

**FORMATION PROCESSES IN UNDERWATER  
ARCHAEOLOGY:  
A STUDY OF CHEMICAL AND BIOLOGICAL  
DETERIORATION**

Thesis submitted for the degree of  
Doctor of Philosophy  
at the University of Leicester

by

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**Abstract**

Archaeological formation processes involve the interactions whereby intrusive human induced evidence reaches a state of balance with its environment. Archaeological material in seawater is subject to a particularly complex set of formation influences because of the range of physical, chemical and biological factors concerned.

Controlled experiments were conducted in the laboratory and on three submerged sites in British waters over 52 weeks to determine which chemical and biological processes were responsible for the deterioration of a range of modern organic materials and metals. Understanding the effects these processes had on the materials would enable better interpretation of the submerged archaeological record. In addition, by understanding the environmental factors which affected the deterioration of the materials, it would be possible to suggest methods to mitigate their effects and facilitate better *in situ* stabilisation and preservation of submerged sites.

The results showed that the two categories of material were deteriorated by quite different processes. Essentially the organic materials were subject to biodeterioration through successive colonisation by micro and macro organisms and algae. The most important environmental parameters governing biodeterioration being seasonality and the presence of oxygen in seawater. The metals mainly underwent electrochemical corrosion, although there was evidence of corrosion induced by micro and macro organisms. The environmental parameters affecting corrosion were mainly the salinity of the seawater.

To mitigate the effects of these environmental parameters it is suggested that organic materials be re-buried and the redox potential and biochemical oxygen demand of the interstitial water of the re-buried sediment be monitored. It is suggested that in order to preserve metal artefacts *in situ* the corrosion potential of the surface of corroding metal artefacts should be measured and sacrificial anodes attached.

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**CHAPTER ONE**  
**FORMATION PROCESSES AND THE *IN SITU* PRESERVATION OF**  
**WOODEN SHIPWRECK SITES**

**1.1 Introduction**

Over the past two decades or so, land archaeologists have realised the need to study the formation of the archaeological record as a prelude to making inferences based upon excavated material; an inference being a statement about the past supported by relevant principles and relevant evidence (Schiffer, 1987: 4).

Binford's ethnoarchaeological work (1972: 89-90; 1978: 3-14; 1989: 190-208) with the discarded faunal remains of the Nunamiut of North America was pivotal in the development of the study of the formation of the archaeological record on land. His work, along with others, became known as "New" or "Processual" archaeology in the late 1960s and early 1970s. According to processual archaeologists, past behaviours of societies were easily provided by a set of principles, known as correlates, which related behavioural phenomena to material and spatial behaviour. In Britain, Clarke (1968) discussed a model to explain archaeological processes:

"The tentative model that we are about to develop arises from the assumption that cultural systems are integral whole units. Material culture, economic structure, religious dogma and social organisation are on this hypothesis merely subsystems arbitrarily extracted from their coupled context by the specialist academic. The socio-cultural system is a unit system in which all the cultural information is a stabilised but constantly changing network of intercommunicating attributes forming a complex whole - a dynamic system." (Clarke, 1968: 43.)

In the decades since Binford's and Clarke's work, processualism has become outmoded in archaeological theory due to its deterministic approach. Such inferential procedures assume that past behaviours of interest, for example material culture, economic structure, religious dogma and social organisation, were the sole determinants of the present day properties of the archaeological record (Schiffer, 1987: 5).

Correlates are clearly necessary for archaeological inference, but these principles are not sufficient. Any inferences must explicitly recognise and take into account the entire range of relevant processes that form the archaeological record.

**1.2 Formation processes of the archaeological record**

The factors that create the archaeological record are known as Formation Processes (Schiffer, 1987: 7). There is a substantial corpus of research related to formation processes operating on land. However, it is beyond the scope of this thesis to discuss this research in depth. Essentially, formation processes can be of two basic kinds: cultural, where the agency of transformation is human behaviour; and non cultural, in which the agencies stem

from processes of the natural environment. Cultural formation processes can be defined as the processes of human behaviour that act or transform artefacts after the initial period of use in a given activity. Such processes are responsible for the initial deposition and hence creation of the archaeological record. For instance cultural refuse disposal constitutes a large proportion of the archaeological record (Schiffer, 1987: 47). Cultural formation processes are also responsible for any subsequent cultural modifications of materials in the archaeological record such as re-use of construction materials like wood and stone; treasure hunting, where materials are removed and collected in a non archaeological manner; building and modern farming methods.

Non-cultural formation processes are simply all and any events and processes of the natural environment that impinge upon artefacts and archaeological deposits.

### 1.3 Formation processes in the marine archaeological record

Both cultural and non-cultural formation processes are operative on underwater sites. Figure 1.1 (Muckelroy, 1978: 158) shows a flow diagram of the development of a wreck site, from functioning ship to a scatter of objects on the seabed. Considering that a ship is a mobile entity which can move freely over global water surfaces, if it should be wrecked the remains are likely to be deposited fortuitously (that is to say the individual has little part in determining where artefacts will be deposited). However, once the ship has sunk to the seabed both cultural and non-cultural post-depositional formation processes, similar to those on land sites, operate on the wreck site.

The effects of cultural formation processes such as salvage, dredging, commercial fishing, waste disposal and sport diving in the sea around the U.K. have been discussed elsewhere (Gregory, 1992; Ferrari, 1994) and their implications on the effects of wreck sites will not be discussed further.

Up to the early 1980s, research into natural formation processes on wreck sites focused on the correlation of the observed seabed distribution of wreck sites with various environmental attributes. Predictive models were developed to explain the preservation of artefacts in terms of the site environment. However, models of relevance to the study of formation processes around the U.K., such as Muckelroy's (1977) and Cederlund's (1980), sought to explain preservation almost exclusively in terms of the physical processes of deterioration.

Muckelroy (1977: 47-57) placed wreck sites into five distinct classes based on their observed preservation. The relevant attributes for the preservation were related to the topography (percent of bottom sedimentary deposit), sedimentary deposit (the range of sediment type), slope (average slope in degrees), sea horizon (sector of open water for 10 +Km) and the fetch (maximum offshore distance) over the site. However, Muckelroy does conclude that the chemistry and biology of the seawater around the sites will potentially play a part in preservation (1977: 56).

Similarly, Cederlund (1980: 102) defines factors which determine the preservation of shipwrecks in Swedish waters. These were: the geographical appearance of the coast; the situation of the wreck in relation to the water surface (at a great depth or in shallow waters); the type of bottom from a geographical point of view (such as a hard bottom with big stones or a sandy bottom); the type of bottom from a geological point (such as sedimentation or erosional bottom); the differences of height in the bottom (for example causing movements in layers of sand on the seabed); the existence of ice; the existence of currents; the existence of heavy wave movements; the existence of special biological conditions (for example ship worm).

As I have argued previously (Gregory, 1992: 22-23), this retrospective approach to the preservation of shipwrecks is dubious. Our knowledge of the shipwreck site is partial due to several factors, therefore retrospective interpretation of the formation processes which have acted upon a site over time may potentially be misleading. Considering the wrecking process from the moment the ship begins to sink, the nature of the wrecking of each ship will vary. Some ships may sink virtually intact, such as the *Vasa* (Kvarning, 1993: 66), others may be damaged prior to sinking and lose many of their contents whilst sinking, such as the *Santa Maria de la Rosa* (Martin, 1975: 96-100). Thus a shipwreck may be deposited in the ideal environment for preservation, yet because of the nature of the wrecking the whole ship may not have been deposited.

Further to this, such classifications do not allow for the temporal difference between the wreckings. For example, in Muckelroy's classification there is a difference of 235 years between the oldest and youngest wreck in his sample (Gregory, 1992: 8-9). This assumes the shipwreck and its surrounding environment have been stable and undisturbed since sinking. However, the environment is dynamic and will change over time. Such an example is provided by *H.M.S. Stirling Castle*, lost on the Goodwin Sands in 1703. In 1979 the wreck was discovered embedded in a deeply shelving bank of sand, her emerging port side disintegrating. The disintegration was the result of recent exposure. The initial transition to a stable wreck formation was quick, as shown by the survival of the hull and contents *in situ*. Re-exposure to a dynamic environment posed the threat of completely destroying the de-stabilised wreck formation (Oxley, *pers. comm.*).

The wreck site will not necessarily have been undisturbed either, since cultural and natural processes may have degraded the shipwreck site in the past and present. Admittedly physical processes do play an important part in the evolution of a shipwreck. However, as Robinson notes (1981: 3) it seems that the incident of the wrecking and the degree of exposure of the site to wind and water contribute to the preservation in the early stages of a wreck's history. Subsequently the features of the burial environment, whether seawater or marine deposit, predominate.

Instead of attempting to predict which natural processes have affected a wreck site from its observed state of preservation, it is argued that the natural environment should be

studied so as to define and understand what processes affect shipwreck sites in the present. This is the first aim of this thesis; to experimentally study the deterioration of a range of modern organic materials and metals. An improved knowledge of the chemical and biological mechanisms governing their deterioration would facilitate a better understanding of the nature of wreck sites and their formation.

The second aim is to apply this knowledge to the preservation of materials *in situ*. Potentially, if the mechanisms which govern deterioration can be understood and monitored, methods to mitigate their effects could be proposed, as will be discussed.

#### 1.4 *In situ* preservation of shipwreck sites

In U.K. waters the main legislative act which affords protection of wreck sites from the effects of cultural formation processes is the 1973 Protection of Wrecks Act. Under this, wreck sites may be designated for protection if they are deemed to be of historic, archaeological or artistic importance. Once a wreck site has been designated it becomes an offence to damage, carry out diving or place obstructions on (or within a restricted area) unless carried out with the authority of a licence granted by the Secretary of State (Chippindale and Gibbins, 1990: 93).

At present there are 41 designated wreck sites (Figure 1.2) in the U.K. (Dean *pers. comm.*). However, this is only a tiny percentage of the total number of potential shipwrecks around the U.K. Figure 1.3 shows the number of ships wrecked around the British Isles in a single year. Potentially there are thousands of shipwrecks around the U.K. and it is unlikely that even a tiny percentage of them will ever be excavated. Thus even if they are not going to be excavated and raised, it is important that effective *in situ* preservation methods are developed so that the wrecks can be monitored and preserved *in situ*, just as the archaeological resource on land, for future generations.

Preservation *in situ* (or site stabilisation) of submerged archaeological sites, or parts of sites, has been demonstrated on numerous sites around the world. In the case of wooden shipwrecks, most instances have involved re-burial either under sediment, excavated spoil, sandbags or artificial seagrasses. For example, after excavation, the remains of the *Rapid*, which sank off western Australia in 1811, were covered with the original overburden, for example ballast, sand, coral etc. The problems were that even though the back fill was the same as the original material, it was not as impervious to marine organisms and oxygen as pre-excavation sediments (McCarthy, 1986: 137).

The Basque whaler, thought to be the *San Juan*, which sank in Red Bay, Labrador in 1565 was excavated between 1979 and 1985. The wreck was completely disassembled and over 2500 timbers were raised to the surface, recorded and placed in temporary underwater burial pits. The timbers were then re-buried. The re-burial plan was to create a sealed and anaerobic environment in which to store the timbers in an attempt to duplicate

the pre-excavation environment of the wreck timbers in terms of light, temperature and gas exposure (Stevens and Wadell, 1987: 4).

The *William Salthouse*, which sank off Melbourne, Australia, in 1841 was initially stabilised using wooden fences and dumping sand on the exposed wreck. Emergency sandbagging was deployed while more long term engineering possibilities were researched. Sand deposition was encouraged by placing barriers across the path of the current consisting of a biodegradable and removable system of sandbag walls (pre-mixed sand and cement in hessian bags (Elliget and Breidhal, 1991: 27)). Later strategies on the *William Salthouse* included the application of a system known as "Cegrass" to create a pattern of artificial seagrass beds around the site. The buoyant plastic strips of the Cegrass remain upright in strong currents and reduce the velocity of the water and encourage deposition of suspended sediment. Forty-six Cegrass mats were constructed by clipping fronds of the artificial grass to mats of concrete reinforcing mesh. The mats were shackled together and then transported to the site, positioned by divers and held down with sections of old railway track. After only two months substantial sediment accumulation was noted around the wreck (Elliget and Breidhal, 1991: 28).

All these methods have consolidated the sites against physical processes of deterioration. However, as McCarthy (1986: 137) and Stevens and Wadell (1987: 4) noted, it is not just physical processes which account for deterioration; chemical and biological factors also play a part. However, research is limited into what chemical and biological parameters should be measured in order to develop mitigation strategies. McCarthy (1982: 50) and Smith *et al.* (1981: 354) suggest that as part of a wreck inspection programme the temperature, salinity, pH and dissolved oxygen content, water movement and purity, and bottom type of a site should be studied. Undoubtedly these parameters play a part in the deterioration of the wreck but the authors' discussions of their importance is not explicit.

A prerequisite for effective *in situ* preservation must be an understanding of the effect the re-buried environment has on the preservation of archaeological materials. Although, as mentioned, the chemistry and biology of the re-buried environment are acknowledged to have an effect on preservation, their consequences have not been systematically studied. Research into these processes has been undirected, attention being to the empirical gathering of data without considering its relationship to the preservation of archaeological materials. This need for systematic study is alluded to when Stephenson (1985) discusses the nineteenth century wooden wreck of the American gunboat the *Chattahoochee*:

"The water quality parameters for which data are readily available are water temperature, dissolved oxygen, pH, turbidity, conductivity, biochemical oxygen demand, total alkalinity, ammonia, oxygen, nitrate-nitrite, total phosphorus, total organic carbon and faecal coliform bacteria. Insofar as the integrity of the wreck is concerned, several of these parameters are important. However, their relationship to the *in situ* preservation or

destruction of wooden hulls and various metals is not well understood." (Stephenson, 1985: 99).

### **1.5 Experimental study of formation processes**

This thesis set out to solve the above problems through practical experimentation. A range of modern organic materials and metals were placed on several submerged sites and their deterioration observed and measured periodically over 52 weeks. Specimens of the same materials under controlled laboratory conditions served to distinguish between biological and chemical deterioration. Various environmental parameters were measured on the sites and in the laboratory to correlate their effects with the deterioration. This approach would satisfy both problems. First, ascertaining the initial rates of deterioration of fresh materials would, to a certain extent, simulate the conditions a shipwreck is exposed to on first sinking and hence the survivability of materials in archaeological context. Second, correlation of the processes of deterioration with environmental parameters on the sites would make it possible to ascertain which parameters were important to measure and monitor in order to develop mitigation strategies for sites which were to be preserved *in situ*.

### **1.6 Conclusion**

Archaeological formation processes involve the interactions whereby intrusive human induced evidence reaches a state of equilibrium with its environment. An understanding of such processes is therefore crucial to the interpretation of such evidence and the preservation of shipwrecks *in situ*. Of the complex range of physical, chemical and biological processes operating in the marine environment research into chemical and biological processes has been limited and unsystematic. As will be discussed, their effects were determined by monitoring the deterioration of a range of modern organic materials and metals over a 52 week period in controlled laboratory and field experiments.

## CHAPTER TWO

### SELECTION AND PREPARATION OF MATERIALS

#### 2.1 Introduction

Controlled laboratory and field experiments were conducted to answer the problems defined in Chapter One. For these a range of modern organic materials and metals, representative of materials typically associated with ancient and modern shipwrecks, were used. The organic materials used were wood, rope, canvas, leather and animal bone. The metals used were steel, bronze, and lead (see Appendix I for the suppliers of the materials).

Non-archaeological artefacts were used purely for practical reasons. First, the availability of the various materials had to be considered. Sufficient amounts of each material were required for the replicate analyses the experiments would entail. Hence the quantities required had to be of a homogeneous nature so as to make the results comparable between experiments. Second, as some of the analytical methods used to monitor deterioration of the materials were destructive, non-archaeological artefacts had to be used. Third, materials had to be obtained as cheaply as possible. Fourth, due to the nature of this research (that is to say a three year study) experimental data could only be collected over a 52 week period. Thus materials which were likely to show some deterioration within this time were chosen. Inorganic materials were not selected for the experiment as potentially they are better preserved in underwater environments than organic materials (Figure 2.1).

Modern materials have their limitations in comparative experiments and it is necessary to state the provisos for their use. Notably, the methods of manufacture of many of the materials have changed since antiquity.

In the case of the metals used in this experiment there are elements, added to improve their performance, which would not have been used in antiquity. For instance the steel used contains manganese and aluminium and this deliberate alloying of iron with other metals is of comparatively modern origin (Hodges, 1988: 80). Similarly, the bronze contained, apart from copper, tin and lead, small amounts of zinc, aluminium and nickel. Until the recent past most copper alloys only consisted of three major constituents (Hodges, 1988: 64).

As for the organic materials the leather used was chromate tanned, a method only introduced after 1880 (Kühn, 1986: 188). In addition the leather, along with the wood, canvas, and rope, had all had synthetic biocides, which would not have been available in the past, added to them during their manufacture (*pers. comm.* with suppliers) to limit the effects of biodegradation. However, the use of modern materials was valid as their basic structures were the same as those used in the past. Consequently an understanding of the chemical and microbial mechanisms causing their deterioration will help further our understanding of how archaeological materials deteriorate in underwater environments.

In addition to the limitations of using modern materials this investigation must be considered to be an overview of the deterioration and corrosion of organic materials and

metals on different underwater sites. A definitive statement as to the preservation of any one of the particular materials cannot be made due to the enormous variety of the types of materials. For example different species and parts of animal, wood, and plant fibre may deteriorate at different rates and by different mechanisms. Metals may contain different elements and may have been manufactured and worked differently, all of which affect their rates of corrosion.

## **2.2 Preparation of materials**

### **2.2.1 Organic materials**

#### **2.2.1.1 Oak wood**

A radially split section of oak planking was cleaned using a mechanical sander and then sawn up into 3.0 x 3.0 x 0.5cm test blocks (Figure 2.2). A thin section was prepared following the method of Purvis *et al.* (1966: 85) and microscopically examined. This section (Figure 2.3) was compared with a reference *Quercus* spp section (Miles, 1978: 65).

#### **2.2.1.2 Sisal Rope**

Ten mm diameter, three strand sisal rope was separated out into the individual strands. Each strand was cut up into approximately 12cm lengths and from each strand six yarns taken and tied with nylon monofilament so as to form a loop (Figure 2.4). Microscopic examination of individual fibres (Figure 2.5) were compared with reference sisal fibres (Florian *et al.*, 1990: 46-48).

#### **2.2.1.3 Canvas**

Seven ounce (302 gm<sup>-2</sup>) cotton canvas was cut up into 4.0 x 4.0cm square test specimens (Figure 2.6). Microscopic examination of individual fibres (Figure 2.7) were compared with reference cotton fibres (Florian *et al.* 1990: 39-43).

#### **2.2.1.4 Leather**

Chrome-tanned leather made from cow hide was cut up into 4.0 x 4.0cm square test specimens (Figure 2.8). Microscopic examination (Figure 2.9) of the grain surface was compared with a reference calf skin (Haines, 1981: 9).

#### **2.2.1.5 Bone**

Sheep radii (separated from their ulna) were formally identified by comparison with a reference collection (School of Archaeological Studies, University of Leicester). They were de-fleshed by simmering in boiling deionised water for 20-30 minutes to loosen any flesh which was subsequently removed by gentle scraping. The proximal and distal epiphyses were removed from a random, representative, sample of the de-fleshed bones and the remaining shafts were sawn up into 0.5cm thick sections using a hand hacksaw.

The marrow was cleaned from the inside of the bone using deionised water and a fine brush. The specimens were air dried and analysed to check that the levels of total nitrogen and calcium:phosphate ratio (see Chapter Four section 4.2.7 for these methods of analysis) did not differ significantly within each bone and between individual sheep. The results (see Appendix II) showed that these values did not differ significantly between individual sheep. However, there were differences within each bone. The proximal ends of the bones showed increased nitrogen levels and calcium:phosphate ratios. The shafts and distal ends had lower, yet similar, levels and ratios. On the basis of these initial experiments 0.5cm thick samples were taken from the shafts of the bones (Figure 2.10 and 2.11). Specimens were not taken closer than 2.5cm to either the proximal or distal ends of the bones.

### 2.2.2 Metals

The metals were prepared as follows and prior to their use, degreased in 1,1,1 trichloroethane liquid/vapour and then in acetone according to British Standard CP3012 (BSI, 1972: 19).

#### 2.2.2.1 Steel

2.5cm diameter rods of low carbon grade steel (BS 970 EN3B) were cut into 2.5mm thick discs (Figure 2.12) using a mechanical band saw with continuous cooling. One face of each of the discs was then polished on a rotary grinder with 120 grade silicon carbide paper to give the samples a uniform surface finish.

Metallographic examination (Figure 2.13) of a specimen polished to 2 $\mu$ m and etched with a solution of 5%v/v nitric acid in ethanol (Richardson, 1971: 432) was compared with an example of a low carbon steel from the literature (Nutting and Baker, 1965: 122). The structure showed polygonal ferrite grains and small regions of the ferrite-iron carbide eutectoid pearlite, indicative of a low carbon (0.1-0.5%), cold drawn, steel.

#### 2.2.2.2 Bronze

2.5cm diameter rods of sand cast, leaded tin bronze (BS 1400 LG4), were cut into 2.5mm thick discs (Figure 2.14) and polished as for the steel specimens.

To check the material was a cast bronze and that the sawing had not affected the metallographic structure - work hardening increases the corrosion rate of metals: Shoesmith, 1987: 48, two samples were further polished to 600 grade silicon carbide paper under water with a final polish to 2 $\mu$ m with alumina on a fast rotary polisher. The specimens were then etched with a 1:1 solution of ammonium hydroxide : 3% hydrogen peroxide (Richardson, 1971: 464). Metallographic examination of one specimen (Figure 2.15) showed its structure to be fully dendritic and compared with a standard reference specimen of the same material (Bower and Randlett, 1985: 639).

The other specimen was annealed at 750°C for 30 minutes and left to cool overnight prior to etching. Metallographic examination showed twinned grains (Figure 2.16) around the outermost surfaces of the bronze, indicating that the sawing had slightly work-hardened the outer surfaces of the metal, (Hosford *et al.*, 1985: 684-91). Micro hardness tests were carried out on both specimens to check that the sawing had not significantly affected the hardness of the bronze (Appendix III). The un-annealed specimen gave an average hardness of 99.4 Vickers hardness. On the annealed specimen, hardness readings were taken in the centre and around the outer surfaces; the average hardness of these areas was 78.0 and 79.6 Vickers hardness respectively. The results indicated that the metallographic structure of the original cast bronze had not been significantly affected by the sawing - a much higher hardness would have been expected if this were the case.

#### 2.2.2.3 Lead

A sheet of rolled lead (BS 1178) was cut into 3.0 x 3.0cm squares using hand cutters (Figure 2.17). The worked state of the metal could not be determined by metallographic examination due to the self annealing nature of lead at room temperature (Greaves and Wrighton, 1957: 200). However, a specimen was polished as for the bronze and iron samples and etched with a solution of 35cm<sup>3</sup> acetic acid : 5cm<sup>3</sup> hydrogen peroxide (30%) : 5cm<sup>3</sup> ethanol and 10cm<sup>3</sup> water to confirm that there were insignificant impurities visible in the lead. The small number of black inclusions visible in Figure 2.18 compared with 99% pure, chemical grade lead (DiMartini, 1985: 418).

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## CHAPTER THREE

### LABORATORY AND FIELD EXPERIMENTS

#### 3.1 Introduction

Laboratory experiments were set up to act as controls for field experiments. Deionised water and artificial seawater were used to investigate, respectively, the chemical and biological effects of seawater on the deterioration and corrosion of the materials. The field experiments were set up on three underwater sites in U.K. waters to simulate the effects of deterioration and corrosion at the artefact/sediment/water and artefact/seawater interfaces.

In both laboratory and field experiments the deterioration or corrosion of each of the materials was looked at in isolation; interactions between them were not investigated. Specimens were taken for analysis at: four, eight, 12, 16, 32 and 52 weeks. This sampling regime was adopted on the pre-supposition that any deterioration and corrosion would be exponential and hence the rates would be greater initially. Due to some of the methods of analysis being destructive, sufficient samples were prepared to allow for four specimens of each material to be taken at each sampling interval. This enabled triplicate analysis, with an additional specimen available if further analysis was required.

#### 3.2 Laboratory experiments

As interactions between the materials were not investigated, specimens had to be isolated from one another. This was achieved through a system of wooden trays which consisted of a lattice work structure of individual 5 x 5 x 10cm compartments (Figure 3.1).

A polythene bag was placed into each compartment and a single specimen was suspended in it (Figure 3.2). This was achieved by drilling or cutting a hole through the specimen's centre and placing a strip of plastic, slightly larger than the diagonal width of the compartment, through the hole. The specimen was then lowered halfway into the polythene bag by gently bending the plastic strip. As the strip was slightly longer than the width of the compartment, when released it pressed against the sides of the bag and supported the specimen above the bottom of the bag. The procedure was repeated for all the materials, sufficient to give four specimens at each sampling interval. Not only did this method keep specimens separate from each other but it also eliminated the problems of contact, and hence interactions, between the materials and the polythene bags.

Prior to placing the specimens into the bags they were weighed on an analytical balance (Organic materials Appendix IV and Metals Appendix V) and surface sterilised by dipping in an aqueous solution of 70%v/v Industrial Methylated Spirits, which had been acidified to pH 2 with hydrochloric acid, for five minutes (Sykes, 1969: 113).

100cm<sup>3</sup> of deionised water or artificial seawater, sterilised by passing through a 0.22µm cellulose-ester filter (Millipore), were added to each of the polythene bags. *Van't Hoff's*

formulation for sea water, supplied by Dr. Paul Ridout (*pers. comm.*), was used (Table 3.1):

Chemical	Grams $\text{dm}^{-3}$
Sodium Chloride	19.00
Magnesium Sulphate	1.50
Magnesium Chloride	2.40
Potassium Chloride	0.53
Calcium Chloride (anhydrous)	0.37

Table 3.1 Formulation for *Van't Hoff's* artificial seawater.

This solution gives a salinity of 34.36 parts per thousand (‰); sufficient was prepared using general purpose reagents (Fisons) which were diluted with deionised water.

Blank solutions, consisting of deionised or artificial seawater in a polythene bag with a plastic strip, were also prepared to check that these did not interfere. The trays were then covered with a layer of polyvinyl chloride cling film, to limit evaporation, and stored in the dark at room temperature.

The laboratory experiments were started on 03.05.93. At each sample interval the specimens were aseptically removed for analysis with flame-sterilised tweezers. They were photographed, their appearance noted, they were then placed into small plastic re-sealable bags labelled with specimen number and sample interval, and they were stored in a domestic freezer ( $-5^{\circ}\text{C}$ ) pending analysis. The pH, voltage and temperature of one of the sets of solutions the materials had been submerged in was measured using a Jenway 105 Combination Meter. The salinity of the artificial seawater was measured initially and at the end of the experiment (Appendix VI)

### 3.3 Field Experiments

#### 3.3.1 Validation of field experiments

There are several environmental interfaces which affect archaeological materials deposited in an underwater environment: the artefact/seawater; artefact/sediment/seawater; artefact/sediment/interstitial water and the artefact/sediment/interstitial water below 50cm (Figure 3.3).

The artefact/sediment/seawater and artefact/seawater interfaces were selected as areas to study as these would most likely represent the scenario when ships first become wrecked or when buried archaeological material becomes re-exposed. The artefact/sediment/interstitial water interface and artefact/interstitial water interface below 50cm were eliminated as areas to study for two reasons. First, we know that archaeological materials tend to be well

preserved in these environments: for instance the *Mary Rose* which sank in the Solent in 1545 (Rule, 1982) yielded an enormous amount and diverse range of organic and inorganic artefacts upon excavation approximately 400 years after she sank. Bearing this in mind it was unlikely that an experiment lasting 52 weeks would yield measurable results. Second, as the samples were to be removed at regular intervals, continual disturbance of the sediments in which the samples were buried would affect the microbial and chemical parameters of the sediment, thus creating an atypical environment.

### 3.3.2 Selection of sites for field experiments

The sites used in the field experiments were chosen for logistical reasons rather than archaeological reasons. First, personal safety was an important factor and sites where diving operations either satisfied the Health and Safety Executive *Diving operations at Work Regulations* (HSE, 1981) and *Diving Operations at Work: Guidance on Regulations* (HSE, 1981), or operated under an archaeological exemption from this in compliance with General Certificate of Exemption number 1/81, were chosen. Second, sampling intervals were frequent so sites which were actively being worked upon throughout the year were needed. Third, incorporating the research into an ongoing project would limit the cost of diving operations. This also had the benefit that should personal collection of the samples prove impossible for any reason, the samples could be collected and forwarded on. Fourth, the logistics and cost of reaching the field sites with full diving equipment had to be taken into account.

Thus archaeological consideration for selection of the field sites was of secondary importance. The three sites selected (Figure 3.4) were: a wreck off Alum Bay in the Solent being surveyed by the Nautical Archaeology Society for the Hampshire and Wight Trust for Maritime Archaeology; a wreck off Duart Point, the Isle of Mull, being surveyed by Dr. Colin Martin of the Scottish Institute of Maritime Studies, University of St. Andrews; and the Albert Dock in Liverpool which is one of the research stations for the Department of Environmental and Evolutionary Biology of the University of Liverpool.

#### 3.3.2.1 Alum Bay, Isle of Wight

The wreck, which lies off of Alum Bay, Isle of Wight (Figure 3.5), was surveyed by the Nautical Archaeology Society under the guidance of the Hampshire and Wight Trust for Maritime Archaeology. Firth (1993: 1-14) documents the survey of the wreck in his interim report. A summary of this report notes that:

“The wreck site consists of a coherent 20m stretch, high on the port side of the vessel, including two deck levels. Structure from the bow area is visible including two hawse holes. Materials used in its construction (copper alloy bolts, lead hawse holes, copper alloy sheathing, iron knees) appear to date the vessel to the late 18th or early 19th century. Reports of broad arrow marks on metal bolts recovered from the site suggest that it is a

Royal Naval vessel. Although there are a number of documented losses from the period in the area, the vessel could not be formally identified as no link could be made between the archaeological material and historical records.

The wreck lies in an area of sand and silt. There is a line of boulders running north east-south west at the foot of a reef of the same orientation. A broad strip of sand runs parallel to the reef and the wreck lies perpendicular to it, across the sand. Much of the adjacent rock is very angular and there are long, narrow features which form fences to the south west.

Sand waves appear to lie over a silty horizon. It appears that the waves move along the top of the silt, as some tags have been buried and there is a "clean" strip around the lowest visible portion of the hawse holes. There appears to be appreciable, though variable, movement of the sand. On 29th May 1993 the sand waves were small (height approximately 50mm) whereas the following week they were approximately 300mm in height, and previously unknown material was exposed in the silty gullies. It is difficult to say how stable the silt is, or which factors are responsible for the movement. There is a variable current over the site, ranging from negligible to perhaps a quarter of a knot on the flooding spring tide. The morphology of Alum Bay appears to cause a back eddy from the generally strong currents through the Needles Channel. Alternatively, swell generated by weather may be responsible for the movement. A more worrying explanation is that the whole seabed is subject to general erosion due to interference in the supply of sand elsewhere."

The specimen trays were placed on the site on the 02.06.93.

#### 3.3.2.2 Duart Point, Mull

The wreck lies slightly to the east of Duart Point (Figure 3.6). The ship was wrecked in 1653, a full account of which is documented by Martin (1995: 15-32). Essentially, Duart Castle, the seat of the Clan Maclean, was one of the few remaining pockets of Royalist resistance to Cromwell's Commonwealth. In a bid to quell the Royalists, Cromwell sent a small fleet of ships to seize Duart Castle, but when they landed at Duart they encountered no resistance - the Maclean chief had decamped to the neighbouring island of Tiree. However, before the fleet could leave, a violent storm blew up and out of the six ships sent to take the castle three were wrecked and the remaining three were disabled.

The wreck site itself lies in 10 metres of water on, and in, a seabed of gravelly sand, silts, shell and some intrusive rocks. Its environment has been categorised by Martin (1995: 17) as follows:

"The general environment of the site, as indicated by its biological regimes, was categorised as including mid-energy and moderately high-energy zones. A considerable and complex tidal set runs from west to east across the site during the ebb and is subject to strong eddying and variable direction at different levels of the depth profile. Sediment

transportation, apparently including both build up and erosion, also influences the morphology of the site. This effect is greatest at spring tides (when there is a vertical range of up to 4.5m), particularly when these coincide with north to north westerly winds. The visible remains comprise seven heavily concreted cast iron guns up to 2.5m in length, a small iron anchor, various iron concretions and concretion complexes, two (or possibly three) concentrations of stone ballast, and considerable quantities of wholly or partially exposed organic material, including elements of structure. The character of the ballast varies considerably between the two mounds. That to the west contains large flat slabs which have evidently been packed together with some care, while the central mound is made up of a tumble of smaller more rounded pebbles.”

The specimen trays were placed on the site on 02.08.93.

### 3.3.2.3 Albert Dock, Liverpool

The Albert Dock in Liverpool (Figure 3.7) was built by Jesse Hartley in 1845. Today the docks are the home of the Liverpool Maritime Museum and Tate Gallery. The docks themselves are used by the Department of Environmental and Evolutionary Biology as part of their research programme, and these experiments were conducted with the aid of Jim McGill, the departmental experimental field officer, and other members of staff from the department. The sample trays were located along the north western wall of the dock in six metres of water. This site had the advantage that certain elements of the physical, chemical and biological nature of the dock environment had been previously characterised by Allen (1992).

The specimen trays were placed in the Dock on 05.10.93.

## 3.4 Field Experimental method

As with the laboratory experiments the various materials were kept separate to avoid problems of interactions. This was achieved by suspending the specimens across box-sided wire mesh trays (Figure 3.8) which were anchored to the seabed, a method suggested by Dr. A Gale (*pers. comm.*).

Four trays were prepared and due to the dynamic environment, each tray was a complete unit containing strings of all the materials for all the sampling time stations. Thus, in the event of a tray(s) being lost it would not jeopardise the whole experiment on a particular site. Twenty-eight strings were prepared, each of which contained separate specimens of the eight types of materials. Each specimen was weighed on an analytical balance (Organic materials Appendix VII and Metals Appendix VIII) prior to threading on to a length of nylon monofilament of 50 lbs breaking strain with a piece of polyethylene tubing in between each one, so as to keep them separate. Eight strings of specimens were tied inside each of the metal trays with re-usable electrical cable ties so that they could be easily removed from the trays. One, two, three or four cable ties were also attached to one side of

each of the tray. This made it easier for the diver retrieving the samples to identify each tray. By placing the strings containing the four week samples nearest the cable tie(s) denoting the tray number, and the remaining strings in sequential order of sample interval, this system also served as a simple way of co-ordinating which strings were to be removed at each interval. In the event of low visibility diving conditions rendering the reading of written signs impossible, this would allow the diver to identify the different trays and retrieve the strings by touch alone.

Prior to immersion in the sea, the strings of specimens were surface-sterilised by spraying them periodically for five minutes with acidified 70%v/v Industrial Methylated Spirits. The trays were then anchored to the sea bed with 30cm steel pins placed in the corners of each of the trays.

At each sampling interval a string of specimens was removed from each of the four trays by undoing the cable ties. If conditions allowed (that is to say the visibility was sufficient and the water current was not too strong) each specimen was taken off the monofilament line and placed in a separate sterile bag labelled with the sampling interval and tray number. If conditions were unsuitable the whole string was placed in a bag labelled with the tray number and the individual specimens placed into separate bags on the surface. The specimens were then stored in a domestic freezer (-5°C) pending analysis. At each sample interval water samples were taken from around the specimens trays and the pH and voltage recorded at the surface. Temperature and depth were recorded *in situ* using a Suunto dive computer. Dissolved oxygen was measured at the surface by a modified *Winkler* Method (Appendix IX). Salinity was measured using the same method as for the Artificial seawater (Appendix VI).

### 3.5 Conclusion

Field experiments were set up on three sites in U.K. waters to monitor the deterioration and corrosion of the materials in conditions which simulated the artefact/sediment/water and artefact/seawater interfaces. Laboratory experiments, using the same range of materials, were set up as controls to investigate the chemical and biological variables in the field experiments.

Specimens were removed for analysis at four, eight, 12, 16, 32 and 52 week intervals to determine their state of deterioration/corrosion. The methods used to monitor their deterioration and corrosion are discussed in Chapter Four.

## CHAPTER FOUR ANALYTICAL TECHNIQUES

### 4.1 Introduction

A combination of quantitative and qualitative analytical methods were used to monitor the progressive deterioration and corrosion of the organic materials and metals. Quantitative methods were used so that the empirical data obtained could be compared with the laboratory experiments and different sites in the field experiments.

### 4.2 Organic Materials

#### 4.2.1 Weight change

The organic specimens were washed in deionised water, gently brushed to remove any superfluous surface matter accumulated during submersion, dabbed dry with a paper towel and dried in an oven at 70°C to constant weight. Their percentage weight change was then calculated.

#### 4.2.2 Scanning Electron Microscopy

The 52-week samples from the laboratory and field experiments were examined using an International Scientific Instruments DS-130 scanning electron microscope (S.E.M.). The materials were freeze dried and splatter coated with gold (50nm thickness) prior to examination at various magnifications.

#### 4.2.3 Analysis of wood

Hoffman's (1981: 77) scheme for the analysis of waterlogged wood (Figure 4.1) was used to monitor the deterioration of the wood specimens. This enabled quantitative results to be obtained on the chemical composition of the cell wall. Of the methods in his scheme the water content, holocellulose, alphacellulose, hemicelluloses (Sodium Hydroxide extractives) and lignin content of the specimens were determined.

Hoffman used the standard methods developed by the *Technical Association of the Pulp and Paper Industry* (TAPPI). Grattan and Mathias (1986: 7) reviewed Hoffman's work and advocated the use of the standard methods of the *American Society for Testing and Materials* (ASTM) noting that, although the two sets of standards were very similar, ASTM methods were easier to use and required smaller amounts of sample.

A combination of both sets of standard methods was used. Hoffman's original TAPPI standard method was used to determine the holo, alpha and hemicellulose content of the specimens as these could be determined from the same sample. ASTM methods were used for the analysis of lignin and extractives.

#### 4.2.3.1 Water content

Hoffman's method for determining the water content of wood was the same as the method used for determining weight change in section 4.2.1.

#### 4.2.3.2 Alcohol and water soluble extractables

Water and alcohol extractables were determined using ASTM D1106-56 (ASTM, 1966a: 391). Hot alcohol removed catechol tannins and hot water soluble material including tannins, gums, sugars, proteins, inorganic salts and starch. Apart from showing an increase or decrease in these extractables preliminary extractive treatments were required for analysis of the lignin content of the wood.

A complete wood specimen, dried and ground to pass a number 40 (420  $\mu\text{m}$ ) sieve, was accurately weighed into a cellulose extraction thimble which was placed into a Soxhlet extraction apparatus and extracted with 95% alcohol for four hours. The thimble and alcohol extracted specimen was then treated as before using deionised water as solvent. The specimen and extraction thimble were dried at 70°C to constant weight and the percentage alcohol and water soluble extractables calculated.

#### 4.2.3.3 Lignin content

The lignin content was determined using ASTM D1106-56 (ASTM, 1966a: 389-390). In addition to alcohol and water extraction, specimens for lignin analysis were further extracted with a 2:1 toluene:alcohol solution according to ASTM D1107-56 (ASTM, 1966b) for six hours. The ASTM method used benzene; however, benzene is carcinogenic and so due to health risks toluene was used instead. The specimen was then washed with alcohol and dried at 70°C to constant weight. Approximately one gram of the extracted specimen was accurately weighed into a 500  $\text{cm}^3$  round-bottomed flask and digested with 400  $\text{cm}^3$  of boiling water under reflux for three hours. The water was decanted and the residue in the flask was air dried. 15  $\text{cm}^3$  of cold (12-15°C) sulphuric acid (72 %v/v) were added, with stirring, to the flask and allowed to stand for two hours at 20°C. The resulting residue was washed into a 1  $\text{dm}^3$  conical flask and diluted with water to give a three percent (by volume) concentration of sulphuric acid. The flask was then boiled under reflux for four hours. After allowing the insoluble material to settle, the solution was filtered into a tared coarse fritted glass crucible (number two) and washed with deionised water until the washings were neutral to litmus. The crucible was then dried at 100°C to constant weight and the percentage lignin calculated.

#### 4.2.3.4 Holocellulose, Alphacellulose, and Hemicellulose content

The TAPPI method developed by Wise *et al.* (1946: 15-17) required five grams of sample for analysis. However, as each wood specimen only weighed approximately three grams,

which was further reduced to two grams after grinding and extracting, the method was adapted for the use of one gram of sample. This also served as a method of investigating the variability between separate wood samples. From the stock of prepared specimens ten were analysed using five grams and ten using one gram. The results (Appendix X) of these analyses showed an insignificant difference between separate samples and sample weight, indicating that these factors did not affect the analysis. Thus the cellulosic fractions were determined using one gram sample weight.

Approximately one gram of alcohol-water extracted wood was accurately weighed into a 50 cm<sup>3</sup> conical flask; two drops of glacial acetic acid were added followed by 35 cm<sup>3</sup> of deionised water containing 0.5 g of sodium chlorite. The flask was then heated to 80°C using a steam bath in a fume cupboard (chlorine dioxide was evolved). After one hour, without cooling, (i) a further two drops of glacial acetic acid were added to the mixture; and (ii) a further 0.5 g of sodium chlorite was added. The suspension was then mixed and heating continued for a further hour after which time steps (i) and (ii) were repeated and heating continued for a third hour. The suspension was then cooled in an ice bath, filtered under vacuum through a tared coarse fritted glass crucible (number two) and then washed repeatedly with iced water and finally with acetone. The white residue, termed holocellulose, was then air dried, weighed and the percent holocellulose calculated.

The holocellulose was transferred quantitatively from the original crucible to a 50 cm<sup>3</sup> conical flask and nitrogen flushed through the flask for 10-15 minutes to displace air. 20cm<sup>3</sup> of 24% aqueous potassium hydroxide were added and nitrogen flushing continued for a further ten minutes. The flask was stoppered, placed at 20°C for 110 minutes, and shaken every ten minutes. The solution was then filtered with suction through the original crucible into a Buchner flask containing an excess of glacial acetic acid. The final residue was washed with five cm<sup>3</sup> of 24% potassium hydroxide followed by 30 cm<sup>3</sup> of water and 25 cm<sup>3</sup> of 10% acetic acid. The filtrate was quantitatively transferred to a 500 cm<sup>3</sup> conical flask, using small amounts of 95% alcohol to ensure complete transference. The solution was tested for acidity and ethanol added to fill the flask. The flask was then stoppered and shaken and the resultant white precipitate, hemicellulose, was left to settle out. The supernatant liquid was then removed as completely as possible by syphon. The remaining supernatant and precipitate was transferred quantitatively to a centrifuge bottle and washed with 95% alcohol to remove water and finally with ether to remove alcohol. The hemicelluloses in ether suspension were then filtered through tared fritted glass crucibles, washed with ether, and dried to constant weight.

Wise *et al.* (1946: 17) noted that hemicellulose fractions isolated in this manner contain appreciable amounts of ash, largely in the form of potassium salts. To correct for these the hemicellulose fractions were ashed by carbonising with one or two drops of concentrated

sulphuric acid, and heating until all fuming had stopped. The ash was then heated to low red heat, cooled, weighed and the fraction corrected for the ash content.

The fibrous residue remaining in the crucible after extracting the hemicelluloses, termed alphacellulose, was washed with water until neutral to litmus and finally washed with acetone and then air dried to constant weight. The percentage alphacellulose was then calculated.

#### 4.2.4 Analysis of Rope

##### 4.2.4.1 Weight change

Weight change in the specimens was determined as for section 4.2.1.

##### 4.2.4.2 Scanning Electron Microscopy

Samples were prepared for S.E.M. as in section 4.2.2.

##### 4.2.4.3 Tensile strength

The tensile strength of separate yarns of the rope specimens was determined using a Housenfield Tensometer (Type W, Engineering Department, University of Leicester). The test pieces were pulled following the instructions set out in the manufacturer's manual. The load at which the strands broke, expressed in Newtons, was recorded.

Specimens from the field experiments were too badly deteriorated after 12-16 weeks submersion to be analysed using this method due to the insensitivity of the tensometer. These specimens were prepared for testing as for those tested by the tensometer. They were then suspended vertically and weights added to the free end, gradually increasing the load, until the strands broke.

#### 4.2.5 Analysis of canvas

##### 4.2.5.1 Weight change

Weight change in the specimens was determined as for section 4.2.1.

##### 4.2.5.2 Scanning Electron Microscopy

Samples were prepared for S.E.M. as in section 4.2.2.

##### 4.2.5.3 Tensile strength

Specimens were cut into 1.25cm wide strips and the tensile strength was determined as for the rope specimens in section 4.2.4.3. following the manufacturer's instructions for testing textiles.

#### 4.2.6 Analysis of leather

##### 4.2.6.1 Weight change

Weight change in the specimens was determined as for section 4.2.1.

##### 4.2.6.2 Scanning Electron Microscopy

Samples were prepared for S.E.M as in section 4.2.2.

##### 4.2.6.3 Nitrogen content

The colorimetric method developed by Havilah *et al.* (1977) to analyse nitrogen in Kjeldhal digests was used.

*Materials and methods* : A salicylate reagent was prepared by dissolving 34 g of sodium salicylate and 0.24 g of sodium nitroprusside in about 500 cm<sup>3</sup> of deionised water, which was then diluted to one dm<sup>3</sup>. A cyanurate reagent was prepared by dissolving 0.25 g of sodium dichloroisocyanate in 200 cm<sup>3</sup> of deionised water, 15 cm<sup>3</sup> of sodium hydroxide (400 g dm<sup>-3</sup>) was added and the solution diluted to 1.0 dm<sup>3</sup> with deionised water.

Nitrogen standard solutions were prepared by weighing 0.5350g of ammonium chloride into a 100cm<sup>3</sup> volumetric flask, which was diluted to volume with deionised water. This was then diluted 100, 200, 300, 400 and 500µdm<sup>3</sup> to 100cm<sup>3</sup> with a Kjeldhal digest solution containing only a catalyst tablet (1g di-sodium sulphate and 0.05 g selenium) and sulphuric acid to give a similar solution matrix to the sample digest; the concentration range of prepared standards was 0.1-0.5mM ammonium chloride dm<sup>-3</sup>.

*Procedure* : A dried sample of leather was finely comminuted and approximately 0.1 g accurately weighed into a Kjeldhal flask. This was digested with one Kjeldhal catalyst tablet and five cm<sup>3</sup> of concentrated sulphuric acid. The cooled digest was quantitatively transferred to a 100 cm<sup>3</sup> volumetric flask and diluted to volume with deionised water. This was diluted 1.0cm<sup>3</sup> to 100.0 cm<sup>3</sup>, and 100 µl aliquots of the diluted digests and nitrogen standards were transferred to separate universal sample tubes using a precision micro-sampler (Gilson P-200 Pipetman). Then five cm<sup>3</sup> of salicylate reagent followed by five cm<sup>3</sup> of cyanurate reagent were added using dispensers (Oxford pipettor) and swirled for a few seconds to mix. The sample tubes were left to stand for at least 30 minutes before their absorbances were read on a spectrophotometer (Beckman, Model 24 UV/Visible) at 667nm, calibrated with the standard solutions to read directly in concentration units. The results were expressed as the percentage total nitrogen content.

#### 4.2.7 Analysis of bone

##### 4.2.7.1 Weight change

Weight change in the specimens was determined as for section 4.2.1.

#### 4.2.7.2 Scanning Electron Microscopy

Samples were prepared for S.E.M as in section 4.2.2.

#### 4.2.7.3 Nitrogen content

Nitrogen content of the bone specimens was determined using the same procedure as for the leather specimens. Dried bone specimens were ground up in a cutter-hammer mill and 0.1g was digested and diluted to 100.0 cm<sup>3</sup>. These were diluted 1.0cm<sup>3</sup> to 100cm<sup>3</sup> and 1.0cm<sup>3</sup> of these solutions analysed as for the leather specimens.

#### 4.2.7.4 Calcium and Phosphate content

The calcium and phosphate content of the bone specimens was determined from the Kjeldhal digest solutions. Calcium was determined using an Atomic Absorption method developed by Dr. D. Ratcliffe (*pers. comm.*) and Phosphate was determined using the method of Kaila (1955).

##### 4.2.7.4.1 Calcium content

A standard solution of calcium carbonate was prepared by accurately weighing approximately 0.1g of dried calcium carbonate into a 100.0 cm<sup>3</sup> volumetric flask dissolving in five cm<sup>3</sup> of concentrated hydrochloric acid, and diluting to volume with deionised water. This solution gave a concentration of 10 mM Calcium per litre. A range of standards, 0.1 - 0.5mM calcium dm<sup>-3</sup>, was prepared by diluting the stock solution 1.0, 2.0, 3.0, 4.0, and 5.0 cm<sup>3</sup> to 100.0 cm<sup>3</sup> adding one cm<sup>3</sup> of blank Kjeldhal digest to each before diluting to volume with deionised water. A blank of one cm<sup>3</sup> of Kjeldhal digest diluted to 100.0 cm<sup>3</sup> with deionised water was prepared as a reference.

Samples were prepared from the Kjeldhal digests by diluting them one cm<sup>3</sup> to 100.0 cm<sup>3</sup> with deionised water.

Samples and standards were analysed using a Varian Techtron S1-R0 Atomic Absorption spectrophotometer. The instrument was fitted with a nitrous oxide-acetylene burner and set up with a calcium hollow cathode lamp, the wavelength set to 422.7nm. The gas flow was set to give a fuel-rich flame and the spectrophotometer was calibrated to read directly in concentration units. The results were expressed as the mM concentration of calcium per specimen.

Ionisation suppressants, to counter the effect of the phosphate, could not be added to the solutions because the Kjeldhal catalyst tablets would cause their precipitation. To ensure that the levels of phosphate present in the samples were not interfering with the analysis, a recovery experiment using calcium carbonate at the approximate level of calcium in the samples was carried out. Approximately 0.1g of calcium carbonate was weighed accurately into a 100.0 cm<sup>3</sup> volumetric flask. This was diluted to volume with water and

one cm<sup>3</sup> pipetted into a 100cm<sup>3</sup> volumetric flask, one cm<sup>3</sup> of blank Kjeldhal digest was added and the flask diluted to volume with deionised water. The results of this recovery test gave an average calcium recovery of 98.41% (Appendix XI) demonstrating that phosphate levels had no appreciable effect.

#### 4.2.7.4.2 Phosphate Analysis

The ammonium vanadate-molybdate method for analysing phosphate developed by Kaila (1955: 42) was used.

*Reagents* : One dm<sup>3</sup> ammonium vanadate-molybdate reagent was prepared containing 300cm<sup>3</sup> of 50% nitric acid, 300 cm<sup>3</sup> of 0.25% ammonium vanadate and 300 cm<sup>3</sup> of 5% ammonium molybdate in deionised water.

A standard solution was prepared by weighing accurately approximately two grams of dried potassium di-hydrogen orthophosphate (Fisons A.R. grade) into a one dm<sup>3</sup> volumetric flask which was diluted to volume with deionised water; one cm<sup>3</sup> of this solution contained one mg of phosphorus pentoxide. A range of calibration standards were prepared by auto pipetting, using a calibrated Gilson P200 pipetman; 100, 200, 300, 400, and 500  $\mu$ dm<sup>3</sup> of the stock solution into separate plastic universal tubes. Five cm<sup>3</sup> of ammonium vanadate-molybdate reagent were added to each and then made up to 15 cm<sup>3</sup> with deionised water. A solution containing 500  $\mu$ dm<sup>3</sup> of blank Kjeldhal digest was prepared in the same way to act as a "zero" phosphate solution for calibration of the spectrophotometer.

Samples were prepared from the Kjeldhal digests by autopipetting 500 $\mu$ dm<sup>3</sup> into separate universal sample tubes. Five cm<sup>3</sup> of ammonium vanadate-molybdate reagent was added and the tubes diluted to 15.0 cm<sup>3</sup> with deionised water.

The tubes containing the samples and standards were shaken and left to stand for at least five minutes before reading their absorbance on a spectrophotometer (Beckman, Model 24 UV/Visible) at 430nm using the blank as reference and calibrated with the standard solutions to read directly in concentration units. Results were expressed as the percentage phosphate content of the specimens.

From both the calcium and phosphate results the calcium:phosphate ratio was calculated.

### 4.3 Metals

#### 4.3.1 Weight loss

Corrosion of the metals was accompanied by the formation of corrosion products. These were loosely adhered to the metal surfaces and often came away during collection of the samples. Drying and weighing the specimens would have yielded erroneous results depending upon how much corrosion product had fallen off. Specimens were chemically cleaned to remove all corrosion products prior to drying and weighing to give a more

accurate determination of weight loss. The International Standard procedure: ISO 8407:1991 (E) *Corrosion of Metals* (ISO, 1991), which specifies procedures for the chemical removal of corrosion products formed on metal and alloy corrosion test specimens during their exposure to corrosive environments, was used to determine the weight loss of the metals. This procedure involves immersion of corrosion test specimens in to a series of chemical solutions which are specifically designed to remove the corrosion products with minimal dissolution of any base metal.

The specimens were initially cleaned with light brushing using a soft bristled brush to remove lightly adherent and bulky corrosion products. The metals were then cleaned chemically using the solutions detailed in Appendix A of the International Standard. The solutions and the order in which the metals were immersed for the steel, bronze and lead are shown in Tables 4.1 to 4.3 respectively. Control specimens of uncorroded steel, bronze and lead were also tested. Following immersion in the solutions the specimens were briefly dipped in acetone and placed in a desiccator to dry prior to weighing and calculating the percentage weight loss of metal.

	<b>Solution</b>	<b>Time</b>	<b>Temp. (°C)</b>
1.	Conc Hydrochloric acid containing 2% w/v Antimony trioxide and 5% w/v Tin chloride	15 minutes	20°C
2.	5% w/v Sodium hydroxide (aq) containing 20% w/v Zinc chips	30 minutes	80°C
3.	20% w/v Sodium hydroxide (aq) containing 2% w/v Zinc chips	30 minutes	80°C
4.	20% w/v Diammonium citrate (aq)	20 minutes	80°C
5.	50% v/v Hydrochloric acid (aq) containing 0.35% w/v hexamethylenetetramine	10 minutes	20°C

Table 4.1 The formulae of the solutions and the sequence and conditions of their use for the removal of corrosion products on steel specimens.

	Solution	Time	Temp. (°C)
1.	50% v/v Hydrochloric acid (aq)	3 minutes	20°C
2.	0.5% w/v Sodium cyanide (aq)	3 minutes	20°C
3.	10% v/v Sulphuric acid (aq)	3 minutes	20°C
4.	12% v/v Sulphuric acid (aq) containing 3% w/v Sodium dichromate dihydrate	10 seconds	20°C
5.	5.4% v/v Sulphuric acid (aq)	60 minutes	50°C

Table 4.2. The formulae of the solutions and the sequence and conditions of their use for the removal of corrosion products on bronze specimens.

	Solution	Time	Temp. (°C)
1.	1% v/v Acetic acid (aq)	5 minutes	Boiling
2.	5% w/v Ammonium acetate (aq)	10 minutes	60°C
3.	25% w/v Ammonium acetate (aq)	5 minutes	50°C

Table 4.3 The formulae of the solutions and the sequence and conditions of their use for the removal of corrosion products on lead specimens.

#### 4.3.2 X-Ray Diffraction

Analysis was carried out on samples of corrosion products scraped from the 52-week samples with a scalpel blade. Micro-layers within the corrosion products were not separated but as far as was possible only one colour sample was taken for each analysis. However, these proved hard to discern even with the aid of a low power microscope due to mixtures of corrosion products or amorphous material. The samples were ground up finely in a glass mortar and pestle and placed into capillary tubes for the Debye-Scherrer powder camera. They were irradiated with Cobalt K Alpha X rays at 30 Kv and 22 mA, with a nickel filter. The resulting diffraction lines were measured manually using a vernier and the  $2\theta$  distances converted to "d" values using Fang and Bloss' (1966) X-ray diffraction tables. Identification was then conducted using JCPDS diffraction reference tables (JCPDS, 1986).

#### 4.3.3 Scanning Electron Microscopy

Scanning Electron Microscopy was determined using the same model as in section 4.2.2. The metals were not gold coated prior to examination. Energy dispersive X-Ray analysis of corrosion products on the surface of the materials was carried out on some of the specimens using a Princetown Gamma Technology 3 Probe.

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**CHAPTER FIVE**  
**INVESTIGATION OF MICROBIOLOGICAL DETERIORATION AND**  
**CORROSION**

**5.1 Introduction**

After eight weeks of submersion on the field sites some of the materials, notably the rope and canvas, had deteriorated to a far greater extent than corresponding laboratory control materials submerged in deionised water and artificial seawater. Unfortunately, by this time the laboratory control materials had become contaminated with micro-organisms from the laboratory atmosphere. Although this contamination meant that the materials in the laboratory experiments did not serve as strict controls, the fact that these were less deteriorated than those from the field sites, indicated that deterioration on the field sites was biologically rather than chemically induced.

Microscopic examination indicated that the field site materials were heavily colonised by micro-organisms. Isolation and identification of the micro-organisms which were causing this deterioration was attempted as correlation of these with the results of the measured deterioration of the materials would provide a better understanding of the environmental conditions necessary for microbiological deterioration to occur. However, by the time the feasibility of this had been assessed, and subsequent training in microbiological methods taken, the 32-week sampling interval was the next time when specimens could be retrieved for study.

By this stage the sample trays on the Alum Bay site had been lost, and the canvas specimens from both the Duart Point wreck site and the Albert Dock had completely deteriorated; however, in anticipation of this the 16 week specimens were aseptically retrieved from these sites and stored at -5°C pending examination. Isolation of micro-organisms from all organic materials was attempted along with those from metals, as there was considerable evidence for microbially-induced corrosion in the literature (Borenstein, 1994: 22 ).

**5.2 Culture of fungi and bacteria on to nutrient media**

The 32-week submerged specimens were taken from the laboratory and field sites aseptically, stored in separate sterile polythene bags and refrigerated (-5°C) prior to isolating the colonising micro-organisms.

Micro-organisms were extracted from the materials by aseptically removing small samples with a scalpel, placing them into sterile universal tubes and adding an aliquot of filter sterilised water taken from the respective field site or laboratory experiment. They were agitated by ultrasonic and vortex mixer and aliquots of the extraction waters spread on to separate agar plates, so as to isolate the micro-organisms.

Due to time constraints, it was not possible to investigate and determine the optimum culture medium for the isolation of the multitude of bacteria and fungi which were potentially colonising and deteriorating the samples of the different materials. Thus general media were used to culture either the bacteria or fungi. A casein-peptone-starch medium (Table 5.1) was used to culture the bacteria (Collins and Willoughby, 1962).

Chemical	Gram dm <sup>-3</sup>
Peptone	0.50
Soluble casein	0.50
Soluble starch	0.50
Glycerol	1.28
Magnesium Sulphate (hepta hydrate)	0.05
Di-potassium hydrogen orthophosphate	0.20
Agar	20.00
Water	to 1.0 dm <sup>-3</sup>

Table 5.1 Formulation of Casein-peptone-starch media used to culture bacteria (Collins and Willoughby, 1962: 297).

Separate batches of this medium were prepared by weighing the above constituents into 1000 cm<sup>3</sup> autoclavable bottles which were diluted to volume with water from the respective laboratory experiment or field site. These were sterilised by autoclave and, after cooling to 60°C, a concentrated solution (sufficient to give a media concentration of 0.1g dm<sup>-3</sup>) of the anti-fungal Nystatin, dissolved in dimethylsulphoxide, was aseptically added through a 0.22µm pore size cellulose-ester filter.

A yeast extract-glucose medium (Table 5.2) was used for the isolation of the fungi (Gareth Jones, 1971a):

Chemical	Gram dm <sup>-3</sup>
Yeast extract	0.1
Glucose	1.0
Agar	20.0
Water	to 1.0 dm <sup>-3</sup>

Table 5.2 Formulation of yeast extract-glucose medium for the isolation of the fungi (Gareth Jones, 1971a: 351).

The fungal media were prepared as for the bacterial media, except a concentrated solution (sufficient to give a media concentration of  $0.2\text{g dm}^{-3}$ ) of the anti-bacterial Chloramphenicol, dissolved in acetone, was added.

The prepared bacterial and fungal media were poured on to petri plates, cooled, dried and  $0.1\text{ cm}^3$  of the water extracts from each of the materials spread onto separate plates. These were incubated at laboratory temperature in sealed plastic bags until bacterial and fungal colonies appeared on the agar surface (between 10 and 14 days). This produced plates with mixed cultures of bacteria and fungi. Colonies showing obvious morphological differences were picked, re-streaked and examined microscopically for purity; this was repeated until they were obtained in pure culture.

### 5.3 Determination of bacteria and fungi causing deterioration

An *in vitro* experiment was set up to check that the micro-organisms isolated were responsible for the deterioration of the various materials, rather than either simply using them as a substrate to colonise or having been a carry over from the water brought up with the samples. For each bacterial or fungal organism cultured, two separate samples of undeteriorated material, from which they were isolated, were weighed (after drying to constant temperature at  $70^\circ\text{C}$ ) and placed into  $150\text{ cm}^3$  conical flasks.  $100\text{ cm}^3$  of water, from the respective site/laboratory experiment, were added to each and to one flask  $0.05\text{g}$  of yeast extract added to provide an additional source of nutrients.

The flasks were sealed with gauze and cotton wool plugs and sterilised by autoclave. Controls of undeteriorated material with and without yeast extract and the various waters were also prepared. The flasks were then inoculated with the separate bacteria and fungi and left at laboratory temperature for 32 weeks.

After this the specimens were removed and a sample of water taken and re-streaked on to agar plates to check the purity of the microbial cultures and the sterility of the controls. Subsequent colonies were stored on media slopes for future identification. The specimens were dried at  $70^\circ\text{C}$  to constant weight and their percentage weight change calculated (Appendix XII) and the microbes from those samples which showed a significant deterioration (25% decrease when compared to the controls) were characterised.

### 5.4 Characterisation of Bacteria

#### 5.4.1 Phase Contrast Microscopy

Fresh cultures of the deteriorative bacteria and fungi were grown from the media slopes in nutrient media solutions, made up to the formulations in Tables 5.1 and 5.2 but without agar. After 7-10 days these were examined as wet mounts using a Leitz phase contrast microscope in order to determine motility and other morphological characteristics.

#### 5.4.2 Gram Staining

Cells were Gram stained according to Collins and Taylor (1966: 85). A small drop of sterile water was placed in the centre of a glass slide. A small amount of bacterial growth was removed with an inoculating loop and emulsified in the liquid. The slide was dried by waving over a bunsen and fixed by passing it down through the bunsen flame. The slide was then stained with crystal violet (0.5%w/v in water) for 30 seconds. This was washed off with water and replaced with an iodine solution (containing 2%w/v potassium iodide and 1%w/v iodine in water) and left for one minute. The iodine was washed off with acetone and water respectively and counter stained with safranin (1%w/v in water). After staining the slide was drained, blotted with filter paper and dried by gentle heating over a bunsen flame. Gram positive bacteria appeared blue/violet and Gram negative bacteria appeared red.

#### 5.4.3 Colony Morphology

Colonies were observed after 7-10 days growth in the laboratory and their characteristics coded according to the system researched by Gerhardt *et al.* (1981) as shown in Table 5.3.

	1	2	3	4	5	6
<b>Colour</b>	white	cream	beige	yellow	pink	red
<b>Size</b>	Size in millimetres					
<b>Form</b>	circular	irregular	filamentous	rhizoid	spindle	punctiform
<b>Elevation</b>	flat	raised	convex	pulvinate	umbonate	-
<b>Margin</b>	entire	undulate	lobate	erose	filamentous	curled

Table 5.3 Observed morphological characteristics of bacterial colonies (Gerhardt *et al.* 1981: 414).

#### 5.4.4 Endospore formation

Spore like structures were observed under the light microscope in some of the bacterial cultures and a heat resistance test, after Gerhardt *et al.* (1981: 422), was performed to confirm their presence. A tube of broth was inoculated with growth from liquid media in which sporulation was suspected. The inoculated tube of broth was placed into a water bath at 80°C along with an un-inoculated tube of broth containing a thermometer. The tubes were incubated for ten minutes, beginning from when the thermometer read 80°C. The tubes were cooled and a sample of the inoculated broth spread onto a plate containing media which had been prepared with water corresponding to the site/laboratory experiment from where the bacteria had been cultured.

#### 5.4.5 Biochemical tests

The following tests were not performed on all the bacteria isolated. Specific tests were required to aid in the identification of certain bacteria.

##### 5.4.5.1 Catalase test

Catalase activity was determined according to Method 2 of Gerhardt *et al.* (1981: 413). A small amount of bacterial growth was emulsified on a glass slide in a drop of 3%v/v hydrogen peroxide. Effervescence of oxygen within the first five minutes indicated the presence of catalase.

##### 5.4.5.2 Oxidase test

Oxidase activity was determined according to Gerhardt *et al.* (1981: 420). A filter paper strip was impregnated with a 1%w/v aqueous solution of N,N,N,N-tetramethyl-p-phenylenediamine (Sigma) and bacterial growth was applied with a wooden toothpick. A pink-purple coloration developing between 10-30 seconds indicated the presence of oxidase.

##### 5.4.5.3 O/129 (Vibriostat) test

The O/129 was determined according to Gerhardt *et al.* (1981: 420). One or two crystals of 2,4-diamino-6,7-diisopropylpteridine phosphate (Sigma) were placed onto an inoculated agar plate and incubated at laboratory conditions. A zone of growth inhibition around the crystals indicated a positive test.

##### 5.4.5.4 Anaerobic growth

Anaerobic jars were set up with GasPak (BBL) H<sub>2</sub> generators using a methylene blue strip to indicate anaerobicity. Plates were incubated in these jars for 7-10 days at laboratory conditions to check for anaerobic growth.

##### 5.4.5.5 API 20NE strip tests

For Gram negative bacteria API 20NE strips were used. These consisted of 20 tests for the identification of non-fastidious, non enteric Gram-negative rods. The API 20NE strip consisted of 20 tubes and cupules containing dehydrated media and substrates. The test substrates were for testing for assimilation of: nitrates, tryptophan, glucose, arginine, urea, esculin, gelatine and p-nitro-phenyl-BD-galactopyranoside, glucose, arabinose, mannose, mannitol, N-acetyl-glucosamine, maltose, gluconate, caproate, adipate, malate, citrate and phenyl-acetate. The strip was prepared according to the instructions (API), inoculated and incubated at 37°C for 48 hours. During the incubation period metabolism of the bacteria on the test substrates produced colour changes that were either spontaneous or developed

upon the addition of a reagent. The reactions were read according to the interpretation table supplied with the kit.

#### 5.4.6 Identification

From the results of the tests the bacteria were identified using *Bergey's manual of Determinative Bacteriology* (Holt *et al.*, 1994), *Bergey's manual of Systematic Bacteriology* (Krieg and Holt, 1984 ) and *The Prokaryotes* (Balows *et al.*, 1992).

### **5.5 Characterisation of Fungi**

Small squares (approximately 0.5 x 0.5 mm) of agar medium with fungal growth were taken with a sterile scalpel blade and placed on a glass slide. A few drops of "Permout" were placed on the block and then covered by a cover-glass tilted so that one side was in contact with the slide. The agar was then gently melted without boiling the permount and the cover glass allowed to fall under its own weight. This resulted in a thin film of agar containing the fungi surrounded by a ring of Permout which hardened within 24 hours. The fungi were then examined under a low powered microscope and classified through their morphology using the dichotomous keys in Domsch *et al.* (1980a and 1980b).

### **5.6 Isolation of true marine fungi**

Gareth Jones (1971a: 351) has shown that the above method is not always successful at isolating true marine fungi. A further method (Gareth Jones, 1971a: 350) to isolate marine fungi growing on the materials from the field experiments was investigated. The specimens were incubated on a layer of tissues in petri plates with the periodic addition of small amounts of artificial seawater added to prevent drying out. The samples were incubated until the fruiting bodies of any fungi were visible on their surface and up to a maximum of 10 weeks. Identification was done by picking up a loopful of ascospores from the spore mass found at the tip of the fruiting bodies. These were placed onto a glass slide with a drop of artificial sea water and covered with a cover glass. The fungi were examined under low powered microscope and classified through the morphology of their ascospores and fruiting bodies using the classifications of Barghoorn and Linder (1944: 395) and the dichotomous keys of Kohlmeyer and Kohlmeyer (1979: 188-211).

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## CHAPTER SIX

### RESULTS

#### 6.1 Introduction

Included within this chapter are the results of the environmental parameters, the microbiological examinations and the observed and measured deterioration of the test materials. There are two points to be considered when examining the results. First, the Alum Bay 12 week samples could not be collected due to bad weather. By the subsequent sampling interval, 16 weeks, two of the sample trays had been completely broken up and lost. Of the remaining trays, a total of only three strings of specimens remained intact. It was decided to collect one string of specimens for the 16 week interval, leaving two strings: one for the 32 week sample interval and one for the 52 week interval. Unfortunately, by the 32 week sampling interval the trays had been completely ripped from the seabed and all specimens lost. Thus only environmental parameters and deterioration of specimens for four, eight and 16 weeks submersion are available from the Alum Bay wreck site. No microbiological work was carried out on the specimens but those macro-organisms which were visibly colonising the specimens were characterised. Second, the results of the 32 week specimens from Duart Point may be ambiguous due to the sample trays having been covered by sediment, due to natural seabed movement, at this sampling interval.

The microbiological methods isolated a total of 40 fungal and 43 bacterial species colonising the test specimens from the laboratory and field experiments (see Table 6.7). Of these, nine of the fungi and 11 of the bacteria were found to cause deterioration of the materials. These were identified to genus level.

The results of the deterioration of the materials have been grouped into organic materials and metals. Within these groups each material is considered separately. Descriptive observations of the materials throughout the experiment are documented along with macro photographs and scanning electron micrographs to illustrate progressive deterioration and the organisms and mechanisms by which this was occurring.

#### 6.2 Environmental parameters

The results of the environmental parameters recorded in the laboratory and field experiments are summarised and include tabular and graphical representations. To highlight any seasonal variations the results have been plotted showing the months at which the laboratory and field sites were sampled, rather than the duration of submersion of the test specimens.

## 6.2.1 Laboratory experiments

### 6.2.1.1 Temperature

The temperature of the deionised water and artificial seawater solutions containing test specimens in laboratory conditions was  $19 \pm 2^\circ\text{C}$  throughout the experiment.

### 6.2.1.2 pH and Voltage measurements

The pH and voltage measurements for the deionised water solutions containing the various test materials are tabulated in Table 6.1 and are graphically represented in Figures 6.1 and 6.2 respectively.

Material		0 Weeks (May)	4 Weeks (June)	8 Weeks (July)	12 Weeks (Aug)	16 Weeks (Sept)	32 Weeks (Jan)	52 Weeks (May)
Blank	pH	8.24	7.80	7.52	7.40	7.45	7.76	7.40
	mV	-083	-046	-036	-031	-035	-040	-031
Wood	pH	8.24	3.45	3.35	3.30	3.05	2.88	2.96
	mV	-083	196	198	196	189	167	203
Rope	pH	8.24	6.91	7.61	7.06	7.89	7.95	7.97
	mV	-083	026	-041	-015	-075	-119	-076
Canvas	pH	8.24	7.11	7.45	7.18	6.96	6.93	6.74
	mV	-083	016	-032	-021	-019	-025	-009
Leather	pH	8.24	3.96	4.05	3.65	3.35	3.47	3.61
	mV	-083	163	161	175	094	134	166
Bone	pH	8.24	7.47	7.92	7.82	8.42	7.72	8.02
	mV	-083	-017	-057	-057	-120	-106	-078
Steel	pH	8.24	5.83	6.83	7.75	8.76	7.60	7.63
	mV	-083	093	003	-051	-140	-099	-053
Bronze	pH	8.24	6.10	6.38	5.78	6.09	6.22	6.18
	mV	-083	078	027	056	-052	-021	-022
Lead	pH	8.24	9.40	9.27	9.05	8.45	8.55	9.05
	mV	-083	-140	-139	-123	-120	-153	-136

Table 6.1 pH and voltage measurements of deionised water containing test materials under laboratory conditions.

Apart from the solutions containing lead, a decrease in pH, when compared to the blank control, was recorded after four weeks submersion. Of the other solutions those containing the leather and wood attained the lowest pHs of 3.96 and 3.45 respectively. The pHs of the other solutions were: rope 6.91, canvas 7.11, bone 7.47, steel 5.83 and bronze 6.10. Thereafter, the pHs fluctuated slightly throughout the experiment. The solution containing the lead specimen showed an initial increase in pH, attaining 9.40 after four weeks submersion. The solution containing steel specimens demonstrated an increase up to pH 8.76 after 16 weeks, which had decreased to pH 7.60 by 32 weeks submersion and remained constant for the remainder of the experiment.

The converse was true for the voltage measurements: apart from the solution containing lead, they all showed an increase in voltage, when compared to the blank control, after four weeks submersion. The leather and wood attained the highest readings

of +163 and +196 mVs respectively. The voltages of the other solutions (in mVs) were: rope +26, canvas +16, bone -17, steel +93 and bronze +78. The solution containing lead showed an initial decrease in voltage reaching -140 mV after four weeks submersion. The solutions showed a decreasing trend in voltage between four and 16 weeks which, apart from the steel, thereafter remained relatively stable. After 16 weeks submersion the voltage of the solution containing steel had decreased to -140 mV and had increased to -99 by 32 weeks. This increasing trend continued to the end of the experiment, whereupon the voltage was -53 mV.

The pH and voltage measurements for the artificial seawater solutions containing the various test materials are tabulated in Table 6.2 and are graphically represented in Figures 6.3 and 6.4 respectively.

Material		0 Weeks (May)	4 Weeks (June)	8 Weeks (July)	12 Weeks (Aug)	16 Weeks (Sept)	32 Weeks (Jan)	52 Weeks (May)
Blank	pH	7.50	7.61	7.43	7.52	7.10	7.16	7.52
	mV	-051	-039	-045	-050	-078	-074	-049
Wood	pH	7.50	2.75	2.82	2.78	2.20	2.40	2.20
	mV	-051	205	227	218	196	193	246
Rope	pH	8.24	6.91	7.61	7.06	7.89	7.95	7.97
	mV	-083	026	-041	-015	-075	-119	-076
Canvas	pH	7.50	7.02	7.15	6.35	5.77	6.76	6.82
	mV	-051	002	-015	023	007	-052	-011
Leather	pH	8.24	3.96	4.05	3.65	4.16	3.47	3.61
	mV	-083	163	161	175	094	134	166
Bone	pH	7.50	7.17	7.56	7.55	7.54	7.49	7.78
	mV	-051	055	-039	-042	-075	-092	-064
Steel	pH	7.50	5.95	5.21	5.46	6.53	3.99	6.21
	mV	-051	024	093	-072	-025	100	024
Bronze	pH	7.50	8.07	8.10	8.38	8.35	7.61	7.66
	mV	-051	002	-069	-086	-117	-100	-059
Lead	pH	7.50	7.34	7.40	7.85	8.25	7.36	7.75
	mV	-051	-003	-031	-057	-118	-085	-063

Table 6.2 pH and voltage measurements of artificial seawater solutions containing test materials under laboratory conditions.

Apart from the solutions containing bronze specimens, a decrease in pH was recorded, when compared to the blank control, after four weeks submersion. Of the other solutions those containing leather and wood specimens attained the lowest pHs of 4.59 and 2.75 respectively. The pHs of the other solutions were: rope 6.03, canvas 7.02, bone 7.17, steel 5.95 and lead 7.34. The solution containing the bronze specimen showed an initial increase in pH, attaining 8.07 after four weeks submersion. Apart from the solutions containing rope, canvas and steel specimens, the pH of the solutions remained relatively stable for the remainder of the experiment. However, the pH of the solutions containing the rope specimens steadily increased after four weeks submersion, and by 52 weeks had reached pH 7.87. The pH of the solutions containing the canvas specimens dropped to

5.77 after 16 weeks, rose to 6.76 after 32 weeks, and was then constant for the remainder of the experiment. The pH of the solution containing the steel specimens reached pH 5.21 after eight weeks submersion, rose gradually to 6.53 after 16 weeks, and was followed by a rapid decrease to 3.99 after 32 weeks. The solution showed a final increase to pH 6.21 after 52 weeks submersion.

The voltage measurements for all the solutions showed an increase, when compared to the blank control, after four weeks submersion. The recorded values were: blank -31, wood +205, rope +96, canvas +2, leather +115, bone +55, steel +24, bronze +2 and lead -3. The values of the solutions containing wood and leather specimens remained relatively stable throughout the experiment and showed a slight increase after 52 weeks submersion. The solutions containing the steel specimens showed an increase up to +93 mV after eight weeks submersion, followed by a drop to -72 mV after 12 weeks and then a gradual increase up to +100 mV after 32 weeks. By 52 weeks submersion this had decreased to +24 mV. The solutions containing the rope, canvas, bronze and lead specimens showed decreases in voltages after eight weeks submersion, which fluctuated slightly throughout the remainder of the experiment.

#### 6.2.1.3 Salinity

The salinities (measured in parts per thousand, ‰) of the artificial seawater solutions containing the various test materials were measured initially and at the completion of the experiment (Appendix VI). The results showed insignificant differences (Table 6.3).

Material	Initial salinity (‰).	Salinity (‰) after 52 weeks.
Blank	34.5	34.3
Wood	34.5	34.3
Leather	34.5	34.9
Canvas	34.5	34.9
Rope	34.5	34.6
Bone	34.5	34.5
Steel	34.5	34.3
Bronze	34.5	34.9
Lead	34.5	34.4

Table 6.3 Salinity of artificial seawater solutions containing test materials in laboratory conditions before and after completion of the experiment.

#### 6.2.2 Field experiments

The environmental parameters for the water of the three field sites are tabulated in Tables 6.4, 6.5 and 6.6.

Time (weeks)	Month	Temp. (°C)	pH	Volt (mV)	Salinity (‰)	Depth (metres)	Oxygen (mg dm <sup>-3</sup> )
0	Jun.	10.5	8.4	-118	33.6	8.2	9.0
4	Jul.	13.0	8.7	-123	33.7	8.0	8.8
8	Aug.	14.0	8.8	-142	34.0	7.9	8.6
12	Sept.	N/A <sub>1</sub>					
16	Oct.	12.5	8.5	-125	33.0	8.6	9.2
32	Feb.	N/A <sub>2</sub>					
52	Jul.	N/A <sub>2</sub>					

Table 6.4 Environmental data for the Alum Bay Wreck site. N/A<sub>1</sub>: results not available as samples were not collected due to bad weather. N/A<sub>2</sub>: results not available as the samples were lost.

Time (weeks)	Month	Temp. (°C)	pH	Volt (mV)	Salinity (‰)	Depth (metres)	Oxygen (mg dm <sup>-3</sup> )
0	Aug.	12.0	8.85	-150	33.8	13.0	8.3
4	Sept.	12.5	8.60	-135	34.0	11.7	7.5
8	Oct.	13.5	8.40	-120	33.2	12.9	8.1
12	Nov.	10.5	8.40	-120	33.0	10.8	9.0
16	Dec.	9.0	7.90	-060	34.0	11.6	9.2
32	Apr.	9.5	8.60	-132	32.5	10.7	10.0
52	Sept.	12.0	8.65	-140	32.1	12.5	8.8

Table 6.5 Environmental data for the Duart Point Wreck site.

Time (weeks)	Month	Temp. (°C)	pH	Volt (mV)	Salinity (‰)	Depth (metres)	Oxygen (mg dm <sup>-3</sup> )
0	Oct.	11.5	8.90	-155	24.50	5.6	4.94
4	Nov.	8.5	8.75	-140	26.80	5.6	5.53
8	Dec.	8.0	9.00	-162	28.00	5.6	6.27
12	Jan.	6.5	8.60	-135	27.50	5.6	8.30
16	Feb.	7.0	8.65	-138	26.00	5.6	7.31
32	Jun.	16.0	7.5	-037	25.50	5.6	3.05
52	Nov.	11.0	8.2	-069	26.00	5.6	4.03

Table 6.6 Environmental data for the Albert Dock.

### 6.2.2.1 Temperature

The temperatures for the water of the three field sites, graphically represented in Figure 6.5, showed seasonal fluctuations. The data available for the Alum Bay and Duart Point wreck sites showed that the temperatures increased to maxima between August and October. The additional Duart Point data showed that minimum water temperatures were reached in December. Thereafter, the temperature increased and a second maximum was recorded in the following September at the completion of the experiment. The water of the Albert Dock showed similar seasonal fluctuations. A maximum was recorded in October followed by a gradual decrease to a temperature minimum in January. An additional

temperature maximum was recorded in June and a further minimum in November at the completion of the experiment.

#### 6.2.2.2 pH and Voltage measurements

The pH and voltage measurements of the water from all of the field sites are shown in Figures 6.6 and 6.7 respectively. The data available for the Alum Bay wreck and Duart Point wreck sites show that the pH reached a maximum in August. The additional Duart Point data showed a minimum pH value in December. Thereafter the pH increased and a further maximum was seen in April. The water from the Albert Dock also showed seasonal trends. Maximum pH was recorded in December, thereafter the pH decreased to a minimum in June and by the following November, at the end of the experiment, the pH had risen again.

The voltage measurements of the water from all of the field sites were the converse of the pH measurements. The values for the Alum Bay and Duart Point wreck sites showed minimum in August. The additional data for Duart Point showed a maximum in December and minima between April and October. Similarly the Albert Dock data was the converse of the pH data. Minimum voltage was recorded in December followed by a maximum in June and tending towards a lower voltage at the completion of the experiment in November.

#### 6.2.2.3 Salinity

Salinity measurements (Appendix VI) for all of the field sites are shown in Figure 6.8. The salinities for the Alum Bay and Duart Point wreck sites showed that maximum levels were recorded between August and September. The additional data for Duart Point showed that lowest salinity was recorded in November. Low salinity levels were also recorded in the following April and September. The Albert Dock showed minimum salinity in October, increasing to maximum salinity in December. Lower salinity levels were recorded in the following June and November.

#### 6.2.2.4 Depth

The average depths for the Alum Bay and Duart Point wreck sites were 8.2 and 11.9 metres respectively, the depth fluctuating slightly due to the state of the tide when the samples were collected. The depth of the Albert Dock was 5.6 metres.

#### 6.2.2.5 Dissolved oxygen content

The dissolved oxygen contents (Appendix IX) for the water from all of the field sites are shown in Figure 6.9. The dissolved oxygen content of the Alum Bay and Duart Point wreck sites showed seasonal fluctuations. Minimum oxygen levels were recorded between August and September. The additional data for Duart Point showed that maximum

oxygen levels were in April. The data for the Albert Dock also showed seasonal fluctuations. Low oxygen levels were recorded in October at the start of the experiment, increasing to a maximum level in January. Minimum oxygen content was recorded in the following June and thereafter a further increase was recorded in November at the end of the experiment.

### 6.3 Microbiological Results

Thirty-nine fungal and 43 bacterial species, distinguished by their morphology, were cultured from test materials submerged for 32 weeks in deionised water and artificial seawater, under laboratory conditions, and on the Duart Point wreck site and in the Albert Dock by the nutrient agar methods (Table 6.7). The fungi isolated by this method were all examples from terrestrial genera. One marine fungus was identified by the sterile tissue method and although its power of deterioration was not tested in the laboratory this is well documented in the literature (Gareth Jones, 1971b: 237).

Material	Deionised water		Artificial seawater		Duart Point wreck site		Albert Dock	
	Fungi	Bacteria	Fungi	Bacteria	Fungi	Bacteria	Fungi	Bacteria
<b>Wood</b>	5	1	2	-	2	1	1	2
<b>Rope</b>	2	2	1	2	1	1	1	3
<b>Canvas</b>	2	2	2	-	2	3	3	2
<b>Leather</b>	-	1	-	5	1	2	2	2
<b>Bone</b>	2	1	4	2	1	2	1	3
<b>Steel</b>	1	3	1	-	1	1	1	2
<b>Lead</b>	-	-	-	-	-	-	-	-
<b>Bronze</b>	-	-	-	-	-	-	-	-

Table 6.7 Table of total number of fungi and bacteria isolated onto nutrient agar from test materials in laboratory and field experiments.

Eight of the fungi and 14 of the bacteria in Table 6.7 were observed to cause deterioration, as recorded by a 25% weight loss when compared to control specimens of materials in sterile solutions, during the *in vitro* experiments. The results of these experiments are tabulated in Tables 6.8 to 6.13. In these tables, \*1/\*2 indicates control specimens of materials in sterile solutions.

Those specimens which showed such a weight loss, indicated in the tables with bold type, were characterised by the methods set out in Chapter Five. As the fungal isolates were identified through their morphology alone they were not given an isolate letter. However, as the bacterial isolates were subject to a number of tests to identify them they were assigned a letter (A-N), which is indicated in brackets next to the weight loss.

Location	Organism	Wt specimen before deterioration (g)	Wt specimen after deterioration (g)	% Wt loss
*1 Deionised water		3.2568	2.8188	13.45
*2 Artificial seawater		3.0160	2.6646	11.65
Deionised water	Fungus	3.0126	2.6812	11.00
"	"	2.7986	2.4331	13.06
"	"	2.7765	1.9669	<b>29.16</b>
"	"	2.8936	2.6317	9.05
"	"	3.1056	2.6348	15.16
"	Bacterium	2.9618	2.6025	12.13
Artificial seawater	Fungus	2.9365	2.1977	<b>25.16</b>
"	"	3.0165	2.6808	11.13
Duart Point	Fungus	3.5168	3.0840	12.32
"	"	2.7986	1.9825	<b>29.16</b>
"	Bacterium	3.0165	2.5845	14.32
Albert Dock	Fungus	2.7689	1.9042	<b>31.23</b>
"	Bacterium	2.5693	2.2186	13.65
"	"	3.1876	2.7994	12.18

Table 6.8 Table of weight loss of wood specimens from *in vitro* microbiological experiments.

Location	Organism	Wt specimen before deterioration (g)	Wt specimen after deterioration (g)	% Wt loss
*1 Deionised water		2.3241	2.0889	10.12
*2 Artificial seawater		1.8652	1.7167	7.96
Deionised water	Fungus	2.0196	1.875	7.15
"	"	1.7985	1.3293	<b>26.09</b>
"	Bacterium	2.1365	1.9637	8.09
"	"	2.0965	1.9449	7.23
Artificial seawater	Fungus	1.9365	1.4489	<b>25.18</b>
"	Bacterium	1.7985	1.6553	7.96
"	"	2.1856	2.0038	8.32
Duart Point	Bacterium	2.1386	1.9878	7.05
"	Fungus	2.1496	1.9753	8.11
Albert Dock	Fungus	2.1259	1.4650	<b>31.09</b>
"	Bacterium	2.1378	1.9636	8.15
"	"	1.8795	1.7092	9.06
"	"	1.9653	1.8252	7.13

Table 6.9 Table of weight loss of rope specimens from *in vitro* microbiological experiments.

Location	Organism	Wt specimen before deterioration (g)	Wt specimen after deterioration (g)	% Wt loss
*1 Deionised water		0.8723	0.8108	7.05
*2 Artificial seawater		0.8165	0.7635	6.49
Deionised water	Fungus	0.8516	0.6035	<b>29.13</b>
"	"	0.8509	0.6366	<b>25.18</b>
"	Bacterium	0.8376	0.7935	5.26
"	"	0.8518	0.6286	<b>26.18 (I)</b>
Artificial seawater	Fungus	0.8616	0.8169	5.19
"	"	0.8403	0.7896	6.03
Duart Point	Fungus	0.8529	0.8107	4.95
"	"	0.8573	0.8142	5.03
"	Bacterium	0.8246	0.5669	<b>31.25 (J)</b>
"	"	0.8715	0.5827	<b>33.14 (K)</b>
"	"	0.8430	0.7995	5.16
Albert Dock	Fungus	0.8555	0.8114	5.15
"	"	0.8976	0.6288	<b>29.95</b>
"	"	0.7856	0.7382	6.03
"	Bacterium	0.8163	0.5868	<b>28.11 (L)</b>
"	"	0.8254	0.7806	5.43

Table 6.10 Table of weight loss of canvas specimens from *in vitro* microbiological experiments.

Location	Organism	Wt specimen before deterioration (g)	Wt specimen after deterioration (g)	% Wt loss
*1 Deionised water		1.7862	1.6924	5.25
*2 Artificial seawater		1.3256	1.2276	7.39
Deionised water	Bacterium	1.5768	1.4978	5.01
Artificial seawater	Bacterium	1.3759	1.3063	5.06
"	"	2.0163	1.9133	5.11
"	"	1.7765	1.6678	6.12
"	"	1.6589	1.5562	6.19
"	"	1.5432	1.4324	7.18
Duart Point	Fungus	1.5432	1.4174	8.15
"	Bacterium	1.6895	1.0867	<b>35.68 (M)</b>
"	"	1.5325	1.0714	<b>30.09 (N)</b>
Albert Dock	Fungus	1.4876	1.4012	5.81
"	"	1.3796	1.2841	6.92
"	Bacterium	2.0165	1.8717	7.18
"	Bacterium	1.6759	1.5785	5.81

Table 6.11 Table of weight loss of leather specimens from *in vitro* microbiological experiments.

Location	Organism	Wt specimen before deterioration (g)	Wt specimen after deterioration (g)	% Wt loss
*1 Deionised water		0.7685	0.7450	3.06
*2 Artificial seawater		0.6987	0.6857	1.86
Deionised water	Fungus	0.8695	0.6507	<b>25.16</b>
"	"	1.0136	0.9626	5.03
"	Bacterium	1.1275	0.7352	<b>34.79 (C)</b>
Artificial seawater	Fungus	0.9369	0.9165	2.18
"	"	0.8752	0.8546	2.35
"	"	0.9516	0.9222	3.09
"	"	1.0129	0.9951	1.76
"	Bacterium	1.0369	1.2737	2.54
"	"	0.8756	0.8549	2.36
Duart Point	Fungus	0.9475	0.9231	2.58
"	Bacterium	1.2365	0.8262	<b>33.18 (E)</b>
"	"	0.7756	0.5355	<b>30.96 (F)</b>
Albert Dock	Fungus	0.9813	0.9553	2.65
"	Bacterium	0.9256	0.6364	<b>31.25 (D)</b>
"	"	0.7575	0.7207	4.86
"	"	0.9836	0.9455	3.87

Table 6.12 Table of weight loss of bone specimens from *in vitro* microbiological experiments.

Location	Organism	Wt specimen before deterioration (g)	Wt specimen after deterioration (g)	% Wt loss
*1 Deionised water		2.0659	1.6614	19.58
*2 Artificial seawater		1.7863	1.5246	14.65
Deionised water	Fungus	2.3016	1.8330	20.36
"	Bacterium	2.1987	1.4032	<b>36.18 (A)</b>
"	"	1.7659	1.2670	<b>28.25 (B)</b>
"	"	1.6896	1.3486	20.18
Artificial seawater	Fungus	2.1101	1.7854	15.39
Duart Point	Fungus	1.7935	1.5288	14.76
"	Bacterium	1.9756	1.3871	<b>29.79 (G)</b>
Albert Dock	Fungus	1.2836	1.1084	13.65
"	Bacterium	1.7579	1.2079	<b>31.29 (H)</b>
"	"	2.0168	1.7919	11.15

Table 6.13 Table of weight loss of steel specimens from *in vitro* microbiological experiments.

### 6.3.1 Identification of bacteria causing deterioration

The results of the various tests carried out on the bacterial isolates causing deterioration are tabulated in Tables 6.14 and 6.15. Table 6.14 includes the results for Gram negative bacteria and Table 6.15 for Gram positive bacteria.

	Isolate A	Isolate B	Isolate C	Isolate D	Isolate E	Isolate F
Colour	1	4	1	3	4	1
Size	0.75	1	05	0.55	0.6	1
Form	1	1	4	1	3	1
Elevation	3	3	1	3	1	2
Margin	1	1	5	4	5	1
Gram stain	-ve rods					
Catalase test	+ve	+ve	+ve	+ve	+ve	+ve
Oxidase test	+ve	+ve	+ve	+ve	+ve	+ve
Motility	+	+	-	-	-	-
Vibriostat (O/129)	N/A	N/A	N/A	N/A	+	+
Anaerobic Growth	N/A	N/A	N/A	N/A	+	+
Denitrification	-	-	+	+	-	-
Tryptophan	-	+	-	-	-	-
Glucose	+	+	+	-	-	-
Arginine	+	+	-	-	-	-
Urea	-	-	+	+	-	-
Esculin	+	+	+	+	+	+
Gelatin	-	+	-	+	+	-
p-nitro-phenyl-BD-galactopyranoside	-	-	-	+	-	-
Glucose	+	+	+	+	+	-
Arabinose	+	+	+	+	-	-
Mannose	+	+	+	+	-	-
Mannitol	+	+	+	+	+	-
N-acetyl-glucosamine	+	+	-	+	+	+
Maltose	-	+	-	+	+	+
Gluconate	+	+	+	+	+	+
Caproate	+	+	-	-	-	+
Adipate	-	+	+	-	+	+
Malate	+	+	+	+	-	+
Citrate	+	+	+	+	+	+
Phenyl-acetate	-	-	-	+	-	-

Table 6.14 Results for the identification of Gram negative bacteria. (+) denotes a positive result and (-) a negative result. N/A denotes that the particular test was not applicable to the identification.

	Isolate G	Isolate H	Isolate I	Isolate J	Isolate K	Isolate L	Isolate M	Isolate N
Colour	6	1	4	4	1	4	1	1
Size	0.6	0.75	0.7	0.8	0.5	0.75	1.0	1.0
Form			3	1	1	1		
Elevation	1	1	3	3	3	3	2	2
Margin			5	1	1	1		
Gram stain	+ve rods	+ve rods	+ve rods	+ve rods	+ve cocci-bacilli	+ve cocci	+ve rods	+ve rods
Catalase test	+	+	+	+	+	+	+	+
Oxidase test	-	-	-	-	-	-	-	-
Motility	+	+	-	+	-	-	+	+
Endospore	-	-	-	-	-	-	+	+
Anaerobic Growth	-	-	-	N/A	N/A	N/A	N/A	N/A

Table 6.15 Results of tests for identification of Gram positive bacteria. (+) represents a positive result and (-) a negative result. N/A denotes that the particular test was not applicable to the identification.

Tentative identifications of the bacterial isolates A-N have been made based on the results of the tests and are discussed under the specific materials they were isolated from in sections 6.4 and 6.5. Ideally further tests would have been carried out to confirm the results; however, this was not possible due to time constraints. On the basis of the results the Gram negative bacteria were identified to species level and where possible the Gram positive bacteria were identified as far as genus. Preliminary identifications were confirmed, as far as was possible, using *Bergey's manual of Determinative Bacteriology* (Holt *et al.*, 1994), *Bergey's manual of Systematic Bacteriology* (Krieg and Holt, 1984) and *The Prokaryotes* (Balows *et al.*, 1992).

### 6.3.2 Identification of fungi causing deterioration

#### 6.3.2.1 Terrestrial fungi

Terrestrial fungi were cultured by both the nutrient agar and sterile tissue methods. They were initially identified to genus level from their morphology using a low powered microscope (x 40) and the dichotomous keys in Domsch *et al.* (1980b: 1-18). Identification to this level indicated fungi of the genera: *Penicillium*, *Cladosporium*, *Trichoderma*, *Aspergillus*, *Verticillium*, and *Ulocladium*. Where possible the fungi were further identified to species level using additional dichotomous keys (Domsch *et al.*, 1980a).

The *Penicillium* spp were identified as: *P. expansum*, *P. frequentans* and *P. citrinum* (Domsch *et al.*, 1980a: 541 - 545). The species of *Cladosporium* isolated was identified as *Cladosporium cladosporoides* (Domsch *et al.*, 1980a: 202). The species of *Trichoderma* found was identified as *Trichoderma koningii* (Domsch *et al.*, 1980a: 799). The species of *Ulocladium* isolated was identified as *Ulocladium botrytis* (Domsch *et al.*, 1980a: 824). The species *Aspergillus* and *Verticillium* could not be identified to species level from the dichotomous keys available.

### 6.3.2.2 Marine Fungi

No marine fungi were cultured on nutrient agar and only one species was cultured on sterile tissue. This corresponded with observations by light and scanning electron microscope that only one fungal species was seen colonising the wood and rope specimens from the Albert Dock, Alum bay and Duart Point wreck sites.

Ascospores of the fungus were viewed by light (Figure 6.10) and scanning electron microscope (Figure 6.11) on wood and rope specimens which had been removed from the field sites and from specimens which had been cultured on sterile tissue. However, as specimens for examination by electron microscopy were freeze dried without preservation the morphological characteristics of the appendages were lost. Perithecia were also viewed by light and scanning electron microscope (Figure 6.12) on rope and wood specimens which had been removed from the sites and from specimens cultured on sterile tissue. The fungus was identified as *Ceriosporopsis halima* using the dichotomous keys of Kohlmeyer and Kohlmeyer (1979: 189-211) and descriptive and pictorial information of Barghoorn and Linder (1944: 409).

### 6.3.2.3 Summary of fungi causing deterioration

The test materials, and the sites where the aforementioned fungi causing deterioration were cultured from, are summarised in Table 6.16.

Material	Deionised water	Artificial seawater	Duart Point Wreck site	Albert Dock
Wood	<i>P. frequentans</i>	<i>P. frequentans</i>	<i>P. expansum</i> , <i>C. halima</i>	<i>P. citrinum</i> , <i>C. halima</i>
Rope	<i>U. botrytis</i>	-	<i>C. halima</i>	<i>C. halima</i> , <i>T. koningii</i>
Canvas	<i>C. cladosporoides</i> <i>P. frequentans</i>		-	<i>Verticillium</i> spp
Leather	-	-	-	-
Bone	<i>Aspergillus</i> spp	-	-	-
Steel	-	-	-	-
Lead	-	-	-	-
Bronze	-	-	-	-

Table 6.16 Summary of fungi isolated from test materials from laboratory and field experiments.

## 6.4 Deterioration of organic materials

### 6.4.1 Wood

#### 6.4.1.1 Observations

The specimens submerged in deionised water (Figure 6.13) and artificial seawater (Figure 6.14) under laboratory conditions showed distinct differences throughout the experiment. Initially, the specimens submerged in deionised water showed little evidence of biocolonisation. However, after 32 weeks specimens had been colonised by several fungal and bacterial species. The specimens in artificial seawater showed bacterial and fungal colonisation after four weeks submersion, which became quite extensive after 52 weeks. Specimens from Alum Bay (Figure 6.15) showed biocolonisation by the barnacle *Balanus crenatus* (Hayward and Ryland, 1990: 354) after four weeks submersion. After 16 weeks the samples showed evidence of colonisation by fungi, the ascospores of the marine fungus *Ceriosporopsis halima* being visible on the wood. No evidence of macro fouling organisms was seen on the specimens.

The specimens from Duart Point (Figures 6.16) did not show biocolonisation until 12 weeks submersion whereupon samples showed fungal colonisation. By 16 weeks the specimens were colonised by the boring crustacea *Limnoria tripunctata* (Figures 6.17 and 6.18) (Kühne, 1971: 70-74). In addition to the colonisers already mentioned, calcareous tubeworms, *Pomatoceros triqueter* (Hayward and Ryland, 1990a: 286) were adhering to the surface of the 32 week submerged specimens. However, colonisation of the 32 week specimens was not very extensive and may have been due to the sample trays being covered by sediment. By 52 weeks the specimens were extensively colonised by fungi and *Limnoria tripunctata*. Also fouling organisms present on the 32 week specimens were present in increased numbers and in addition the specimens had been used as hold fast for juvenile kelp, *laminaria hyperborica* (Martin, 1995: 29) and the barnacle *Balanus crenatus*. The wood specimens were very fragile and, as shown in Figure 6.16, often broke during removal from the site.

The specimens from Albert Dock (Figure 6.19) showed extensive fungal colonisation and deterioration and their wasexfoliation of the surface layers after eight weeks submersion which had become extensive by 52 weeks. No boring, or other macro-fouling organisms, were seen to colonise the wood specimens from the Albert Dock after 52 weeks submersion.

#### 6.4.1.2 Weight change

The percentage weight change results (Appendices IV and VII) for the specimens from the laboratory and field experiments are graphically represented in Figure 6.20.

The control specimens submerged in deionised water showed a rapid rate of loss up to four weeks submersion. Between four and 12 weeks weight loss was at a much slower rate and thereafter remained relatively stable throughout the rest of the experiment. Those

controls submerged in artificial seawater showed an initial decrease in weight loss up to 16 weeks; thereafter this continued at a much slower rate throughout the rest of the experiment.

Specimens from Alum Bay showed no weight change up to eight weeks but between eight and 16 weeks submersion there was a rapid increase in weight loss. Specimens from Duart Point showed an initial decrease in weight loss. After eight weeks there was a sharp increase in weight loss which stabilised after 16 weeks and stayed level to 32 weeks. After 32 weeks there was a sharp increase in weight loss. Specimens from the Albert Dock showed little weight change after eight weeks submersion. Between 8 and 16 weeks there was rapid weight loss. Between 16 and 32 weeks this rate slowed and finally between 32 and 52 weeks the rate of weight loss increased.

#### 6.4.1.3 Alcohol and water extractables

The results of the percentage alcohol and water extractables (Appendix XIII) of specimens from the laboratory and field experiments are graphically represented in Figure 6.21.

The control specimens submerged in deionised water showed an initial decrease in extractables up to 12 weeks submersion. Between 12 and 16 weeks a slight increase was recorded and thereafter a gradual decrease in the levels of extractables was recorded for the remainder of the experiment. The control specimens submerged in artificial seawater showed an increase in extractables up to 16 weeks and then gradually decreased throughout the remainder of the experiment.

The specimens from the Alum Bay wreck site showed an initial increase in extractables and a subsequent steady decrease up to 16 weeks submersion. The specimens from the Duart Point wreck site showed an increase in extractables up to eight weeks and then a decrease which stabilised after 16 weeks. Thereafter the level of extractables decreased up to 32 weeks, and rose again after 52 weeks. The specimens from Albert Dock showed an exponential increase in extractables up to 16 weeks submersion; thereafter increased levels of extractables were recorded at a much slower rate for the remainder of the experiment.

#### 6.4.1.4 Lignin content

The percentage lignin content of the specimens from the laboratory and field experiments (Appendix XIV) is graphically represented in Figure 6.22.

The lignin content of both sets of control specimens fluctuated throughout the experiment, and only showed a slight loss by the end of the experiment.

The loss of lignin from specimens from all of the field sites showed an initial decrease: up to eight weeks in Alum Bay, and 12 weeks in both Duart Point and Albert Dock specimens. Thereafter an increase in lignin content was recorded in specimens from all field sites for the remainder of the experiment.

#### 6.4.1.5 Holocellulose content

The percentage holocellulose content of the specimens from the laboratory and field experiments (Appendix XV) is graphically represented in Figure 6.23.

The specimens in deionised water showed a gradual loss of holocellulose throughout the experiment. Those in artificial seawater showed a greater rate of loss up to 12 weeks which slowed thereafter. Overall those specimens in artificial seawater showed a greater loss of holocellulose than those in deionised water.

The specimens from the Alum Bay wreck site showed a rapid rate of holocellulose loss up to eight weeks submersion, which slowed after 16 weeks. The specimens from the Duart Point wreck site showed an initial rapid rate of loss up to 16 weeks, after which there was no apparent loss up to 32 weeks and then a further period of loss up to 52 weeks. The specimens from the Albert Dock showed an initial rapid rate of loss up to four weeks. Holocellulose loss was slower between four and 16 weeks and again slower between 16 and 52 weeks.

#### 6.4.1.6 Alphacellulose content

The percentage alphacellulose content of the specimens from the laboratory and field experiments (Appendix XVI) is graphically represented in Figure 6.24.

The specimens in deionised water showed fluctuations up to 16 weeks, which may have been due to analytical error. As Figure 6.48 shows, the standard deviation error bars for the replicate analysis of the four and 16 week specimens are large. After 16 weeks a gradual rate of loss of alphacellulose was recorded up to 52 weeks. Those in artificial seawater showed a gradual rate of loss up to 16 weeks, which slowed thereafter. Overall, the specimens in artificial seawater showed a greater loss of alphacellulose than those in deionised water.

The specimens from all the field sites showed similar initial rates of alphacellulose loss. Data for the Alum Bay specimens suggest the rate of loss was greater than that of the Albert Dock up to 16 weeks. The rate of loss for the Albert Dock specimens slowed after 16 weeks, yet loss of alphacellulose still continued up to 52 weeks. The Duart Point specimens showed a greater rate loss than the other sites up to 16 weeks, whereafter the rate of loss was at a much slower rate up to 52 weeks.

#### 6.4.1.7 Hemicellulose content

The percentage hemicellulose content of the specimens from the laboratory and field experiments (Appendices XVII and XVIII) is graphically represented in Figure 6.25.

The rate of loss of hemicellulose from specimens in deionised water was rapid up to 16 weeks, whereupon the hemicellulose content remained relatively stable for the remainder of the experiment. Those specimens in artificial seawater showed a greater and faster rate of

loss up to 12 weeks than the deionised water specimens. Between 12 and 52 weeks hemicellulose loss was still apparent but at a slower rate.

The rate of loss of hemicellulose from the field sites was very similar up to four weeks. Thereafter those specimens from the Alum Bay and Duart Point wreck sites showed a similar rate of loss up to 16 weeks. Between 16 and 52 weeks the rate of loss for specimens from the Duart Point wreck site continued at a slower rate. The initial rate of loss was greatest for those specimens from the Albert Dock, the rate slowing after eight weeks and continuing at a slower rate between eight and 52 weeks.

#### 6.4.1.8 Identification of microbial isolates causing biodeterioration

One fungus, *Penicillium frequentans*, was identified to be causing deterioration of the laboratory control specimens in deionised water and artificial seawater. No bacterial isolates were found to cause deterioration of the laboratory controls.

The terrestrial fungus *Penicillium expansum* and the marine fungus *Ceriosporopsis halima* were identified to cause deterioration of the specimens on the Duart Point wreck site. On specimens from the Albert Dock the terrestrial fungus *Penicillium citrinum* caused deterioration as did the marine fungus *Ceriosporopsis halima*. No bacterial isolates were found to cause deterioration of the specimens on the field sites.

### 6.4.2 Rope

#### 6.4.2.1 Observations

The control specimens submerged in deionised water (Figure 6.26) did not show biocolonisation until after 12 weeks submersion, whereupon several bacterial and fungal colonising species were observed. Increased colonisation, in particular by fungi, was observed throughout the remainder of the experiment. The control specimens submerged in artificial seawater (Figure 6.27) showed biocolonisation by bacterial and fungal species after four weeks submersion which increased progressively throughout the experiment. Samples of the 32 week specimens cultured onto nutrient agar in the laboratory yielded several bacterial and fungal groups. However, they showed no evidence of causing significant deterioration.

The specimens from the Alum Bay wreck site (Figure 6.28) showed little signs of biocolonisation up to eight weeks. After 16 weeks colonisation by a marine species of fungus was apparent. No macro organisms were observed to be colonising the specimens. The specimens from the Duart Point wreck site (Figure 6.29) did not show biocolonisation until after 12 weeks submersion, whereupon specimens showed colonisation by a marine fungus, however, no deterioration was visible. Discoloration was seen on the 16 and 32 weeks specimens. Samples of the 32 week specimens cultured onto nutrient agar in the laboratory yielded several bacterial and fungal groups; however, none were found to cause deterioration. The 52 weeks specimens were quite broken down and were observed to be

extensively colonised by the marine fungus and *Limnoria tripunctata*. The specimens from the Albert Dock (Figure 6.30) showed evidence of colonisation by marine fungus after eight weeks submersion. Deterioration was visible by 12 weeks and by 16 weeks there was virtually a complete breakdown of the rope strands. Samples of the 32 week specimens cultured onto nutrient agar in the laboratory yielded several bacterial and fungal groups, of which one fungus caused deterioration in the laboratory. By 52 weeks, breakdown of the specimens was to such an extent that specimens had fallen from the strings and been lost from the sample trays.

#### 6.4.2.2 Weight change

The percentage weight change results for the specimens from the laboratory and field experiments (Appendices IV and VII) are graphically represented in Figure 6.31.

Both sets of control specimens showed a similar trend of increasing weight loss with time, the rate of loss being greater in those specimens kept in deionised water. The specimens from the Alum Bay wreck site showed a slight increase in weight loss up to eight weeks and a rapid increase in weight loss by 16 weeks. The specimens from the Duart Point wreck site showed a small increase in weight loss up to 12 weeks and then a rapid increase in weight loss up to 16 weeks. The rate of weight loss was slower up to 32 weeks; thereafter there was an increased rate of weight loss up to 52 weeks. The specimens from the Albert Dock showed a rapid rate of weight loss throughout the experiment. Specimens deteriorated to such an extent that they were lost some time between 32 and 52 weeks submersion.

#### 6.4.2.3 Tensile strength

The tensile strength results for the specimens from the laboratory and field experiments (Appendix XIX) are graphically represented in Figure 6.32.

Both sets of controls showed similar trends in loss of tensile strength: an initial rapid rate of loss after four weeks submersion; a further slight loss between four and eight weeks; and a greater loss between eight and 12 weeks. After 12 weeks there were only slight fluctuations in the tensile strength. The tensile strength of specimens in both laboratory experiments was very similar by the end of the experiment.

The rate of deterioration of the specimens from all the field sites was rapid. If the results are compared at eight weeks, when specimens were available from all sites, it can be seen that the specimens from the Albert Dock showed the fastest rate of loss of tensile strength followed by specimens from the Duart Point and Alum Bay wreck sites respectively. It was not possible to measure the tensile strength of the specimens from the Albert Dock after 12 weeks submersion as the strands of rope had broken down.

#### 6.4.2.4 Identification of microbial isolates causing biodeterioration

One fungus, *Ulocladium botrytis*, was identified to be causing deterioration of control laboratory specimens in deionised water. No bacteria were identified to cause deterioration. No bacteria or fungi were identified to be causing significant deterioration of those control specimens in artificial seawater.

The marine fungus found to cause deterioration of rope on all field sites was identified as *Ceriosporopsis halima*. The terrestrial fungus isolated from specimens in the Albert Dock was identified as *Trichoderma koningii*. No bacteria were identified to be causing significant deterioration of specimens on any of the field sites.

### 6.4.3 Canvas

#### 6.4.3.1 Observations

The control specimens submerged in deionised water (Figure 6.33) showed colonisation by fungi after two weeks which progressed steadily throughout the experiment. In addition to the fungi a bacterial species, Isolate I, which colonised and deteriorated the canvas, was cultured from a specimen submerged in deionised water. The 52-week specimens were completely covered by fungal hyphae and spores.

The control specimens kept in artificial seawater (Figure 6.34) showed no deterioration by bacteria or fungi and appeared the same throughout the experiment.

The specimens from the Alum Bay wreck site (Figure 6.35) did not show signs of deterioration up to the 16-week sample interval. The 16-week specimens showed a blackening; it was not certain whether this was staining due to sediments or biological activity. No microbial activity was discernible under the light microscope. Structurally the specimens appeared intact.

The specimens from the Duart Point wreck site (Figure 6.36) showed no signs of deterioration up to eight weeks. The 12-week specimens, although not showing any signs of deterioration or colonisation by marine fungi similar to those seen on the wood and rope specimens, were tacky to the touch. By 16 weeks, specimens were very tacky and the fibres of the fabric were beginning to separate. Several bacterial and fungal groups were colonising the specimens, of these two bacterial isolates, isolates J and K, were found to cause deterioration. No specimens were found after the 16 week sample interval.

The specimens from the Albert Dock (Figure 6.37) showed no sign of deterioration after four weeks submersion. After eight weeks the specimens had a tacky texture similar to the Duart Point specimens. As with the Duart Point specimens, light and scanning electron microscopy revealed no evidence of bacteria or marine fungi. After 12 weeks the fibres of the specimens were starting to separate and were very tacky. After 16 weeks the specimens had almost completely broken down and had the consistency of a gel. Samples of the 16 week specimens yielded several bacterial and fungal groups colonising the specimens. One fungus and the bacterial isolate L, were found to cause deterioration. No evidence of

marine fungi was visible by light or scanning electron microscope, nor was their presence indicated by culture onto sterile tissue in the laboratory. No specimens remained intact after the 16 week sample interval.

#### 6.4.3.2 Weight change

The percentage weight change results for the specimens from the laboratory and field experiments (Appendices IV and VII) are graphically represented in Figure 6.38. Both sets of controls showed an insignificant weight change throughout the experiment.

The specimens from the Alum Bay wreck site showed an insignificant weight change up to eight weeks. The 16 week specimen showed a dramatic rate of weight loss. The specimens from the Duart Point wreck site showed a steady rate of weight loss up to 16 weeks; no specimens were found after this sampling interval. The specimens from the Albert Dock showed the most rapid rate of weight loss of all the field sites, specimens being completely broken down by 16 weeks and no specimens being found after this sample interval. Weight loss could only be determined up to 12 weeks submersion as by 16 weeks the specimens had broken down to such an extent it was impossible accurately to determine the weight.

#### 6.4.3.3 Tensile strength

The tensile strength results for the specimens from the laboratory and field experiments (Appendix XX) are graphically represented in Figure 6.39. Both sets of controls showed similar trends in loss of tensile strength: an initial rapid rate of loss up to 16 weeks submersion, after which time there was a slight fluctuation in the tensile strength throughout the remainder of the experiment. Those specimens in deionised water showed a greater loss of tensile strength.

The rate of loss of tensile strength from all of the field sites was rapid. The rate of loss of tensile strength was fastest for the specimens from the Albert Dock, followed by those from the Duart Point and Alum Bay wreck sites respectively.

#### 6.4.3.4 Identification of microbial isolates causing biodeterioration

Bacterial isolate I was cultured from a specimen submerged in deionised water and was assigned to *Group 22 Nocardioform Actinomycetes* of Bergey's manual (Holt *et al.*, 1994: 625) on the basis that it formed branching filaments. Tentative identification based on the morphology and colour of the colonies was made to the genus *Nocardia*. The colonies were irregular and aerial hyphae were visible by low powered microscope (x 40) observations which corresponded with Korn-Wendisch and Kutznevs' observations of the genus (1992: 936). In addition they were yellow in appearance (a characteristic often associated with members of this genus), non-motile, without endospores, and they were catalase positive (Lechevalier, 1984: 1459). The fungi found to cause deterioration were

identified as *Penicillium frequentans* and *Cladosporium cladosporoides*. The latter produces a profuse amount of black hyphae (Domsch *et al.*, 1980a: 204) and it was thought that this fungus was the main coloniser of the canvas in this case.

Isolates J and K, cultured from specimens submerged on the Duart Point wreck site and L, from specimens submerged in the Albert Dock, were assigned to *Group 20: Irregular, nonsporulating Gram positive rods*, of Bergey's manual (Holt *et al.*, 1994: 571). They were further identified to the genus *Cellulomonas* based on the fact that they were convex yellow colonies, characteristics often associated with growth of species of this genus on peptone-yeast extract agar, and they were catalase positive (Holt *et al.*, 1994: 575). In addition no endospores were formed (Stackerbrandt and Keddie, 1984: 1325). The fungus which caused deterioration of specimens in the Albert Dock was identified as *Verticillium* sp.

#### 6.4.4 Leather

##### 6.4.4.1 Observations

The control specimens submerged in deionised water (Figure 6.40) showed few signs of deterioration or biocolonisation throughout the experiment. The control specimens submerged in artificial seawater (Figure 6.41) showed biocolonisation by bacterial and fungal species after four weeks submersion which increased progressively throughout the experiment. However, none of the micro-organisms cultured from specimens appeared to cause deterioration.

The specimens from the Alum Bay wreck site (Figure 6.42) showed few signs of deterioration or biocolonisation after four weeks submersion. By eight weeks submersion they were colonised by the barnacle *Balanus crenatus*. However, no evidence of biocolonisation was seen on the 16 week specimens. The specimens from the Duart Point wreck site (Figure 6.43) showed little evidence of biocolonisation or deterioration until 52 weeks submersion, at which time specimens were extensively colonised by a wide range of micro and macro-organisms. The specimens had been colonised by barnacles (*Balanus crenatus*), kelp (*Laminaria hyperborea*) and calcareous tubeworms (*Pomatoceros triqueter*). In addition, bacterial isolates M and N were found to cause deterioration.

Apart from a slight covering of sediment, the specimens from the Albert Dock (Figure 6.44) showed few signs of deterioration or biocolonisation throughout the experiment.

##### 6.4.4.2 Weight change

The percentage weight change results for the specimens from the laboratory and field experiments (Appendices IV and VII) are graphically represented in Figure 6.45. The control specimens in deionised water showed a weight loss up to eight weeks submersion. This was followed by an increase in weight up to 12 weeks submersion whereupon insignificant weight change was recorded throughout the remainder of the experiment. The specimens in artificial seawater showed an increase in weight up to 12 weeks submersion

followed by a weight loss up to 32 weeks whereupon an insignificant weight change was recorded throughout the remainder of the experiment.

The specimens from the Alum Bay wreck site showed an increase in weight up to four weeks submersion and a subsequent weight loss up to 16 weeks submersion. The specimens from Duart Point showed an initial rapid weight increase up to four weeks submersion which then stayed stable up to 32 weeks submersion. Subsequent samples showed a further increase in weight. The specimens from the Albert Dock showed an increase in weight up to four weeks submersion followed by a decrease in weight up to eight weeks. This was followed by a further increase in weight up to 12 weeks, a subsequent weight loss up to 16 weeks and a final increase in weight up to 32 weeks submersion which stayed level for the remainder of the experiment.

#### 6.4.4.3 Nitrogen content

The percentage nitrogen content results for the specimens from the laboratory and field experiments (Appendix XXI) are graphically represented in Figure 6.46. The control specimens, both in deionised and artificial seawater, showed an insignificant change in their total nitrogen content throughout the experiment.

The specimens from the Alum Bay wreck site showed an exponential decrease in nitrogen content for the samples available. Results for specimens from the Duart Point and Albert Dock sites showed fluctuations throughout the experiment: a period of nitrogen loss, followed by an increase, then a further decrease, and finally an increase. This made the determination of rates of deterioration difficult by this method as it was not simply exponential. It was thought that these fluctuations reflected an effect of seasonality rather than poor analytical results. Although the specimens from Duart Point may have been covered and uncovered sometime between the 16 and 32 week sampling intervals, they gave good replicate analyses, as shown by the small error bars in Figure 6.46.

#### 6.4.4.4 Identification of microbial isolates causing biodeterioration

Isolates M and N, both cultured from specimens submerged on the Duart Point wreck site, were assigned to *Group 18: Endospore forming Gram positive rods and cocci*, of Bergey's manual (Holt *et al.*, 1994: 559). They were further identified to the genus *Bacillus* (Holt *et al.*, 1994: 559) on the basis that they were catalase positive whereas other members of the group were catalase negative.

### 6.4.5 Bone

#### 6.4.5.1 Observations

The control specimens from deionised water (Figure 6.47) and artificial seawater (Figure 6.48) showed little sign of deterioration or biocolonisation throughout the experiment. However, several bacterial and fungal groups were identified, of which one fungus and

bacterial Isolate C, were identified to cause deterioration of those specimens which had been submerged in deionised water.

The specimens from the Alum Bay wreck site (Figure 6.49) softened and became "chalky" after four weeks submersion. Softening worsened with time, although the surface morphology did not appear to be affected. The specimens from the Duart Point wreck site (Figure 6.50) also showed softening after four weeks submersion which worsened with length of submersion, the 52 week specimens having a paste-like consistency. In addition to the softening, the specimens began to show surface pitting after eight weeks which was appreciable by 52 weeks submersion. Several bacterial and fungal groups were isolated from specimens, of which bacterial Isolates E and F were found to cause deterioration. The specimens from the Albert Dock (Figure 6.51) also showed softening after four weeks which worsened with the length of submersion. However, it was not as extensive as on the specimens from the Alum Bay and Duart Point wreck sites. Several bacterial and fungal groups were isolated from specimens, of which bacterial Isolate D, was found to cause deterioration.

#### 6.4.5.2 Weight change

The percentage weight change results for the specimens from the laboratory and field experiments (Appendices IV and VII) are graphically represented in Figure 6.52. Both sets of control specimens showed insignificant weight loss throughout the experiment.

The specimens from the Alum Bay wreck site showed a gradual weight loss up to 16 weeks submersion. The specimens from the Duart Point wreck site showed a greater overall weight change when compared to the specimens from the Albert Dock. However, the pattern of weight change was very similar. Specimens from both sites showed an increase in weight after four weeks submersion, followed by a decrease in weight up to 16 weeks submersion. In both cases the rate of weight loss slowed and was more gradual for the remainder of the experiment.

#### 6.4.5.3 Nitrogen content

The percentage nitrogen content of the specimens from the laboratory and field experiments (Appendix XXII) is graphically represented in Figure 6.53. The control specimens submerged in deionised water showed a slight decrease in their nitrogen content throughout the experiment. Those submerged in artificial seawater showed an insignificant change.

The specimens from the Alum Bay wreck site showed a gradual decrease in nitrogen content throughout the experiment. The specimens from the Duart Point wreck site showed a rapid rate of loss of nitrogen up to 16 weeks, whereupon the nitrogen content appeared to increase for the remainder of the experiment. The specimens from the Albert Dock showed fluctuations in nitrogen content throughout the experiment: decrease up to four weeks, followed by an increase up to eight weeks, a further decrease up to 12 weeks, a larger

increase up to 16 weeks, followed by a final decrease up to 32 weeks, after which the nitrogen content remained stable.

#### 6.4.5.4 Calcium and phosphate analysis

The results of the calcium and phosphate analyses (Appendix XXIII) are reported as a ratio of calcium:phosphate in Figure 6.54. Both sets of control specimens showed slight yet insignificant fluctuations in their calcium:phosphate ratio throughout the experiment.

The specimens from the Alum Bay wreck site showed an initial decrease in the ratio up to eight weeks submersion, followed by an increase up to 16 weeks. The specimens from the Duart Point wreck site showed little change in the ratio up to eight weeks submersion, followed by a rapid increase up to 16 weeks, followed by a decrease up to 32 weeks, and then a further increase up to 52 weeks. The specimens from the Albert Dock showed a similar trend of peaks and troughs in the ratio: a gradual increase up to 12 weeks submersion followed, by a decrease up to 16 weeks, followed by an increase up to 32 weeks, and a final decrease up to 52 weeks.

#### 6.4.5.5 Identification of microbial isolates causing biodeterioration

Isolate C was cultured from a specimen submerged in deionised water. Isolate D was cultured from a specimen submerged in the Albert Dock and isolates E and F were cultured from specimens submerged on the Duart Point wreck site. Isolates C and D were assigned to *Group 4: Gram negative aerobic rods and cocci*, of Bergey's manual (Holt *et al.*, 1994: 71). Further correlation of the data identified them as most likely to be members of the family *Pseudomonadaceae* and the genus *Pseudomonas*. Although the biochemical test results (Table 6.8) do not completely match Bergey's characterisations (Krieg and Holt, 1984: 140-218) for this genus, the majority of the results correlate with the proposed identifications that both isolates were *P. aeruginosa* (Krieg and Holt, 1984: 165-168).

Isolates E and F were assigned to *Group 5: Facultatively anaerobic Gram negative rods*, of Bergey's manual (Holt *et al.*, 1994: 175) Further correlation of the data identified them as most likely to be members of the family *Vibrionaceae* and of the genus *Vibrio*. Correlation of the biochemical tests with Bergey's characterisations (Krieg and Holt, 1984: 531-538) limited the likelihood of isolate E to two species: *V. fischeri* or *V. logei*. The results for isolate F correlated with the characterisations for *V. marinus* (Krieg and Holt, 1984: 2979-2982). The fungus found to cause deterioration of specimens in deionised water was identified as *Aspergillus* sp.

## 6.5 Deterioration of metals

### 6.5.1 Steel

#### 6.5.1.1 Observations

All specimens were progressively covered by dark red/brown corrosion products: deionised water, Figure 6.55; artificial seawater Figure, 6.56; Alum Bay wreck site, Figure 6.57; Duart Point wreck site, Figure 6.58 and Albert Dock, Figure 6.59.

Apart from the control specimens in deionised water, very little difference was apparent between them. The corrosion products on those specimens in deionised water, although looking similar to the artificial seawater controls when submerged, did not adhere strongly to the surface. They were extremely flocculated and fell from the metal when touched. The corrosion products on the other specimens tended to be solid and well adhered to the surface. Scanning electron microscopy of the surfaces of the uncleaned specimens showed one discernible form of corrosion product. At low magnifications this appeared as globose groups encrusting the surface (Figure 6.60). At higher magnifications the globose structures appeared to be made up of small plates (Figure 6.61)

After chemical cleaning all of the specimens showed pitting, an example of this is shown in Figure 6.62. The extent of this ranged from slight, in the case of specimens submerged in deionised water and the Albert Dock, to considerable in those submerged in artificial seawater and the Alum Bay wreck site, to extreme in the specimens submerged on the Duart Point wreck site.

Samples of the 32 week specimens cultured onto nutrient agar in the laboratory yielded bacteria and fungi from each of the experimental laboratory and field sites. Only certain bacteria were found to promote corrosion. Of these, bacterial Isolates A and B from control specimens in deionised water and Bacterial Isolates G and H, from specimens from the Albert Dock and Duart Point respectively, were found to affect the corrosion rate under laboratory conditions.

#### 6.5.1.2 Weight loss

The percentage weight change results for the specimens from the laboratory and field experiments (Appendices V and VIII) are graphically represented in Figure 6.63. The control specimens submerged in deionised water showed a continual rate of weight loss throughout the experiment. The controls in artificial seawater showed an initial rapid weight loss up to eight weeks which tailed off by 12 weeks, increased again up to 16 weeks and then remained stable for the remainder of the experiment.

The specimens from the field sites showed similar trends: an initial rapid weight loss up to four weeks, a decrease in weight loss up to eight weeks and then a gradual increase in weight loss up to 16 weeks. Up to this point the specimens from the Alum Bay and Duart Point wreck sites showed very similar rates of weight loss. Additional data for Duart Point showed a constant increase in weight loss for the remainder of the experiment. The rate and extent of weight loss of the specimens from the Albert Dock were less.

### 6.5.1.3 Identification of microbial isolates causing biodeterioration

Isolates A and B were cultured from steel specimens which were submerged in deionised water. They were assigned to *Group 4: Gram negative aerobic rods and cocci* of Bergey's manual (Holt *et al.*, 1994: 71). Further correlation of the data identified them as most likely to be members of the family *Pseudomonadaceae* and of the genus *Pseudomonas* (Holt *et al.*, 1994: 93). Although the biochemical test results (Table 6.8) do not completely match Bergey's characterisations (Krieg and Holt, 1984; 140-218) for this genus, the majority of the results correlate with the proposed identifications that isolate A was *P. putida* and isolate B was *P. aeruginosa* (Krieg and Holt, 1984: 165-168).

Isolates G and H were cultured from specimens submerged in the Albert Dock and on the Duart Point wreck site respectively. They were assigned to *Group 20: Irregular, non sporing Gram positive rods*, of Bergey's manual (Holt *et al.*, 1994: 571). As the isolates stained as Gram positive rods were catalase positive and non sporing they were tentatively identified as belonging to the genus *Arthrobacter*. Speculatively, one of them may be the species *A. siderocapsulata* as this is known to oxidise iron (Krieg and Holt, 1984: 1299).

## 6.5.2 Bronze

### 6.5.2.1 Observations

The control specimens submerged in deionised water (Figure 6.64) showed no evidence of corrosion throughout the experiment. Those in artificial seawater (Figure 6.65) initially showed a red corrosion product on their surface, which was followed by a series of green and green/blue corrosion products as the experiment progressed.

The specimens from the Alum Bay wreck site (Figure 6.66) were similar to the artificial seawater control specimens in that after four weeks a red corrosion product was seen which was followed by a series of green and green/grey/blue corrosion products. The specimens from the Duart Point wreck site (Figure 6.67) showed a layer of red corrosion products after four weeks submersion which persisted throughout the experiment. As with the Alum Bay and artificial seawater control experiments, blue/green corrosion products were observed, but not to the same extent as the red corrosion products. The specimens from the Albert Dock (Figure 6.68) showed a dark green/blue coating of corrosion products after four weeks which progressed throughout the experiment.

Scanning electron microscopy of the surfaces of the uncleaned specimens from those conditions which promoted corrosion showed two discernible forms of corrosion product which correlated with the red and blue/green corrosion products seen. The red corrosion product appeared as octahedral crystals (Figure 6.69). The blue/green corrosion product appeared as globular crystals made up of plates (Figure 6.70). On cleaning, all of the specimens from artificial seawater and the field sites exhibited localised pitting, an example of which is shown in Figure 6.71.

No bacteria or fungi were isolated from the bronze specimens.

#### 6.5.2.2 Weight loss

The percentage weight change results for the specimens from the laboratory and field experiments (Appendices V and VIII) are graphically represented in Figure 6.72. The control specimens submerged in deionised water showed an insignificant change in weight throughout the experiment. Those in artificial seawater showed a gradual increase in the amount and rate of weight loss throughout the experiment.

The specimens from the Alum Bay wreck site showed an initial rapid rate of weight loss up to four weeks followed by a period of decrease in weight loss up to eight weeks and then an increase in weight loss up to 16 weeks. The specimens from the Duart Point wreck site and the Albert Dock both showed similar trends in the rate of corrosion although the amounts of metal lost were different. In both cases the rate of weight loss was rapid up to four weeks submersion and thereafter was at a much slower rate for the remainder of the experiment. Those specimens from Duart Point showed the greatest weight loss.

#### 6.5.3 Lead

##### 6.5.3.1 Observations

Corrosion products were observed on the deionised water control specimens within a few days of the experiments starting (Figure 6.73). After four weeks these corrosion products were fine white granular crystals, which were loosely attached to the specimen surface. With time the corrosion products became larger, more angular and adhered more strongly to the surface of the specimens. After chemical cleaning the specimens exhibited extensive pitting. The control specimens in artificial seawater (Figure 6.74) showed very little change in appearance throughout the experiment and there was no change on chemical cleaning.

The specimens from the Alum Bay wreck site (Figure 6.75) showed very little change throughout the experiment. However, by eight weeks the specimens were colonised by the calcareous tubeworm, *Pomatoceros triqueter*, which progressed up to 16 weeks. No change was seen after chemical cleaning of the specimens. The specimens from the Duart Point wreck site (Figure 6.76) showed little change up to eight weeks submersion. By 16 weeks they were extensively colonised by the calcareous tube worm, *Pomatoceros triqueter*. By 32 weeks bryozoans (Hayward and Ryland, 1990b: 797) were also present on the lead. After 52 weeks there was an apparent decrease in the amount of colonisation by macro fauna and the lead appeared appreciably darker. Very little change was seen after chemical cleaning of the metals. The specimens from the Albert Dock (Figure 6.77) showed no evidence of change throughout the experiment.

Scanning electron microscopy of the uncleaned surfaces of all specimens from the field sites revealed no discernible corrosion products, neither was there any change in the surface of the metals after chemical cleaning. No bacteria or fungi were isolated from the lead specimens.

### 6.5.3.2 Weight loss

The percentage weight change results for the specimens from the laboratory and field experiments (Appendices V and VIII) are graphically represented in Figures 6.78 and 6.79. The control specimens submerged in deionised water showed extensive weight loss, and at a rapid rate, throughout the experiment. Those in artificial seawater showed an insignificant change in weight throughout the experiment.

The weight changes of those specimens submerged in artificial seawater and on the field sites were much less. Weight loss of the lead specimens from the field sites was very slight, but trends were apparent. The rate and extent of weight loss was initially faster in specimens from the Alum Bay wreck site, followed by those from the Duart Point wreck site and the Albert Dock respectively. At the end of the experiment greatest weight loss was recorded in those specimens from the Duart Point wreck site.

## 6.6 X-Ray Diffraction results for metal corrosion products

Identification of corrosion products in some cases was tentative as, due to the sampling method, mixtures of corrosion products were analysed. The results tabulated throughout the chapter, show the diffraction lines for the reference compounds and corrosion products; those in bold type indicate the most intense lines. The reference diffraction lines were listed as far as the measured  $d$  values of the corrosion products.

### 6.6.1 Steel

Discerning, sampling and analysing the corrosion products on the steel specimens from both laboratory and field experiments proved extremely hard due to their amorphous nature. In some instances the  $d$  values of the corrosion products were mixtures and interpretation of the data was based on the most intense diffraction lines.

As analytical samples may have been mixtures of compounds the  $d$  values of a control (untreated) steel specimen were determined in order to eliminate these values from the corrosion product values (Table 6.17).

The corrosion products on the 52 week control specimens in deionised water were a flocculated, reddish brown mass, the  $d$  values of which best compared with the Iron Oxide Hydroxide *lepidocrocite*,  $\text{FeO}(\text{OH})$  (Table 6.18). The corrosion products on the 52 week control specimens in artificial seawater were a dark brown amorphous mass. Analysis showed several intense  $d$  values which indicated a mixture of iron compounds. These data best compared with the Iron Oxide Hydroxides *goethite*,  $\text{Fe}_2\text{O}_3 \cdot \text{H}_2\text{O}$  (Table 6.19) and *lepidocrocite* (Table 6.18).

The surfaces of the 16 week submerged specimens from the Alum Bay wreck site were covered with a fine grained brown/black corrosion product, the  $d$  values of which best compared with the Iron Oxide *magnetite*,  $\text{Fe}_3\text{O}_4$  (Table 6.20). The corrosion products observed on the 52 week specimens submerged on the Duart Point wreck site were an

amorphous mass. Although samples taken for analysis were a mixture of compounds, several intense  $d$  values showed that the bulk of the corrosion product compared with the Iron Oxide Hydroxide *goethite* (Table 6.19). In addition there were a number of less intense  $d$  values which indicated the presence of *lepidocrocite* (Table 6.18). Two discernible corrosion products were observed on the 52 week specimens submerged in the Albert Dock. Adjacent to the metal was a black powder product, the  $d$  values of which best compared with Iron Sulphide, *pyrite*,  $\text{FeS}_2$  (Table 6.21). This was overlain by a dark red-brown product which was in fact a mixture of the Iron Oxide Hydroxides *lepidocrocite* and *goethite*, (Tables 6.18 and 6.19).

#### 6.6.1.1 $d$ values for iron corrosion products from steel specimens

The  $d$  values for the aforementioned corrosion products from the steel specimens are summarised in Tables 6.17-6.21.

Reference Iron	Control specimen of steel
2.01	2.03
1.43	1.47
1.17	1.20
1.01	0.99

Table 6.17  $d$  values for reference iron (JCPDS, 1986: 550), and control specimen of steel.

Reference Iron Oxide Hydroxide ( <i>lepidocrocite</i> )	Corrosion product from specimen in deionised water	Corrosion product from specimen in artificial seawater	Corrosion product from specimen in Albert Dock
6.26	6.20	6.24	6.16
		4.96	4.85
		4.19	4.21
3.29	3.25	3.30	3.28
	2.92	2.94	2.93
		2.70	2.69
2.47	2.50	2.52	2.50
2.36			2.44
2.09	2.11	2.10	2.07
1.94	1.91	1.94	1.92
1.85			1.83
1.73	1.69	1.72	1.73
1.57			1.60

Table 6.18  $d$  values for reference Iron Oxide Hydroxide, *lepidocrocite* (JCPDS, 1986: 656), and corrosion products from steel specimens submerged for 52 weeks in deionised water, artificial seawater and the Albert Dock.

Reference Iron Oxide Hydroxide (goethite)	Corrosion product from specimen in artificial seawater	Corrosion product from specimen on the Duart Point wreck site	Corrosion product from specimen in Albert Dock
	6.24		6.16
4.98	4.96	4.90	4.85
4.18	4.19	4.20	4.21
		4.14	
3.38			
	3.30	3.29	3.28
		3.17	
	2.94	2.86	2.93
2.69	2.70	2.65	2.69
2.58			
2.49	2.52	2.50	2.50
2.45		2.45	2.44
2.25		2.30	
2.19	2.10	2.19	2.07
1.92	1.94	1.97	1.92
1.80		1.78	1.83
	1.72	1.70	1.73
	1.61	1.59	1.60
	1.48	1.50	1.47

Table 6.19 *d* values for reference Iron Oxide Hydroxide, *goethite* (JCPDS, 1986: 435), and corrosion products from steel specimens submerged for 52 weeks in artificial seawater and the Albert Dock.

Reference Iron Oxide (magnetite)	Corrosion product from specimen on the Alum Bay wreck site
4.85	
	4.04
	3.23
2.97	2.93
2.53	2.60
2.42	2.48
2.10	2.06
	1.91
1.71	1.77
1.62	1.59
1.48	1.50
1.42	1.46

Table 6.20 *d* values for reference Iron Oxide, *magnetite* (JCPDS, 1986: 702), and corrosion product from steel specimen submerged for 16 weeks on the Alum Bay wreck site.

Reference Iron Sulphide (pyrite)	Corrosion product from specimen in the Albert Dock
	3.89
3.13	3.07
2.71	2.78
2.56	
2.43	2.41
	2.34
2.21	2.26
	2.12
	2.01
1.92	1.88
1.63	1.68

Table 6.21  $d$  values for reference Iron Sulphide, *pyrite* (JCPDS, 1986: 702), and corrosion product from steel specimen submerged for 52 weeks in the Albert Dock.

### 6.6.2 Bronze

No corrosion products were observed on the specimens submerged in deionised water. Two discernible corrosion products were seen on the 52 week control specimens submerged in artificial seawater. One, adjacent to the metal surface, was red and its  $d$  values correlated with the Copper Oxide corrosion product Cuprite,  $\text{Cu}_2\text{O}$  (Table 6.22). The second corrosion product, overlaying the first, was a dark green/blue. Several intense  $d$  values indicated a mixture of corrosion products which best compared with data for the Copper Chloride Hydroxides: *paratacamite*,  $\text{Cu}_2\text{Cl}(\text{OH})_3$  (Table 6.23) and *atacamite*,  $\text{CuCl}_2 \cdot 3\text{Cu}(\text{OH})_2$  (Table 6.24).

The specimens submerged for 16 weeks on the Alum Bay wreck site appeared different to all other bronze specimens. Whereas the specimens from the other field experiment sites had an outer coating of a blue/green corrosion product, the Alum Bay specimens were predominantly grey. The  $d$  values for this corrosion product correlated with the  $d$  values of the Tin Oxide *cassiterite*,  $\text{SnO}$  (Table 6.25). In addition there were areas of blue/green corrosion product whose  $d$  values correlated with those of *paratacamite* and/or *atacamite* (Tables 6.23 and 6.24). The specimens submerged for 52 weeks on the Duart Point wreck site showed three discernible corrosion products: a red product nearest the metal surface which, in some areas, was overlain by a dark green/blue corrosion product. In other areas the red corrosion product was overlain by a mauve/purple product. The  $d$  values for the red corrosion product correlated well with the Copper Oxide, *cuprite* (Table 6.22). The  $d$  values of the blue/green product correlated with *paratacamite* and/or *atacamite* (Tables 6.23 and 6.24). As with the red corrosion product, the  $d$  values for the mauve/purple corrosion product also correlated with *cuprite*. It was thought this was a genuine result as an uncontaminated sample of each corrosion product had been taken from the specimen for analysis. The specimens submerged for 52 weeks in the Albert Dock showed three

corrosion products: red nearest the metal surface, overlain by a light green product which in turn was overlain by a dark green product. The  $d$  values for the red product correlated well with those for *Cuprite* (Table 6.22). The  $d$  values for the light green and dark green products indicated *paratacamite* and/or *atacamite* (Tables 6.23 and 6.24).

#### 6.6.2.1 $d$ values for copper and tin corrosion products from bronze specimens

The  $d$  values for the aforementioned corrosion products from the bronze specimens are summarised in Tables 6.22-6.25.

Reference Copper Oxide (cuprite)	Corrosion product from bronze specimen in artificial sea water	Corrosion product from bronze specimen from Duart Point wreck site		Corrosion product from bronze specimen from the Albert Dock
		(Red)	(Mauve)	
	5.29	4.49	4.55	3.83
	3.34		4.10	
			3.89	
<b>3.02</b>	2.98	3.01	<b>3.05</b>	3.00
	2.84			
	2.71	2.72		2.73
<b>2.47</b>	<b>2.48</b>	2.48	<b>2.48</b>	<b>2.49</b>
	2.42		2.36	
			<b>2.26</b>	
2.14	<b>2.22</b>	<b>2.13</b>	<b>2.15</b>	<b>2.12</b>
		1.94	1.96	
1.74	1.69	1.72	1.75	1.74
<b>1.51</b>	<b>1.48</b>	<b>1.50</b>	<b>1.59</b>	1.50
1.29				
1.23				

Table 6.22  $d$  values for reference Copper Oxide, *cuprite* (JCPDS, 1986: 277), and corrosion products from bronze specimens submerged for 52 weeks in artificial seawater, on the Duart Point wreck site and in the Albert Dock.

Reference copper Chloride Hydroxide (paratacamite)	Corrosion product from specimen from Artificial seawater	Corrosion product from specimen from the Alum Bay wreck site	Corrosion product from specimen from Duart Point wreck site	Corrosion product from specimen from the Albert Dock
				6.55
5.44	5.45	5.47	5.42	5.46
4.69	4.65			
		4.50	4.55	
			3.86	
3.41	3.38	3.40		
			3.25	3.21
	3.00	2.98		
2.89	2.87	2.85		
	2.80			
2.78	2.79	2.77	2.75	2.82
2.76				
2.74				
2.72		2.70	2.65	
			2.50	2.54
2.34	2.32	2.33		2.37
2.27	2.24	2.21	2.27	2.23
2.25	2.19			2.11
2.03	2.02	2.00	2.05	2.04
1.83	1.89	1.86		1.81
1.81	1.80			
1.71	1.74	1.71	1.73	1.70

Table 6.23 *d* values for reference Copper Chloride Hydroxide, *paratacamite* (JCPDS, 1986: 888), and corrosion products from bronze specimens submerged for 52 weeks in artificial seawater, on the Alum Bay and Duart Point wreck sites and in the Albert Dock.

Reference Copper Chloride Hydroxide (atacamite)	Corrosion product from specimen from Artificial seawater	Corrosion product from specimen from the Alum Bay wreck site	Corrosion product from specimen from Duart Point wreck site	Corrosion product from specimen from the Albert Dock
				6.55
5.49	5.45	5.47	5.42	5.46
5.04				
	4.65	4.50	4.55	
			3.86	
4.54				
3.44	3.38	3.40		
3.21			3.25	3.21
3.02	3.00	2.98		
2.84	2.87	2.85		
	2.80			
2.77	2.79	2.77	2.75	2.82
2.75		2.70		
2.65			2.65	
2.52			2.50	2.54
	2.32	2.33		2.37
2.27	2.24	2.21	2.27	2.23
2.20	2.19			2.11
2.13	2.02	2.00	2.05	2.04
1.91	1.89	1.86		1.81
1.83	1.80			
1.72	1.74	1.71	1.73	1.70
	1.67			1.68
	1.65	1.62		
1.63	1.63			
1.61	1.59			
			1.50	

Table 6.24 *d* values for reference Copper Chloride Hydroxide, *atacamite* (JCPDS, 1986: 72), and corrosion products from bronze specimens submerged for 52 weeks in artificial seawater, on the Alum Bay and Duart Point wreck sites and in the Albert Dock.

Reference tin oxide (cassiterite)	Corrosion product from specimen from the Alum Bay wreck site
	6.86
	3.65
3.35	3.31
	3.25
	3.11
	2.87
2.64	2.63
2.37	2.35
2.31	2.33
	2.29
	2.26
2.12	2.06
	1.93
	1.81
1.77	1.77
1.68	1.70
1.59	
1.50	1.49
1.44	
1.42	1.41

Table 6.25  $d$  values for reference Tin Oxide, *cassiterite* (JCPDS, 1986: 191), and corrosion product from a bronze specimen submerged for 16 weeks on the Alum Bay wreck site.

### 6.6.3 Lead

The  $d$  values for a control sample of lead were determined to eliminate these values from the corrosion product values (Table 6.26).

Three discernible corrosion products were seen on the 52 week control specimens submerged in deionised water. The first, adjacent to the metal surface, was silver and its  $d$  values compared with those of the Lead Carbonate, *cerrusite*,  $\text{PbCO}_3$  (Table 6.27). The second and third corrosion products, which were both patchily overlaying the first, were red and green respectively and plate-like in appearance (Figure 6.73). The  $d$  values of both of these products compared with the Lead Oxides,  $\text{PbO}$ , *litharge* (red, Table 6.28) and *massicot* (green, Table 6.29). No corrosion products were seen on the control specimens submerged in artificial seawater.

Analysis was carried out on a specimen submerged for 16 weeks on the Alum Bay wreck site. The surface was covered with a fine grained black corrosion product, the  $d$  values of which compared with data for Lead Sulphide *galena*,  $\text{PbS}$  (Table 6.30). Two discernible corrosion products were seen on the specimen submerged on the Duart Point wreck site. The first, adjacent to the metal surface, was a fine white powder and its  $d$  values compared with data for the Lead Chloride Hydroxide *laurionite*,  $\text{PbClOH}$  (Table 6.31). This was overlain by a fine grained black powder, the  $d$  values for which compared

with data for *galena* (Table 6.30). No corrosion products were seen on the specimens submerged in the Albert Dock.

#### 6.6.3.1 *d* values for lead corrosion products from lead specimens

The *d* values for the aforementioned corrosion products from the lead specimens are summarised in Tables 6.20-6.25.

Reference Lead	Control specimen of lead
2.86	2.83
2.48	2.47
1.75	1.94
1.49	1.53
1.43	1.47
1.24	
1.14	
1.11	1.10
1.01	
0.95	

Table 6.26 *d* values for reference (JCPDS, 1986: 652), and control lead.

Reference Lead Carbonate ( <i>cerrusite</i> )	Corrosion product from lead specimen from deionised water
4.43	
4.26	
3.59	3.56
3.50	3.45
3.07	2.96
2.89	2.74
2.52	
2.49	2.50
2.21	2.31
2.13	2.14
2.08	
2.01	
1.98	1.96
1.93	
1.86	1.86
1.80	
1.75	1.75
1.69	1.68
1.64	
1.59	1.59
1.50	1.52
1.48	1.48
1.45	1.44
1.43	1.43
1.33	1.37

Table 6.27 *d* values for reference Lead Carbonate, *cerrusite* (JCPDS, 1986: 198), and corrosion product from lead specimen in deionised water.

Reference Lead Oxide (litharge)	Corrosion product from lead specimen from deionised water
5.02	4.94
3.12	3.10
2.81	2.79
	2.61
2.51	2.50
2.12	
1.99	1.99
	1.95
	1.93
1.87	1.80
	1.71
1.68	1.67
	1.63
1.56	1.54
1.54	
	1.47
1.44	1.44
1.41	1.40

Table 6.28 *d* values for reference Lead Oxide, *litharge* (JCPDS, 1986: 671), and corrosion product from lead specimen in deionised water.

Reference Lead Oxide (massicot)	Corrosion product from lead specimen from deionised water
5.89	5.88
	5.03
3.07	3.10
2.95	2.95
	2.84
2.74	2.72
	2.53
2.38	2.38
2.01	2.02
1.96	1.96
1.85	1.86
1.80	1.80
1.72	1.73
	1.68
1.64	1.63
	1.59
1.53	1.55
1.51	1.52
1.47	1.48
	1.41

Table 6.29 *d* values for reference Lead Oxide, *massicot* (JCPDS, 1986: 407), and corrosion product from lead specimen in deionised water.

Reference Lead Sulphide (galena)	Corrosion product from specimen from Alum Bay	Corrosion product from specimen from Duart Point
3.57	3.43	3.43
2.94	2.97	2.97
2.47		
2.23		
2.09	2.10	2.10
1.94		
1.86		
1.75	1.79	1.79
1.65	1.71	1.71
1.50	1.48	1.48
1.45		
1.43	1.37	1.37

Table 6.30 *d* values for reference Lead Sulphide, *galena* (JCPDS, 1986: 407), and corrosion products from lead specimen on the Alum Bay and Duart Point wreck sites.

Reference Lead Chloride Hydroxide (laurionite)	Corrosion product from lead specimen from Duart Point
5.7	
4.88	
4.01	4.07
3.71	
3.56	3.56
3.33	
3.30	3.30
	3.17
2.93	
2.87	
2.84	2.84
2.66	
2.57	2.60
2.51	2.47
2.43	2.46
2.39	
2.30	2.23
2.06	
2.00	1.96
1.88	
1.79	1.80
1.75	1.75
1.69	1.66

Table 6.31 *d* values for reference Lead Chloride Hydroxide, *laurionite* (JCPDS, 1986: 646), and corrosion product from lead specimen submerged for 52 weeks on the Duart Point wreck site.

### 6.7 Conclusion

The results show that the two categories of material, organic and metal, underwent deterioration by two different mechanisms. Although the control laboratory experiments did not strictly serve as control (in the sense that they were not sterile and were themselves susceptible to biodeterioration) they showed that the organic materials under the experimental conditions on the field sites were colonised and deteriorated by micro, macro and mega-organisms. Under such circumstances, chemical deterioration was of secondary importance in governing the rate of deterioration. Interestingly, not only did the results show that biodeterioration was the main mechanism for deterioration, but that the rates of deterioration were governed by the seasonal production of the many organisms involved.

The metals, were not greatly affected by micro and macro organisms and, apart from the lead specimens from the Duart Point wreck site, nor were they affected by macro organisms. The mechanism of deterioration in the case of the metals was mainly electrochemical corrosion, the rates of which seem to have been affected by the initial physical and chemical parameters of the sites.

The following chapter interprets the mechanisms of deterioration and corrosion which were indicated by these results.

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## CHAPTER SEVEN

### INTERPRETATION OF RESULTS

#### 7.1 Introduction

The results indicated that the organic materials and metals predominantly underwent biodeterioration and corrosion respectively. This chapter interprets the results in terms of these mechanisms, and considers the two broad groups of materials in separate sections.

#### 7.2 Organic materials

##### 7.2.1 Introduction

As the Figures of the organic materials in Section 6.4 show, biological organisms were the major agents causing the deterioration of the organic materials. The control specimens showed that for the duration of this experiment, water and salts did not affect the materials as much as the various organisms present in the marine environment.

The succession of events that occurs during the biocolonisation of materials is well documented: Floodgate (1971), Gareth Jones *et al.* (1976), Edyvean *et al.* (1985), Cundell and Mitchell (1977), Zachary *et al.* (1982). Although the majority of their research is concerned with the economic implications of biodeterioration and does not consider the colonisation of all the materials under study in this research the succession of biocolonisation does follow a definite order. It is initiated by the attachment of bacteria to the surface, followed by other micro-organisms including diatoms, fungi, micro algae and protozoa and boring crustacea and mollusca. Bacteria and fungi produce extracellular enzymes which destroy the material on which they grow, while the crustaceans and mollusca bore into the materials which they ingest and may subsequently utilise. Secondly, there are fouling organisms, such as algae, polysoa, tunicata, and mollusca which use the materials as a substrate to grow upon (Gareth Jones *et al.*, 1976: 120). Although the organic materials did not all show this full succession of colonisation, they all showed some element of colonisation, and subsequent deterioration, by fouling organisms.

The results indicate the importance of seasonal production and distribution of the micro-organisms which cause the primary colonisation and, in certain cases, secondary fouling of the materials. This seasonal production affected the initial rates of deterioration depending on when the sample trays were placed on the different field sites. Rheinheimer (1992: 96) states that in temperate climate zones the sea shows seasonal fluctuations in microbial numbers. Saprophyte numbers in the western Baltic showed two maxima (Figure 7.1): one in spring (April/May), the other in autumn (October/November). Due to time constraints it was not possible to generate similar data for the sites in the field study. Neither was it possible to obtain data from the literature or oceanographic institutes around the areas under study. However, under the supposition that Rheinheimer's research holds true for the sites under study, it will be shown that seasonal production of micro-organisms

affected the colonisation of the organic materials. In addition, the results indicate that the environmental parameters measured on each of the sites were important in terms of their effect on the ecology of the colonising organisms.

#### 7.2.1.1 Wood

The weight change of the wood specimens proved the most useful method of determining the rate and extent of deterioration. Determination of the levels of extractables, celluloses and lignin only showed the state of the wood *left behind* and thus served as a poor method of determining the overall rate and extent of deterioration.

The laboratory control specimens submerged in deionised water and artificial seawater became contaminated with micro-organisms from the laboratory atmosphere, but showed minimal deterioration by them, indicating that the action of water and salt alone did not seriously degrade the wood over the period of this experiment. This was supported by the minimal deterioration of control specimens submerged in sterile conditions as part of the *in vitro* microbiological experiments. The important factor was the presence of macrobiological organisms.

As noted in the introduction to this section, bacteria and fungi are the primary colonisers of materials submerged in aquatic environments and cause their initial deterioration. Bacteria were cultured from specimens submerged for 32 weeks on the field sites. However, none were found to cause deterioration. This does not preclude the fact that the methods used may not have been suitable to isolate those bacteria responsible for deterioration. The mechanisms of bacterial degradation of wood have been described by Kohlmeyer (1969: 20-30), Kirk and Farrell (1987: 465-505) and Coughlan and Ljungdhal (1988: 11-17). Figure 7.2 shows the structure of the wood cell wall. Essentially, deterioration of the wood cells begins at the lumen or along the rays with the bacteria attacking the cell wall. The tertiary wall (S3) is penetrated, the secondary wall (S2), which contains mainly cellulose, swells and then disintegrates as the cellulolytic bacteria create cavities in the wall. )

The first indication of an organism which caused deterioration was the presence of the marine fungus *Ceriosporopsis halima*, which was observed on specimens from all field sites. Wood submerged in the marine environment is attacked by fungi, whether marine or terrestrial, which in most cases causes "soft-rot". The wood loses mechanical strength and becomes wet and spongy. Such rot is caused by Ascomycotina and Deuteromycotina (fungi imperfecti) and involves the utilisation of the cellulosic content of the wood (Dix and Webster, 1995: 145). Fungal hyphae grow in the lumen of the wood cells and these penetrate to the highly lignified S2 layer of the secondary cell wall where, using extracellular enzymes, they disintegrate the cellulosic content (Dix and Webster, 1995: 161-163). Appearance of *Ceriosporopsis halima* on the specimens was observed after the following lengths of submergence: Alum Bay between eight and 16 weeks, Duart Point

after 12 weeks and in the Albert Dock after eight weeks. Considering these observations in the light of Rheinheimer's research, the Albert Dock specimens were placed on the site at a time when it can be postulated that saprophytic productivity was reaching the autumnal maximum whereas the Alum Bay and Duart Point samples were sited in the summer when saprophytic productivity was low. The Albert Dock samples were therefore submerged for a shorter period of time before colonisation. However, these observations do not explain the weight changes recorded as fungi and bacteria were not the only organisms causing deterioration. Weight loss was recorded between eight and 16 weeks for the Alum Bay specimens and after eight weeks on the Duart Point specimens. Only slight weight loss was recorded between eight and 12 weeks on the Albert Dock specimens.

The weight loss between eight and 12 weeks at both the Duart Point wreck site and in the Albert Dock could have been due to bacteria. After 16 weeks the Duart Point specimens were colonised with *Limnoria tripunctata* and their presence greatly affected not only the rate but the extent of deterioration. No macro-organisms were seen on the Albert Dock specimens and deterioration was through the action of bacteria and fungi. By 16 weeks the extent of weight loss for the Alum Bay specimens was similar to that of the Duart Point specimens. The rate of weight loss was slower and no *Limnoria* were observed to be colonising the specimens. It is unclear why they were apparent on the Duart Point specimens and not the Alum Bay specimens as, environmentally, the seawater conditions at the two sites were very similar and attack by *Limnoria* spp on the timbers of the Alum Bay shipwreck was evident. The absence of *Limnoria* spp on the Albert Dock specimens throughout the experiment was probably due to the water conditions over the site. Becker (1971: 310), discusses the ecology of *limnoria* and, based on the environmental data available for the sites, the pH and dissolved oxygen content are unlikely to be the cause of the differences. However, the salinity of the sites may be a factor.

Becker (1971: 314) notes that the optimum salinity for *Limnoria tripunctata* was 25-35‰. The average salinity for the Alum Bay and Duart Point sites was, on average, 33.6‰ and 33.23‰ respectively but only 26.33‰ was recorded for the Albert Dock. Although this is within the optimum range for survival it may have been too marginal and may have affected the ability of the organism to survive.

In addition to the marine fungi, the terrestrial soil fungi *Penicillium expansum* and *Penicillium citrinum* were isolated from specimens submerged for 32 weeks on the Duart Point wreck site and in the Albert Dock respectively and were found to cause deterioration under laboratory conditions. The results of the cellulose analysis reflect the deterioration through bacteria, fungi and chemical dissolution (which may in turn have been affected by pollution and other nutrients). Considering the holocellulose, alphacellulose and hemicellulose results of the wood analyses, the rate and extent of deterioration of those wood specimens submerged in the Albert Dock was the greatest. As was noted at the beginning of this section, these analyses did not show the true rate of deterioration as

different suites of organisms were operating on each of the sites. The predominant organisms on the Albert Dock were fungi. Qualitatively assessing the extent of colonisation, the Albert dock specimens showed more perithecia and ascospores on the surface of the wood than did specimens from the other sites. This may explain why the cellulosic content of the Albert Dock specimens was less than for specimens from the other sites. However, the hemicellulose analyses do not directly reflect the holo and alphacellulose trends and the levels of hemicellulose in the Albert Dock specimens was higher than for the other field sites.

Lignin is relatively resistant to bacterial deterioration (Kirk and Farrell, 1987: 469). Lignin coats the cell wall polysaccharides and chemically combines with them to form lignocellulose, a substance very resistant to microbial degradation (Dix and Webster, 1995: 145). However, as Kirk and Farrell (1987: 470-472) and Kirk *et al.* (1978:235) note, soft-rot wood decay caused by various ascomycetes and fungi imperfecti involves lignin degradation, although wood polysaccharides tend to be preferentially degraded. White-rot basidiomycetes degrade lignin more rapidly and extremely than any other studied microbial group. They invade the lumens of wood cells, where they secrete enzymes that degrade the wood components.

This was reflected in the lignin analysis with some initial loss recorded from specimens from all field sites. However, after eight weeks in the Duart Point, and 12 weeks in the Albert Dock, an increase in the lignin content of the specimens was recorded. The 16 weeks Alum Bay specimens, which were the last samples to be analysed from the site, still showed a decrease in lignin. This phenomenon was thought to be due to the analytical method rather than the true lignin content of the specimens. This was a gravimetric method which determined the lignin content of the specimens after removal of cellulose. The problem with such a method is that if the level of cellulose in the specimen has been reduced by microbial degradation the sample weight taken for analysis will contain an artificially high level of lignin compared with specimens sampled earlier in the experiment.

No other organisms were observed on the specimens submerged in the Albert dock. The Alum Bay specimens showed colonisation by barnacles after four weeks. The specimens from Duart Point showed colonisation by a whole host of macro and mega fouling organisms such as barnacles, kelp, and calcareous tubeworms. Again it is argued that these were seasonally and environmentally induced. The barnacles (*Balanus crenatus*) have a complex settling and shell formation cycle dependant on geographical distribution, salinity, temperature, light, water current and nutrient concentrations. The breeding habit of the species is such that eggs are deposited in the mantle cavity and fertilised in November; the eggs develop *in situ* and hatch and settle in early spring (Houghton, 1971: 197-215).

Seaweeds, such as kelp, show definite seasonal cycles in growth and reproduction due to light intensity, temperature and less often nutrient concentration. Growth rate usually

begins to increase in late winter or early spring and declines in the summer or autumn (Darley, 1982: 191).

#### 7.2.1.2 Rope

The weight change and tensile strength methods used to determine the rates and extent of deterioration yielded similar results. The laboratory control specimens showed that those specimens submerged in deionised water deteriorated to a greater extent than those submerged in artificial seawater. Although these control specimens were somewhat contaminated by micro-organisms from the laboratory atmosphere, as with the wood specimens, the results showed that deterioration was not greatly affected by water and salts. This was further supported by the minimal deterioration of control specimens submerged in sterile conditions as part of the *in vitro* microbiological experiments. Thus deterioration was mainly through the action of organisms present in the marine environment.

As was noted in Section 7.2.1.1 discussing wood, the primary colonisers were likely to be bacteria. However, by the methods used, those bacteria isolated from the 32 week specimens from each site were not found to cause deterioration. The marine fungus, *Ceriosporopsis halima*, was isolated from all rope specimens: after 16 weeks on the Alum Bay (colonised between eight and 16 weeks), 12 weeks on the Duart Point specimens and eight weeks on the Albert Dock specimens. In addition, the terrestrial fungus, *Trichoderma koningii*, was isolated from specimens submerged for 32 weeks in the Albert Dock and was found to cause deterioration under laboratory conditions. These colonisation times corresponded with those seen on the wood specimens. In addition to the fungus, *Limnoria tripunctata* were observed on the Duart Point specimens after 52 weeks. What is interesting here is that the Albert Dock specimens were degraded far more quickly, and to a greater extent, by the action of micro-organisms alone than were the Duart Point specimens which were colonised by both micro and macro-organisms. As with the wood specimens, when the extent of colonisation of the 16 weeks submerged specimens was qualitatively compared, the specimens from the Albert Dock were far more extensively colonised by *Ceriosporopsis halima* than were specimens from the other sites. As for the wood, the cause of the different rates of deterioration recorded on the sites was due to the seasonal production of the microbes.

The chemical structure of sisal rope is similar to wood in that it is mainly cellulosic with some lignin. The distribution of these polymers in the cell walls (Figure 7.3) is similar to that for wood with the cellulose and hemicellulose in the primary and secondary walls. Some lignin is deposited in the primary wall but the majority occurs in the secondary wall. Observation made by scanning electron microscope revealed helical strips separating from the main visceral bundle sheath (Figure 7.4). The manufacture of sisal rope is discussed by Florian *et al.* (1990: 123). The leaf fibres from which it is extracted comprise vascular

bundles (xylem, phloem, sclerenchyma fibres) and fibre bundles (sclerenchyma fibres). As the fibres grow in discrete fibre bundles, the leaves are processed to extract the whole bundle, which is a compact complex of many cells. Further processing removes vascular bundles, leaving compacted bundles of sclerenchyma fibre cells. Even after processing there are remnants of the vascular cells. These are the protoxylem cells with spiral thickenings (Florian *et al.* 1990: 48). These spiral thickenings are helical shaped and account for the helical strips observed separating from the visceral bundle sheath. The inside of the primary wall is covered with the helical secondary thickenings. That these had separated from cell wall indicates that the secondary wall (S2), containing the majority of the celluloses and hemicelluloses, had been degraded. Figure 7.5 shows a specimen from artificial seawater where deterioration was limited. Figure 7.6 shows a specimen from the Albert Dock which exhibits a series of ridges and furrows. The ridges indicate the remains of the cell wall and the furrows indicate where the secondary cell wall once was. The fungus *Ceriosporopsis halima* has known cellulolytic activity (Gareth Jones, 1971b: 252-255). Based on the fact the specimens submerged in artificial seawater showed very little/no helical structures or series of ridges and furrows, and combined with the results in the loss of tensile strength of the specimens, it is assumed that micro-organisms were the main cause of deterioration.

#### 7.2.1.3 Canvas

The weight change and tensile strength methods showed similar results overall.

The laboratory control specimens showed little deterioration and, as with the results for the wood and rope specimens, indicate that deterioration was not greatly affected by the presence of water and salt. This was supported by the work of Doree (1920: 709-714) who, in his study of the deterioration of canvas in seawater, concluded that destruction was due to micro-organisms and not oxygen, light or salts present. However, those control specimens in deionised water were greatly colonised and discoloured by the terrestrial fungus *Cladosporium cladosporoides* although significant deterioration was not recorded. In addition a bacterium of the genus *Nocardia* was identified to cause deterioration.

Of the field experiments, the Albert Dock specimens were the most rapidly deteriorated (lost after 12 weeks), followed by Duart Point (16 weeks), with the Alum Bay specimens showing extensive deterioration after 16 weeks submersion.

However, the rates recorded by the two analytical methods were different. Deterioration by weight change showed that the control specimens were only slightly deteriorated throughout the experiment. The specimens from the Alum Bay and Duart Point wreck sites did not show deterioration until after eight and four weeks respectively. The specimens from the Albert Dock showed exponential deterioration upon submersion.

However, the tensile strength method was more indicative of the mechanism and rate of deterioration. As the canvas was made of cotton, which consists of more than 90% cellulose (Florian, 1987: 30), it was extremely susceptible to microbial decay. Raschle (1989: 238) notes that microbial enzymes expedite the hydrolytic cleavage of the links between the glucose monomers which constitute the cellulose polymer. The result is a shortening of the polymeric chain length which leads to a decrease in tensile strength. Thus the cellulolytic activity can best be determined through measurement of this parameter. The tensile strength results showed that the control specimens deteriorated up to 16 weeks submersion and thereupon deterioration virtually stopped. The Alum Bay specimens lost over 50% of their tensile strength within four weeks, whereas the Duart Point specimens took eight weeks to reach the same level of deterioration. The Albert Dock specimens had lost over 80% of their tensile strength after just four weeks submersion. Bacteria of the genus *Cellulomonas* were cultured from the Duart Point and Albert Dock specimens which, under laboratory conditions, were found to cause deterioration. Cellulomonads have the enzyme cellulase which is capable of breaking down cellulose (Stackerbrandt and Prauser, 1992: 1328). However, these microbes did not have the same effect of total destruction as was observed in the field experiments. Thus it may be either that different suites of microbes were operating on the different field sites, and the cellulomonads isolated were not responsible for causing the rapid deterioration seen in the field samples, or that they did not exhibit the same type of deterioration under laboratory conditions. Scanning electron microscopy of the deteriorated specimens did not reveal any obvious fungal or bacterial organisms (interestingly, *Ceriosporopsis halima* was not observed on any specimens) which may have caused deterioration.

Having established that micro-organisms were the main cause of deterioration the different rates, based on the tensile strength results, were, as with the wood and rope, due to variations in the seasonal production of micro-organisms.

#### 7.2.1.4 Leather

Of all the organic test materials the leather specimens exhibited the least structural deterioration. The reason that very little deterioration was seen was probably because of the tanning process which effectively cross-links the collagen molecules present, rendering the leather extremely resistant to biodeterioration (Atlas and Bartha, 1993: 352). However, the collagen is not fully cross-linked and a small portion remains open to attack. The weight change method did not really show the extent or mechanism of deterioration. The analysis of total nitrogen content of the specimens not did not reveal the true rate or extent of deterioration but instead recorded deterioration of the collagen which had not cross-linked during the tanning process. As this does not constitute much of the leather, it would not be greatly affected, hence little change was seen.

Considering weight change, the field specimens showed an initial weight increase after four weeks submersion. This was attributed to the uptake of salt from the seawater. However, the rates and extent of this weight increase were not reflected in the seawater control specimens which did not show a comparable weight increase even after 12 weeks submersion. It is hard to determine what the mechanism of deterioration was for the remaining results as they are quite different for each site. Alum Bay and Albert Dock showed an overall weight loss up to 16 weeks submersion. However, the Albert Dock specimens from 32 to 52 weeks show no deterioration. The Duart Point specimens showed no weight change after the initial weight increase up to 32 weeks, whereupon there was a rapid increase in weight due to colonisation by fouling organisms which could not be totally removed before determining the weight change.

Although the weight change did not conclusively reflect the deterioration of the specimens, observation of the Duart Point specimens indicate that these were the most highly affected specimens. Deterioration was not revealed by weight change, but the fouling organisms would affect the long term stability of the leather, if not in terms of the chemical and physical structure, then through disruption due to the dynamic nature of the marine environment.

The total nitrogen content did reveal a little more in terms of deterioration. As with the bone specimens there was a definite seasonal trend. Minimum nitrogen contents were recorded in spring and maximum contents were in winter. As with the bone it was thought these fluctuations may have been due to nitrifying, denitrifying and nitrogen fixing bacteria. Species of the genus *Bacillus* were isolated from the Duart Point specimens. Singleton and Sainsbury (1987: 492) note that, in relative humidities greater than 80%, *Bacillus sphaericus* had been found to cause deterioration of leather. Certain *Bacillus* species fix nitrogen under anaerobic conditions and as with the bones this may have been the case after 32 weeks submersion when the specimens were covered with sediment.

The cyclic series of nitrification and denitrification, leading to nitrogen loss, followed by nitrogen fixation may have affected the total nitrogen content of the specimens. If the total nitrogen contents were reflecting the changes in the collagen the mechanism for the deterioration was due to the molecular structure of the collagen (Haines, 1981: 137). The conformation of the collagen molecule makes it highly resistant to enzymes but at the non-helical end of the molecule, the telopeptide, the amino acid sequence is different from the main helical portion and is more vulnerable to enzyme attack. The intra and inter molecular bonds are sited in the telopeptide region and if these bonds are removed from the molecule as a result of enzyme action, the collagen fibres swell, to a greater extent in acid or alkaline conditions, which results in the breakdown of the leather.

#### 7.2.1.5 Bone

The analytical methods of weight change, nitrogen content and calcium:phosphate ratio, showed differences in deterioration of the bone specimens from the laboratory and field experiments. The weight change reflected the rate and extent of deterioration but did not provide a conclusive understanding of the mechanism. Deterioration by weight change showed that the control specimens in deionised water and artificial seawater were relatively undeteriorated. Alum Bay specimens showed exponential deterioration throughout the experiment and for the data available were the least deteriorated. The Duart Point and Albert Dock specimens showed weight increases up to eight weeks submersion and then exponential weight loss thereafter, the extent and rate of weight loss being greater in the specimens from Duart Point.

The methods of total nitrogen content and calcium:phosphate ratio of the bone specimens did not show an exponential change of the rate and extent of deterioration. However, the pattern of change yielded evidence for the possible mechanism of deterioration, when combined with microscopic observations of the bone surface and bacteria isolated from the field specimens. The specimens were extremely crumbly and showed exfoliation. By scanning electron microscopy the surfaces of the specimens appeared extremely pitted (Figures 7.7-7.8). Berg (1963: 237) discusses the deterioration of bones in terrestrial soils and notes that such observations are the result of both the decomposition of connective tissue and the removal of inorganic salts. As with the other organic materials, it was the presence of micro-organisms which was the key factor in the deterioration of the bone specimens.

Nitrogen analysis showed the effect of deterioration on the connective collagen tissues, and the calcium:phosphate ratio the effect of deterioration of the inorganic salts. The fluctuations in the nitrogen content recorded in the bone specimens from the field sites were thought to be due to the two processes of nitrification/denitrification and nitrogen fixation. A loss in the nitrogen content of the specimens was due to micro-organisms with proteolytic enzymes capable of decomposing the organic nitrogen elements of the bone (such as collagen) and liberating ammonia. The ammonia was subsequently oxidised to nitrate via nitrite in a two step process called nitrification. First the ammonia is oxidised to nitrite and the nitrite is further oxidised to nitrate. Nitrates are highly soluble and therefore easily leached from the specimens leading to a decrease in the total nitrogen content. In addition nitric acid is produced as part of this process, and this would have solubilised inorganic salts.

Subsequently, denitrification may have also taken place. Many aerobic bacteria can use nitrate as a final electron acceptor in place of oxygen if conditions are anaerobic. As a consequence, nitrate is reduced. Some bacteria are able to mediate two subsequent anaerobic respirations by which nitrite ion is reduced to nitrous oxide gas ( $N_2O$ ) and

subsequently nitrogen gas ( $N_2$ ). By this process (denitrification) combined nitrogen may have also been removed from the specimens in the form of nitrogen gas.

The increase in nitrogen content of the specimens may be explained by the converse process of nitrogen fixation in which nitrogen is reduced to form ammonia which micro-organisms utilise in their respiration. In terms of the micro-organisms which were isolated from the field specimens a pseudomonad (*P.aeruginosa*) was isolated from a bone specimen in the Albert Dock. Two species of the genus *Vibrio* were isolated from the Duart Point specimens. *P. aeruginosa* has been found to cause denitrification (Zumft, 1992: 554). No bacteria were isolated which may have been responsible for nitrogen fixation. It must be stated that the culture medium used was not ideal for their isolation. However, the reason may also have been that, as the specimens were covered with sediment between 16 and 32 weeks submersion, there was a period of nitrogen loss and thus nitrogen fixing bacteria were not present.

In terms of the specimens from the Duart Point wreck site, nitrogen fixing bacteria, *Vibrio* spp, were isolated from the 32 weeks submerged specimens. Nitrogen fixation under anaerobic conditions, or environments of very low dissolved oxygen contents, by facultative anaerobes is restricted to some *Bacillus* and *Vibrio* species (Eady, 1992: 536). Two different species of the genus *Vibrio* were isolated from the specimens submerged for 32 weeks. The nitrogen content of the Duart Point specimens had increased after 32 weeks; the *Vibrio* species thriving due to the anaerobic environment which may have been created when the specimens were covered between 16 and 32 weeks.

As with the nitrogen content, the calcium:phosphate ratio of the bone specimens from the field sites fluctuated throughout the experiment. The control specimens showed slight fluctuations in the ratio throughout the experiment, indicating that water and salt were not the main cause of deterioration. The fluctuations seen in the field specimens were thought to have been due to the effect of the aforementioned nitrifying/denitrifying and nitrogen-fixing micro-organisms. A decrease in the calcium:phosphate ratio was due to the solubilisation of the inorganic bone mineral caused by nitrifying bacteria. During the phase of nitrification, acids are produced (Stanier *et al.*, 1987: 549) and the solubility of calcium is increased at the resulting lower pH. Conversely an increase in the calcium:phosphate ratio is due to the effect of denitrification. Stanier *et al.* (1987: 549) notes that denitrification increases the pH of the environment; alkaline pHs favour the precipitation of calcium carbonate in the marine environment. Thus the calcium:phosphate ratio would seem to be higher. It may be that fluctuations in the calcium:phosphate ratio were as a result of seasonal trends. Maximum calcium:phosphate ratios were seen in the specimens from Duart Point and Albert Dock in winter and in summer whereas minimum ratios were recorded in spring. Again such trends follow Rheinheimer's seasonal saprophyte production results; these results indicating that the solubilisation and precipitation of

calcium salts in the bone may be governed by the production of nitrifying and denitrifying bacteria.

### 7.3 Metals

#### 7.3.1 Introduction

Only those corrosion mechanisms which were apparent on the metals in the experiment will be discussed. These were mainly electrochemical. However, there was some evidence to suggest that biologically-induced corrosion was apparent and was brought about by the effects of micro and macro-organisms.

These mechanisms manifested themselves in the form of localised pits on the surface of the metals and, in the case of the microbiologically-induced corrosion, were associated with overlying tubercles.

Before considering the interpretation of the results two fundamental aspects need to be discussed. First, the basic theory of electrochemical corrosion and in particular pitting corrosion. Second, the use of Pourbaix Diagrams to interpret electrochemical corrosion mechanisms.

#### 7.3.2 Electrochemical corrosion and the formation of localised pits

When a metal is placed in an aqueous environment, such as seawater, it undergoes electrochemical corrosion. This process is considered by Evans (1981: 32-61), North and MacLeod (1987: 69 - 73), Robinson (1982: 221-231) and Uhlig (1963: 6-16). A synopsis of their work is presented here.

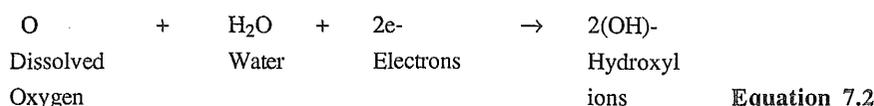
Electrochemical corrosion involves the flow of electrons (which carry a negative electrical charge), from one location to another. These electrons emanate from the atoms of the metal itself. They are released from the metal and enter the system at the anode and leave it by combining with other substances at the cathode; the complete system is called a corrosion cell and is summarised in Figure 7.9.

The reactions which take place at the anode and cathode of a corrosion cell are essentially the same for all metals. At the anode electrons are released by the metal and give rise to an oxidation reaction, by chemical definition meaning the loss of electrons, to form an electrically charged atom, an ion. Metal ions carry a positive electrical charge and are called cations, for example

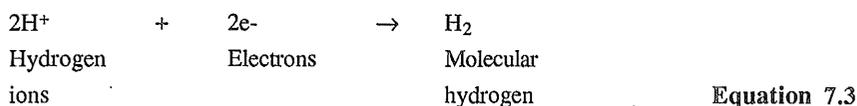


Equation 7.1

The complement of this reaction takes place at the cathode and is termed reduction, or the gaining of electrons. Electrons become involved in one of two reactions according to whether oxygen is present or not. When atmospheric oxygen is present electrons combine with water and dissolved oxygen to form hydroxyl ions



When oxygen is not present hydrogen ions (a constituent of water) act as electron acceptors and they are reduced to molecular hydrogen



This hydrogen may disperse, although it sometimes accumulates at the cathode. If sufficient of it accumulates an electrochemical force is exerted, so as to stay electrically neutral, and there is an apparent increase in resistance at the cathode and the corrosion cell becomes polarised. Although polarisation by the accumulation of hydrogen is theoretically possible, it rarely occurs due to the intervention of anaerobic sulphate reducing bacteria. These bacteria upset the equilibrium, either directly by removing hydrogen from the cathode, or indirectly by releasing sulphides which can be powerful stimulators of anodic reactions. Polarisation cannot occur when oxygen is present because hydroxyl ions (OH<sup>-</sup>) are formed instead of hydrogen. These do not exert an electrochemical counterforce, as they are removed as FeO(OH).

In addition to this general movement of ions there must be a specific movement of reactants and products to and from the reactive areas if corrosion is to continue. The metal ions can be removed by diffusion into the surrounding solution by formation of insoluble precipitates, or by combination with other species to form complex ions. The course followed will depend on the metal and the solution composition. If a precipitate is formed (corrosion product), this may coat over the reactive sites and so reduce the corrosion rate.

There are many forms of corrosion. Borenstein (1994: 122) lists eight classifications: general or uniform, pitting, galvanic, cracking phenomena, velocity effects, interangular, dealloying and high temperature corrosion. Of these, pitting was the most prominent on the metals which corroded in this experiment.

Pitting is a localised type of attack, the rate of corrosion being greater at some areas than at others. Pitting generally occurs when the stability of a passivating film (such as an oxide) is reduced. In media such as seawater the chloride ions present penetrate and destroy the passivating film, thus decreasing its stability.

After a pit is initiated, it may grow or repassivate. In an aerated, neutral solution, the initial oxidation and reduction reactions are as for Equations 7.1 and 7.2:

When a pit is initiated the condition illustrated in Figure 7.9 occurs. Initially, reactions 7.1 and 7.2 occur uniformly over the whole surface. During the initial pit formation, possibly due to a weak spot in the passive film, the oxygen within the pit is depleted by the reduction reaction. The restricted conditions in the pit do not allow replenishment of the oxygen. On the unpitted areas, the rate of corrosion remains the same as the passive current. However, to support the oxidation reaction within the pit, the rate of oxygen reduction increases on the passive surface and within the pit, an excess of metal ions ( $M^+$ ) is produced which is balanced by chloride ions, as shown in Figure 7.10. The resulting reaction is:



The result is a high concentration of chloride ions in the pit and a correspondingly lower pH in comparison with the bulk solution. These effects further accelerate corrosion within the pit.

Although ultimately it is an electrochemically governed phenomenon pitting can be affected by micro-organisms (Borenstein, 1994: 22). If pitting has been instigated by micro-organisms it is usually associated with tubercles. In these situations pitting results from concentration cells set up by tubercles. The influences of chemical reactions are also a factor in forming tubercles. Influences such as oxygen depletion, concentration of chlorides and reduction of sulphates increase the susceptibility of specific metals to corrosion, and these influences, combined with microbial activity, can enhance microbially induced corrosion.

Borenstein (1994: 31) notes that aerobic bacteria such as *Gallionella* and *Leptothrix* are generally thought to contribute to corrosion by forming differential aeration cells. Aerobic corrosion occurs when the oxide film is damaged or oxygen is kept from the surface by the micro-organisms. A tubercle forms and corrosion occurs underneath this. As oxygen-starved conditions develop, corrosion occurs underneath. In addition, the micro-organisms formed on the surface of the metal seem to concentrate chlorides and manganese ions in their metabolic process. *Gallionella* and *Leptothrix* are suspected (Borenstein, 1994: 27) of converting the soluble ferrous ion to the less soluble ferric ion, a chemical reaction that results in ferric hydroxide. The result of these reactions is thought to encourage the growth of the micro-organisms and provide an environment for other organisms. Aerobic organisms form and grow on the water side of the tubercle, and anaerobic organisms do so on the metal surface. The tubercle protects the colony of organisms. *Pseudomonas* spp is an aerobic slime-former and often forms thin films combined with corrosion deposits on metal surfaces. Figure 7.11 shows how pits can grow under tubercles. Pits grow as ferrous ions collect at the base of the pit and chloride ions concentrate (to stay electrically neutral). Pit growth is a two step process: initiation and propagation. The microbes form a

film and an oxygen concentration cell initiates a pit. Localised corrosion drives the pit to propagate deeper. The pit growth is unaffected by the presence of microbes.

For iron bacteria on stainless steel, the deposits are typically brown or red-brown mounds. These pits may often be low and cone shaped; the iron oxidising bacteria oxidise the ferrous ion to the insoluble ferric state; the resulting deposits create a mound.

Corrosion reactions are essentially oxidation and reduction reactions, regardless of whether they are electrochemically or microbiologically induced, and like all reactions involving electrons the tendency for these reactions to occur is expressed by their reduction potential. These reactions are always tabulated as reduction reactions, that is:

Reactants + Electrons  $\rightarrow$  Products

Reduction potentials are measured experimentally by placing two separate electrodes in solution. The oxidation reaction occurs at one electrode and the reduction reaction at the other. The voltage between the electrodes, when no current is flowing, is equal to the difference in reduction potentials of the oxidation and reduction reactions. The reduction potentials of all reactions depend on the activities of the species taking part in the reaction. If all of these activities are equal to unity, then the potential is called the *standard reduction potential* ( $E^0$ ). This is the potential which normally appears in tables such as Table 7.1.

Volts versus hydrogen at 25°C	Volts
$\text{Au(OH)}_3 + 3\text{H}^+ + 3\text{e}^- \rightarrow \text{Au} + 3\text{H}_2\text{O}$	1.45
$\text{O}_2 + 4\text{H}^+ + 4\text{e}^- \rightarrow 2\text{H}_2\text{O}$	1.229
$\text{AuCl}_4^- + 3\text{e}^- \rightarrow \text{Au} + 4\text{Cl}^-$	0.994
$\text{Ag}^+ + \text{e}^- \rightarrow \text{Ag}$	0.800
$\text{Fe}^{3+} + \text{e}^- \rightarrow \text{Fe}^{2+}$	0.770
$\text{Ag}_2\text{O}_3 + \text{H}_2\text{O} + 2\text{e}^- \rightarrow 2\text{AgO} + 2\text{OH}^-$	0.74
$\text{Cu}^+ + \text{e}^- \rightarrow \text{Cu}$	0.522
$\text{H}_2\text{SO}_3 + 4\text{H}^+ + 4\text{e}^- \rightarrow \text{S} + 3\text{H}_2\text{O}$	0.45
$\text{O}_2 + 2\text{H}_2\text{O} + 4\text{e}^- \rightarrow 4\text{OH}^-$	0.401
$\text{Ag}_2\text{O} + \text{H}_2\text{O} + 2\text{e}^- \rightarrow 2\text{Ag} + 2\text{OH}^-$	0.342
$\text{Cu}^{2+} + 2\text{e}^- \rightarrow \text{Cu}$	0.340
$\text{AgCl} + \text{e}^- \rightarrow \text{Ag} + \text{Cl}^-$	0.2223
$\text{SO}_4^{2-} + 4\text{H}^+ + 2\text{e}^- \rightarrow \text{H}_2\text{SO}_3 + \text{H}_2\text{O}$	0.20
$\text{Cu}^{2+} + \text{e}^- \rightarrow \text{Cu}^+$	0.158
$\text{S} + 2\text{H}^+ + 2\text{e}^- \rightarrow \text{H}_2\text{S aq}$	0.141
$2\text{H}^+ + 2\text{e}^- \rightarrow \text{H}_2$	0.000
$\text{Ag}_2\text{S} + 2\text{H}^+ + 2\text{e}^- \rightarrow 2\text{Ag} + \text{H}_2\text{S}$	-0.037
$\text{Pb}^{2+} + 2\text{e}^- \rightarrow \text{Pb}$	-0.126
$\text{Sn}^{2+} + 2\text{e}^- \rightarrow \text{Sn}$	-0.136
$\text{Cu(OH)}_2 + 2\text{e}^- \rightarrow \text{Cu} + 2\text{OH}^-$	-0.224
$\text{PbCl}_2 + 2\text{e}^- \rightarrow \text{Pb} + 2\text{Cl}^-$	-0.262
$\text{PbSO}_4 + 2\text{e}^- \rightarrow \text{Pb} + \text{SO}_4^{2-}$	-0.356
$\text{Cu}_2\text{O} + \text{H}_2\text{O} + 2\text{e}^- \rightarrow 2\text{Cu} + 2\text{OH}^-$	-0.361
$\text{Fe}^{2+} + 2\text{e}^- \rightarrow \text{Fe}$	-0.409
$3\text{FeO(OH)} + \text{e}^- \rightarrow \text{Fe}_3\text{O}_4 + \text{H}_2\text{O} + \text{OH}^-$	-0.49
$\text{PbO} + \text{H}_2\text{O} + 2\text{e}^- \rightarrow \text{Pb} + 2\text{OH}^-$	-0.576
$\text{Ag}_2\text{S} + 2\text{e}^- \rightarrow 2\text{Ag} + \text{S}^{2-}$	-0.705
$\text{Zn}^{2+} + 2\text{e}^- \rightarrow \text{Zn}$	-0.763
$\text{Fe}_3\text{O}_4 + 4\text{H}_2\text{O} + 8\text{e}^- \rightarrow 3\text{Fe} + 8\text{OH}^-$	-0.91
$\text{SnO}_2 + 2\text{H}_2\text{O} + 4\text{e}^- \rightarrow \text{Sn} + 4\text{OH}^-$	-0.945
$\text{Al}_2\text{O}_3 \cdot 3\text{H}_2\text{O} + 6\text{e}^- \rightarrow 2\text{Al} + 6\text{OH}^-$	-2.33

Table 7.1 Standard reduction potentials of metals. After North and MacLeod, 1987: 70.

Metals are commonly described as active if they have a negative  $E^0$  value for their reductive reaction in a solution of the metal ions at an activity equal to 1.0. Such metals tend to corrode easily. Examples relevant to this study from Table 7.1 are iron ( $E^0 = -0.409$  volts), tin ( $E^0 = -0.136$  volts) and lead ( $E^0 = -0.126$  volts). Metals with positive  $E^0$ s are regarded as noble and they do not corrode readily; an example is copper ( $E^0 = +0.522$  volts).

However, the  $E^0$  value for the corrosion reaction only shows *if* a particular reaction can occur; it does not indicate the *rate* at which a reaction will occur. This is because when a piece of metal is placed in seawater, a large number of different oxidation and reduction reactions can occur on the metal surface. Consequently the metal requires a potential of all these reactions. This potential is thus a mixed electrode potential and in corrosion studies is referred to specifically as the *corrosion potential*,  $E_{\text{corr}}$ . All reactions occurring at the metal surface which have an  $E^0$  more positive than  $E_{\text{corr}}$  will proceed in the reduction direction (accept electrons from the metal) and so collectively form the cathodic reactions. Conversely all reactions with  $E^0$  more negative than  $E_{\text{corr}}$  will proceed in the oxidation direction (donate electrons to the metal) and collectively form the anodic reactions. For an inert electrode in the solution (such as platinum) the measured potential will depend solely on the reactions between species in the solution. The measured potential in this case is referred to as the *Eh* of the solution.

### 7.3.3 Pourbaix Diagrams

Pourbaix Diagrams are widely used in the study of the corrosion of metals (Pourbaix, 1974: 70). These are graphs with solution potential (*Eh*) plotted on the vertical axis and pH on the horizontal. All other possible factors are constant over the whole of the diagram. Pourbaix Diagrams are a chemical stability map showing which compound or ionic species is the thermodynamically stable species at particular *Eh* and pH. In Pourbaix Diagrams the overall composition of the solution is specified, such as pure water, seawater, seawater with  $10^{-3}$  molar total sulphide added and so forth. Each metal in each solution produces a different diagram and when studying metal corrosion in a specific solution it is generally necessary to construct a Pourbaix Diagram from known thermodynamic data.

Figure 7.12 shows the Pourbaix Diagram for iron at 25°C in pure water (Pourbaix, 1974: 312). The sloping broken lines in the diagram, a) and b), give the potentials of the solutions in equilibrium with oxygen and hydrogen respectively. If the potential of the system rises above line a), oxygen evolution will commence; if the potential of the system drops below line b), hydrogen evolution will occur. Of the other lines on the diagram each one represents a balanced reaction. A horizontal line represents an equilibrium involving electrons but not  $H^+$  or  $OH^-$  ions; a vertical line represents one involving  $H^+$  or  $OH^-$  ions but not electrons; a sloping line represents one involving  $H^+$  or  $OH^-$  ions and also

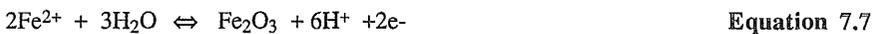
electrons. For example, in Figure 7.12 the family of horizontals marked 23 show the potentials of the electrode equilibria



at ferrous ion activities of  $10^0$ ,  $10^{-2}$ ,  $10^{-4}$ , and  $10^{-6}$  times normal according as to whether the number attached is 0, -2, -4 or -6. The vertical marked 20 represent the hydrolysis

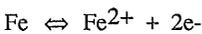


whilst the family of sloping curves marked 22 represent the equilibria deciding the possibility of "reductive dissolution" of solid ferric oxide to ferrous ions in the liquid



The curves on the diagram effectively delimit the conditions under which immunity, corrosion and passivation may reasonably be expected. The immune region is that region in which the metal itself is the most stable species, that is corrosion cannot occur. In this case, below one of the horizontals 23, corrosion is impossible once the liquid has come to contain  $\text{Fe}^{2+}$  ions at the concentration appropriate to the line selected; if a higher concentration of  $\text{Fe}^{2+}$  existed, metallic iron would be deposited; the area below the line can be regarded as the region of immunity.

Conversely, at potentials raised above the line the change the change as defined in Equation 7.5,



will take place and this is the region of corrosion. In this region the most stable species are either the metal ions in solution or non protective insoluble compounds and corrosion will occur and will continue until the metal is consumed.

Above the sloping lines 28 the formation of a solid corrosion product becomes possible but the formation of  $\text{Fe}^{2+}$  ions in the liquid also remains possible, so far as the energy considerations are concerned. However, as soon as a solid film has been produced, the entry of iron in solution is likely to be obstructed, and we may therefore consider this area the region of passivation. Thus, the passive regions are those in which an insoluble protective compound is the most stable product. In these areas some initial corrosion will occur until a surface film is formed to protect the underlying metal. The passive regions for the metal are defined as thermodynamically unstable but kinetically inert with regard to metal corrosion.

A simplified form of the Pourbaix Diagram for iron showing the regions of immunity, corrosion and passivity is shown in Figure 7.13; the small "corrosion" triangle on the right represents the formation of ferroates in a strongly alkaline solution.

The Pourbaix theoretical potential-pH diagrams, whilst being an efficient means to collect and represent thermodynamic data for corroding systems, do have certain limitations. Bockris (1970: 891) highlights four such limitations. 1) The Pourbaix Diagram presupposes equilibria between the metal and its ions in the solution and corrosion products containing these ions. In practical corrosion cases conditions may be far from equilibrium; 2) The term passivity in the diagram is applied to the field of existence for oxides, hydroxides or other sparingly soluble substances, irrespective of their prospective properties; 3) The pH value referred to in the diagram is the one prevailing at the surface of the metal considered. This often varies from point to point and is usually lower at the anode surface and higher at the cathode surface than the measured values in the bulk of the corrosive solution; 4) The Pourbaix Diagram gives no information on the corrosion rate, since it is based on thermodynamic and not kinetic data.

Of particular importance to this experiment is point three. It was only possible to measure the pH and the voltage of the solutions in the laboratory experiments or seawater from the field sites and not the pH and Eh. Dr. Ian MacLeod of the Western Australian Maritime Museum has conducted extensive research on measuring the potential-pH of corroded artefacts *in situ* on shipwreck sites (MacLeod, 1981: 291-303, 1987: 49-56, 1989: 7-16, 1992: 45-51, 1995: 53-59). Personal communication with Dr. MacLeod at an early stage in the experiments revealed that, although in practice it was relatively simple to measure such parameters, the cost of the equipment required would be too high for this research. However, using the data available from the measured environmental parameters and observations and identification of the corrosion products, interpretations regarding how and why the metal specimens corroded under the various experimental conditions will be discussed.

#### 7.3.4 Interpretation of results

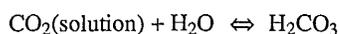
##### 7.3.4.1 Steel

The macro appearance of the uncleaned specimens from all the experimental conditions showed amorphous red/brown corrosion products. The only noticeable differences between them was that the corrosion products on those specimens in deionised water appeared flocculated and did not adhere to the metal surface very well. This was due to the nature of the deionised water used. The deionised water solutions showed an initial decrease in pH which was probably due to the chemical reaction of atmospheric carbon dioxide with the water as follows (Riley and Chester, 1971: 129):



Equation 7.8

and this then reacts with water to form carbonic acid



Equation 7.9

the carbonic acid initially lowering the pH of the solution as was observed. This lowering of pH could explain the formation of the iron oxide hydroxide corrosion product *lepidocrocite*, (FeO(OH)). Based on this identification it is postulated that the low pH placed the metal surface in the region of passivity on the corresponding Pourbaix Diagram for iron in pure water (Figure 7.13). However, as was noted in the introduction to this section, passivity does not necessarily imply the absence of corrosion, only that insoluble corrosion products are formed making passivity theoretically possible. In this case corrosion was not abated. The rate and extent of corrosion, as recorded by weight loss, were the greatest in deionised water, and it is argued that passivation did not occur because the corrosion products did not form a protective layer over the metal surface, but instead they tended to fall away from the surface after any significant build up. Thus, effectively, the surface of the metal was being continually exposed to the corrosive nature of the deionised water. That a passivating layer was not formed was further attested by the fact that, upon chemical cleaning, little evidence of pitting was apparent. This indicated that corrosion was uniform over the specimens and not localised.

Although two species of the genus *Pseudomonas* were identified to cause deterioration of steel specimens in deionised water under laboratory conditions, no evidence of characteristic tubercles was present on the specimens, presumably because of the absence of a solid corrosion layer.

The specimens in artificial seawater showed a similar initial rate of weight loss to the deionised water specimens. However, after eight weeks submersion relative stability was achieved. Based on the identification of the corrosion products: *lepidocrocite* (FeO(OH)), and *goethite* (Fe<sub>2</sub>O<sub>3</sub>. H<sub>2</sub>O), it is postulated that the initial low pH of the artificial seawater solutions placed the metal surface in the region of passivity on the corresponding Pourbaix Diagram for iron (Figure 7.13). As with those specimens in deionised water, *lepidocrocite* was formed which did not passivate the specimens and corrosion continued until eight weeks submersion. After this point corrosion effectively stopped and thereafter remained relatively stable throughout the experiment. North (1982: 76) suggests the reason is that, at this point, the Fe<sup>2+</sup> ions formed in the anodic reaction are readily oxidised to Fe<sup>3+</sup> ions as they move away from the metal ions. Fe<sup>3+</sup> species are much less soluble than Fe<sup>2+</sup> in seawater and this oxidation results in Fe (III) oxide and hydroxide formation. In the case of the artificial seawater specimens, *goethite* (Fe<sub>2</sub>O<sub>3</sub>.H<sub>2</sub>O) was identified as a corrosion product. The covering of the specimens with this corrosion product effectively served to passivate corrosion. On chemical cleaning of the specimens, appreciable pitting was

observed. There was no evidence to suggest that this had been initiated by microbial activity and it was thought the corrosion was purely electrochemical.

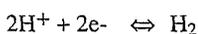
Those specimens submerged on the Alum Bay and Duart Point wreck sites showed similar initial rates and extent of weight loss up to four weeks submersion. Between four and eight weeks corrosion effectively stopped, the reason being similar to that for specimens in artificial seawater. It is postulated that the surface potential-pH of the specimen was in the passive region for iron in seawater, with initial corrosion taking place until a passivating film was formed after four weeks. This film came about from the oxidation of  $\text{Fe}^{2+}$  to  $\text{Fe}^{3+}$  which oxidised to Fe (III) oxides and hydroxides. In the case of the Duart Point specimens *lepidocrocite* ( $\text{FeO}(\text{OH})$ ) and *goethite* ( $\text{Fe}_2\text{O}_3 \cdot \text{H}_2\text{O}$ ), were the passivating films and *magnetite* ( $\text{Fe}_3\text{O}_4$ ) the passivating film for the Alum Bay specimens. Between eight and 16 weeks submersion the rate of weight loss slowed and for the additional information for the Duart Point specimens the rate of weight loss slowed even more between 32 and 52 weeks.

Examination of the uncleaned 52 week specimens from Duart Point revealed the presence of tubercles, indicative of microbially induced corrosion, randomly over the surface of the specimens (Figure 7.14). A species of the genus *Arthrobacter* was identified to cause corrosion under laboratory conditions and this, combined with the chloride content of the seawater, initiated pitting corrosion as was observed on the cleaned specimens.

Those specimens from the Albert Dock showed a similar profile of weight loss to the Alum Bay and Duart Point wreck sites. However, the rate and extent of weight loss were less than that recorded for those specimens submerged on the Alum Bay and Duart Point wreck sites. The reason for this is thought to be the fact that the salinity of the Albert Dock was on average 26.3‰ as opposed to the average salinity for the Alum Bay and Duart Point wreck sites of 33.6‰ and 33.2‰ respectively. The cleaned specimens showed evidence of pitting corrosion, to a lesser extent than the other field sites. It is thought that the lower salinity, and in particular, the chloride ion concentration, slowed the propagation phase of the pitting process.

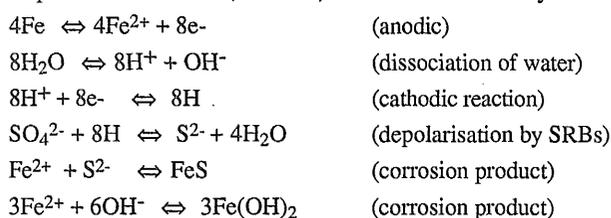
Uncleaned specimens revealed the presence of tubercles like those observed on the Duart Point specimens. Thus pitting was probably affected by micro-organisms. As with the Duart Point specimens, a species of the genus *Arthrobacter* was identified to promote corrosion of specimens under laboratory conditions.

One of the corrosion products on the specimens was different to the types seen on the other field and laboratory specimens. Areas of the specimens, adjacent to the metal surface, were covered in iron sulphide ( $\text{FeS}$ ). North and MacLeod, (1987: 75) note that, if the oxygen content of the water immediately in contact with the metal becomes very low, then the Eh of the water can fall below the hydrogen evolution potential. Once this happens the main cathodic reaction in the metal corrosion process is:

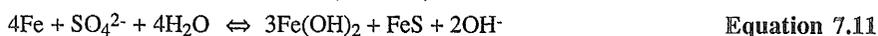


Equation 7.10

In the absence of catalysis this reaction is generally fairly slow on most metals in the seawater pH range and consequently the corrosion rate is slow. The action of sulphate reducing bacteria (SRBs) speeds up this reaction since their metabolism converts sulphate to sulphide ions which can subsequently react with the metal ion to produce metal sulphides. Borenstein (1994: 23) details the reactions by which these sulphide form:



The overall reaction can be summarised as:



At certain times of the year the dissolved oxygen content of the Albert Dock approached anoxia, for example, in June, levels were recorded as low as  $3\text{mg cm}^{-3}$  as opposed to approximately  $9\text{mg cm}^{-3}$  at the other field sites at this time.

In addition to the iron sulphide corrosion product were *lepidocrocite* and *goethite* which, it is assumed, were formed when the potential-pH of the metal surface was able to undergo aerobic corrosion, and were formed by the same reactions as those specimens from the Alum Bay and Duart Point wreck sites.

#### 7.3.4.2 Bronze

The specimens submerged in deionised water showed no signs of corrosion. Those submerged in artificial seawater and on the Duart Point wreck site and in the Albert Dock showed red and blue/green corrosion products which were indicative of copper corrosion products. Similarly those specimens submerged on the Alum Bay wreck site showed red and blue/green corrosion products and in addition a grey corrosion product identified as being a tin corrosion product.

The pH of the deionised water containing bronze specimens was 6.10 after four weeks and thereafter fluctuated slightly throughout the experiment. It was assumed that, for there to be no corrosion, as was observed, the electrode potential coupled with the pH was sufficient to place the copper, tin and lead in the immunity region of the respective Pourbaix Diagrams at  $25^\circ\text{C}$  in pure water (Figures 7.15, 7.16 and 7.17 respectively). Thus corrosion was thermodynamically impossible.

The corrosion products on the specimens submerged in artificial seawater, on the Duart Point wreck site and in the Albert Dock, were essentially the same and were all identified as being copper-based. Specimens from these experimental conditions showed

the formation of the red copper oxide, *cuprite*, which was overlain by the green/blue copper chloride hydroxides *atacamite* and/or *paratacamite*. The bronze specimens placed in these experimental conditions were initially subjected to pHs of between 8 and 9 and were under slightly oxidising conditions. Correlating this with the Pourbaix Diagram for copper in seawater (Figure 7.18) and the occurrence of the corrosion product *cuprite* it is postulated that the specimens were initially in the region of passivity. Chemical cleaning of the specimens revealed pitting and this, along with the identification of the corrosion products, gave the key to the mechanism of corrosion. Macleod (1981: 16-26) discusses the conditions for pit formation of bronzes from the marine environment. Pitting requires the presence of cuprous chloride under a layer of cuprite. Corrosion (anodic) occurs on the metal side of the cuprite layer while oxygen reduction (cathodic) occurs on the seaward side (see Figure 7.19) The existence of the cuprous chloride layer in the pit depends on the relative rates of copper corrosion:



and the subsequent hydrolysis of the cuprous chloride



The driving force for the pitting reaction is the concentration gradient of copper (I) species between the bottom of the pit and the corrosion mound above the *cuprite* film. In the presence of chloride ions, CuCl can form a series of soluble complexes such as  $\text{CuCl}_2^-$ ,  $\text{CuCl}_3^-$  in the pit. These copper (I) species can diffuse through cracks in the  $\text{Cu}_2\text{O}$  membrane and are oxidised by molecular oxygen to cupric ions



while oxygen is reduced to hydroxide ions



Equation 7.15 causes localised increase in pH and some calcium carbonate may be precipitated. Some of the cupric ions formed by Equation 7.14 will be precipitated in the form of the basic copper chlorides, such as *atacamite* and/or *paratacamite*. Comparing the rates and extent of corrosion of the specimens, those in artificial seawater were the worst affected. The extent of the surface pitting was also greatest on these specimens and it can be postulated that the concentration gradient of copper (I) species was such that the formation of chlorides resulted which deepened the pits by promoting anodic corrosion.

The profiles for the Duart Point wreck site and the Albert Dock were similar in that, after four weeks submersion, the rate of corrosion was much slower due to the formation of passivating  $\text{Cu}_2\text{O}$ . The reason that the specimens from Duart Point showed a greater weight loss may be that the chloride content of the water over the site was higher, a fact reflected in the greater level of surface pitting. One difference between the artificial seawater, Albert Dock and the Duart Point wreck site was the predomination of the red corrosion product *cuprite* on the Duart Point wreck site specimens. Macleod and North (1987: 84) and Evans (1981: 125) note that copper and its alloys are susceptible to increased corrosion attack by water movement. On the Duart Point wreck site there was a complex tidal set and a strong current running from east to west over the samples was prevalent during the ebb tide (it was not possible to quantify this current). If water rapidly passes over the surface at one point but is relatively stagnant over the rest of the surface localised attack can prevail. The removal of copper ions from the point of rapid motion keeps the ionic concentration locally low and this point will be permanently anodic to areas where copper ions can accumulate. The flow of water can also remove the protective oxide film and at high flow rates the concentration of copper in the vicinity of the artefact surface is not sufficient to allow reformation of the protective film and film free corrosion rates prevail.

Although the corrosion rates and the extent of corrosion were not greatly higher in the Duart Point specimens, the observation that the major corrosion product observed was *cuprite* is important when considering the formation processes operating on the wreck site.

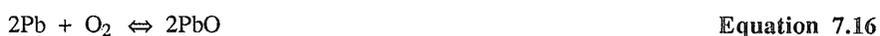
Although the corrosion rates and the appearance of the specimens from the Alum Bay wreck site were similar to those from the Duart Point wreck site up to eight weeks submersion, the 16 weeks submerged specimens were completely different. Up to eight weeks they showed similar corrosion; red *cuprite* overlain by blue/green *atacamite* and *paratacamite* and cleaned specimens showed pitting. However, on the 16 week specimens the predominant corrosion product was grey and was identified as the tin oxide, *cassiterite* ( $\text{SnO}_2$ ). Along with the observed corrosion products on the surfaces of the other metal specimens from the Alum Bay wreck site, it is thought this was due to the covering of the specimens with sediment and hence anaerobic corrosion. North and MacLeod (1987: 88) comment that under deaerated seawater, the tin rich  $\alpha$  plus  $\gamma$  eutectic in a bronze bell (20% tin 3% lead which was of a similar composition to the bronze used in this experiment) was preferentially attacked, with *cassiterite* being deposited on the surface of the artefact.

#### 7.3.4.3 Lead

Those specimens submerged in artificial seawater and in the Albert Dock showed no signs of corrosion. The specimens submerged in deionised water and on the Alum Bay and Duart Point wreck sites all showed corrosion.

Of the specimens which showed corrosion, those in deionised water were by far the most heavily attacked. Macroscopically the uncleaned specimens showed patchy areas of red and green plate-like corrosion products which were identified as the lead oxides *litharge* and *massicot* respectively. These overlay a finer grained white corrosion product identified as the lead carbonate, *cerrusite*.

Corrosion took place in this environment due to the presence of dissolved atmospheric carbon dioxide and oxygen (Smith, 1987: 784). The Pourbaix Diagram for the lead in the presence of carbon dioxide (Figure 7.17) indicates that at the initial solution pH the metal would be in the region of passivation by the formation of lead carbonate. Liddiard and Bankes (1944: 39-48) examined the effects of distilled water on lead and they suggested that in water which was substantially free from carbon dioxide the heavy initial attack is due to the reaction of lead to form lead oxide(s);



This heavy initial attack causes saturation of the solution with lead hydroxides (this was attested in this experiment by the increase in the pH of the solution) according to the reaction:



The lead ions then react with the hydroxyl ions (and any traces of carbon dioxide) to give insoluble basic lead carbonate, giving rise to the white precipitate noted in the initial stages of corrosion. Mayne (1946: 353) reported on the formation of lead oxides. A super-saturated solution of lead oxide tends to deposit solid upon existing crystals rather than start new ones; this may explain why they were seen overlying the lead carbonate. However, on chemical cleaning the specimens were extremely pitted, indicating localised pitting corrosion. This, coupled with the fact that the rate and extent of weight loss at the end of the experiment had not stabilised, suggest that the potential and pH at the metal surface was such that corrosion was favoured over passivation.

The specimens from the Alum Bay and Duart Point wreck sites showed different rates of corrosion. Those from the Alum Bay site corroded exponentially upon submersion up to 16 weeks. The Duart Point specimens showed little corrosion up to eight weeks submersion, followed by exponential corrosion for the rest of the experiment. Such observations are thought to be due to the fact that the Duart Point specimens were not only corroded by electrochemical mechanisms but also through the effect of macro fouling organisms.

The lead specimens submerged on the Alum Bay wreck site were patchily covered in a fine grained black corrosion product which was identified as the lead sulphide, *galena*.

This is often associated with lead artefacts from anaerobic marine environments (MacLeod and North, 1987: 89). Although it was not apparent when the site was visited, it is possible that due to the dynamic nature of the Alum Bay wreck site the sample trays were covered and uncovered with sediment in between sampling intervals. As with the iron sulphide corrosion products the lead sulphide was probably formed by the action of sulphate reducing bacteria (SRBs); in a similar way to that of the iron sulphide:

1. Cathodic polarisation via biological oxidation of hydrogen formed on a polarised metal surface.



2. Anodic stimulation by precipitation of ion plumbous ions produced at the anode with biological sulphur.



Those specimens submerged on the Duart Point wreck site were corroded not only through pure electrochemical mechanisms but also through the effect of fouling organisms.

In terms of pure electrochemical corrosion, two corrosion products were identified on the lead specimens. The first, adjacent to the metal surface was the lead chloride hydroxide, *laurionite* ( $\text{PbClOH}$ ). Beccaria *et al.* (1982: 88), when discussing the effects of 3.5% Sodium Chloride solutions on the corrosion of lead, concluded that (under their experimental conditions) the potentials of the lead specimens were such that they were passivated by the formation of a lead oxychloride such as  $\text{Pb}(\text{OH})\text{Cl}$ , according to the reaction:



The other corrosion product, *galena*, was probably formed as a result of anaerobic corrosion due to sediment cover, following the same mechanism, Equations 7.19-7.21, as for the specimens on the Alum Bay wreck site.

In addition to these corrosion products there was extensive evidence of macro fouling organisms on the lead and these too had an effect on the corrosion of the specimens. As Figure 6.76 shows, bryozoans and calcareous tubeworms were heavily encrusting some of the specimens, removal of these, and examination of the metal surface where they were affixed, indicated that the surface had been somewhat affected (Figure 7.20). X-Ray probe analysis of the area indicated high levels of sulphur (Appendix XXIV). It is postulated that under the macro organisms an anaerobic reducing environment was produced. This reduced any hydrogen sulphide produced by the aerobic or anaerobic bacteria (as for the

formation of *galena*) on the metal surface and contributed to the corrosion. This hypothesis is supported by the fact that significant corrosion did not occur until after eight weeks submersion when the specimens were beginning to be colonised heavily by the various macro-organisms.

#### 7.4 Conclusion

The interpretation of the analytical results confirms the observations that the organic materials were deteriorated by biological organisms and the metals, by and large, underwent electrochemical corrosion.

Colonisation of the organic materials was sequential beginning with bacteria and fungi followed by macro and mega fauna and algae. In some cases the action of the primary colonisers was sufficient to cause total deterioration, in others it was advanced by the secondary colonisation of organisms.

The metals mainly underwent electrochemical corrosion, although there was evidence of biological colonisation. Importantly the rates of deterioration and corrosion were different on each site and this was related to the differences in the environmental parameters on the sites and the different seasonal times when the specimen trays were placed on the sites, as will be discussed in Chapter Eight.

## CHAPTER EIGHT

### PRESERVATION AND MONITORING OF ARCHAEOLOGICAL ARTEFACTS ON UNDERWATER SITES *IN SITU*

#### 8.1 Introduction

This chapter considers the effect the environmental parameters measured in the field experiments had on the biological and electrochemical processes responsible for deterioration and corrosion of the organic materials and metals.

Based on this knowledge methods will be proposed of preserving *in situ* and monitoring the stability of artefacts made from each group of material.

#### 8.2 Organic materials

The significance of the environmental parameters measured in the field experiments (pH, voltage, temperature, salinity and dissolved oxygen) was not their direct effect on the materials but rather their effect on the ecology of the organisms which caused deterioration.

Of particular importance was the salinity, temperature and dissolved oxygen. For instance, the major reason for the different organisms seen colonising specimens from the Duart Point wreck site and the Albert Dock was the salinity of the water. Organism productivity and colonisation of specimens were affected and/or governed by temperature fluctuations which were seasonally induced. Dissolved oxygen was essential for the fungi (Barghoorn and Linder, 1944: 435), *limnoria* (Becker, 1971: 314), barnacles, (Houghton, 1971: 199) and calcareous tubeworms (Mill, 1972: 14) as they are all dependant upon oxygen for their respiration.

As discussed in section 7.2.1, the rate of deterioration of most of the materials was governed by the speed of their colonisation by bacteria and fungi. Primary colonisation of the materials was followed by secondary colonisation by various macro and mega-algae and fauna.

To mitigate these stages of colonisation and prevent deterioration, the primary colonisation has to be slowed down and/or stopped. Considering the parameters which affect the ecology of the micro-organisms causing deterioration, limiting their oxygen supply would seem to be the most obvious parameter to regulate, as this would create an unfavourable environment for their survival. This could be achieved through re-burial of previously surveyed and excavated artefacts. By re-burying artefacts, establishing and maintaining an anaerobic environment within the sediment, the effects of aerobic microbial deterioration will be slowed. In addition, re-burial would consequently limit secondary colonisation.

As will be discussed, any programme of re-burial should be systematic and take into consideration factors such as the sediment used, depth of re-burial, stabilisation of the

overlying sediment and, importantly, monitoring the burial environment in order to check the long term stability of artefact materials.

### 8.2.1 Re-burial of organic materials

Only the top few centimetres of marine sediments contain micro-organisms (Zobell, 1946: 24). Saprophytic micro-organism numbers are often several tens of thousands to several hundred thousand per gram of sediment due to the high content of organic nutrients in this level (Jones, 1980: 285-292). Below the first few centimetres the bacterial content of the sediment is reduced, the saprophyte numbers falling more rapidly than the total numbers of micro-organisms. At a depth of below one metre the bacterial counts are only a fraction of those on the surface of the sediments and then slowly decrease further. Rheinheimer (1992: 87) states that this holds equally for both aerobic and anaerobic forms. Thus re-burial to a depth of at least one metre would create an overlying deposit which, in time, would be relatively sterile.

In addition, a deep layer of sediment would be required to mitigate the effects of bioturbation by the various epifauna whose habitat is on or in the seabed. Ferrari and Adams (1990: 139) noted that a range of mollusca, fish and crustacea inhabit soft sediments and firmer substrates. Of the main species operating in softer sediments the Red Band fish, *Cepula Rubescens*, readily illustrates the problem of burrowing fauna: its burrows have been observed penetrating almost one metre into the sediment. On firmer substrates the crab, *Cancer pagumis*, uses its claws as a bulldozer when excavating its pits.

The actual sediment used for re-burial would be important. Weier (1974: 135) notes the physical preservation afforded by various types of sediment. The grain sizes quoted are from Leeder's categorisation (1982: 36). Gravel (2.00-4096mm) provides the greatest cause of physical break-up, whereas a sandy bottom (0.0625-1.68mm) swept by currents causes rapid erosion of anything in or above the shifting sand. Silt (0.0039-0.053mm) can be protective due to its small grain size and clay (0.00006-0.0020mm), due to its colloidal properties and small grain size, binds tightly any object buried in it.

Based on purely physical properties re-burial in fine grained sediment would seem to be the best option. However, particle size affects the abundance of bacteria within the sediment. Greater abundance of bacteria are present in finer grained sediment. This is due to the sorting action in sedimentation which tends to segregate particles of similar size, so the tendency is for any free floating bacteria to be deposited with other particles of colloidal dimensions (Zobell, 1946: 94). This relationship can be seen in Table 8.1, which shows the average nitrogen, water and bacteria content of sediment samples from the Channel Islands.

Deposit	Nitrogen content	Water content (%)	Bacteria (per gram)
Sand	0.09	33	22,000
Silt	0.19	56	78,000
Clay	0.37	82	390,000
Colloid	1.00	98	1,510,000

Table 8.1 Average nitrogen, water and bacteria content of sediments from the Channel Islands (Zobell, 1946: 94).

From this evidence, sand would appear to be the most suitable medium for re-burial, as it shows a low propensity for microbial activity and is of a relatively low particle size.

Within the sediment it would be imperative to develop an anaerobic, hence reducing, environment in order to inhibit the growth of aerobic organisms. It is postulated that covering the sediment with a layer of impermeable material, such as polythene, would inhibit the diffusion of oxygen into the sediment. Any oxygen remaining in the sediment below the polythene would be utilised by aerobic micro-organisms until anaerobic conditions prevailed.

Admittedly, deterioration would still occur in anaerobic environments; however, canvas, the material which was degraded most quickly in this experiment, was found on the wreck of the *Vasa* (sank 1628). It was in a relatively poor state of preservation, but it had been encapsulated in anaerobic sediment on sinking (Kvarning, 1993: 71-72). This implies that, even though deterioration still occurs in such an environment, it is preferable to an aerated environment.

Overlying the polythene sheeting, a system of artificial seagrass mats, as described in Chapter One and used on the *William Salthouse* (Elliget and Breidhal, 1991: 27), could be placed to act as an additional sediment trap and further enhance stabilisation of the site. Establishing an artificial seabed over a deep layer of sediment would mitigate the effects of bioturbation by creating an artificial habitat for epifaunal organisms and would protect the re-buried material from other potentially destructive physical processes.

However, having re-buried and stabilised a site *in situ*, it is imperative that monitoring is carried out, if not in perpetuity, then at least for a sufficient length of time to check that the rate of deterioration had been significantly slowed.

### 8.2.2 Monitoring the deterioration of organic artefacts preserved *in situ*

Any programme which sets out to monitor the deterioration of organic artefacts preserved *in situ* should focus upon three aspects. First, measuring the aerobic microbial activity would determine the deterioration potential of the re-burial sediment. Second, as anaerobic

and consequently reducing conditions are favourable for preservation, the oxidising/reducing nature of the re-burial sediment should be measured. Third, placing samples of sacrificial material(s) within the re-buried sediment and monitoring their deterioration periodically would serve to check that the environment was genuinely preserving artefacts. The implications of these aspects will be discussed.

Organic matter oxidises as it decays and as it does so in an aerobic environment carbon dioxide, water and new organic matter are produced. The organic matter is gradually broken down into simpler molecules (Borenstein, 1994: 16). Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) are methods used to determine the amount of oxygen used in the aerobic biological decay of organic matter. Specifically, the BOD is the amount of dissolved oxygen required by aerobic and facultative micro-organisms to stabilise organic matter (HMSO, 1983: 14). The COD is the amount of oxygen required to oxidise completely organic matter (HMSO, 1983: 14). These methods are used mainly in the sewage and water treatment industry to understand how much oxygen would be used if waste-water was diluted into fresh water (HMSO, 1983: 6-14).

Their potential archaeological use was fortuitously discovered during this experiment. While attempting to interpret the nature of deterioration of the leather and bone specimens the graphs of nitrogen content (Figures 6.46 and 6.53) of these materials were compared with BOD measurements of the Albert Dock taken by Allen (1992: 55). Figures 8.1 and 8.2 shows the results of the bone and leather experimental data with the BOD measurements for the Albert Dock; as can be seen, they all show similar peaks and troughs. The explicit relationship between the results and the BOD is not certain. However, the series of seasonal peaks and troughs seen in Figures 8.1 and 8.2 imply that there is a relationship between the BOD and the deterioration of these materials. The principle of the BOD method is to measure the oxygen consumed by micro-organisms in a diluted sewage or water sample during five days of incubation at 20°C. After incubation the decrease in dissolved oxygen is measured (British Standards Institute, 1990: BS 6068: Section 2.14). The implications for the sewage industry are that if the dissolved oxygen content is low then aerobic bacteria cannot survive and the purification of water is slowed as the organic materials cannot be broken down as easily. For the archaeologist these are the ideal conditions: a low BOD would imply low aerobic deterioration.

Admittedly, the BOD in this case was of the water in the Albert Dock and extrapolation to interstitial water in sediments may be untenable. However, it can be postulated that measuring the BOD and/or COD in sediments could give an indication of the microbial activity and, depending on a high or low reading, whether organic materials were potentially being deteriorated.

The oxidative nature of the sediment could be determined by measuring its redox potential. Parkes *et al.* (1978: 113) discuss a method for determining the redox potential in

their work on bacterial activity in estuarine sediments. A core sample of sediment was taken and the redox potential measured with a platinum electrode and a double junction reference electrode containing 3.5 Molar potassium chloride. The reference electrode was inserted vertically into the sediment surface and the platinum electrode inserted horizontally into the core at a specific depth through pre-drilled holes in the core tube. Figure 8.3 illustrates the coring apparatus used.

By measuring the pH of the sediment as well as the Eh, Baas Becking *et al.* (1960: 259-260) were able to construct Eh-pH potential graphs, similar to Pourbaix diagrams, and characterised marine sediments in terms of their Eh-pH. They found that the Eh of various sediments ranged from oxidising in coarse grained and shelly sediments, with Eh values up to +350 mV, to highly reducing black and green clays, with Eh lower than -400 mV. The lowest potentials were found in deep basins where organic matter had accumulated due to the restricted circulation of the water which prevented aerobic decomposition. The pH of the large majority of sediments was found to be between 7.0 and 8.5. In general there was a decrease in the pH of the surface sediments relative to that in the overlying water. They found that there was usually a slight increase in pH with increase in depth of the core.

By monitoring the Eh-pH of the sediments around the re-buried materials, the oxidising or reducing nature of the sediment could be determined. The restricted diffusion of oxygen through the impermeable layer of polythene would promote reducing conditions which would be more favourable to preservation.

Baas Becking *et al.* (1960) also discuss the limits of Eh-pH for sedimentary micro-organisms as shown in Table 8.2.

The implications of this information are that by establishing a reducing environment, not only would oxygen be eliminated from the re-burial sediment, but if a reducing redox potential of greater than -300mV could be obtained, the activity of many bacteria would be reduced.

Organism	pH limits	Eh limits (mV)
Eukaryotic Algae	1.20-11.72	+630 - -220
Sulphate reducers	4.15-9.92	+115 - -450
Purple phototrophic bacteria	4.92-9.75	+328 - -230
Sulphur oxidising bacteria	1.00-9.20	+855 - -190
Green phototrophic bacteria	6.15-9.78	+7 - -293
Iron bacteria	2.00-8.90	+850 - +60
Denitrifiers	6.20-10.20	+665 - -205

Table 8.2 Milieu limits and ranges for various organisms (after Baas Becking *et al.*, 1960: 264).

Measuring the microbial activity and redox potential of the sediment would give information about the deterioration potential of the re-buried environment. In addition, monitoring the actual deterioration of sacrificial materials would be beneficial to check that the monitoring methods did work. Considering that, in this research, canvas was the most susceptible to deterioration it would be a good sacrificial material to use. The *Cotton Strip Assay* (Harrison *et al.*, 1988: 6-46) is a method which tests the deterioration of cotton materials, such as canvas, in soils. By burying a cotton textile and subsequently monitoring its deterioration through change in tensile strength, this method assesses the resistance of the textile to microbial activity. Similarly, burying cotton textiles within the re-burial sediment and retrieving them periodically and measuring their deterioration would serve as a method to determine if the environment was conducive to preservation.

### 8.3 Metals

In the field experiments, the corrosion rates of the various metals were quite different. The steel specimens were the most rapidly corroded, followed by bronze and lead. The mechanism for the steel was mainly electrochemical, although the activity of micro-organisms did appear to play a part. The mechanism for the bronze specimens was electrochemical. Lead was relatively unaffected, but where corrosion was recorded, it occurred through the effects of macro-organisms.

In terms of electrochemical corrosion, several of the environmental parameters measured in the field sites were important. The salinity affected the corrosion rate: for instance corrosion was slower in the Albert Dock than it was on the Alum Bay and Duart Point wreck sites due to the lower salinity of the former sites. In addition, water movement was important in the corrosion of the bronze specimens. Corrosion appeared worse on the Duart Point site compared with the other sites, as there was an appreciable current. The pH at the surface of the metal was important but not in the bulk of the seawater. Similarly, instead of measuring the voltage of the seawater the corrosion potential voltage,  $E_{\text{corr}}$ , should have been measured at the metal surface. MacLeod *et al.* (1986: 117) acknowledge these factors as important in the corrosion of metals in seawater and in addition note that the temperature, dissolved oxygen content and the inherent reactivity of the metal in relation to water are important factors.

#### 8.3.1 Corrosion potential measurement and cathodic preservation systems

Measurement of the pH and redox potential at the metal surface so as to determine the corrosion potential,  $E_{\text{corr}}$ , of the metal would be the most important parameters to measure in order to mitigate the effects of electrochemical corrosion and thus provide effective *in situ* preservation.

MacLeod (1995: 53-54) discusses the method for measuring these parameters *in situ*. Measurements are obtained by drilling through the marine growth and corrosion products and inserting a platinum electrode into the hole to establish electrical contact with the object. The reference electrode is placed adjacent to the point of measurement and the voltage,  $E_{\text{corr}}$ , is read on a volt-meter in an underwater housing. Having measured the corrosion potential, the pH of the metal surface is measured using a flat-tipped pH electrode. The  $E_{\text{corr}}$  is the voltage of the corrosion cell that exists between oxidation of the metal surface and the reduction of the dissolved oxygen in oxygenated seawater. The voltage measured by the volt-meter refers to the difference in electrical potential of a silver chloride reference electrode (Ag/AgCl, seawater) and the platinum electrode in contact with the corroded metal. As discussed in Chapter Seven, by plotting the  $E_{\text{corr}}$  and the corresponding pH values for the artefact on a Pourbaix diagram, it is possible to determine the nature of the processes that control the stability of the object. Knowing the state of stability, for instance whether the metal is actively corroding or is passive, corrosion protection systems can be developed.

Corrosion protection is based on preventing one or more of the four requirements of the corrosion cell. As discussed in section 7.3.2, these are metal dissolution, or oxidation, the complimentary reaction of reduction, electrical conductivity and ionic conductivity. Modifying the environment to reduce its conductivity and/or eliminate the cathodic reactants will provide corrosion protection (Kentish, 1995: 7-12). The application of a coating system will separate the environment from the metal surface, preventing the cathodic reactions occurring. An insulating paint coating will prevent the completion of the electrical circuit by resisting ionic movement to the metal surface. Some corrosion inhibitors operate by preventing the anodic or cathodic reactions. Another method of corrosion prevention is to ensure that the entire metal surface, which previously accommodated anodic and cathodic areas on the surface, is maintained cathodic relative to a remotely located anode. This last method is the basis of cathodic protection.

Shrier (1976) discusses cathodic protection in detail, but in general terms a cathodic protection system consists of a highly reactive disposable metal which is in electrical contact with a less reactive metal. This forms a galvanic couple with the more reactive (and expendable) metal suffering increased corrosion attack and the less reactive metal being protected against corrosion. North and MacLeod (1987: 72) discuss the principle. When two metals with different  $E_{\text{corr}}$  values are brought into electrical contact, electrons will flow from the metal with the lower  $E_{\text{corr}}$  value into that with the higher  $E_{\text{corr}}$  value. With good electrical contact the metals are at the same potential, which is between the values of the individual uncoupled metals. This electron flow will be sustained by a change in the rates of the anodic and cathodic reactions on the metal surfaces. On the metal with the lowest  $E_{\text{corr}}$  value, the anodic reaction rates will increase while the cathodic reaction rates decrease.

This generates a surplus of electrons, which then flow into the metal with higher  $E_{\text{corr}}$  value. At this metal surface the cathodic rates increase, the anodic rates decrease and the surplus electrons are consumed. As the anodic reactions are usually metal dissolution reactions, the coupling of two pieces of metal with different  $E_{\text{corr}}$  values will cause an increase in corrosion rate of the piece with the lowest  $E_{\text{corr}}$  value and a decrease in that with the highest  $E_{\text{corr}}$ . If the two metals so coupled together have different compositions, this is known as galvanic coupling, with the noble (higher  $E_{\text{corr}}$ ) metal corroding less (galvanic protection) and the active (lower  $E_{\text{corr}}$ ) metal corroding more (galvanic corrosion). Table 8.3 shows the corrosion potentials of some metals in seawater (volts versus hydrogen at 25°C); this is known as the *Galvanic series*.

Thus, for example, if mild steel ( $E_{\text{corr}}$  -0.21 volts) is coupled with zinc ( $E_{\text{corr}}$  -0.86 volts) in seawater the zinc will corrode faster than normal, but the steel will be protected and corrode less. Apart from corrosion control the action of cathodic protection generates alkali at the metal seawater interface and thus reduces the corrosive chloride content. Raising the pH of the seawater also causes calcium carbonate to precipitate, adding to the protective layer of corrosion products on the metal.

Metal	$E_{\text{corr}}$ (Volts)
Silver	0.26
Admiralty brass	0.10
Red brass	0.09
Copper	0.07
Yellow brass	0.04
Lead	-0.06
Mild steel	-0.21
Aluminium (+4% copper)	-0.36
Zinc	-0.86

Table 8.3 Corrosion potentials of some metals and alloys in seawater, volts vs hydrogen at 25°C. (After North and MacLeod, 1987: 72).

Cathodic protection systems using sacrificial anodes have been used successfully to conserve metal artefacts from the wrecks of the *Rapid* (1811) (MacLeod, 1981: 72) *SS Xantho* (MacLeod *et al.*, 1986: 113-130) and *HMS Sirius* (1790) (MacLeod, 1993: 221-245). In the case of *HMS Sirius*, anchor and carronades were preserved by a cathodic protection system. Corrosion potential measurements on one of the carronades showed

that it was actively corroding. A 20 kilogram piece of aluminium alloy (a truck engine block) was attached to the concreted carronade. Within ten minutes of the anode being connected, the  $E_{\text{corr}}$  had fallen by 165mv, which showed that a good current flow had been established and chloride and acid removal had commenced. Measurements one year after the anode had been connected showed that the surface pH had increased and that  $E_{\text{corr}}$  had shifted to more negative values (less reactive).

### 8.3.2 Re-burial of Metals

It may be argued that electrochemical corrosion is the most important mechanism of corrosion of metals. However, as these experiments have shown, the action of water movement and micro and macro-organisms played a part in corrosion. In certain instances, cathodic protective systems alone would be insufficient to afford protection against corrosion.

The bronze specimens at the Duart Point wreck site showed that corrosion was affected by the current which flowed across the site. Thus, if a site with a strong current running over it contained bronze or copper artefacts and was to be preserved *in situ*, the current would have to be taken into consideration. It would be necessary to re-bury the artefact to mitigate the effect of the current. As was shown by some of the Alum Bay specimens which had been covered with sediment during this experiment, the anaerobic environment would inevitably produce a new corrosive environment. The Eh-pH at the re-buried metal artefact surface would need to be monitored to determine the corrosion potential and consequently develop cathodic protection systems.

Micro-organisms which affected the corrosion of the steel specimens were apparent from the Albert Dock and Duart Point sites. However, as discussed, their corrosive mechanism was essentially electrochemical. Thus measuring the corrosion potential and establishing suitable cathodic protective systems would mitigate their effect.

The lead specimens showed little sign of electrochemical corrosion in these experiments. The most significant corrosion was recorded on the Duart Point specimens and was due to the surface colonisation by macro-fauna. Admittedly, the overall effect of these organisms was to promote electrochemical corrosion at the metal surface. However, to prevent corrosion the colonisation by the macro-organisms needs to be prevented. As with the organic materials the solution lies with re-burial to limit the oxygen required for the organism's respiration. However, as some of the Alum Bay and Duart Point wreck site specimens which had been covered with sediment showed, re-burial establishes a corrosive anaerobic environment. As with the re-buried copper/bronze artefacts, the corrosion potential would still need to be monitored and, if need be, a suitable cathodic protection system established.

#### 8.4 Conclusion

Re-burial of organic materials with subsequent monitoring of aerobic microbial activity and the reducing nature of the environment would seem the best options to stabilise and slow the rate of deterioration by aerobic organisms. However, further practical work is required to validate the methods proposed.

The successful preservation of archaeological metals *in situ* by cathodic protection systems has been documented in tropical waters. However, practical work of this nature is required in temperate waters, such as around the United Kingdom. These implications will be discussed in Chapter Nine.

## CHAPTER NINE CONCLUSION

### 9.1 Introduction

The impetus for this thesis came from Keith Muckelroy's seminal paper, *Historic wreck sites in Britain and their environments* (1977: 47-57), which discusses the formation of wreck sites through a study of their environments. In summary this paper attempted to categorise twenty wrecks in British waters into five discrete classes by assessing various physical environmental attributes relevant to wreck preservation. The basis for Muckelroy's classification stems from the fact that the environment is external to the wreck system and directly influences the process of wrecking, the disintegration of perishables and seabed movement. In order to classify the wreck sites Muckelroy selected eleven environmental attributes all of which affect a site. The twenty sites were ranked from 1 to 20 (1 being the best preserved, 20 the worst) for each attribute. By statistical correlation of the survival characteristics of each wreck site and the eleven environmental attributes the environmental attributes which most affected the site were identified. Through this Muckelroy placed wreck sites into five distinct classes. A class one wreck is the best preserved with extensive structure and organic remains with many other objects present all of which were deposited in an ordered and coherent manner. At the other end of the scale a class five wreck had few structural and organic remains with few other objects surviving, all of which were deposited in a scattered and disordered manner. The relevant attributes for the preservation were related to the topography (percent of bottom sedimentary deposit), sedimentary deposit (the range of sediment type), slope (average slope in degrees), sea horizon (sector of open water for 10+ Km) and the fetch (maximum offshore distance) over the site.

In a reassessment of Muckelroy's paper I have previously argued (Gregory, 1992) that such classifications are potentially misleading due to the incomplete nature of the observed seabed distribution. Due to several factors our knowledge of the seabed is partial. Considering the wrecking process from the moment the ship begins to sink we see that the nature of the wrecking of each ship will vary. Some ships may sink virtually intact, such as the *Mary Rose*, while others may be damaged prior to sinking and lose many of their contents whilst sinking, such as the *Santa Maria de la Rosa*. Thus a shipwreck may be deposited in the ideal environment for preservation, yet because of the nature of the wrecking the whole ship may not have been deposited. Further to this, such classifications do not allow for the difference in time between the wreckings. For example, in Muckelroy's classification there is a difference of 235 years between the oldest and youngest wreck in his sample. This assumes the shipwreck and its surrounding environment have been stable and undisturbed since its sinking. However, the site

environment is dynamic, due to both cultural and natural formation processes, and will change over time.

While I believe this kind of retrospective approach to the study of shipwrecks and their environments may be ambiguous, I support a systematic approach to the study of the site environment. Muckelroy himself was the first to acknowledge that further work was required, indeed, in his paper Historic wreck sites in Britain and their environments he states, "... in the mean time, the ideas presented above now await confirmation, refutation or most likely, modification in the light of future finds around the coast of Britain" (1977: 56).

A systematic approach to the problem is essential but research should tackle the problem from an opposing view point - predicting the preservation and/or degradation of shipwrecks by understanding the processes operating in the present. Monitoring and management programmes which have sought to assess the environment systematically have tended to focus on the various processes in isolation, for example Muckelroy, in his 1977 paper, considers the physical processes rather than the complex interactions of physical, chemical and biological processes. Those programmes which do seek to monitor physical, chemical and biological processes, such as McCarthy (1982:50), state which parameters should be measured and/or monitored but do not adequately state the reasons for measuring them, or what the consequences of these measurements are.

This thesis has attempted to re-dress the balance by empirically measuring the deterioration of materials and monitoring various chemical and biological parameters operating in the natural marine environment, in order to quantify which of these processes were important in terms of the formation of the submerged archaeological record and its future preservation *in situ*. This was achieved using a range of modern organic materials and metals on three underwater sites in British waters over a 52-week period. It was postulated that understanding the effects that these processes had on the materials would enable a better interpretation of the submerged archaeological record upon excavation. In addition, by understanding the factors which affected the chemical and biological deterioration of the materials, it was hoped to be able to propose methods to mitigate their effects and facilitate better *in situ* stabilisation and preservation of submerged sites.

## **9.2 The implications of chemical and biological processes of deterioration to the interpretation and *in situ* preservation of the archaeological record**

Although physical processes, either cultural or natural, were not measured in this thesis, it must be stated that they play an important part in the formation/stabilisation of a wreck site. This was attested by the fact that the specimen trays on the Alum Bay wreck site were ripped from the seabed between 16 and 32 weeks due to the dynamic nature of the site, sport diving, or anchor-drag through the site. If it were mainly the physical processes

which caused this loss Muckelroy's classification would put the site into a Class Two state of preservation: elements of structure, some organic materials, and objects of other types of materials would be preserved. They would be scattered but in an ordered position. However, as the site has only been surveyed and not excavated it would be impossible to conclusively assign this classification. The major factor which I feel may have governed the loss of the sample trays was the particularly bad weather which prevailed during the period of this experiment. On both the 16 and 32 week sampling intervals the swell over the site was considerable. Muckelroy does note (1977: 55) that there are low correlations between survival characteristics and storm conditions, tidal currents and depth. If the losses were due to cultural processes this would make classification of the wreck site even harder.

Physical processes were also apparent on the Duart Point site, the most important being the tidal set which ran from west to east across the site during the ebb tide. As already noted, sediment transportation, which included both erosional and depositional cycles, was evident. The effects were greatest at spring tides (when there was a vertical range of up to 4.5m), particularly when these coincided with north to north westerly winds. Correlating the site with Muckelroy's classification would put the site between a Class One and Class Two category. However, one of the reasons for destabilisation of the site is thought to be the activities of sports divers (Colin Martin pers. comm.), who are known to have removed artefacts from the site. This initial destabilisation of the site was subsequently exacerbated by the aforementioned tidal set.

Thus although physical processes are evidently important the regimes which operate inter and intra site are, in my opinion, too complex to construct a classification system based on the observed seabed distribution of different shipwrecks. Instead classification should concentrate on regimes operating in the present over individual sites so as to understand what factors are important to specific sites and may have affected them in the past and will affect them in the future.

As has been discussed, previous research into chemical and biological processes has been limited to making qualitative statements, for example anaerobic conditions favour preservation, or has concentrated on empirical data gathering without concern for the implications of the measurements. An experimental approach to chemical and biological formation processes, such as this thesis, serves to semi-quantify which of these processes are important in terms of why shipwreck materials have been preserved and what factors affect their subsequent preservation. The results of this thesis have shown what processes are likely to affect organic materials and metals. Of those specimens which survived physical deterioration, the organic materials underwent deterioration by biological mechanisms and the metals were corroded through electrochemical mechanisms. This is of

course a broad generalisation, the mechanisms vary depending upon the different material within each category.

The organic materials were colonised and underwent deterioration by biological organisms in a two stage process. Primary colonisation was by terrestrial and marine bacteria and fungi and secondary colonisation was by marine macro fauna and algae. In the Albert Dock, canvas and rope were totally destroyed by the effects of primary colonisers. In the Albert Dock and on the Duart Point wreck site, bone specimens were affected by primary colonisers, although deterioration was not to the same extent as the rope and canvas specimens.

Wood and leather on the Duart Point wreck site were susceptible to secondary colonisers. The metals underwent electrochemical corrosion. However, there was evidence to suggest that micro-organisms affected the steel specimens in the Albert Dock and on the Duart Point wreck site. Macro-organisms affected lead specimens on the Duart Point wreck site. As has been discussed, such mechanisms have been documented in literature pertaining to the study of biodeterioration. However although the above principles were true for the sites under study, the rate, extent and organisms involved in deterioration and corrosion on each were different. This was due to the different environmental parameters operating on the sites. Each of these parameters affected the deterioration of the organic materials and corrosion of metals differently.

In relation to the deterioration of the organic materials, the parameters measured (dissolved oxygen content, temperature, salinity, pH, voltage) did not appear to directly affect the deterioration of the materials, but instead they had an effect on the ecology of the organisms which caused deterioration. Of the parameters, the dissolved oxygen would affect the respiration of the macro-organisms which caused deterioration; temperature, due to seasonal fluctuations, which would affect the productivity of the various micro and macro-organisms causing deterioration and salinity would affect the tolerance of various organisms to sites.

In relation to those metals which showed corrosion, the salinity was the most important parameter measured. The higher the salinity, the greater the rate of corrosion. Recording the pH and voltage of the water did not prove useful. However, measuring the pH and the corrosion potential at the metal surface would be useful in interpreting the mechanism of corrosion and state of preservation of the metal.

Significant differences in deterioration and corrosion were recorded between specimens from the Duart Point wreck site and the Albert Dock. The higher salinity at the former site allowed the secondary colonisers to thrive on the organic materials and lead and increased the corrosion rates of the metals.

A parameter which was not measured on the sites but was observed to have an effect on the bronze specimens at the Duart Point wreck site was the current. The strong current

prevalent on this site adversely affected the specimens by removing protective corrosion products thereby increasing the rate of corrosion. Thus, although this research has not totally quantified the effects, it has shown the importance and implications of monitoring various chemical and biological parameters.

The most significant implication of the results to the archaeological record was the rapid deterioration of some of the organic materials.

Canvas and rope underwent complete deterioration within 32 weeks due to the action of micro-organisms present in aerobic seawater. Thus the presence of artefacts manufactured from these materials on wreck sites would imply that the ship had stabilised within an anaerobic environment relatively rapidly and had subsequently not been re-exposed for a long period of time.

Under the right environmental conditions, deterioration of those materials which survived the primary colonisation was greatly affected by the colonisation of macro-organisms. Wooden artefacts which have been submerged within anaerobic sediments for any length of time tend to consist mainly of lignin, the cellulose having been removed by micro-organisms and chemical dissolution (Squirrell and Clarke, 1987: 153-162; Mouzouras *et al.*, 1990: 173-188). The remaining structure, which is predominantly lignified, can subsequently be consolidated and conserved and, archaeologically, little information is lost. However, the effects of colonisation of exposed wooden artefacts made from oak, by boring and surface encrusting organisms, such as *limnoria* and barnacles, would potentially lose not only the artefact itself but the technological evidence contained within the artefact. Consider, for example, the carved wooden cherub from the Duart Point wreck site (Figure 9.1); the age of the barnacles was found to be less than six months indicating the rapid rate of colonisation.

In addition, the presence of such organisms on artefacts which had been excavated from sealed archaeological deposits would indicate that there had been previous episodes of covering and uncovering of the wreck site. This information would help in providing a better understanding of the wrecking process, subsequent stabilisation and interpretation of the shipwreck.

Similarly, the macro-algae which grew on the wood would affect the long term stability of artefacts. I measured the length of mature kelp fronds on the Duart Point wreck site, of the kind found on the wood and leather, and they were often in excess of two metres and would be capable of transporting exposed artefacts with the current. I witnessed this phenomenon on the Duart Point wreck site when an exposed Hebridean *crogan* pot had been used as a kelp hold-fast (Figure 9.2), the fronds of which acted as a "sail" and transported the pot down-current.

The salinity, type of metal and water current were factors which were observed to affect the corrosion rate and, hence, what would potentially survive in the archaeological record.

Salinity affected all the metals used in this experiment. The higher the salinity the faster the steel and bronze specimens corroded, whereas lead specimens corroded in low salinities. However, the conditions for corrosion of the lead may not occur in the natural environment as, even in "fresh water", dissolved salts would potentially inhibit corrosion. Paradoxically, the salinity of the water over a site may enhance the long-term preservation of steel/iron artefacts. It was apparent that the salinity of the control laboratory experiments enhanced the formation of consolidating corrosion products, which effectively slowed the rate of corrosion. The corrosion rate of those specimens in deionised water progressed unabated, because no solid corrosion products were formed. Thus the rate of corrosion of steel/iron artefacts in fresh water may potentially be greater over time than those in seawater due to the lack of protective corrosion products.

Conversely, corrosion of the bronze specimens was negligible in deionised water and this may be the case for artefacts in fresh water. Corrosion did increase with increasing salinity, but the greatest effect on the bronze specimens appeared to be the water current. Those specimens on the Duart Point site showed fresh surface corrosion products at each sampling interval due to repeated surface scouring. Thus bronze or copper artefacts which are exposed to strong currents will potentially be corroded at a faster rate due to the absence of protective corrosion products.

Apart from the deionised water adversely affecting the corrosion rate of the lead, macro-fouling organisms increased the initial corrosion of the exposed lead. However, in the long term these may protect the lead as, once the metal has been colonised and an equilibrium established under the colony, corrosion would be minimal. However, artefacts themselves may be damaged by the effects of the adherence of the organisms.

Summarily the results have shown that chemical and biological processes play as equal a part as the physical processes in the formation of a wreck. The presence or absence of certain types of artefact material, and organisms on artefacts will indicate the rate of stabilisation of a wreck site and through an understanding of the environmental constraints of different organisms it may in addition tell us about the physical processes that have operated on a site prior to excavation. Such factors show that each site should be considered on its own terms; obviously there would be similarities in the processes acting on sites, but only by systematically analysing the processes operating within a site would we be able to obtain a clearer picture of the formation of a wreck upon its excavation.

Environmental monitoring for *in situ* preservation of excavated material is complimentary with the study of the formation of a shipwreck. The results of this thesis have shown which chemical and biological parameters are important to measure for the stabilisation and preservation of artefacts excavated, recorded and then preserved *in situ*. As with the interpretation of the archaeological record the correlation of the chemical and biological parameters with measured deterioration of materials has indicated **what**

parameters are important to measure and **why** they need to be monitored to effectively preserve and manage sites which are preserved *in situ*.

### 9.3 Evaluation of this research project

Completion of a Masters degree prior to this thesis was exceedingly beneficial as it enabled me to familiarise myself with the literature regarding formation processes and *in situ* preservation of materials. However, as discussed I felt that the majority of this material was based on observational interpretation of chemical and biological processes. A more empirical approach was required in order to quantify which of these processes were important in terms of the formation of the submerged archaeological record and its future preservation *in situ*.

In hindsight a smaller cross section of materials would have made the logistics of the experiments easier. However, as there was very little archaeological literature to suggest rates of deterioration of various materials and most biodeterioration literature considered modern synthetic materials it was a matter of trying to incorporate a broad cross section in the hope that some of the materials would deteriorate within the short period of this experiment. In hindsight a further initial period of literature research into early literature on biodeterioration, of the period when natural materials were still used extensively in maritime contexts, would have indicated the rapid rates of deterioration of certain materials. However, due to the limited length of a PhD, that is a three year project, it was of paramount importance to get the experimental element of the research up and running. In this way I could, and did, research the aforementioned literature as the experiments were running.

Regarding the setting up of the experiments, the specimen sizes chosen could usefully have been much larger. Although it was possible to collect and analyse the specimens at the size used, it would have been easier if the specimens had been larger. I would suggest for future experiments the following for specimen size: wood blocks to be at least 10cm x 10cm x 1cm; rope (three strand 1cm diameter worked well) to be at least 30cm long; canvas and leather to be in 30 x 10cm strips and to use complete bone shafts. The size of the metals was sufficient for the analytical method but a slightly bigger size would make collection easier.

Because the importance of the microbiological parameters had not been recognised the initial laboratory experiments did not strictly serve as controls due to contamination by airborne organisms. This could have been limited if they had been prepared as for the microbiological controls in sterile water with plugs which allowed oxygen to pass but not microorganisms. Bearing this in mind, the laboratory experiments served to act as a control for chemical processes, despite microbial contamination. Simulating seasonal change and the various parameters operating in the marine environment is extremely

difficult and the validity of small scale laboratory experiments such as this is therefore questionable.

The field experiments ideally would have been conducted on three archaeological sites, or at least on sites which were all in the marine environment (the Albert Dock was brackish). In this way the results would have been more comparable and the effects of the seasonality on the fouling organisms could have been assessed more readily. Nevertheless the Albert Dock did provide some extremely interesting results. The fact that it was not possible to work on three archaeological sites does reflect the problem that there is a dearth of ongoing field research around the UK at present.

The strategy of placing the specimens onto the sediment rather than within it was the best option bearing in mind that continual disturbance due to sampling would have rendered the sediments atypical. The method of stringing specimens across trays and anchoring these to the seabed worked in the sheltered environment of the Albert Dock yet as was shown on the Alum Bay, sea movement in the more capricious marine environment could unfortunately cause their loss. To prevent this from happening a much heavier duty system would be required, such as attaching specimens to stakes constructed from inert materials which were set well into the seabed or concrete. The initial expense of procuring, preparing and deploying such a system would be offset by the peace of mind the dynamic nature of the underwater environment was unlikely to cause the loss of a series of experiments.

The analytical methodology applied varied for each of the materials. For the general determination of the deterioration of the materials, weight loss proved to be the best method while tensile strength was predictive for textiles and fibrous materials. The chemical assay methods used for the bone and leather specimens did yield interesting results about the potential importance of the Biochemical Oxygen Demand. However such methods only tell us the state of the material left behind - not the actual rate of deterioration. This fact was highlighted by the various analyses of the wood specimens. Although the methods recorded the loss of various fractions of the cell wall they did not show the true rate of deterioration. As was seen on the Duart Point site in particular serious deterioration was caused by *Limnoria*, in some cases to the extent of virtually destroying experiments.

The microbiological work was limited. The major problem being time limits and having to gain sufficient training in the appropriate methods and interpretations of results. Ideally culture media would have been selected which were best suited to isolating the likely microorganisms colonising the various materials rather than a single "general purpose medium". For example, a medium for cellulose degraders could have been used to isolate the bacteria colonising the wood, canvas or rope.

#### 9.4 Future work

Although the areas of site formation and *in situ* preservation are closely related in terms of the study of chemical and biological processes, the recommendations for future work based on the results of this thesis are slightly different.

In the first instance, regarding formation of the submerged archaeological record, there are four avenues of research which would require further investigation. First, to look at the whole gamut of physical, chemical and biological formation processes operating on a single wreck site as it is being excavated. Quantifying the physical processes, such as those suggested by Muckelroy (1977), the rates of colonisation and deterioration of various organic materials and establishing the corrosion processes of metal artefacts through their corrosion potentials and correlating these with the observed excavated artefacts. In this way a better understanding of the formation processes operating on sites would be gained and a systematic model developed which takes into account and interrelates all the various natural physical, chemical and biological processes.

Second, again considering a single site at a time, placing specimens of materials such as canvas, rope and wood on to the seabed and sampling them at the same time intervals as in this experiment but placing sets of specimens at monthly intervals throughout the year so as to correlate the rates of deterioration with the effects of seasonality. The implications of this would have a bearing on the formation of the stabilised wreck and in addition would be useful when considering the management of long term excavation projects which are excavated over a number of years and re-buried between excavation seasons.

Third, this research considered materials in isolation, the next step would be to consider the effects of interactions between materials, to see what effect they have on the deterioration and / or preservation of materials.

Fourth, a similar experiment to this but placing materials within different sediments and at different depth levels to ascertain the effects of depth and sediment type on deterioration and/ or preservation. The materials would need to be placed into the sediment in such a way as to cause minimal disturbance and the specimens should be removed at longer sample intervals than in this experiment. Depending on the nature of the sediment chosen, the sampling intervals may need to be extended to accommodate an anticipated slower rate of deterioration.

Need for continuing work on *in situ* stabilisation and preservation of sites stems from the fact that the proposals set out in section 9.2 are at this stage theoretical and require validation. As has been discussed in Chapter Eight, methods already exist to measure the redox potential (Eh) and pH of soil and shallow marine sediments. Similarly, there are methods which measure BOD and COD of aquatic environments and methods to determine the effect of soil on cotton materials such as canvas. However, all these methods tend to have been developed for purposes other than archaeology. Thus all these proposed

methods need to be researched further and developed for use as monitoring tools in management of underwater archaeological sites preserved *in situ*.

The effective *in situ* preservation and conservation of metal artefacts, predominantly iron and steel, have been successfully demonstrated in tropical climates. However, limited work has been carried out in UK waters (MacLeod, 1995: 53). The corrosion rates of such metals in these waters may be significantly different due to the lower temperatures and seawater conditions (MacLeod, 1995: 57-58). Systematic measuring and monitoring of the corrosion potentials of iron and steel artefacts on the seabed around Britain would greatly add to the corpus of material on corrosion and subsequent conservation of archaeological artefacts of these kinds *in situ*. Subsequently the feasibility, effectiveness, practicality and ethical implications of all these methods need to be assessed before they could be applied to an archaeological site.

#### **9.5 Wider implications of this research**

The wider implications of such research for archaeology are that it forces the issue of the subject becoming more interdisciplinary and demonstrates the need to call on the assistance and co-operation of other fields of expertise if we are to obtain as full an interpretation of the archaeological record as possible and / or if we are to successfully preserve it *in situ*. This is true for both land and underwater sites since there are similar problems experienced with both.

A further implication of this work is the need to study formation processes from the outset of an excavation and the processes should be integrated within a site report as part of the interpretation of the excavation, rather than as an appendix as is often the case with specialist reports.