. If this correction factor is applied to the dry season samples in Table 1 the average count of $447,000 \pm 67,000$ bacteria per ml for moderate levels of activity is normalised to $128,000 \pm 19,000$. The previous high level of $733,000 \pm 104,000$ becomes $209,000 \pm 30,000$ and so it becomes possible to make a better overall

comparison of the levels of activity in the two seasons. The results of this *normalisation* procedure for the Napier Range sites are summarised in Table 5.

Analysis of the data shows that the overall level of bacterial activity is significantly higher in the wet than in the dry season with a 65% increase in the *moderate* and more than a nine fold increase for the *high* levels at the various sites. Since the average shelter relative humidity reading for *Bilyarra* and *Barralumma I* was $22 \pm 3\%$ RH at a temperature of $31.6 \pm 0.5^\circ\text{C}$ this equates with a mean water vapour pressure of 7.6mm Hg; clearly insufficient moisture to allow a high level of activity to develop. The environment at *Barralumma II* was even more desiccating with a mean vapour pressure of 4.2mm Hg. During the wet season the average humidities, $74.4 \pm 4.4\%$, were much higher but the temperatures were much the same and the mean water vapour pressure was 25.8 ± 0.2 mm Hg. Inspection of the data in Table 5 for the relative number of bacteria at *high* activity levels during the wet season shows a very comparable level of increased biological activity compared with the increased availability of water.

When confronted with a management decision regarding control of vegetation in the area, it is now apparent that any factors that can lower the effective concentration of water available to the microflora will lower the level of biodeterioration. Any planting would have to be done with an understanding of the shading effects that would lower surface temperatures and so affect the overall moisture balance. These factors are counteracted by the fact that increased levels of vegetation will decrease the amount of dust depositing on the images. Control of visitor access is also important as this will also minimise dust containing nutrients from excreta being transferred to the images on

the rock surfaces.

Deterioration of the Pigments and Rock Surfaces

The higher level of biological activity in the wet season is a major cause of the changes in the surface pH. Since the vast majority of the pigments identified in the Napier Ranges involve carbonaceous species such as dolomite, calcite and huntite the increased level of acidity will result in increased rates of acid dissolution. Laboratory studies on the dissolution of huntite (Ford et al:1994) indicate that huntite dissolves according to the relationship,

$$\log_{10}[\text{Mg}^{2+}] = 3.94 - 0.38 \text{ pH}$$

The relationship shows that a change in pH from 7.63, the dry season mean at *Barralumma II*, to the mean wet season value of 5.42 at *Barralumma I* causes an increase of 230% in huntite solubility for a change of 2.2 in the pH. Owing to the stoichiometry of the $\text{Mg}_3\text{Ca}(\text{CO}_3)_4$, the overall solubility changes by one third of the increase in solubility of magnesium. Part of the change in the pH during the wet season may well be due to the absorption of sulphur dioxide, SO_2 , that results from the oxidation of hydrogen sulphide, H_2S , which is the end result of the breakdown of organic materials that have been oxidised during thunderstorms⁴. The characterisation of so many of the surface layers and pigment contaminants as being gypsum, $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$, is a clear indication of the major impact that the airborne sources of sulphate have on the deterioration of rock paintings.

Where there are high levels of biological activity at a site, the changes in pH may well assist the disbondment of the multi-layered pigment samples. From our analysis of the subsurface of exfoliated pigments, there is evidence of morphological changes in the pigments in the layers immediately adjacent to the exfoliated

Table 5: Comparison of "normalised" bacterial counts in the wet and dry seasons

Activity 10 ³ bacteria/ml	Dry Season	Normalised dry season	Wet season
Sterile	85	24	24
Moderate	447	128	212
High	733	209	1920

surface. These changes are typical of the dissolution/precipitation phenomena observed in marine concretions which encapsulate corroding metal artefacts on historic shipwrecks (MacLeod:1982). These observations indicate that part of the mechanism of disbondment involves the inward diffusion of an acidic "front" of metabolites percolating through the pigment and into the host rock surface. Flowing in the opposite direction is an alkaline diffusion gradient which arises from the interaction of acidic rains with the calcareous substrate. As the diffusion fronts interact there will be a series of dissolution and precipitation reactions which can ultimately lead to disbondment.

At *Bilyarra* a freshly exfoliated surface had a pH of 8.80 which is consistent with the pH of calcium carbonate. It may well be that crystallisation forces readily cleave off degraded interfaces from the host rock.

Conclusion

Our analyses demonstrate a greatly increased level of biological activity characterises the wet season. The shift to more acidic pH values for pigment and substrate surfaces in the wet season can be used as a good indicator of the overall impact of the microflora. Future work in the field could be facilitated using sites that have been cleared for painting images in the traditional manner for use in experiments. This would allow statistically valid sampling methodologies for pigments, substrates and microflora. From such future data we may be able to quantify the effect of various activities on the sites and be able to develop appropriate management strategies for culturally significant sites.

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Biography

Ian MacLeod*: Apart from his work on corrosion of metals on historic shipwrecks he has spent the past ten years working on the problems of deterioration of Aboriginal rock paintings and applying mineralogical and biological approaches to the classical chemical problems.

Philip Haydock*: After a degree in Environmental Sciences from Murdoch University he has worked in the area of Aboriginal rock paintings and their preservation, which saw him complete the Post Graduate Diploma at the University of Canberra. He has applied the techniques of micrometeorology to the problems of pigment deterioration and exfoliation.

Don Tulloch*: After more than thirty years professional experience as a chemist Don brought his skills with growing, identifying and culturing bacteria, yeasts and moulds to the project. He is currently a volunteer in the Conservation Department.

*Western Australian Maritime Museum

Bruce Ford is a conservation scientist with a wide range of analytical and practical skills, which have been applied to the problems of characterisation of the processes of deterioration of aboriginal rock paintings. Bruce also completed the Post Graduate Diploma Course in Rock Art at the University of Canberra.

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