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LUCAS HEIGHTS RESEARCH LABORATORIES

MICROBIAL ECOLOGY OF RUM JUNGLE
PART I. ENVIRONMENTAL STUDY OF SULPHIDIC OVERBURDEN
DUMPS, EXPERIMENTAL HEAP-LEACH PILES AND
TAILINGS DAM AREA

by

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LEACHING; OXIDATION; PYRITE; RUM JUNGLE; SEASONAL VARIATIONS; TAILINGS; THIOBACILLUS FERROXIDANS; PH VALUE; COPPER COMPOUNDS; HUMIDITY; EXPERIMENTAL DATA

<u>ABSTRACT</u>

The microbial ecology of the abandoned uranium mine at Rum Jungle, Northern Territory, was investigated to determine the nature and extent of microbial populations occurring in sulphidic waste areas. Several groups of bacteria were identified and population sizes were estimated, using selective media techniques. Various physicochemical parameters of each sample were determined and correlated with the occurrence of bacteria. A medium giving a high percentage recovery of Thiobacillus ferrooxidans colonies was developed.

Sulphidic waste areas were found to support a large and diverse microbial flora, with To ferrooxidans consistently occurring, although microorganisms were isolated only from the far eastern end of the tailings dam area. In White's dump, relatively low numbers of To ferrooxidans and high numbers of acidophilic heterotrophs occurred with no seasonal variation, whereas sulphur-oxidising bacteria were absent at the end of the dry season and increased to high numbers during the wet. Desulfovibrio sppowere isolated only from a zone, less than one metre high, at the very base of the dump within which conditions otherwise were aerobic. The dump supported different microbial populations in localised areas and, in two areas where To ferrooxidans was virtually absent, little pyritic oxidation appeared to be occurring.

Intermediate dump was found to differ significantly from White's. <u>T</u>. <u>ferrooxidans</u> was the major microbial species, numbers of which increased from the end of the wet season to the early dry. Other bacterial types were scarce and no anaerobic bacteria were isolated. Relatively crude temperature measurements indicated that, in the top of the dump, pyritic oxidation may be occurring more homogeneously and at higher rates than in White's dump.



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GLOSSARY

Acidophiles - organisms which live at low pH

- (a) Facultative acidophiles organisms which tolerate high concentrations of H⁺ ions.
- (b) Obligate acidophiles organisms which require high concentrations of H⁺ ions (possibly because H⁺ ions may be required for the membrane stability of these organisms).

Brock [1979]

Aerobes

- (a) Facultative aerobes organisms which are able to use alternative electron acceptors when oxygen is not available and do not require oxygen for biosynthesis.
- (b) Obligate aerobes organisms which require oxygen for growth (i.e. cannot use alternative electron acceptors) and biosynthesis.

Brock and Brock [1973]; Brock [1979]

Anaerobes - organisms which are unable to use oxygen as the terminal electron acceptor

- (a) Aerotolerant anaerobes organisms which do not use oxygen, but are not harmed by it and thus are able to grow in the presence or absence of oxygen.
- (b) Obligate anaerobes organisms which do not use oxygen and to which oxygen is toxic.

Brock and Brock [1973]; Brock [1979]

Autotrophs - organisms which obtain all their cellular carbon by the fixation of ${\rm CO_2}$ using the Calvin cycle, which is unique to them, for ${\rm CO_2}$ reduction.

- (a) Chemoautotrophs organisms which obtain their cellular carbon from CO2 and obtain all their energy requirements by the oxidation of inorganic substrates.
- (b) Facultative autotrophs organisms which use CO_2 as the carbon source and organic compounds as the energy source.

Brock [1979]

Bacteria - a group of diverse and ubiquitous procaryotic single-celled organisms, generally about 1-10 microns and having a spherical, rod or spiral shape.

Singleton and Sainsbury [1978]

Bacterial leaching - the solubilisation of metals from dumps of low-grade ore (the 'waste' from the mining of richer ore) by percolation of acidic Within such dumps, bacterial activity results in the oxidation of mineral sulphides and solubilisation of metals.

Kelly et al. [1979]

- "the bacterial leaching of metal sulphides can be considered as a bjochemical oxidation process which is catalysed by microorganisms." Berry and Murr [1978]
- Ecology "... organisms interact with each other and also with the physical conditions which are present. Thus, organisms and the physical features of the habitat form ... an ecosystem.... In every natural situation, the environment affects the organisms present and, to a greater or lesser extent, the organisms affect the environment." Clarke [1954]
 - "that branch of biology entirely abandoned to terminology." Clark [1968]
- Heterotrophs organisms which use organic compounds both for cellular carbon and as an energy source.
 - mixotrophs : organisms which use organic compounds as the carbon source and inorganic compounds as the energy source.

Brock [1979]

Metabolism - "all of the reactions by which an organism converts nutrients into cell material and energy."

Brock and Brock [1973]

Microaerophilic

(a) "refers to an environment in which the partial pressure of oxygen is lower than that which occurs under normal atmospheric conditions - but which is not quite an anaerobic environment."

Singleton and Sainsbury [1978]

(b) organism - one which requires oxygen at pressures lower than that found in air (i.e. lower than 0.2 atmospheres).

Brock [1979]

(c) microorganisms requiring high carbon dioxide concentration rather than low oxygen tension have been 'misnamed' as microaerophiles.

Hawker and Linton [1971]

Pollution - the appearance of some environmental quality to which the exposed community (or organisms) has an inadequate response.

Cairns and Lanza [1972]

Procaryotes - cellular microorganisms, consisting of the bacteria and bluegreen algae, which have a nuclear region not bounded by a membrane.

Brock and Brock [1973]

Thermophile - an organism which has an optimum growth temperature above 45°C.

Singleton and Sainsbury [1978]

Water activity (a_W) - an expression for the amount of available water in a given substrate, " a_W is 1/100th of the relative humidity of air which is in equilibrium with that substrate... Most bacteria fail to grow if the water activity of the medium is below about 0.92 ... while the growth of some halophilic bacteria continues at an a_W of 0.75."

Singleton and Sainsbury [1978]

- relative humidity (RH) is given by the expression

RH = $P/P_0 \times 100$, where P = vapour pressure of the solution and P_0 = vapour pressure of pure water.

Hawker and Linton [1971]

1. INTRODUCTION

The release of acid and heavy metal pollutants into the surrounding environment in drainages from mine workings, waste ore dumps, tailings areas and coal heaps has been a worldwide problem for many years, but the essential role of bacteria in the process has only been recognised in the past thirty years [Kelly et al. 1979].

Microbial involvement in the leaching of sulphide ores was clearly demonstrated in the 1950s [Bryner et al. 1954; Bryner and Jameson 1959]. Since then many mining companies, especially copper mining ones, have incorporated in their operations leaching procedures which are becoming increasingly important in the extraction of minerals from low-grade ores [Brierley 1978]. For example, 11.5 per cent of the total copper production in the United States is realised from the leaching of low-grade copper waste ores [Wadsworth 1975]. Various techniques used in commercial leaching operations are described by Zajic [1970]. However, the generation of sulphuric acid and release of heavy metals from sulphidic mine sites still generally remains a considerable pollution problem and ways of controlling these processes, both during the life of the mine and after operations have ceased, are becoming increasingly essential.

The offending material responsible for the release of acid and heavy metals, which have such deleterious effects on the viability of flora and fauna in the environs of these mines - for example, at the site of the abandoned uranium mine at Rum Jungle, Northern Territory - is the sulphuric material (especially iron pyrites) associated with the particular mineral being mined. Iron pyrites is a ubiquitous mineral, often found in association with other minerals of economic significance (e.g. copper/lead/zinc/nickel sulphides, coal, uranium).

Pyrite in underground sulphidic ore bodies is stable over long periods of geological time [Brock 1978], although most sulphidic ore bodies have a 'cap' consisting of a zone of oxidised minerals which have resulted from the oxidation of the upper layer of the ore body by percolation of oxygen-containing surface waters [Kusnetsov et al. 1963]. When such ore bodies are disturbed (e.g. by mining operations) the material is fractured and the excavation of the ore body and the piling up of 'waste' material into large dumps allows vast surface areas of sulphidic material to be exposed to air and water, and allows access of microorganisms to such surfaces.

Where it has access to air and water, pyrite reacts to form soluble iron sulphate and sulphuric acid:

$$FeS_2 + \frac{7}{2}O_2 + H_2O \rightarrow Fe^{2+} + 2SO_4^{2-} + 2H^+$$
 (1)

Ferrous iron may then be oxidised to ferric iron:

$$Fe^{2+} + H^{+} + \frac{1}{4}0_{2} \rightarrow Fe^{3+} + \frac{1}{2}H_{2}0$$
 (2)

Ferric iron is an oxidising agent and may react with solid sulphides:

$$8Fe^{3+} + MS + 4H_2O \rightarrow M^{2+} + SO_4^{2-} + 8H^+ + 8Fe^{2+}$$
 (3)

where M is a divalent metal.

Reactions (1), (2) and (3) appear fairly simple, straightforward chemical However, the mechanisms of pyrite oxidation, especially in dump equations. situations, are extremely complex and not well understood biogeochemical processes. It is known that the oxidation of pyrite is greatly accelerated in presence of microorganisms, particularly the bacterial Thiobacillus ferrooxidans. Winchell, as described by Smi rnov [1955],suspended powdered pyrite in air-saturated water for 10 months after which less than 0.1 per cent of the pyrite was solubilised [Kusnetsov et al. 1963]. However, Atkins [1978] leached pyrite in the presence of T. ferrooxidans, and achieved 100 per cent solubilisation of pyrite at 2 per cent pulp density in about 21 days.

Living organisms are catalysts of chemical reactions, and microorganisms, especially bacteria, are essential catalysts of many important geochemical reactions (see, for example, Brock [1979] who has given a detailed description of the role of bacteria in biogeochemical 'cycles', e.g. the carbon, sulphur and nitrogen cycles in the transformation of metals). Biological catalysis is rapid and effective and occurs at low temperatures. "Low temperature reactions are of great geochemical significance, because most of the earth is at a low temperature (i.e. the surface crust) and many important reactions would probably not occur at all (or at least, at very minimal rates) if it were not for living organisms." [Brock 1980]. Microorganisms cannot change thermodynamic relationships, but their action as catalysts increases the rates of chemical reactions [Brock 1977]. For example, at acidic pH the rate of oxidation of soluble ferrous to ferric iron by <u>T. ferrooxidans</u> has been shown

to be one million times faster than the chemical reaction rate [Singer and Stumm 1970]. Also, different microorganisms (and their interactions with each other) may utilise various degradative pathways for a particular substrate whose chemical degradation, under a given set of conditions, is always the same [Brock 1978].

Microorganisms are ubiquitous and one of their most fundamental characteristics is their ability to adapt to changing environmental conditions [Le Roux et al. 1978] and to flourish in environments which may be toxic to organisms of greater cell complexity [Brock 1969]. It would be expected. therefore, that in sulphidic, especially pyritic, ores exposed to air and water, certain microbial populations, tolerant to high metal and acid concentrations and able to grow without organic carbon, could rapidly become established as the major biological component of the system. This has indeed been found to be the case. One of the major groups of microorganisms isolated by many workers from such environments throughout the world, consists of several bacterial species belonging to the genus Thiobacillus (thio Greek for sulphur and bacillus Latin for small rod). Of this group, the most widespread and important microorganism in leaching processes and therefore the most extensively studied, is $\underline{\mathsf{T}}_{ullet}$ ferrooxidans which was originally isolated and classified by Colmer and Hinkle in 1947 (see Kusnetsov et al. [1963], pp.136-138 on the occurrence of T. ferrooxidans in nature).

T. ferrooxidans obtains its carbon for cellular constituents by fixing (dissolved) carbon dioxide [Schnaitman et al. 1969]. In the autotrophic bacterial cell, ${\rm CO_2}$ is 'fixed' via the Calvin reductive pentose phosphate cycle. This is illustrated by a simple block diagram in Figure 1. more detailed description of the reactions of the Calvin cycle, see Elsden [1962] and Peck [1968]). Autotrophic Thiobacillus spp. use this pathway for CO₂ fixation; the ATP (i.e. energy) required is generated directly from the oxidation of inorganic growth substrates and the NADH (i.e. reducing power), by the reduction of NAD by reverse electron transport, the energy required for this coming from ATP [Silver 1978]. That reduced sulphur compounds and ferrous iron are used by Thiobacillus spp. as growth substrates in this cycle has been shown by Aleem [1977] and Aleem et al. [1963], as reported in Silver [1978]. The energy required for CO_2 fixation and other metabolic processes is obtained by $\underline{\mathsf{T}}_{\bullet}$ ferrooxidans solely by the oxidation of the following inorganic compounds: soluble ferrous iron; elemental sulphur; reduced sulphur compounds (soluble); mineral sulphides containing iron (by the simultaneous oxidation of the insoluble ferrous and sulphide components of the ore matrix [Duncan et al. 1967]; and non-iron-containing mineral sulphides by oxidation of the sulphur moiety [Torma 1971].

The oxidation of soluble ferrous to ferric iron by $\underline{\mathsf{T}}$. $\underline{\mathsf{ferrooxidans}}$ occurs as in reaction (2) but in two components, spatially separated in the cell envelope: the reaction

$$2e^{-} + 0_2 + 2H^{+} \rightarrow H_20$$
 ,

probably occurs within the inner membrane, whereas the reaction

$$2Fe^{2+} \rightarrow 2Fe^{3+} + 2e^{-}$$

occurs at the outer membrane [Ingledew et al. 1977]. This means that the ferric iron produced never enters the bacterial cell [Lundgren and Tano 1978].

The pairs of electrons (e⁻) from the ferrous ions are transported to a terminal electron acceptor (oxygen) via the cellular cytochrome transport chain as follows [Silver 1978]:

2Fe²⁺ cytochrome c (ox.) cytochrome a (red.)
$$^{1/2}0_2$$
+H⁺
2Fe³⁺ cytochrome c (red.) cytochrome a (ox.) $^{1/2}0_2$ +H⁺
energy

Fe²⁺-cytochrome c oxidoreductase ADP+Pi \longrightarrow ATP

The action of the enzyme iron-cytochrome c-oxidoreductase [Yates and Nason 1966; Din et al. 1967] has been shown by Din and Suzuki [1967] to be of the Ping Pong Bi Bi type; that is, each substrate molecule (in this case, Fe^{2+} ion) is bound and modified sequentially by the enzyme which is correspondingly altered.

The free energy of the above reaction is about 15 kcal/ $2Fe^{2+}$ at pH 1.5 and about 12 kcal/ $2Fe^{2+}$ at pH 3.3 [Lees et al. 1969]. The energy required for the phosphorylation of one ADP to form one ATP is between 8 and 14 kcal [Silver 1978]. The oxidation of $2Fe^{2+}$ ions to $2Fe^{3+}$ ions generates about 12 to 15 kcal, of which no more than 30 to 35 per cent is then available for ATP

formation [Tuovinen and Kelly 1974b]. \underline{T} . $\underline{ferrooxidans}$ must, therefore, oxidise large amounts of ferrous iron, not only to grow [Brock 1979] but also to maintain the metabolic processes necessary for cell survival during periods of non-growth, since CO_2 has been shown to be fixed by non-growing cultures of \underline{T} . $\underline{ferrooxidans}$ [Tuovinen and Kelly 1972].

Since the ${\rm Fe}^{2+}$ is thought to be complexed with an organic molecule (such as a protein probably secreted from the bacterial cell) before electron transfer to cytochrome c, the potential of the ${\rm Fe}^{2+}/{\rm Fe}^{3+}$ couple may be lowered from +0.77 volts to around 0 [Blaylock and Nason 1963; Dugan and Lundgren 1965] and may even be negative [Tuovinen and Kelly 1972]. Oxidised compounds, other than ${\rm O}_2$, may possibly react as terminal electron acceptors for the oxidation of ferrous to ferric iron by ${\rm T}$. ferrooxidans (whereas oxygen is the only electron acceptor of the pure chemical ferrous to ferric iron oxidation).

According to Silver [1978] this mechanism could be used by $\underline{\mathsf{T}}$. ferrooxidans to oxidise any iron in the +2 valence state, such as the insoluble Fe²⁺ in the pyrite matrix.

The ability among <u>Thiobacillus</u> spp. to oxidise ferrous to ferric iron is unique to <u>T. ferrooxidans</u>. <u>T. ferrooxidans</u>, however, may also obtain energy similarly to the other <u>Thiobacillus</u> spp. by the oxidation of reduced sulphur compounds. Most research on oxidation mechanisms has been carried out with these other spp. For comprehensive reviews of this area, see Roy and Trudinger [1970], Tuovinen and Kelly [1972], Silver [1978] and Tuovinen [1977] for studies specifically involving <u>T. ferrooxidans</u>, and Lundgren and Tano [1978] for the oxidation of matrix Fe^{2+} and Se^{2-} by <u>T. ferrooxidans</u> at the surfaces of insoluble metal sulphides.

The characteristics of Thiobacillus spp. are summarised in Table 1.

It has been well established that <u>Thiobacillus</u> spp., particularly <u>T. ferrooxidans</u>, catalyse the solubilisation of metals and the generation of sulphuric acid from sulphide ores, and their role in this process, while the mechanisms are not yet clearly understood, has been well documented [Tuovinen and Kelly 1972; Tuovinen and Kelly 1974b; Torma 1977; Brierley 1978; Kelly et al. 1979].

While Thiobacillus spp. are the only microorganisms to have been used for metal recovery on a commercial scale [Le Roux et al. 1978], many other

types of microorganisms have been isolated from acidic sulphide and sulphur environments (for example, Groudev et al. [1978] and Kelly et al. [1979]). Such microorganisms include:

- . fungi;
- heterotrophic bacteria similar to common soil bacteria but extremely tolerant to high metal and acid concentrations;
- anaerobic sulphate-reducing bacteria;
- thermophilic iron-oxidising bacteria [Brierley 1978];
- extreme thermophilic iron/sulphur oxidisers able to leach sulphide minerals at 60°C and oxidise sulphur anaerobically (<u>Sulfolobus</u> spp.)
 [Berry and Murr 1978].

Laboratory studies of such organisms have not been as extensive as those of <u>Thiobacillus</u> spp. and little is known of their role in the degradation of mineral sulphides. Even less is known of the activities of such microorganisms in the natural environment, although the 'classical' view that <u>T. ferrooxidans</u> is the only important mineral-leaching microorganism [Kelly 1976; Torma 1977] is now being modified [Kelly et al. 1979].

Although laboratory studies of bacterial leaching are numerous, studies of the microbial ecology of natural sulphidic areas, from which most of the species and strains used in such laboratory work have been isolated, are considerably fewer [see, for example, Beck 1967; Groudev et al. 1978; Murr and Brierley 1978]. There are several reasons for this; the underlying factor being the great complexity of the microbial ecology of soils generally and of sulphidic waste rock dumps in particular. Although such a dump may seem to be an extreme environment, it can be considered as only 'moderately' extreme since microorganisms show an abundance and variety of species, even though more complex organisms are extremely few or absent [Brock 1979].

The rates of microbial activity in soils are much lower and activity occurs over much longer periods than under optimised laboratory conditions and, because of this, microbial activity may be extremely difficult to measure in situ [Brock 1971].

Microorganisms in soils behave as colloidal particles and, because of their small size (about 1 μm long and 0.5 μm wide) and the generally negative electric charge on the exterior of bacterial envelopes, tend to be adsorbed onto mineral surfaces [Marshall 1976]. Also, nutrients collect in greater concentrations at interfaces and, with water, are adsorbed onto particle surfaces, and so attached bacterial cells may develop into microcolonies [Nikitin 1973; Brock 1980]. These factors make direct observation of individual cells difficult and direct microscopic identification of a particular bacterial species almost impossible.

The amount and physical nature of the material of such environments (waste rock dumps may contain millions of tonnes of material) make the cost of thorough and systematic sampling of such areas prohibitive, so that sampling is at best only patchy.

The activities of microbial populations in the field depend upon, and also affect, the physicochemical parameters of their micro-environments (such as temperature, pH, water availability, nature and concentration of soluble gases, etc.). Therefore, in ecological studies these factors should also be measured at sites sampled for microorganisms; furthermore many of these measurements are difficult, especially in situ.

Because climatic factors are an important component of the field situation and affect leaching processes, ecological studies should be conducted over long periods (i.e. several years) to determine their influence on microorganisms. Such long-term field studies, although highly desirable, may not produce results as quickly as laboratory studies and considerable foresight is needed by a funding organisation to undertake them.

Finally, the estimation of numbers of $\underline{\text{Thiobacillus}}$ spp., especially $\underline{\text{T}}$. ferrooxidans, is difficult and special media and techniques must be used for their enumeration.

However, despite the difficulties listed above, information from such studies is of vital importance to the understanding of the mechanisms of leaching of mineral sulphides in the field.

Bacterial leaching has become an increasingly important technique in the extraction of metals from low-grade ores [Brierley 1978] and most of the research into the bacterial species responsible and the mechanisms of the

biogeochemical reactions has been concerned with optimisation of the leaching process rather than its inhibition. This has occurred because such commercial operations may be extremely profitable [Lowson 1975; Brierley 1978; Kelly et al. 1979] and therefore most research has been funded by mining companies and government agencies with a view to maximising profits from the mining of lowgrade ores. Comparatively few investigations, however, into the inhibition of leaching of sulphidic waste materials have been undertaken, even in areas which have become extremely polluted by acid and heavy metal discharges from This is because research into pollution problems is generally such wastes. Effective rehabilitation of profit-consuming. mine sites, once mining operations have ceased, can be a costly process and may require long-term monitoring to ensure pollution problems do not recur. Legislation has become necessary to ensure that mine owners participate in environmental protection beyond cessation of operations. This is particularly important where pollution from pyritic oxidation can continue long after mining operations have ceased.

In 1973, the Australian Atomic Energy Commission, in conjunction with the Territory, Australia, then Department of the Northern investigation into the extent of the pollution arising from the abandoned uranium mine site at Rum Jungle, Northern Territory. One of the main aims of this program was to gain an understanding of the complex mechanisms of the generation and dispersal of acid and heavy metal pollutants. The initial results of this study are contained in the publication. Environmental Studies [Davy 1975]. In 1974, as part of this program, the AAEC (by a research contract with the School of Biotechnology, University of New South Wales) initiated an investigation into the microbial ecology of the mine site, in particular the sulphidic waste rock dumps and flooded opencuts, to determine the nature and extent of microbial activities contributing to the pollution problem.

The first objective of this study was to determine the distribution and size of populations of microbial species, known to be either directly involved in leaching mechanisms or associated with other leaching environments, within the overburden dumps and flooded opencuts. It was also the aim to correlate microbial population levels with as many physicochemical characteristics of the sample location as possible, and with climatic factors. This involved the standardisation of procedures such as sampling, analysis of isolation and enumeration of <u>Thiobacillus</u> spp. Also, it was necessary to develop a reproducible and reliable method for the isolation of colonies of <u>I. ferrooxidans</u> as an indication of viable cell numbers in the sample. The growth of this organism as single colonies on plates containing agar as the gelling agent was, at the commencement of study, more an 'art' than a routine technique [Tuovinen and Kelly 1973], and the method using silica gel was not satisfactory for the determination of viable numbers of this organism [Corrans 1970].

The second, and long-term, objective of the microbial study was

- (a) to determine whether sulphur-oxidising processes resulting in acid and heavy metal release, were chemical or biological, or a combination of these, and to devise specific methods for the control of these processes, and
- (b) to model the behaviour of sulphidic overburden dumps and flooded opencuts so that further such pollution problems might be avoided when proposed large-scale uranium mining and processing was begun at a number of locations in the Northern Territory; (see, for example, the Ranger Uranium Environmental Inquiry [1977]).

It is essential that pollution control measures are included at the beginning of future mining ventures where mineral sulphides, especially iron pyrites, are associated with the orebodies (for example, the new uranium deposits in the Alligator Rivers region of the Northern Territory and coal deposits in New South Wales and Queensland) to ensure that the pollution problems of Rum Jungle do not occur elsewhere.

The results of the first objective of the study of the microbial ecology of the Rum Jungle mine site between 1975 and 1979 are presented here for the sulphidic waste areas, and results for the flooded opencuts are presented in Goodman, Khalid and Ralph [1981].

The distance of Rum Jungle from Sydney (over 3000 km) limited field expeditions to one or two each year. The remoteness of the mine site from adequate laboratory facilities unfortunately meant that most analyses were performed some time after sample collection.

2. THE RUM JUNGLE MINE SITE

A major uranium mining operation in the Northern Territory, Australia, was commenced in 1953 at Rum Jungle, 64 km south of Darwin. The area is highly mineralised and uranium, copper, lead, zinc, nickel and cobalt ores occur [Roberts 1960]. The uranium deposits were mined by opencut operations and processed on site for uranium oxide and copper. During the operation of the mine, 1953 to 1971, the release of process effluents and, from 1966, the leaching of sulphidic waste rock dumps and sulphidic stockpiles caused considerable sulphuric acid and heavy metal pollution of the water systems of the area. In 1971, commercial operations ceased and the mine was abandoned, leaving three overburden dumps, an experimental heap leach pile, an extensive tailings dam area and three opencuts which are now flooded [Davy 1975], all of which are contributing to continued acid and heavy metal pollution of the surrounding areas.

The climate at Rum Jungle is tropical and monsoonal with two distinct seasons: a dry season from April to October (with virtually no rain from May to September) and a wet season from November to March (with about 89 per cent of the annual rainfall occurring between December and March), during which the mean annual rainfall is about 1.5 m with most of the rain falling heavily in short thunderstorms [Daniel, Harries and Ritchie 1980b]. However, rainfall data from Darwin have shown quite large fluctuations in annual rainfall in particular wet seasons.

A summary (taken from Davy [1975]) of the major areas responsible for the generation of pollutants, and consequently the areas most intensively investigated in the microbial ecology study, is given below.

2.1 White's Overburden Dump

The dump, of 6.9×10^6 tonnes, was completed in 1958. It contains a heterogeneous mixture of carbonaceous slates, graphitic schists, phosphate, sulphate and sulphide minerals, with about 3 per cent total sulphur content. It is assumed that the sulphur is in the form of mineral sulphides, principally pyrite (FeS₂). Other mineral sulphides present include chalcopyrite (CuFeS₂), chalcocite (Cu₂S), covellite (CuS) and galena (PbS). The dump is 13 to 18 m high, with steep sides, and has a porosity of about 0.4 [Ritchie 1977]. By the middle of the wet season, well defined springs develop near the base of the eastern wall and continue to flow into the early part of

the dry season. This dump is the largest single source of acid and heavy metal pollution of the river flowing through the mine site (the east branch of the Finniss River).

2.2 Intermediate Overburden Dump

This dump, of 1.1×10^6 tonnes, was completed in 1964. The Intermediate orebody differed from White's in that it was mined only for copper. The orebody consisted of sulphide minerals, mainly chalcopyrite and with some chalcocite and bornite (Cu_5FeS_4) in a graphitic schist, and was covered by an extensive cap of copper oxides. The host rock was principally pyritic graphitic shale. The overburden dump is approximately square and about 11 m high, although the south-west area is about 5 m higher than the rest of the dump. Pyrite is visible all over the dump. The walls of the dump are fairly steep and represent 30 per cent of the total area and, because of their open nature, probably absorb most of the rain falling on them during the wet season. The dump is thought to have a porosity of about 0.4, similar to White's dump. Small springs have been noted at the base of the heap, but flow from these ceases shortly after storms. It is thought that water draining through the dump eventually enters the East Finniss River system.

2.3 Copper Sulphide Heap

This heap was constructed in 1965 to recover copper, by bacterial leaching, from the rejected mill grades of copper ore from the Intermediate orebody. This subgrade ore contained about 0.7 to 2.0 per cent copper (as copper sulphides) in a host rock of pyritic graphitic shale. The heap was constructed on top of a 10 cm layer of bituminous concrete (a material known to have high seepage rates). It is rectangular, 10 m high, with steep walls and a flat top, of which the surface 2 m consists of material unavoidably crushed and compacted by trucks during construction. Drainage from the heap was collected in a series of ponds constructed at its base; (for a detailed description of the operation of the leaching experiment, see Davy [1975], pp.6.15-6.19).

2.4 Tailings Dump Area

During 1954-1961, about 6.4 x 10^5 tonnes of tailings material from the processing of all the orebodies were discharged into a dispersal area north of the treatment plant, and this area is now known as the Old Tailings Dam.

Runoff from this area is contaminated by acid and heavy metals.

MATERIALS AND METHODS

3.1 Field Work

3.1.1 Sampling procedures

During 1975 and early 1976, rock and soil samples were collected from holes dug at, or near, the surface by means of a pick and shovel. Penetration to a depth greater than 1 m was not practicable by this method.

During late 1976, 1977 and 1979, sampling trenches were cut in White's and Intermediate overburden heaps with a backhoe, and a penetration of between 3 and 5 m at each site was achieved. Systematic sampling was then carried out at various levels.

3.1.2 Collection of solid samples

The material was collected with either a trowel, spatula or spoon and transferred directly into sealable, polyethylene bags (radiation-sterilised) or screw-top polyethylene containers (250 mL capacity; pre-sterilised with 12 per cent w/w ethylene oxide in dichlorodifluoromethane - Sterigas 27, CIG, Australia). After collection, the samples were stored at 4°C in the field laboratory, then airfreighted to Sydney and stored at 4°C in the main laboratory, pending examination. All samples, solid and liquid, were stored in this manner.

3.1.3 In situ measurements

During 1978 and 1979, the relative humidity in small holes, about 4 cm diameter, 30 cm deep, which had been made by jabbing with a crowbar in the sampling trenches of the overburden heaps, was measured using a portable Shaw (England) Varsity Hygrometer model SVH with gas sensor, adapted to battery operation. Temperature was measured in these small holes using a calibrated, portable thermometer fitted with an extension resistance probe.

3.1.4 Collection of water samples

- (i) Samples for microbiological analysis were transferred directly into pre-sterilised 150 mL or 250 mL screw-top polyethylene containers and stored at 4°C .
- (ii) Samples for chemical analysis were collected as in (i) above and filtered, using a $100\,$ mL syringe, through pre-sterilised pre-filters (Sartorius glass fibre, No. SM13400) into sterile screw-top polyethylene containers, at the field laboratory as soon as practicable after collection. The samples were then stored at 4°C .

3.2 Laboratory Work

3.2.1 Preparation of solid samples

Solid samples were ground with a sterilised mortar and pestle, under clean-air conditions, to a particle size of approximately 120 microns and restored in sterile polyethylene containers at 4°C.

For solid samples collected in 1978 and 1979, small particle size material only was used for analysis; cohesive rock material larger than 3.5 mm was excluded.

3.2.2 Moisture content of solid samples

Weighed aliquots containing 3 to 7 g of ground samples, were dried to constant weight at $105 \pm 0.5^{\circ}\text{C}$ in tared sintered glass crucibles (50 mL Pyrex No.3). Each determination was made in triplicate and the mean moisture loss value for each sample was recorded.

3.2.3 Water-solubles content of solid samples

Dried samples from 3.2.2, retained in sintered glass crucibles, were washed with 200 to 300 mL distilled water under vacuum suction, until filtrates were free of chloride and sulphate ions. Washed samples were dried to constant weight at $105 \pm 0.5^{\circ}\text{C}$. Loss in weight was recorded as water solubles, expressed as mg/kg dry weight (i.e. $\mu\text{g/g}$).

The washings were evaporated to dryness and the residue re-dissolved in 50 mL 2N HCl. These solutions were analysed for Fe, Cu and Zn by atomic absorption spectrometry, using a Varian instrument with modular updating to the A.A.5 level of performance.

3.2.4 Solute content of water samples

Five mL aliquots of water samples, treated as in 3.1.4, were dried to constant weight in small tared Pyrex beakers at $105 \pm 0.5^{\circ}$ C. The weight of the residues was recorded as water solubles, expressed as mg/L.

Further aliquots of the above samples were diluted to an appropriate volume in 2N HCl and analysed for Fe, Cu and Zn by atomic absorption spectrometry.

3.2.5 pH of solid samples

Weighed aliquots (2 to 5 g) of ground, solid samples were agitated in a test tube by a Vortex mixer (Touch-Plate Supermixer, Lab-Line Instruments), with 9 mL of distilled water. The slurry was allowed to stand for 1 to 3 hours. The pH of the supernatant was measured using a Philips digital pH meter (model PW 9408) fitted with a combined KCI-glass probe and calibrated using standard buffers.

3.2.6 pH of water samples

The pH of 5 mL aliquots of water samples, treated as in 3.1.4, was measured using a Philips pH meter as in 3.2.5.

3.2.7 Preparation of solid samples for microbiological examination

Accurately weighed amounts (2 to 5 g) of ground, solid samples were transferred to sterile, capped test tubes and 9 mL of sterile distilled water was added. The slurry was agitated for several minutes by a Vortex mixer and allowed to settle for 2 to 3 hours (stored at 4° C). The supernatant was then diluted in the salt solution appropriate to the particular group of microorganisms; 0.1 mL of several dilutions was then added to the appropriate plates and spread uniformly with a sterile glass rod.

3.2.8 <u>Preparation of water and sediment samples for microbiological</u> examination

Samples were shaken thoroughly and then allowed to settle for 1 to 3 hours at 4°C. Supernatant samples were then diluted in the salt solution appropriate to the particular group of microorganisms and plated out as in 3.2.7. Several attempts were made in the field to transfer small aliquots of water samples directly onto pre-sterilised membrane filters, store the filters at 4°C and transfer them again to the appropriate media plates in Sydney. However, this proved unsuccessful as the numbers of microorganisms were generally too high to allow plating without dilution. The cost of membranes for analysis of several dilutions of the number of types of microorganisms required would have proved prohibitive.

3.2.9 Enumeration of microorganisms

At the beginning of the project A.M. Khalid spent considerable time developing simple, reliable methods for the determination of the viable counts of acidophilic <u>Thiobacillus</u> species, as no completely dependable methods had been reported in the literature.

It was decided to use media which would select for several groups of microorganisms, both known and thought to be important in the leaching of mineral sulphides. Also, the numbers of heterotrophs were examined for comparison and, initially, two media were used: nutrient agar and Czapek-Dox agar. As there appeared to be little difference in the types or numbers of microorganisms growing on these plates, Czapek-Dox agar was not used after 1977. (Czapek-Dox data are not presented here.)

In 1977, a medium for isolating acidophilic heterotrophs [Manning 1975] was added, and in 1978 this medium was modified and used to select for autotrophic sulphur-oxidising microorganisms at pH 4.8.

The counting method employed was the standard microbiological method of plating out various dilutions of samples onto selection plates, either in duplicate or triplicate, incubating these at 30°C, counting the number of colonies growing and then recording the mean value for the appropriate dilution.

(i) T. ferrooxidans

This species is one of the most important of the bacteria involved in mineral sulphide degradation. These microorganisms were grown on silica gel plates, since numerous attempts to make consistent and reliable counts of $\underline{\mathsf{T}}$. ferrooxidans on several agar media were unsuccessful. The method of counting these microorganisms and also the method of obtaining consistently high quality silica gel plates, as developed by A.M. Khalid for this study, has been reported by Goodman et al. [1980].

(ii) Acidophilic sulphur-oxidising microorganisms (representative type T. thiooxidans)

These microorganisms were selected on 9K [Silverman and Lundgren 1959] thiosulphate agar; the method of preparation follows:

Solution A: The basal 9K medium salts, without ferrous sulphate, of Silverman and Lundgren [1959] were dissolved in 500 mL distilled water and the pH adjusted to 3.5 with 10N ${\rm H_2SO_4}$.

Solution B: 7.5 g of Kobe agar (White Bear brand) was suspended in 400~mL distilled water.

Solution C: 10 g of ${\rm Na_2S_2O_3.5H_2O}$ (analytical reagent grade) was dissolved in 100 mL distilled water.

Solutions A and B were sterilised by autoclaving at 15 psi for 15 minutes, and solution C, by filtering through a membrane filter (Sartorius, SM-11106, 0.45 μ). When solutions A and B were at about 45°C, A was added to B asceptically and then solution C with vigorous mixing. The medium was poured quickly into sterile plastic petri dishes (Medical Plastics brand). The plates were dried at 30°C for several hours and stored at 4°C prior to use. It is important that the mixture of solutions A and B is not too hot when solution C is added; if it is, the thiosulphate will decompose producing a white precipitate of elemental sulphur and the pH of the plates will be above 5. The pH of these plates was between 3.5 and 4.0.

(iii) <u>Intermediate pH sulphur-oxidising microorganisms</u> (representative type <u>T. intermedius</u>)

These microorganisms were selected on thiosulphate agar plates with a pH

of 4.8 and incubated for 1 to 2 weeks at 30°C . The method of preparation of the plates was as in (ii) above, except that the basal salts solution was that of Manning for acidophilic heterotrophs [Manning 1975] without pH adjustment, yeast extract or glucose. The mixture of solutions A and B was allowed to cool to about 55°C before the thiosulphate solution was added.

(iv) Non-acidophilic sulphur-oxidising microorganisms (representative type T. thioparus)

These microorganisms were selected on thiosulphate agar plates with a pH of 6.2 and incubated for 1 to 2 weeks at 30°C. The plates were prepared as in (ii) above, except that the basal salts solution was that of Vishniac and Santer [1957], with the modification that only 5 mL/L of trace element solution was used. This trace element solution was sterilised by filtration and added aseptically to solution A when it had cooled to about 50°C. This method prevented the precipitation of heavy metal salts, which would have occurred if the trace element solution had been added to solution A before autoclaving. Solution B was then added, followed by thiosulphate solution.

(v) Acidophilic heterotrophs

These microorganisms were selected on glucose plates having a pH of 3.5. The plates were prepared as in (ii) above, except that the basal salts solution was that of Manning's acidophilic heterotroph medium, without yeast extract or glucose [Manning 1975]. The pH of solution A was adjusted with 2N ${\rm H}_2{\rm S0}_4$ to give a pH of 3.5 in the final plates. Solution C consisted of 10 g of D-glucose (analytical reagent grade) instead of the thiosulphate. If solutions A and B are mixed before autoclaving, the acidic component of A will hydrolyse the agar of B and the resulting sterile solution will not gel when cool. The glucose solution was sterilised by filtration to prevent possible caramelisation during autoclaving.

(vi) Other autotrophic microorganisms

<u>Desulfovibrio</u> spp. and <u>T. denitrificans</u> were isolated on media described by Pankhurst [1971] (modified Baar's medium) and Hutchinson et al. [1965] respectively.

Aliquots of samples were transferred directly (in the field) to 5 mL McCartney bottles, containing the appropriate sterile medium, and subsequently incubated at 30°C in the laboratory in Sydney. The presence of these microorganisms in the samples was recorded as either positive (+) or negative (-). Plates containing 0.1 mL aliquots of sample dilutions, for counting and identification purposes, were incubated anaerobically at 30°C for 1 to 2 weeks.

Algal colonies, identified as a species of <u>Chlorella</u>, were observed growing on the media for acidophilic heterotrophs and intermediate pH, sulphur oxidisers. After 2 weeks' growth at 30° C, the colonies were small (less than 1 mm in diameter), round and dark green.

4. RESULTS

All samples collected from the Rum Jungle mine area between 1975 and 1979 were analysed for pH; total soluble solid concentration; soluble copper, iron and zinc concentrations; numbers of specified bacterial species and also moisture content in the case of rock soil samples. These results are contained in Appendices A, B, C and D, together with the locations of all sampling points, and short descriptions of the areas. The data on sample locations immediately precede the results of analysis on the samples. Particular areas are grouped together chronologically.

The logarithm (base 10) of all variables except pH, moisture content and temperature, was taken to reduce the variability, to equalise variances especially of bacterial numbers and to normalise the distributions [Niemala and Tuovinen 1973; Weir 1978]. It should be noted that the mean (x) value of data transformed to logarithms is the geometric mean of the original data.

4.1 <u>Initial Investigations During September 1975 and March 1976</u>

Results of all samples analysed and sample locations are contained in Appendices A and C.

The mean values (and standard deviation, s.d.) of the transformed data for samples collected from White's and Intermediate dumps and the sulphide and oxide heap leach piles are shown in Tables 2 and 3.

4.2 Tailings Dam Area

Results of sample analysis and their locations are contained primarily in Appendix B, with a few in Appendices A and \mathbb{C}_{\bullet}

The mean values (and s.d.) of the transformed data for these samples are shown in Tables 4 and 5. The distribution, with depth, of parameters of samples taken during a dry season, September 1976, is illustrated in Figures 2-4.

4.3 White's Overburden Dump, Sampled September 1976 and March 1977

Details of the location of each sample together with field observations are set out in Appendix C together with the physicochemical and microbiological data for these samples.

Mean values (and s.d.) of the data, transformed to logarithms (base 10) where necessary, are contained in Tables 6-10. Statistical comparison of the means is also shown in Tables 6-9. Table 11 contains the means of the variables for the three areas sampled in the top of the dump during the late dry season in 1976 (September) and during the late wet season the following year (March). Where the probability (P) indicated that the means of a particular variable in a particular area were not significantly different, i.e. $P \ge 0.1$, they were combined to give one new mean value for that area. Note that the March sampling holes were deeper than the September ones and so the two levels in the March holes were treated separately and compared statistically (Table 9).

The distribution of several parameters with depth is illustrated in Figures 5-10. Data from samples collected from White's Spring No.4 at various seasons (only one sample was taken each time) are shown in Table 12.

The sites of wet and dry season trenches are shown in Map 1, along with the sites of some of the AAEC probe holes used for temperature measurements.

4.4 Intermediate Overburden Dump, Sampled June 1978 and May 1979

Details of the location of each sample and relevant field observations are set out in Appendix D, together with the physicochemical and microbiological data for these samples.

Mean values (and s.d.) of the data, transformed to logarithms where necessary, are contained in Tables 13-16. Statistical comparison of the means is also shown in these tables. Table 17 contains the means of the variables for Intermediate dump; where the probability (P) indicated that the two means of a particular variable (i.e. that of the June and May values) were not significantly different, i.e. $P \ge 0.1$, these two means were combined to give one new mean value for the June and May samples. Temperature and relative humidity measurements are shown in Tables 18 and 19 for June 1978 and May 1979 respectively. The distribution of several parameters with depth is illustrated in Figures 11-14. The site of each trench in shown in Map 2.

5. DISCUSSION

5.1 <u>Initial Investigations During September 1975 and March 1976</u>

The soil samples taken during this time were all from a depth of less than 1 m at any location, since sampling holes were dug by hand and penetration to greater depths was not practicable. The results of these initial investigations illustrate the extreme heterogeneity of the sulphidic waste rock areas of the mine site (see Appendix A), both between and within particular heaps.

However, several factors emerged from a qualitative comparison of the means of parameters from the four heaps mainly responsible for the acid and heavy metal pollution (Tables 2 and 3), i.e. White's, Intermediate, sulphide and oxide heaps.

It seemed that the pH of soil samples from White's heap was higher, during both the dry and the wet, than the pHs of soil samples from Intermediate, sulphide and oxide heaps.

Also, concentration levels of total soluble solids, soluble copper and iron were lower in soil samples from White's heap than in soil samples from Intermediate, sulphide and oxide heaps. However, during the wet season, the levels of these in springs flowing from White's heap were comparable to water samples from Intermediate, sulphide and oxide heaps.

Numbers of <u>T. ferrooxidans</u> were generally found to be between 10^2 and 10^3 cells/g (D.W.) or /mL, in both the wet and dry seasons. These levels are

comparable to those found by Beck [1967], who studied $\underline{\mathsf{T}}_{ullet}$ ferrooxidans numbers in various leaching solutions from several mining sites. Since the amount of energy available to T. ferrooxidans from the oxidation of inorganic substrates, especially ferrous iron, is low [Lees et al. 1969], relatively low numbers of bacteria must be able to oxidise quite large amounts of inorganic substrates, both to grow [Brock 1979] and to maintain viability by fixation of CO_2 even when the cells are not growing [Tuovinen and Kelley 1972]. appeared that population levels of $\underline{\mathsf{T}}$. $\underline{\mathsf{ferrooxidans}}$ did not change from the dry to the wet seasons. $\underline{\mathsf{T}}$. $\underline{\mathsf{ferrooxidans}}$ might be regarded as a 'steady state' population which loses cells (e.g. by death, 'migration' or washing away) at the same rate at which new cells are being generated. Bacteria in such a steady state, although degrading large amounts of substrates, may not show much change in population level over long periods and thus counting of their numbers gives no indication of their growth rates, nor the rates of degradation of their substrates [Brock 1971].

It was somewhat surprising to find populations of sulphur-oxidising Thiobacillus spp. often at levels 10 to 100 times higher than those of $\underline{\mathbf{I}}$. ferrooxidans. In White's heap, high pH, sulphur oxidisers were isolated, along with $\underline{\mathbf{I}}$. ferrooxidans, from acidic areas containing large amounts of soluble solids, during both the dry and wet seasons. However, population levels of acidophilic S-oxidising bacteria were high during the wet for all four heaps and negligible during the dry. Although several studies have shown that these types of $\underline{\mathbf{I}}$ high are able to oxidise the sulphide moiety from mineral sulphides [Goroll 1976; Khalid and Ralph 1977; Khalid 1978], their growth on these substrates is slow compared to growth on sulphur compounds such as $\underline{\mathbf{S}}^0$, $\underline{\mathbf{H}}_2\mathbf{S}$, $\underline{\mathbf{S}}_2\mathbf{O}_3^2$ and other polythionates [Khalid 1978]. For populations of these types of bacteria to have been so high, their growth substrates would probably have been reduced sulphur compounds, rather than solid sulphides. This observation raised two questions:

- (a) What is the nature of the growth substrates of these bacteria?
- (b) What is the source of these substrates?

At this stage of the investigation, no answers to these questions could be drawn from the data available. However, because of the occurrence and abundance of these types of bacteria (and also heterotrophic populations, see Appendices A and C), it was concluded that these four heaps were aerobic to a depth of at least 1 m.

The results of this initial study showed that White's heap contained fairly large and diverse bacterial populations, and results from Davy [1975] showed that White's heap was the largest single source of acid and heavy metal pollution to the East Finniss River flowing through the mine site. It was, therefore, decided to investigate several specific areas on White's heap and to sample these intensively and as deeply as possible during the following dry and wet seasons. The results of that sampling program are discussed in Section 5.3.

5.2 Tailings Dam Area

It was assumed that material in this area would have been fairly homogeneous and inactive, because of the way in which the tailings material was processed and then deposited in the dam area (see Davy [1975] for full details). Consequently, this area was not sampled intensively, except for one hole (E), dug in September 1976 to the base of the fill and sampled about every 10 to 20 cm. However, from the results of the few samples that were taken (Tables 4 and 5 and Figures 2-4; and Appendix B) it appeared that physicochemical parameters and bacterial populations varied with location, depth and season.

Table 4 shows the results of surface samples taken between 1975 and 1979. The material taken from the dry stream bed at the extreme west of the tailings area was probably washed there by heavy rain during wet seasons. relatively high and it contained no soluble copper or iron, nor detectable T. ferrooxidans populations, although low numbers of high pH sulphur oxidisers It is most probable that any soluble copper had already been washed out of this material by heavy rain. Samples taken from the eastern end of the tailings area showed a different composition. The pH appeared higher during the wet than the dry seasons. The concentrations of soluble solids, and soluble copper in particular, were higher during the dry than the wet seasons. T. ferrooxidans populations were isolated in numbers comparable to other sulphidic areas (see Tables 2 and 3), in conjunction with high pH sulphur-oxidising bacteria. However, low pH sulphur-oxidising bacteria were isolated only during the wet season, at a level 100 times higher than that of the high pH sulphur-oxidising bacteria. The samples taken during May 1979 were from an area roughly in the centre of the tailings dam. Although the physicochemical parameters of these samples showed somewhat similar values to those of samples taken further east during March 1976, no bacterial species were isolated from this area. At present it is not known whether this small

area was in some way inhibitory to microbial populations, or if the tailings dam area is generally devoid of bacterial species, except at the far eastern end.

Table 5 shows the results of samples taken to a depth of 150 cm at the end of a dry season, September 1976. The distribution of the parameters with depth is shown in Figures 2-4. Except for a very dry surface sample, the moisture content was high and uniform between 30 and 140 cm and decreased slightly at 150 cm (Figure 2). The pH increased steadily from the surface to a depth of 90 cm, then decreased slightly to 110 cm and then increased again to 150 cm. The pH rose by almost one unit from the surface to 150 cm (Figure The concentration of total soluble solids steadily increased by about four times from the surface to 70 to 90 cm depth, while the concentration of soluble copper at 50 cm was double that at 90 cm. However, from 90 to 150 cm the soluble solid concentration decreased by four times and the soluble copper level also decreased (Figure 3). Although only four samples showed T. ferrooxidans populations to a depth of 110 cm, high pH sulphur oxidisers were isolated from seven samples to a depth of 150 cm and acidophilic heterotrophs were found in every sample to a depth of 140 cm. Bacterial numbers did not show any marked variation with depth (Figure 4). Because of the physical nature of the tailings material, i.e. poorly structured and finely divided material with a high moisture content, atmospheric conditions below the first few centimetres at the surface were most probably microaerophilic or even anaerobic as the diffusion rate of oxygen into such 'waterlogged' soils is known to be extremely low [Griffin 1972].

A study, under controlled environmental conditions, of the leaching of a bulk sample of tailings material taken from the centre of the tailings dam area in May 1979 is presented in Babij et al. [1981].

5.3 White's Overburden Dump, September 1976 to March 1977

In late 1976, a decision was made to sample intensively White's dump in several areas at depth within the dump, since studies by the AAEC of the water movement caused by rainfall, and temperature distributions were being concentrated on White's dump [Daniel et al. 1980a]. During the late dry season in September 1976, a backhoe was used to dig a series of holes or trenches into the top and the base of the eastern and southern walls of the dump. In this way, penetration to a depth of about 4 m was possible and the exposed vertical faces were systematically sampled at 30 cm intervals to a

height of about 2.5 m from the bottom of each hole. (Sampling nearer the surface was not possible because no ladder was available to reach the higher levels of the holes and the material was too unstable to allow climbing.) The holes cut into the top of the dump (D, E and G), were dug close to three of the probe holes drilled by the AAEC team for temperature studies (their holes a, c and d respectively); the holes cut into the base of the eastern wall (A and F) were close to the main wet-season spring outflows; hole C, at the base of the southwest wall, was dug also at a point where drainage from the dump occurred, and hole B, further south on this wall, was dug in an area which appeared to have been a high area of the original surface contours; (the location of holes D, E, G, A, F, C and B and AAEC probe holes is shown on Map 1).

At the end of the following wet season (March 1977), further holes were mechanically excavated at the top of White's dump, this time to a depth of about 5 m. These holes were cut as close as possible, and at 90°, to those cut at the end of the preceding dry season, and labelled accordingly. Thus holes D', E' and G' were cut next to holes D, E and G respectively. Samples of the exposed vertical faces were taken at closer intervals than previously (about 7.5 cm) and sampling right to the surface of the holes was achieved using a ladder for support. Unfortunately, conditions at the base of the dump at this time made it impossible to dig holes into the base of the walls of the dump (the ground was far too wet to support the weight of the backhoe), and so a comparison of the base of the dump between wet and dry seasons was not achieved.

One further hole (J) was mechanically dug about one third of the way up the eastern wall of the dump during June 1978. The material was very dry and the rocks were loose and extremely unstable, and only three samples, about 0.5 m apart, were taken. Also, during May 1979, several surface mud samples were collected from the base of the dump, along the flow path of one of the main springs, Spring No.4 (these samples were labelled area S4).

The results indicated that a seasonal difference was occurring in White's dump, to a depth of about 3.6 m into the top of the dump and at the three areas sampled and that there were also non-seasonal differences between these areas (Tables 6-8 and 11). Little variation was found between the depths 1.2 to 3.6 m and 3.6 to 4.7 m in the three areas sampled on the top of the dump during the wet season (Table 9). Also, it was found that there was some variation in the soils between the top and base of the dump during the dry

season (Tables 10 and 11), and that areas of the base of the dump were variable during the dry (Table 10). The results shown in these tables are discussed in detail below.

5.3.1 Moisture content

At the top of the dump, the moisture content of the soil in all three was fairly low (< 10 per cent) in the dry season and increased with depth (Figure 5). During the wet season, the moisture content of the soil from each area was about double that in the dry season and there was no marked variation with depth (Figure 5). The moisture content of the soil, during the dry season, at the base of the dump at holes A, F and C was about as high as that of the soil on top of the dump during the wet season. Holes A, F and C were dug near known drainage areas and were probably indicative of the water-holding capacity of the dump as a whole. The low moisture content of the soil at hole B, even at depth (see Figure 10), probably resulted from water draining away from this area which was a high point of the original surface contour of the land.

These results, which are in agreement with those of Daniel et al. [1980a] who measured rainwater movement through White's dump using neutron and gamma ray scattering techniques, show that the dump dries out in only the surface 1 to 1.5 m during the dry season (see Figure 10 for moisture content distribution with depth at holes A, F and C). The rest remains quite wet, with water accumulating towards the base, and in some areas the base is still saturated at the end of the dry season.

5.3.2 pH

The pH of the soil on the top of the dump was significantly lower during the dry than during the wet. The higher pHs during the wet were most probably caused by a continual washing out of hydrogen ions by rainfall. Also, areas D and G were about 1 and 1.5 to 2.5 pH units, respectively, lower than area E during the wet and dry seasons. (This point will be referred to later in Section 5.3.5.) The pH of soil at the base of the dump was found to be low at drainage areas A and F and also at hole B. The high pH of soil at hole C (\bar{x} pH = 5.97) indicated that leaching processes were probably no longer occurring to any extent in this area, as reflected in the nature of the material observed - the bulk of material in the area of hole C was in the form of a heavy red clay with numerous salt encrustations.

5.3.3 Total soluble solids, soluble copper and iron

In areas D and G, the concentration of total soluble solids in the soil was not significantly different between the dry and wet seasons. However, in area E, the concentration was significantly higher in the dry than in the wet. The distribution with depth at the top of the dump is shown in Figure 6. The concentrations of soluble solids in soil at the top and base of the dump during the dry season were found to be fairly similar. concentrations of soluble solids in the water draining from the base of the dump at Spring No.4 was about three to ten times higher than that in the soil (compare Table 12 with Tables 10 and 11). Soluble copper and iron concentrations in the soil were relatively low and variable in all areas sampled, but the percentage of soil samples taken during the dry and containing soluble copper was higher than that of soil samples taken in the wet (81 per cent and 50 per cent respectively). Again, the concentration of soluble copper in the water from Spring No.4 was consistently about ten times higher than that in the soil of the dump (Table 12). Concentrations of soluble iron in the dump soil were also low and it is thought that most of the iron solubilised from pyrite oxidation was probably re-precipitated in the soil, although water from Spring No.4 consistently contained soluble iron (both ferrous and ferric iron).

5.3.4 Bacterial populations

Populations of <u>T. ferrooxidans</u> were found in the top of the dump at areas D and G in the wet and dry seasons and virtually none at area E (only three out of the 45 samples from the wet and dry seasons contained low numbers of this organism). Numbers were fairly low (between 100 to 1000 bacteria/g soil); the distribution of this organism with depth at areas D and G is shown in Figure 7. <u>T. ferrooxidans</u> was also isolated from the holes at the base of the dump, in numbers similar to those found at the top. <u>T. ferrooxidans</u>, in similar numbers, was isolated in all samples from the springs at the base of the dump, particularly Spring No.4. The population levels of <u>T. ferrooxidans</u> were similar to those found by Beck [1967], as previously mentioned.

A striking feature of the bacterial analysis was the abundance of relatively large populations of high pH sulphur-oxidising Thiobacillus spp. (10 to 1000 times higher than numbers of $\underline{\mathsf{T}}$. ferrooxidans) in the soil at the top of the dump during the wet season, and the virtual absence (4 out of 18 samples positive) of this type of microorganism in the dry. The distribution

of this organism with depth in the top of the dump is given in Figure 8, which shows that there was no marked variation with depth, although at area D this organism was not detected during the wet until a depth of about 2.5 m was It is thought that the build-up of high pH sulphur-oxidising reached. bacteria may have been 'controlled' by the availability of one or more nutrients (whose nature at present is unknown) generated during the dry Elemental sulphur or reduced sulphur compounds, released by the season. oxidation of mineral sulphides, may have been involved as growth substrates. However, some species of Thiobacillus probably isolated in this group, i.e. high pH, sulphur oxidisers (the pH of the selective isolation medium was about 6), are known to be facultatively autotrophic (see Table 1). This means that they can both use organic material for growth when inorganic substrates are not available, and fix carbon dioxide. Thus organic compounds also may have been utilised as growth substrates, giving this group of microorganisms the greatest adaptability, with a wide 'choice' of substrates, and therefore the greatest likelihood of growth success. To know whether these microorganisms increased rapidly at the beginning of the wet season and maintained viability throughout it, or their population levels gradually increased throughout the whole of the wet season, would help determine the nature and source of substrates and thus their contribution to the degradation of mineral sulphides.

High pH S-oxidising bacteria were also isolated from several samples from the base of the dump during the dry season and from Spring No.4 water in May only.

The lack of acidophilic S-oxidising bacteria, i.e. \underline{T} . $\underline{thiooxidans}$ type, was a somewhat surprising result, as this type of organism had previously been isolated from surface samples of sulphidic waste rock heaps (Tables 2 and 3). However, \underline{T} . $\underline{thiooxidans}$ is a strict autotroph (i.e. it cannot use organic carbon for growth) and other work in this program [Babij et al. 1981] has shown that acidophilic S oxidisers rapidly lose viability under atmospheric conditions of low oxygen tension. The combination of these factors (and perhaps others) may have resulted in the \underline{T} . $\underline{thiooxidans}$ type of bacteria being unsuccessful competitors with the higher pH S-oxidising organisms. These can use organic substrates for growth, (as discussed above) tolerate and also grow under conditions of low oxygen tension (see Babij et al. [1981] and Table 1) and, in the case of organisms such as \underline{T} . $\underline{denitrificans}$ they do not need ammonium ion as a nitrogen source. All these factors make the high pH sulphur-oxidising bacteria, as a group, much more genetically versatile than

the <u>T. thiooxidans</u> type, and therefore more successful at establishing themselves as the principal group of S oxidisers when conditions become favourable. Note that such genetic versatility is also possessed by <u>T. ferrooxidans</u>, which can utilise a wide range of inorganic substrates for growth. These include soluble and solid ferrous iron, a growth substrate unique to this organism among the <u>Thiobacilli</u>. <u>T. ferrooxidans</u> grows under conditions of low oxygen tension [Babij et al. 1981; Ralph et al. 1981; Razzell and Trussel 1963; Mackintosh 1978] and fixes atmospheric nitrogen [Mackintosh 1978].

The presence of organic carbon within the dump is indicated by the isolation of fairly large populations of heterotrophic bacteria (i.e. those bacteria which can use only organic carbon for growth). Numbers of heterotrophic bacteria growing at about pH 7 were fairly high in most locations, being more abundant in the wet than in the dry in the top of the dump (data presented in Appendices A and C). Fungi were also often isolated from these soil samples.

Heterotrophic bacteria able to grow well at pH 3.5, and thus labelled acidophilic heterotrophs, were fairly abundant throughout the dump, except in area E in the wet season and hole C in the dry. In both these cases the mean soil pH was about 6 and it is most probable that this group of bacteria could not tolerate such a high pH. Numbers and occurrence of these microorganisms did not otherwise appear to have been influenced by wet or dry season conditions. These bacteria were isolated from Spring No.4 during June, but not during May. The variable distribution of acidophilic heterotrophs with depth in the top of the dump is shown in Figure 9.

One of the most significant results of this investigation was the isolation of anaerobic sulphate-reducing bacteria (most probably <u>Desulfovibrio</u> spp.) only from holes A, F, B and C (i.e. all the holes at the base of the dump) in an area some 10 to 70 cm from the bottom of the dump (Table 10). These bacteria are heterotrophic and strict anaerobes (i.e. they can only grow in the absence of oxygen), and they produce sulphides (usually $\rm H_2S$) from sulphate and frequently occur in waterlogged soils [Brock 1973]. From this microbiological evidence it can be concluded that conditions within the soil in White's dump were either aerobic or microaerophilic and that oxygen was completely absent only in a small zone at the very base of the dump. These microbiological data are also in agreement with the results of Daniel et al. [1980a] who have shown the base of the dump to be water saturated in some

areas.

5.3.5 The top of White's dump

The results of this study indicated that the areas chosen for intensive sampling, on the top of the dump, were highly heterogeneous in the wet and dry seasons. While areas D and G were fairly similar, both differed significantly from area E. These differences have been mentioned in the discussion of each particular variable and will be summarised here. The most significant difference was the virtual absence of populations of $\underline{\mathsf{T}}$. $\underline{\mathsf{ferrooxidans}}$ in area E. This was probably not caused by the higher pH at E, since the maximum pH tolerated for growth by this organism is about 6 [Buchanan and Gibbons 1974] and $\underline{\mathsf{T}}$. $\underline{\mathsf{ferrooxidans}}$ grows well on reduced sulphur compounds at pH 6 [Tuovinen and Kelly 1972]. Some other factor, therefore, was responsible for the absence of $\underline{\mathsf{T}}$. $\underline{\mathsf{ferrooxidans}}$ at area E.

This pH difference was another major variation in the soil from areas D, G and E. During the dry season, the pH of soil at area E was about 1 and 1.5 pH units higher than that at areas D and G, respectively, and similarly during the wet, that of area E was also higher by about the same amount than that of areas D and G. The higher pH at area E during the wet season (i.e. 5.9) was probably the reason why acidophilic heterotrophic bacteria were not isolated at this time, but were found during the dry when the pH was lower (about 4.6).

The other difference apparent from these results was that in the dry, the mean concentration of soluble copper at area D was significantly higher than that at area E (P = 0.005). While the concentration at areas G and E could not be compared statistically (because the difference of the variances was too great, as determined by the F test), the concentrations of soluble copper at areas D and G were not significantly different (P = 0.1), thus indirectly indicating that the concentration at area G was also higher than that at area E. This indicated that, in the dry, copper was being solubilised at area E more slowly than at areas D and G. All these differences indicated that degradative processes (i.e. pyritic breakdown) were probably occurring more slowly in area E than they were in areas D and G.

This was confirmed by the temperature distributions within the dump at these areas [Daniel et al. 1980b]. Daniel et al. found that at area D (which corresponded to AAEC probe hole a) the temperature within the dump rapidly rose to about 50°C at a depth of 5 m and was about 56°C at a depth of 14 m.

At area G (AAEC probe hole d) the temperature rose to about 37°C at 3 m and gradually declined to about 34°C at 17 m depth. The corresponding heat production was calculated and, assuming association of heat source with the oxidation of pyrites, it was shown that significant pyritic oxidation was occurring at these areas throughout the dump. Area E (AAEC probe hole c), however, was found to be cooler, indicating less pyritic oxidation in this area. (See also Harries and Ritchie [1981] for an account of the use of such temperature profiles in estimating areas of pyritic oxidation and oxidation rates in White's dump.)

5.4 Intermediate Overburden Dump, June 1978 and May 1979

The results of the intensive sampling program on White's dump led to the decision to pursue a similar intensive investigation of Intermediate dump during the following dry and wet seasons. It was decided to sample Intermediate dump towards the middle of the next dry season (late June 1978) and to follow this with a mid to late wet season sampling period early in The first of these expeditions was achieved at the time desired, but the second was delayed until the first week in May, several weeks after the end of the wet season. One of the main reasons for this delay was the remoteness of Rum Jungle from the laboratory in Sydney. Because of this. field expeditions had to be carefully planned and organised and necessary equipment had to be collected, tested and usually repaired well in advance. In 1979, difficulties encountered in the organisation and preparation of material and equipment necessary for the field trip delayed departure for several weeks. The wet season in 1979 ended relatively late, about late April to early May (Darwin was still receiving rain in May). The samples taken during the first week of May, therefore, are thought to be indicative of late wet season. In 1978, the wet season ended in early April and samples taken in mid-June probably represent the dump about two months into the dry season.

During these sampling periods, six holes were mechanically cut (as described in Section 5.3) to depths of between 3 and 4.5 m. Holes X, Y, Z and Z* were dug during the third week of June 1978 and holes S, T and V were dug during the first week of May 1979. These holes were all located in the southwestern 'third' of the dump as shown in Map 2. (See Davy [1975] for a full description of the dump.)

Holes X and S were dug straight down into the top of the highest part of the dump, several metres apart, to depths of about 3 and 3.5 m respectively.

Holes Y and T were cut about 3 m into the side of the top part of the dump (directly below holes X and S respectively) using the road at this middle level as the base for the holes, and were about 4 and 3.5 m deep respectively. Note that most of Intermediate dump is about 11 m high, whereas the southern 'tip' is about 5 m higher again. Holes Z and V were cut about 3 m into the side at the base of the dump, directly in line with holes X, Y and S, T respectively. Hole V was extended 3.5 m from the top of the cut to about 0.5 to 1 m from the base of the heap. The material was loose and porous, consisting mainly of large boulders. The surfaces exposed by the cut were very wet and free-flowing water seepage was collected near the base.

Several difficulties were encountered when cutting hole Z as it was the first hole dug on this dump. The material consisted mainly of large rocks and was very dry and loose. As the backhoe driver was digging the hole, he placed the material out of the hole underneath him as a 'platform' to enable him to penetrate deeper into the side of the dump. However, because of this building up of rock and soil, he was unable to dig the base of the hole at this point and was about 1.5 m from the base of the dump. Therefore, he moved about 3 m southwest and began hole Z* at the level at which hole Z ended and continued hole Z* to the base of the dump. Hole Z* was considerably wetter than hole Z, through which draughts of air could be felt by anyone standing next to the walls exposed by the cut.

The results indicated little variation in the top of the southwestern area (Table 13), a slight variation at the middle level (Table 14) and further variation at the base of the dump (Table 16). However, it cannot be determined, from the present data, whether the variation at the base of the dump was caused by climatic factors or the heterogeneity of the dump. This heterogeneity at the base was highlighted by the variation found between holes Z and Z* (Table 15), dug only several metres apart.

The only effect most probably caused by climatic factors was the population levels of \underline{T} . $\underline{ferrooxidans}$. At all levels in the dump, \underline{T} . $\underline{ferrooxidans}$ numbers were consistently found to be 100 times higher in late June than in early May. This may indicate increasing activity of this organism during the dry season.

Another indication that microbial activity was increasing as the dry season progressed was the result that, during May, only six samples out of 75 contained S oxidisers and acidophilic heterotrophs (i.e. about 8 per cent).

However, during June, 24 samples out of 81 contained populations of these types of microorganisms (i.e. about 30 per cent). Nevertheless, further sampling throughout the dry season would be needed to verify this. Anaerobic bacteria were not isolated from any of the trenches, even at the base of the dump.

The distribution of several parameters with depth within the dump is illustrated in Figures 11-14. The moisture content of the soil samples was uniform to about 3 m on the top of the dump, increased slightly (the surface samples were very dry) to about 3.5 m at the middle level and was variable at the base of the dump, although the general trend was increase with depth (again the surface samples were very dry (Figure 11). The moisture content in the bottom 1.5 m at the base of the dump (holes V and Z*) was about double that at the top (holes S and X, Figure 11).

The concentration of total soluble solids is shown in Figure 12. was found to be extremely high at the top of the dump and fairly uniform to a depth of about 3 m, although in May the samples from the top 1.5 m contained slightly higher concentrations than samples from the next 1.5 m. concentrations of soluble solids at the middle level and at the base were considerably lower than at the top of the dump. There was no marked variation with depth at these levels, although May samples contained slightly higher concentrations in the top 1.5 m of the holes than June samples (this is reflected in the mean values, as shown in Table 17). The soluble copper and concentrations tended to follow the same pattern (Table 17). Concentrations of copper and iron in the top of the dump were less variable, and 10 to 100 times higher than those at the middle level and base.

The distribution of \underline{T} . $\underline{ferrooxidans}$ with depth is shown in Figure 13. Numbers are 10 to 100 times higher in June than in May, and fairly uniform to depths of 3 and 3.5 m at the top and middle level, respectively. The middle-level holes (Y, T) were the only ones which contained samples in which \underline{T} . $\underline{ferrooxidans}$ was not detected. Numbers of \underline{T} . $\underline{ferrooxidans}$ at the base of the dump were slightly variable with depth, but with no distinct pattern. The highest numbers of this organism were isolated in the June samples taken from the bottom 1.5 m at the base of the dump (hole Z^*) (Table 17).

The temperature distribution within these holes is shown in Figure 14. At the top of the dump, the temperature within the holes was considerably higher than ambient and increased from about 41 to 47°C to a depth of about

Although there was an ambient temperature difference of over 10°C 3.5 m. between May and June, temperature differences within the dump were much smaller. At the middle level, there was little temperature difference between May and June within the dump, compared to an ambient difference of about 9°C. At this level, temperatures again increased from about 35 to 43°C to a depth of about 4.0 m. However, the pattern at the base of the dump was completely An ambient temperature difference of over 10°C between May and June was reflected in the temperature difference within the dump between holes V and Z, although temperatures in Z* were similar to those in V. already been noted that hole Z was very dry and porous (i.e. with large holes between boulders and large rocks) and draughts of air could be felt coming from these voids, even though there was no wind at the base of the dump at the area where the holes were cut. Air circulation within the dump at about 4 m to the base would explain this observation and also the decrease in temperature in May (hole V) from about 36 to 29°C with depth. (The higher moisture content with depth at the base of the dump would tend to cool the circulating air.) Note, however, that the absolute temperature measurements reported here are very rough and give only an indication of temperature patterns rather than absolute magnitudes. It is most probable that more sophisticated temperature measurements (as conducted in White's dump by Daniel et al. [1980b] and Harries and Ritchie, [1981]) would show even higher temperatures than those reported here, within the dump.

Tables 18 and 19 show that the atmosphere within the dump in both May and June had a high relative humidity, often 100 per cent (except for hole Z), while the ambient atmosphere outside the dump had a low relative humidity (8 to 10 per cent in June, 15 to 24 per cent in May). The significance of relative humidity in relation to bacteria in soils is discussed in Section 5.6.1.

5.5 Differences Between White's and Intermediate Overburden Dumps

5.5.1 Constructional differences

White's dump was completed in 1958, contains about 6.9×10^9 t of material and is about 13 to 18 m high. The dump is roughly heart-shaped and the eastern, southern and western portions of the top slope gently in towards the centre, causing a main drainage channel (during the wet) which discharges into the East Finniss River to the north. Rainwater penetrating the dump in the wet season drains out through a series of well-defined springs (which flow

even in June) along the base of the eastern wall, but most of it drains directly into the groundwater, all of which finds its way into the East Finniss River system.

Intermediate dump was completed in 1964 and contains about 1.1×10^6 t of material. The dump is approximately square and constructed in three distinct areas: an eastern zone, a southwest square and a northwest square. Most of the dump is about 11 m high, with the southwest square being about a further 5 m higher. The drainage pattern is complex and it is thought that the walls (which represent about 30 per cent of the total area of the dump's surface), because of their open nature, absorb most of the rainwater falling on them. Springs at the base of the dump flow only for very short periods immediately after a storm. Most of the rain falling on the heap drains through it and into the groundwater.

5.5.2 Moisture content

The pattern of moisture content of the soil was roughly similar for both dumps. During the dry (June samples for Intermediate and September samples for White's), the base of both heaps was wetter than the top, but there was an indication that the surface few metres of the walls of Intermediate did not dry out as rapidly as those of White's during June (compare Table 17 with hole J in Table 10).

5.5.3 pH

The pH of soil from both dumps was fairly similar in most of the areas sampled, although White's dump was more variable between seasons and also had a greater pH range than Intermediate dump (mean pH 2.77 to 5.97 for White's and mean pH 2.77 to 3.98 for Intermediate). Although the pH of the soil in the base of Intermediate dump varied from May to June, this probably reflected the heterogeneity of the dump rather than a seasonal difference, since the mean pHs of the soil at the top and middle were not significantly different between May and June. It is most probable that the pH of the soil of Intermediate dump would show a similar trend between wet and dry seasons (i.e. the pH of the soil rising during the wet season because of the continued washing away of H⁺ ions by rainwater), but further sampling at appropriate times would be needed to verify this.

Sampling in White's dump indicated at least two areas (area E and hole C) in which leaching processes had either ceased or were considerably lower than other areas and in these former areas the pH of the soil was found to be considerably higher (pH >4). However, all of the areas sampled in Intermediate dump had a soil pH of <4 and all areas were thought to be actively leaching.

5.5.4 Total soluble solids and soluble copper and iron

Whereas the concentration of total soluble solids was similar between the top and base of White's dump, that of Intermediate dump was considerably higher (almost 10 times) at the top than at the middle level or base. Also, the concentration at the top of Intermediate dump was between 10 to 30 times higher than that at the top of White's, and the concentration at the middle level and base of Intermediate was also higher than that at the base of White's. However, water seeping from the base of hole V in Intermediate dump (sample No.436 in Appendix D) had a soluble solids concentration similar to that in the water flowing from the base of White's (Spring No.4, Table 12) and the surface mud from the flowpath of Spring No.4 at the base of White's (Table 10, area S4).

The soluble copper content of the soil at the top of Intermediate dump was between 25 and 400 times greater than that at the top of White's. Also, 100 per cent of the samples from the top of Intermediate dump contained soluble copper, whereas only about 50 per cent of wet season and 80 per cent of dry season samples from the top of White's contained soluble copper. The concentrations of soluble copper at the middle level and base of Intermediate dump were variable and of a similar magnitude to those found at the base of White's. Seepage water from the base of Intermediate (sample No.436) contained a concentration of soluble copper similar to that in the water drainage from the base of White's (Spring No.4, Table 12).

Soluble iron was not detected in many soil samples from White's dump, and not detected in any taken during the dry season from the top at areas D and E and at the base of hole C. At the top of White's, the concentration of soluble iron, when detected, was generally less than 100 mg/kg (D.W.) whereas soil sampled at the top of Intermediate dump always contained soluble iron, the mean value of which was about 80 to 150 times greater than the mean value of that of the soil from the top of White's. The concentration of soluble iron in soil at the middle level and base of Intermediate dump was as variable

and of the same order of magnitude as that of the soil at the base of White's. The concentration of soluble iron in the water seeping from the base of Intermediate dump during May 1979 (sample No. 436) was about the same as that in the water of Spring No.4 flowing from the base of White's (Table 12, May 1979 sample).

It is significant that water drainage from the base of Intermediate dump contained similar concentrations of soluble solids and soluble copper and iron and also a similarly low pH to the water draining from the base of White's east wall. Because runoff and water draining as springs from the base of White's dump flows into the East Finniss River system, it is considered that water drainage through the dump to the local groundwater also eventually enters the East Finniss. The drainage pattern from Intermediate dump, however, is less well-defined, but it is thought that water draining through the dump also passes into the East Finniss River system.

5.5.5 Temperatures within the dumps

AAEC results [Daniel et al. 1980b; Harries and Ritchie 1981] showed White's dump temperatures in excess of 35 and 40°C within the first 5 m only at areas G and D respectively (AAEC probe holes d and a respectively), although six areas were selected for measurement. This suggested that pyritic oxidation was occurring within the dump at different rates in distinct areas.

Temperature measurements in Intermediate dump showed temperatures in excess of 40°C within about the first 4 m at the top and middle level (with the top being slightly hotter). If high temperatures are assumed to be indicative of comparatively rapid rates of pyritic oxidation, this result would indicate that pyritic oxidation was occurring most rapidly in the top southwest square of the dump. This is in agreement with the other results for the top of the dump. A more sophisticated program of temperature measurements (such as that conducted by the AAEC on White's dump) is needed to determine whether pyritic oxidation is occurring uniformly or only in discrete areas, and to compare the rates of pyritic oxidation in Intermediate and White's dumps.

Because of the open nature of the side walls of Intermediate dump, especially near the base, it is possible that a 'chimney' effect is being created by air which enters the dump from the sides, is heated within it and then rises up through it. There was some indication that this may have been

occurring, as temperatures within the dump near the base decreased below ambient and draughts could be felt at the surfaces exposed by cutting the holes.

5.5.6 Bacterial populations

The pattern of bacterial population types within the soil (and water) samples from the two dumps was very different, which in itself is sufficient indication that Intermediate and White's dumps were behaving differently.

T. ferrooxidans was found in about 90 per cent of soil samples from Intermediate dump, whereas this organism was isolated from only about 35 per cent of soil samples from White's dump. Those areas of White's dump which did contain most of the populations of T. ferrooxidans (i.e. areas D and G on the top and holes A and F at the base of the east wall), contained population levels similar to those of samples from Intermediate dump during May 1979. Whereas areas D and G, on the top of White's dump, showed no significant difference in population levels of this organism between the wet and dry seasons, all the areas sampled on Intermediate during June 1978 contained populations of T. ferrooxidans at levels about 100 times higher than those found from samples taken in May 1979. Samples would need to be taken late in the dry season (about September) from Intermediate dump to show whether population levels of T. ferrooxidans remain high during the dry, or if they decline towards the end of the dry. Furthermore samples from White's dump during the early dry season are needed to see if T. ferrooxidans populations undergo a similar rapid increase during this time; there is some indication that this may be the case, since three samples taken in June from the middle of the east wall (hole J, Table 10) contained T. ferrooxidans in numbers about 100 times higher than samples taken in March or September.

Autotrophic sulphur-oxidising bacteria were isolated from only about 12 and 40 per cent of samples taken in May and June, respectively, from Intermediate dump, whereas this group of bacteria was the most abundant and contained the highest numbers of microorganisms in White's dump during the wet season (about 85 per cent of samples contained this bacterial group). If the appearance of such large populations of sulphur oxidisers in White's in the wet season was caused by one or more nutrients (probably generated during the dry) becoming available because of water transport in the wet, then it is most probable that such nutrients were not as available in Intermediate dump (especially in the top).

Acidophilic heterotrophs were abundant and in high numbers in most of the samples from White's dump, both during the wet and the dry (except for hole C in the dry and area E in the wet); about 80 and 70 per cent of samples respectively, contained these bacteria. Acidophilic heterotrophs were scarcer in Intermediate dump, with only about 12 and 30 per cent of samples, taken in May and June respectively, containing them.

Strict anaerobes (i.e. <u>Desulfovibrio</u> spp.) were isolated from each hole cut into a zone about 10 to 70 cm from the base of White's dump, indicating that the zone contained no oxygen at all. Such an anaerobic region was not identified in Intermediate dump, as no anaerobic bacteria were isolated.

The microbiological results suggest that <u>T. ferrooxidans</u> was the principal type of bacterial species in Intermediate dump, whereas in White's dump the most numerous types of bacteria were sulphur oxidisers and acidophilic heterotrophs, although lower levels of <u>T. ferrooxidans</u> were consistently present. White's dump thus contained a greater diversity of microorganisms than Intermediate and it is suggested that conditions within White's were less extreme and more heterogeneous (i.e. a greater range of micro-habitats and energy sources must have been available to support the different bacterial groups) than those within Intermediate.

The factors responsible for all of the above differences between the dumps are at present unknown, but the relatively small age difference between White's dump (completed in 1958) and Intermediate dump (completed in 1964) does not provide sufficient explanation of the observed differences.

5.6 Factors Affecting Bacterial Activity in Leaching Environments

5.6.1 Water availability

Water is essential for bacterial activity and growth. The water activity (a_W) of a solution or substance is related to the water vapour pressure in the air around it and is estimated by measuring the relative humidity when the system is at equilibrium [Brock 1979]. Whereas relative humidity is expressed as a percentage, a_W is given as the fraction (i.e. a_W x 100 = relative humidity). The a_W of pure water is 1 and decreases as salts are dissolved in the water. For example, seawater (which contains approximately 3.5 NaCl/100 mL water) has an a_W of 0.98 at 25°C [Brock 1979]. Most bacteria exist between 0.995 to 0.990 a_W , although halophilic bacteria can exist down

to about 0.75 a_W [Hawker and Linton 1971]. In soil microbiology, the concept of water available to bacteria is usually expressed as 'water potential', an energy term defined as "the free energy between the system under study (e.g. soil, food) and a pool of pure water at the same temperature" [Brock 1979]. The unit used to express water potential is usually the 'bar' which is equal to 10^6 dyne/cm² = 0.986 atmospheres = 100 joules/kg m⁻¹ = 75 cm mercury = 1022 cm water [Griffin 1972]. The lower the water potential (i.e. the less the amount of water available to microorganisms) the lower is the bar value of the soil. Because water potentials of natural systems are always less than that of pure water, bars have negative values. At 25°C, an a_W of 0.995 corresponds approximately to -7 bar, and in the range of 0.75 to 1.0 a_W a decrease of 0.01 a_W corresponds approximately to a decrease of about 15 bar potential [Brock 1979].

The potential of water within soil consists of the matric potential (the water within voids adsorbed onto soil and particle surfaces) and the osmotic potential (water containing dissolved salts) which decreases as the concentration of solutes in the water increases [Griffin 1972]. The water vapour in air within voids in a dump, which is in a state of equilibrium with its water-holding capacity, will be in equilibrium with the water in the voids. The $a_{\rm w}$ (or relative humidity) of the atmosphere in voids in such a dump will be the same as the $a_{\rm w}$ of the water adsorbed onto rock surfaces in the voids [Brock 1979].

Water, or water vapour, is essential for the oxidation of pyrite. It has been found that pyrite in an atmosphere of 100 per cent relative humidity has a rate of oxidation the same as that of pyrite immersed in water, and that as the relative humidity decreases, the rate of oxidation of non-immersed pyrite also decreases [Glover 1973].

It is the water potential, or water activity (a_W) , of the soil that is important to microbial activity, since the activity of bacterial enzymes depends on water, and the DNA molecule (i.e. the chromosome) is highly hydrated until the water potential decreases to about -115 bar. The DNA molecule loses water molecules with further reduction in water potential until the helical structure is distorted (reversibly) at a water potential between -400 and -800 bar [Griffin 1972].

The moisture content of the soil, although easily determined, has little significance as a factor in estimating microbial activity. The relationship

between the moisture content and water potential of soils varies and so water potential must either be measured directly (such as by the methods outlined in Griffin [1972]) or equated to the moisture content of a particular soil by determining a calibration curve for that soil [Brock 1975].

Brock [1975] found that the lower limit of water potential for iron oxidation by T. ferrooxidans in soils was about -23 bar and that soils with similar moisture contents varied with respect to their water potential. Clay in pyritic soils greatly decreases the water potential (while maintaining the moisture content) because it binds water very tightly and thus greatly decreases the matric potential of water within soil voids. Kennedy and Stahl [1974] determined the water potential, as a function of water content, in leach dump material at the Chino mine site (Santa Rica, New Mexico) and their results suggest that the dump material held water tightly and that fine material held water more tightly than coarse material. Bhappu et al. [1969] correlated numbers of T. ferrooxidans, within the same dump, with moisture content of the soil and found that where bacterial numbers were low the moisture content was less than 10 per cent. Brock [1975] suggested, from the above results, that T. ferrooxidans was active in soil with a water potential of about -15 bar, but was not active in soils where the water potential was considerably lower.

In the present study, the water potential of the soil samples, which was not determined, may possibly have been a limiting factor in bacterial activity in various sulphidic waste areas (for example, the tailings material sampled in May 1979) and isolated sampling sites within the holes cut in the dump, especially White's. It is probable that the different groups of bacteria isolated from Rum Jungle have different tolerances to water potential. This may partly explain the variation of microbial species between dumps, between different areas of White's dump and between wet and dry seasons.

Both White's and Intermediate dumps (with porosities of about 40 per cent) are thought to be in a state of equilibrium with respect to their water-holding capacities, so that rainwater drains through them during the wet and start of the dry season (with only the base metre or so of White's being saturated). This means that there is little or no change in total water content of the dumps between seasons. Only the surface of White's dump to a depth of about 2 m has a lower moisture content at the end of the dry season than at the end of the wet (Figure 5; Daniel et al. [1980a]).

However, the osmotic potential of water within the voids in the dumps probably decreases as the dry season progresses and, by the end of it, may be too low for the growth of certain types of microorganisms. The autotrophic sulphur-oxidising bacteria may be much less tolerant to low water potential than are $\underline{\mathsf{T}}$. $\underline{\mathsf{ferrooxidans}}$ and acidophilic heterotrophs, which may explain the almost total absence of the sulphur oxidisers in soil from White's dump at the end of the dry season. Because of the monsoonal climate at Rum Jungle, rainwater drains through the dumps each wet season, washing out solubilised salts and replenishing water with a high \mathbf{a}_{W} , thus allowing those microorganisms which may require a high \mathbf{a}_{W} for growth, such as sulphur oxidisers, to flourish.

Relative humidity measurements in Intermediate dump at the end of the wet season indicate that air within the dump contained water vapour at or near saturation levels during May and, by June, air in some areas was below saturation (Tables 18 and 19). The accuracy of the instrument used, however, was about \pm 5 per cent which was not sufficient to correlate humidity measurements sensibly with the presence, or absence, of certain bacterial species. To determine whether bacteria can grow in a particular soil, one must measure the water potential of that soil, as well as the lowest water potential tolerance of the particular bacterial species of interest.

5.6.2 Metal tolerance

Among microorganisms there is a wide range of sensitivity to metal ions and it is well known that the <u>Thiobacilli</u> have a high tolerance to soluble metals, especially <u>T. ferrooxidans</u>. Factors affecting the toxicity of metal ions to microorganisms are complex and not well understood. For example, it has been found that the anion of metal salts affects toxicity to <u>T. ferrooxidans</u>; sulphate salts are less toxic than chloride salts, which are less toxic than nitrate salts [Tuovinen and Kelly 1972; Barbic 1977]. Also, the combination of several metal ions may have a synergistic toxic effect, reduce the toxicity of one particular ion, or have no interaction at all [Sadler and Trudinger 1967]. The toxicity of a particular metal ion varies between microbial species and even between strains of a particular species, and is also affected by environmental conditions.

In a natural leaching environment, the concentration of soluble metals increases over fairly long periods, thus allowing a gradual selection of bacteria increasingly tolerant to higher concentrations of metal ions. This

was demonstrated in the laboratory by Tuovinen and Kelly [1974a] who adapted $\underline{\text{T}} \cdot \underline{\text{ferrooxidans}}$ to increasing concentrations of uranium by successive subculture. Cultures tolerant to 5 mM UO $_2^{2+}$ were developed, whereas this concentration was immediately lethal to non-adapted cultures.

Pure strains of T_{\bullet} ferrooxidans and T_{\bullet} thiooxidans were individually tested in our laboratory for tolerance to various metal ions. The results are contained in Table 20. The extreme toxicity of molybdenum (as the molybdate ion) to T. ferrooxidans has been reported previously, and is thought to be due to its competition with sulphate which is required by T. ferrooxidans [Lazaroff 1963; Schnaitman et al. 1969; Steiner and Lazaroff 1974]. The high toxicity of mercury to T. ferrooxidans was also reported by Barbic [1977]. It is interesting to note that our results showed T. thiooxidans to be far less tolerant to cobalt, copper, lead, manganese and nickel than T. ferrooxidans. Tuovinen et al. [1971a] found that \underline{T} . ferrooxidans was less tolerant to various metal ions, especially cobalt, when grown on thiosulphate or elemental sulphur rather than on ferrous sulphate. These authors also found low concentrations of silver and the anions of tellurium, selenium and arsenic ferrooxidans. There is little information on the tolerance of toxic to T. Thiobacillus spp., other than T. ferrooxidans, to individual metal ions, although Brock [1969] noted that copper resistance may be a general property of acidophiles and that resistance to heavy metals may be one of the selection pressures for acidophilic bacteria in acid mine drainages.

It is possible that higher pH sulphur-oxidising bacteria (i.e. not $\underline{\mathsf{T}}$. $\underline{\mathsf{thiooxidans}}$ type) may be less tolerant to metal ions than are $\underline{\mathsf{T}}$. $\underline{\mathsf{ferrooxidans}}$ and acidophilic heterotrophs. This may explain the absence of high pH, sulphur oxidisers from the top and middle level of Intermediate dump and White's dump (except for hole C which contained low concentrations of soluble copper and no iron or zinc) during the dry season when increasing solubilised metal ions may have reached toxic levels. The continued washing out of solubilised metals and other ions by rainwater in the wet season may have then allowed the high pH, sulphur oxidisers to grow without inhibition.

5.6.3 pH

<u>Thiobacillus</u> spp. are able to grow over a wide range of pH, although \underline{T} . <u>ferrooxidans</u> and \underline{T} . <u>thiooxidans</u> are termed acidophilic because an acidic pH is optimum for their growth (Table 1). Identification of autotrophic sulphuroxidising bacteria, beyond the division into groups of low pH, intermediate pH

and high pH, was not attempted because the taxonomy of the <u>Thiobacilli</u> is not yet well defined nor completely understood [Parker and Prisk 1953; Hutchinson et al. 1965, 1966, 1967; Khalid 1978]. In the environment of Rum Jungle mine site it is probable that most, if not all, of the now recognised species of <u>Thiobacillus</u>, occurred, as well as some novel species.

The determination of the pH of the soil in this study was done on the assumption that the pH would not change with dilution (samples were diluted about 1 in 10) because of the buffering capacity of the soil. However, it has been shown [Doemel and Brock 1971; Brock 1978] that, in the case of sulphuric acid soils, this assumption may be misleading and that the pH of the actual soil water is often lower than the pH of the supernatant of diluted soil. It is possible, therefore, that the pHs we observed for acidic soils are slightly higher than the pH of the soil water before dilution. Therefore, the pHs of microbial habitats within the acidic soils may be slightly lower than those reported here.

Sulphuric acid attacks mineral-containing rocks and solubilises the minerals. The degradation of large boulders because of sulphuric acid attack may be very rapid and large rocks and boulders at the base of the dumps (and probably within) were found to be disintegrating and collapsing in a similar manner to rocks in solfatara regions, as described by Brock [1978].

5.6.4 <u>Temperature</u>

Microorganisms are isothermal with their environments and it is probable that the combination of acid and high temperature provides a more restrictive environment than high temperature alone [Brock 1969]. Acidophilic <u>Thiobacilli</u> have an upper limit for growth of about 50 to 55°C [Brock 1978]. Bacteria which have an optimum-growth temperature of about 50°C, or above, are termed thermophiles and several thermophilic <u>Thiobacillus-like</u> types of bacteria have been isolated from acidic, sulphurous environments at temperatures above about 45°C [Brierley and Le Roux 1977; Brierley 1978; Brierley et al. 1978; Norris et al. 1980], and these bacteria have been shown to leach mineral sulphides and oxidise ferrous iron at acidic pH at temperatures in excess of 50°C [Brierley 1980; Le Roux and Wakerley 1980; Norris et al. 1980].

Another autotrophic, low pH, thermophilic organism was isolated by Brock and co-workers from hot sulphur springs (pH 1.55 to 3.5, 76 to 90°C) and named <u>Sulfolobus</u> (for a full description of this organism see Brock [1978], Ch.6).

As well as obtaining energy from the oxidation of ferrous iron, elemental sulphur (aerobically and anaerobically) and other reduced sulphur compounds, <u>Sulfolobus</u> has been found to leach mineral sulphides at 60°C [Berry and Murr 1978].

In the present study, species of <u>Sulfolobus</u> could not have been isolated because incubation at temperatures above 60°C is necessary for their growth and our methods employed an incubation temperature of about 30°C . Similarly, the ability of isolated strains of $\underline{\text{T}}$. <u>ferrooxidans</u> to grow at temperatures above about 37°C was not tested. However, it is most probable that thermophilic strains of $\underline{\text{T}}$. <u>ferrooxidans</u> may have occurred in the top and middle level of Intermediate dump, since large populations of $\underline{\text{T}}$. <u>ferrooxidans</u> were isolated from soils with temperatures above 40°C .

The temperature within a dump is an important parameter in assessing the oxidation rates of pyritic (and other sulphidic) material within the dump. because areas with high rates of pyritic oxidation are generating heat, which does not escape because of the low thermal conductivity of the soil, and thus the temperature rises in such areas [Harries and Ritchie 1981]. generation of heat within a dump promotes air convection and, further, affects the rates of chemical reactions and also bacterial activity (i.e. metabolic Temperature measurements of White's dump [Daniel et al. 1980b] have shown that, in the areas tested, temperatures do not exceed 60°C and that there has been little change in temperature in each area tested over several years. Murr and Brierley [1978], from the results of their work which used large-scale leaching tanks (each holding about 200 t of waste rock), suggested that as temperature within waste rock bodies reached various plateaus, 'regimes', which were related to the activity of reaction microorganisms, developed and that these microorganisms, by controlling their own rates of sulphide degradation, may be able to control temperatures at these plateau levels.

5.6.5 Oxygen and carbon dioxide availability

Several workers have found that $\underline{\mathsf{T}}$. $\underline{\mathsf{ferrooxidans}}$ can fix CO_2 , oxidise ferrous to ferric iron, metabolise elemental sulphur and oxidise sulphide minerals under anaerobic or microaerophilic conditions, with increased CO_2 [Pugh and Umbreit 1966; Baker and Wilshire 1970; Brock and Gustafson 1976; Mackintosh 1978; Babij et al. 1981]. It is most probable, therefore, that low oxygen tension would not stop $\underline{\mathsf{T}}$. $\underline{\mathsf{ferrooxidans}}$ from leaching sulphides,

although it may lower the rate of leaching (which in the long-term situation of sulphidic waste dumps may not be significant). However, CO_2 is essential to these bacteria and increased CO_2 concentrations increase their activity in sulphide degradation [Torma et al. 1972]. Although CO_2 has a low solubility in acidic solutions, carbonate gangue material within the dumps would provide CO_2 in solution [Brierley 1978].

During this study, $\mathbf{0}_2$ and $\mathbf{C0}_2$ levels within the dumps were not measured, although such information would be useful in determining possible mechanisms of pyrite oxidation.

5.6.6 Nutrients

As well as ${\rm CO}_2$ and energy substrates, $\underline{\rm T.}$ <u>ferrooxidans</u> requires other inorganic compounds for metabolic function. It is known that nitrogen, phosphorus, sulphate and magnesium are essential for the growth of $\underline{\rm T.}$ <u>ferrooxidans</u> [Tuovinen et al. 1971b]. It has been shown that $\underline{\rm T.}$ <u>ferrooxidans</u> can fix nitrogen [Mackintosh 1978] and therefore a low supply of ammonium ion in the dump would probably not affect this organism. Phosphorus, sulphate and magnesium would be expected to be available in sulphidic leaching environments and also any trace elements required by $\underline{\rm T.}$ <u>ferrooxidans</u>.

Other species of <u>Thiobacilli</u> probably have similar nutrient requirements to \underline{T} . <u>ferrooxidans</u>, although they would require nitrogen in the form of the ammonium ion. Several soil samples from White's dump were repeatedly subcultured in nitrogen-free salts solutions, and several heterotrophic types of bacteria (at least one from each sample) were found to be able to fix atmospheric nitrogen (as determined by the specific reduction of acetylene to ethylene [Mackintosh 1978]. In environments where ammonium would otherwise not occur, such nitrogen-fixing bacteria, as well as \underline{T} . <u>ferrooxidans</u>, may provide a source of ammonium ion to those bacteria which cannot fix nitrogen. As mentioned previously, the nature and source of the energy substrates of autotrophic sulphur-oxidising bacteria are not known.

Heterotrophic bacteria, as well as requiring inorganic nutrients, require organic carbon compounds. Since vegetation on the dumps is scarce, it may be expected that there would have been little organic matter within the dumps. However, microorganisms are themselves organic material and, when growing, secrete organic compounds (often in large amounts, as in the case of $\underline{\mathbf{I}}$. ferrooxidans [Agate et al. 1969]) and when they die, their cells lyse and

release a number of diverse organic compounds. Heterotrophic bacteria would, therefore, have a relatively wide range of organic compounds (produced by autotrophs and other heterotrophs) available for growth.

Note that nutrients accumulate at interfaces and are adsorbed onto surfaces [Marshall 1976]. Therefore, in water adsorbed onto soil surfaces, concentrations of nutrients will occur which are higher than those in the liquid phase surrounding soil particles. Because of such adsorption effects, microbial numbers and activity would be much higher in soil particle surfaces than in free water [Brock 1979]. Bacteria on soil surfaces may be either reversibly or irreversibly attached [Marshall 1976]; in the former case, agitation in a slurry, such as used in our methods, would probably be sufficient to remove undamaged cells from particles and place them in solution, whereas in the latter it would not.

5.6.7 <u>Difficulties of estimating microbial numbers</u>

The groups of bacteria listed in the tables in Section 4 were isolated on specific selection media which could support the colony growth only of the types of bacteria desired. It was assumed that colonies were formed from single bacterial cells.

A medium completely free of organic carbon (which is known to inhibit growth of \underline{T} . $\underline{ferrooxidans}$, [Tuttle and Dugan 1976]) was developed during the first year of this project by A.M. Khalid by the 'trial and error' modification of several existing, but not wholly satisfactory, techniques. This proved to be extremely time-consuming, but resulted in an easily reproducible, consistently high-quality medium (as reported in detail by Goodman et al. [1980]), which gave reproducible counts of \underline{T} . $\underline{ferrooxidans}$ and a percentage recovery of over 70 per cent.

All attempts to use agar as the gelling agent for a \underline{T} . ferrooxidans medium (as reported by Tuovinen and Kelly [1973] and Manning [1975]) were completely unsuccessful in our laboratory, giving less than 0.1 per cent recovery of \underline{T} . ferrooxidans. However, one disadvantage of our silica gel medium was that it took about two weeks' incubation (at 30°C) to count colonies using a binocular microscope. The method of incubating agar plates in an atmosphere of $\text{air/N}_2/\text{CO}_2$ (1:94:5 by volume) in an anaerobic jar at 30°C for two or three days, as developed by Mackintosh [1978], produced the typical rusty-brown colonies (due to the precipitation of ferric salts in the colony

[Goodman et al. 1980]).

The numbers of microorganisms reported here accounted for free-swimming bacteria and those not firmly attached to mineral particles. It has been shown that \overline{I} . ferrooxidans and \overline{I} . thiooxidans attach firmly (i.e. irreversibly) to mineral and sulphur particles and cannot be dislodged even by extreme agitation [Brierley et al. 1973; Karavaiko and Pivarova 1977; Berry and Murr 1978; Baldensperger et al. 1974]. In the case of \overline{I} . ferrooxidans, Le Roux et al. [1973] found that there were three to four times as many bacteria attached to solid particles as there were free-swimming, and other work in our laboratory [Babij et al. 1981] showed that the number of cells attached to mineral particles was about five to six times higher than those in the supernatant.

numbers indicate the activities bacterial However, do not Low numbers of T. ferrooxidans may degrade large microorganisms in situ. amounts of pyritic material because the relative amount of energy acquired from pyrite oxidation by bacterial cells is low compared to the amount of energy required by these cells for CO2 fixation, which must proceed even in non-growing cells to maintain metabolic function and cell viability. Bacteria in soil may grow rapidly when conditions become favourable (for example, when a substrate becomes available) and then remain inactive for long periods if conditions are not favourable for growth. Thus, high cell numbers found at a particular time may not necessarily indicate high microbial activity at this time, although an increase in numbers between two sampling periods does indicate that at some time between the sampling periods there has been extensive microbial activity. One method of determining the activity of autotrophs in situ is to measure the uptake of radioactively labelled CO2 into organic cell material, as developed by Brock and co-workers [Smith et al. 1972; Belly and Brock 1974].

6. CONCLUSIONS

Areas containing sulphidic waste material at the Rum Jungle mine site support a diverse and abundant microbial flora. Populations of \underline{T} . $\underline{ferrooxidans}$, one of the most important microorganisms responsible for catalysing the oxidation of many mineral sulphides, particularly pyrite, are associated with all areas where sulphidic degradation is occurring, and are the major bacterial component in Intermediate dump. In White's dump,

acidophilic heterotrophic bacteria consistently occur, usually in high numbers, and autotrophic sulphur-oxidising bacteria flourish in the wet season.

Conditions in White's dump are heterogeneous and different areas support different types of microorganisms. Pyritic oxidation probably occurs in discrete areas rather than homogeneously through the dump. The dump is aerobic except for an anaerobic zone less than 1 m high at the very base of the dump. Because of the low levels of soluble iron found, it is thought that any ferric iron, formed from the oxidation of ferrous iron by <u>I. ferrooxidans</u>, precipitates within the dump (probably as complex jarosites), and therefore pyritic oxidation by ferric iron may not be an important mechanism in White's dump.

Conditions in Intermediate dump are more homogeneous than in White's and it supports a more restricted range of microbial species than does White's. Intermediate dump has no anaerobic zone supporting strict anaerobic bacteria, even at the base, and it is possible that a chimney effect is occurring. The top of the southwest square of the dump contains far higher concentrations of total soluble solids, including copper and iron, than any area sampled in White's. There is some indication that temperatures within the first 5 m from the surface of this region are higher than temperatures in White's and it is possible that pyritic oxidation is occurring more uniformly and at higher rates in Intermediate dump than in White's.

Conditions within Intermediate and White's dumps are different, as are the leaching behaviours of the two dumps. The reasons for this are as yet undetermined, but it is unlikely that the relatively small age difference between the dumps (White's was completed in 1958 and Intermediate, in 1964) is sufficient explanation.

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TABLE 1 GROWTH CHARACTERISTICS OF THIOBACILLI

terlai Species

terial Species	рН	Temperature (°C)	Inorganic Substrates	$\infty_2^{}$ Fixation	⁰ 2 Requirement	Growth On Organic CPDS
dophilic						
n Oxidiser						
• ferrooxidans*	2.5	28-35	Ferrous,	Positive	Facultatively ⁽¹⁾	Autotrophic
	(<1.4-60)	50	MS, RSC		aerob1c	•
dophilic						
phur Oxidisers						
• thiooxidans*	2.0-3.5	28 - 30	MS, RSC	Positive	Aerobic	Autotrophic
(2)	(<0.5-6.0)	55				, , , , , , , , , , , , , , , , , , ,
• acidophilus (2)	3.0	25-30	Sonly	Positive	Apparently	Facultatively
	(1.5-6.0)				aerobic	autotrophic
• ferrooxidans						
(as above)						
ermediate pH						
ohur Oxidisərs						
Intermedius*	ND	30	RSC	Pos Itive	Aerobic	Facultatively
	(1.9-7.0)					autotrophic
neapolittanus*	6.2-7.0	28	R \$ C	Positive	Aerobic	Autotrophic
	(3.0-8.5)					,
perometabolis*	ND	30	RSC + organic	ND	Aerobic	Mixotrophic
	(2•6-6•8)		CPD			
рН						
hur Oxidisərs						
thioparus*	6.6-7.2	28-30	RSC	Positive	Aerobic	Autotrophic
	(4.5-10)					Auton ophic
denitrificans*	7.0	30	RSC	Positive	Facultatively	Autotrophic
					aerobic	,
novel[us*	7.8-9.0	30	RSC	Positive	Aerobic	Facultatively
	(5.0-9.2)		·····	<u> </u>		autotrophic

Values in brackets indicate growth range, top value is laboratory optimum; Lower value is maximum tolerated [Brock 1978], top value is laboratory optimum; = metal sulphide; RSC = reduced sulphur compounds (including elemental sulphur); = elemental sulphur; CPDS = compounds; ND = not determined; From R.E. Buchanan and N.E. Gibbons [1974]; Experimental evidence in text pp.45,6 and Babij et al. [1981]; Guay and Silver [1975].

TABLE 2
COMPARISON OF DATA: INITIAL INVESTIGATION
SEPTEMBER 1975

		White'	s Dump		Intermo		Sulph: Hea		Oxio Hea	
	soil,	n=23 s.d.	water*,	n=2 s.d.	soil,	n=4 s.d.	soil,	n=5 s.d.	soil,	n=3 s.d.
Moisture %	7.2	4.5			9.9	3.2	8.2	3.0	11.4	2.2
рН	3.98	1.2	2.85	0.4	2.55	0.4	2.34	0.7	2.67	0.3
Log soluble solids (ppm)	3.5	0.5			4.4	0.5	4.4	0.7	4.3	0.04
Log soluble copper (ppm)	0.5	0.7	1.5	0.5	1.8	1.3	2.9	0.4	3.1	0.04
Log soluble iron (ppm)	0.3	0.8	1.4	0.1	2.4	0.8	2.2	1.4	1.8	0.6
Log no. T.ferrooxidans	2.2	0.9	3.0 (2)	0.2	2.1 (3)	0.5	2.4 (2)	0.6	2.9	0.2
Log no. S oxidisers pH 3.5	NG		(1)		NG		NG		NG	
Log no. S oxidisers pH 6.2	2.8 (13)	0.7	NG		2.9 (2)	0.1	2.8 (2)	0.2	NG	

 $^{^{\}infty}$ Log no. bacteria = log organisms/g dry weight, or /mL;

n = number of samples; \bar{x} = mean; s.d. = standard deviation;

NG = no growth detected (or at a low level in only 1 sample);

⁽q) = only q samples positive for bacterial species;

^{* =} Sampled at Spring No.4, holes dug and allowed to fill with seepage.

TABLE 3
COMPARISON OF DATA: INITIAL INVESTIGATION
MARCH 1976

	W	hite'	s Dump	·	Interm Du	ediat mp	e Sı	ılphi	de He	ар		xide	Heap	
	soil x	,n=12 s.d.		ngs, 5 s.d.	wate x	r,n=5 s.d.	soil,				_	n=6 s.d.	I —	r,n=7
Moisture %	11.3	4.2					14.6	2.2			18.3	3.0		
PH	4.38	3 1.0	3.14	0.36	2.70	0.3	2.85	0.5	2.60	0.3	3.42	0.3	2.74	0.4
Log soluble solids (ppm)	2.9	1.0	4.3	0.1	4.1	0.6	4.1	0.3	3.7	0.6	2.9	0.4	3.6	0.9
Log soluble copper (ppm)	0.6	0.8	1.8	0.3	2.1	0.5	2.2	1.4	1.9	1.2	1.6	0.4	2.7	1.1
Log soluble iron (ppm)	0.3	0.4	1.7	0.5	2.1	1.0	2.2	1.1	2.0	1.3	0.3	0.5	1.8	1.5
Log no. T. ferrooxidans	1.8 (10)	- 1	1.9 (4)	1.1	2.4 (5)	0.4	2.3 (12)	0.3	3.0 (5)	0.4	2.2 (5)	0.2	2.7 (5)	0.5
Log no. S oxidisers OH 3.5	3.3 (9)	1.2	2.8 (4)	1.8	4.2 (5)	1,1	3.0 (9)	0.7	3.7 (2)	1.4	NG		3.6 (4)	8.0
Log no. S oxidisers DH 6.2	3.0 (8)	1.2	1.2 (2)	1.7	NG		NG		NG		(2)		NG	
			7				· • · · · · · · · · · · · · · · · · · ·							

 $^{^{\}alpha}$ Log no. bacteria = log organisms/g dry weight, or /mL;

n = number of samples; \bar{x} = mean; s.d. = standard deviation;

NG = no growth detected (or at a low level in only 1 sample);

⁽q) = only q samples positive for bacterial species.

TABLE 4
TAILINGS MATERIAL: SURFACE SAMPLES

			Sı	rface S	amples		·	
	Septemb	er 19 7 5	March n=2		Septemb n=		May n=	
	West n=1	East n=1	Depth:2	20-50cm	Depth:		_	20-60cm
	25cm	35cm	х	s.d.	x	s.d.	х	s.d.
Moisture %	7.2	11	26	7.0	18	18	15	9.0
рН	4.4	3.0	4.1	0.3	3.7	0.2	4.2	0.2
Log soluble solids (ppm)	3.0	4.1	3.5	0.9	3.9	0.4	4.2	0.4
Log soluble copper (ppm)	ND	3.3	1.1	0.1	2.7	0.5	2.7	0.3
Log soluble iron (ppm)	ND	1.0	1.0	0.4	1.1	0.1	ND	
Log no. T. ferrooxidans	ND	1.6	2.6	0.1	3.0		ND	
Log no. S oxidisers pH 3.5	ND		5.2	0.1	ND		ND	
Log no. S oxidisers pH 6.2	2.6	2.7	3.2	1.4	3.4		ND	
Log no. acidophilic heterotrophs pH 3.5					3.6	0.6	ND	

 $^{^{\}alpha}$ Log no. bacteria = log organisms/g dry weight, or /mL;

n = number of samples; \bar{x} = mean; s.d. = standard deviation;

ND = not detected.

TABLE 5 TAILINGS MATERIAL: SEPTEMBER 1976

		Septemb	er 1976	
	Depth Sa	ŀ	Surfac	ce Samples n=2
	Depth:50-	I.	Depth	:0-30 cm
	x	s.d.	x	s.d.
Moisture %	29			<u></u>
Нq	1 29	1.0	18	18
	4.2).1	3.7	0.2
Log soluble solids (ppm)	4.2	_		***
	7.2	-1	3.9	0.4
Log soluble copper (ppm)	3.1 0	.1		
)	• 1	2.7	0.5
Log soluble iron (ppm)	1.5 0.			
	2.5 0.	. 3	1.1	0.1
Log no. ^α L <u>ferrooxidans</u>	2.8 0.			
	2.8 0.	3	3.0	
og no.				
Н 3.5	ND		ND	
og no.				:
oxidisers	2.5 0.4	,		
H 6.2	2.5 0.2	*	3.4	j
og no.				
cidophilic eterotrophs	3.7 0.4			l
1 3.5	3.7 0.4		3.6	0.6
				İ

Log no. bacteria = log organisms/g dry weight, or /mL;

οc

⁼ number of samples; \bar{x} = mean; s.d. = standard deviation; n = not detected. ND

TABLE 6 TOP OF WHITE'S DUMP: COMPARISON OF WET AND DRY SEASONS - AREA D

			A:	rea D	
	Dry Hole D Depth:1		Wet Hole D' Depth:1.	, n=21	Probability (P) that x is same
Moisture %	9.7	4.1	14.5	1.7	*
pH	3.58	0.3	3.83	0.3	p = 0.05
Log soluble solids (ppm)	3.5	0.3	2.8	1.0	p = 0.1
Log soluble copper (ppm)	1.6	0.4	0.4	0.6	p = 0.001
Log soluble iron (ppm)	ND		0.3		
Log no.	2.2 (³ / ₉)	0.2	(13/ ₁₇)	0.9	*
Log no. S oxidisers	(1/9)		3.9 (11/ ₂₁)	0.8	
pH 6.2 Log no. Acidophilic heterotrophs	2.5 (⁶ / ₉)	0.2	4.0 (¹⁵ / ₁₉)	1.4	*

Log no. bacteria = log organisms/g dry weight;

⁼ number of samples; \bar{x} = mean; s.d. = standard deviation; n

⁼ probability that the means are the same, as determined by Students' t test;

⁼ P could not be determined (difference in variances, i.e. s.d. 2 too large, as determined by the F test);

⁽p/q) = p out of q samples positive;

⁼ not detected. ND

TABLE 7 TOP OF WHITE'S DUMP: COMPARISON OF WET AND DRY SEASONS - AREA E

				Area E	
	Hole	Dry E, n=9 :1.2-3.6 m s.d.	Hole	Vet E', n=18 1.2-3.6 m s.d.	Probability (P)
Moisture %	8.6	3.1	13.8	2.5	P = 0 000
PH Log gold 1	4.56	1.0	5.91	0.7	P = 0.001 P = 0.001
Log soluble solids (ppm)	3.8	0.2	3.2	0.5	P = 0.005
Log soluble copper (ppm)	0.8	0.6	0.5	0.7	P = 0.5
Log soluble iron (ppm)	ND		0.7	0.8	- 0.5
Log no. T. ferrooxidans	(¹ / ₉)		(² / ₁₈)		
Log no. S oxidisers PH 6.2	(¹ / ₉)	(4.5 ¹⁸ / ₁₈)	0.7	
og no. cidophilic eterotrophs	3.6 (⁵ / ₉)	0.4	1/18)		

Log no. bacteria = log organisms/g dry weight; n

⁼ number of samples; \bar{x} = mean; s.d. = standard deviation; Р

⁼ probability that the means are the same, as determined by

⁼ P could not be determined (difference in variances, i.e. $s.d.^2$ too large, as determined by the F test); (p/q) = p out of q samples positive;

⁼ not detected. ND

TABLE 8 TOP OF WHITE'S DUMP: COMPARISON OF WET AND DRY SEASONS - AREA G

			Area	G 	
	Dr Hole G, Depth:	y , n = 9 1.2-3.6 m	Wet Hole G', Depth: 1		Probability (P) that x is same
	- x	s.d.	×	s.d.	
Moisture (%)	6.2	2.0	14.7	3.2	P = 0.001
	3.0	0.4	3.42	0.4	P = 0.02
pH Log soluble solids (ppm)	3.7	0.3	3.6	0.2	p = 0.2
Log soluble copper (ppm)	1.3	0.2	0.8	0.8	*
Log soluble iron (ppm)	ND		0.4	0.4	
Log No. ^α T. ferrooxidans	2.1 (⁵ / ₉)	0.9	2.3 (⁶ / ₁₈)	0.4	p = 0.6
Log No. S oxidisers	(2/9)		5.5 (14/ ₁₈	0.7	
Log No. Acidophilic heterotrophs	3.5 (⁸ / ₉)	0.5	3.6 (¹⁶ / ₁₆		P = 0.8

Log no. bacteria = log organisms/g dry weight;

⁼ number of samples; \bar{x} = mean; s.d. = standard deviation;

⁼ probability that the means are the same, as determined by the n Р Students' t test;

⁼ P could not be determined (difference in variances, i.e. $s.d.^2$ too large, as determined by the F test);

⁽p/q) = p out of q samples positive;

ND = not detected.

WITHIN THE HOLES DUG IN THE MARCH 1977 WET SEASON TOP OF WHITE'S DUMP: COMPARISON OF TWO DEPTHS TABLE 9

		-	Hole D'			Hole F	ļ.		-			
	1.2-3.6 m		3.6-4.4 m				,		==	Hol	Hole G'	
	n = 21	# #			1.2-3.6 m	n 3.6-4.7 m	.7 m		1.2-3 6 #			
	ı×	; ;		<u>п</u>	o d	II	15		n = 18		3.6-4.7 m	
Moisture (%)		<	s.d.			ı×	ν. (γ.	д			1	
,	14.5	16.5	3.5	P=0.1	2 -];	;		×	· ×	s.d.	ц ——
	3.83	3.52	0,3	DIO 000	0 (14.6	2.8	₽ = 0.3	14.7	16.2	1.8	P=0 3
Log soluble solids (ppm)	2.8	3.4	0.2	*	I6 o	5.83	0.4	P = 0.7	3.42	3.64	0.3	7 C
Log soluble copper (ppm)	0.4	0.5	0.5	С Д	3,2	3.4	0.4	P = 0.4	3.6	3.6	0.5	, E
Log soluble iron (ppm)	0.3	(1/10)	•	0	S 0	6.0	8.0	P = 0.1	0.8	1.1	0.7	
Log no. lpha T. ferrooxidans	2.4	2.3			0.7	0.8	6.0	P = 0.7	0.4	0.4	0.7	5.01.4 M
	(13/17)	(3/10)			(-/18)	(1/15)			2.3	2.8	9.0	, j
Log. no. S oxidisers		4.4	1.1	P=0.3	4.5	v			(6/18)	(10/15)	•	69
	(**/21)	(10/10)			(18/18)	$(^{15}/_{15})$	· · ·	₽ 0.0	5.5	5.5	6.0	P=1
Acidophilic heterotrophs pH 3.5	(15/19)	5.0 (⁹ / ₁₀)	1.4	P=0.1	(1/18)	Q			3.6	4.1	·	4
									(16/16)	$(^{10}/_{12})$		F=0.3
								=	_	!	-	

= number of samples; \tilde{x} = mean; $s \cdot d \cdot = standard deviation;$ Log no. bacteria = log organisms/g dry weight;

= P could not be determined (difference in variances, i.e. s.d.² too = probability that the means are the same, as determined

ģ

large, as determined by the F test); (p/q) = p out of q samples positive;

= not detected.

TABLE 10 WHITE'S DUMP: EAST WALL (HOLES A, F, J AND AREA S4) AND SOUTHWEST WALL (HOLES B AND C)

								_	Z C C C C C C		Hore C) ' -	
		1	7 O COT	Į.	Hole J, n = 3		Area S 4, n = 3		Depth: 0.6-3.6 m		Depth: 1.2-3.6	2-3.6 m	
	Hole A, 1	E	Depth: 1.2	<u> </u>	Depth: 0-	_	uriace sa	+	\ ,	-	ı×	s.d.	
	Depth: 1.2-3.0	_		-	1,	s.d.	ı×	s.d.	×	S.a.	:		
	ı×	s.d.	×	s.d.	٠	+-		1	, ,	2.3	15.6	1.1	
	11.7	4.5	14.2	5.3	5.2	0.3	44	- T	, 6	0.5	5.97	1.0	
Moisture (%)	2.77	0.2	3.12	0.3	0.0	0.1	3.30	4	, 6	0.3	3.6	0.1	
Bg (maa) spilos (maa)	3.6	0.2	2.9	1.1	2.2	1.9	4. 6	n 5	1.2	1.0	0.4	9.0	
Log soluble solubra (mag)	1.0	0.7	1.3	8.0	2.3	0.4	ж. °	, r.	6.0	9.0	g		
Tod sormpre control (mom)	1.0	0.7	1.2	8.0	QN		2.5		2.6	0.5	(6/2)		
Log soluble iton vrr	2.8	0.4	2.9	0.2	4.5	0.5	4.4	†	(3/11)				
Log no. Tr. ferrooxidans	(6/9)		(6/5)			c c	, 5, 4, 5, 5, 5, 5, 5, 5, 5, 5, 5, 5, 5, 5, 5,	0.2					
					o. 	r. O	$(\frac{2}{4},\frac{2}{3})$						
Log no. S oxidisers PH4.8	<u></u>		(2/6)		2.2	4.0	(1/3)		2.8	0.4	3.5	6.0	
Log no. S oxidisers pH6.2	<u> </u>			•	<u> </u>		4.6	0.2	4.1	0.4	(1/6)		
Log no. Acidophilic heterotrophs	3.8	9.0	3.2 (6/9)	r	•		(5/3)		2.2	0.3	1.7	1.0	
Log no.	2.5	0.2	$\binom{2.6}{(^2/9)}$	0.1	∄ 				(*/11)		5		
Desulfovibrio SPP.					-	·		• • • •					

Log no. bacteria = log organisms/g dry weight; = number of samples; \bar{x} = mean; s.d. = standard deviation; = number of samples;

= probability that the means are the same, as determined by

= P could not be determined (difference in variances, i.e. $s.d.^2$ too large, as determined by the F test); Students' t test;

(p/q) = p out of q samples positive; ND = not detected.

TABLE 11 TOP OF WHITE'S DUMP - AREAS D, E, G

Area D Area E	Dry Mar	76) (March 1977) (Sept. 1976) (Mar 1977) (1.2-4.7 m 1.2-3.6 m 1.2-3	9.7 15.5 8.6 14	:	3.5	1.6	ND 0.3	$\frac{2.2}{(3/9)}$ $\frac{2.5}{(16/2)}$ ND 0.4	$\binom{1/9}{21/2}$ $\binom{1/9}{21/2}$ $\binom{1}{2}$ $\binom{1}{9}$ 4.5 $\binom{2}{9}$	$\begin{array}{cccccccccccccccccccccccccccccccccccc$
		-	Moisture (%)		нd	Log soluble solids (ppm)	Log soluble copper (ppm)	Log soluble iron (ppm)	Log no. $^{\infty}$ T. ferrooxidans	Log no. S oxidisers pH6.2	Log no. Acidophilic heterotrophs pH3.5

~ Log no. bacteria = log organisms/g dry weight; ND = not detected; (p/q) = p out of q samples positive.

TABLE 12
WHITE'S DUMP: SPRING NO.4

		•	White's Spri	ng No.4	
	Septemb	er 1975	March 1976	May 1979	June 1978
	Mud n=1	Seepage Water n=1	Water n=1	Water n=1	Water n=1
•	3.1	2.6	2.9	3.4	3.4
pH Log soluble solids (ppm)			4.3	4.5	4.3
Log soluble copper (ppm)	1.1	1.9	2.0	2.2	2.0
Log soluble iron (ppm)	1.5	1.3	1.7	2.8	2.1
Log no.	3.1	2.8	2.1	2.9	1.8
T. ferrooxidans	4.9	ND	2.1	ND	NID
S oxidisers pH3.5				3.6	2.6
Log no. S oxidisers pH4.8	ND	ND	ND	3.8	ND
Log no. S oxidisers pH6.2				ND	2.5
Log no. Acidophilic heterotroph pH3.5	s				

[∝] Log no. bacteria = log organisms/mL;

n = number of samples;

ND = not detected.

TABLE 13
TOP OF INTERMEDIATE DUMP: COMPARISON OF
EARLY MAY 1979 AND LATE JUNE 1978 TO A
MAXIMUM DEPTH OF 3.5 m

	· · · · · · · · · · · · · · · · · · ·		Top			
	May 1979 Hole S, x=8 Depth 0-3.5 m		June Hole X, Depth 0-	1978 n=7	Probability (P)	
	x	s.d.	×	s.đ.	The same	
Moisture (%)	12.8	1.2	13.4	1.3	P = 0.2	
рН	3.33	0.4	3.26	0.3	P = 0.7	
Log soluble solids (ppm)	4.9	0.1	4.9	0.1	P = 0.9	
Log soluble copper (ppm)	3.1	0.4	2.9	0.6	P = 0.5	
Log soluble iron (ppm)	2.6	0.5	2.6	0.3	P = 1	
Log no. ^α T. ferrooxidans	2.8	0.3	4.9	0.4	P = 0.001	
Log no. S oxidisers pH4.8	ND		(¹ / ₇)			
Log no. S oxidisers pH6.4	ND		(¹ / ₇)			
Log no. Acidophilic heterotrophs pH3.5	ND		ND			

Log no. bacteria = log organisms/g dry weight, or /mL; n = number of samples; $\bar{x} = mean$; s.d. = standard deviation (p/q) = p out of q samples positive; ND = not detected.

TABLE 14
MIDDLE LEVEL OF INTERMEDIATE DUMP: COMPARISON OF
EARLY MAY 1979 AND LATE JUNE 1978 TO A MAXIMUM
DEPTH OF 4 m

		M	iddle Leve	1		
-	May 1979 Hole T, n=8 Depth: 0-3.5 m		June 1 Hole Y, Depth: (n=9	Probability (P) that x is same	
	x	s.d.	x	s.d.		
Moisture (%)	14.8	6.4	14.4	4.4	P = 0.98	
рН	3.14	0.2	3.30	0.2	P = 0.3	
Log soluble solids (ppm)	4.0	0.3	3.7	0.3	P = 0.05	
Log soluble copper (ppm)	1.7	0.1	1.3	0.5	P = 0.05	
Log soluble iron (ppm)	1.4	0.7	(² / ₉)			
Log no. T. ferrooxidans	2.6	0.2	4.8 (⁶ / ₉)	0.5	p = 0.001	
Log no. S oxidisers pH4.8	(1/8)		2.8 (⁴ /9)	0.4		
Log no. S oxidisers pH6.2	ND		(1/9)			
Log no. Acidophilic heterotrophs pH3.5	ND	·	2.6 (⁴ / ₉)			

Log no. bacteria = log organisms/g dry weight, or /mL; n = number of samples; \bar{x} = mean; s.d. = standard deviation; (p/q) = p out of q samples positive; ND = not detected.

TABLE 15
BASE OF INTERMEDIATE DUMP: COMPARISON OF
TWO HOLES DUG ABOUT 3 m APART IN JUNE 1978

		Base			
	Hole Z, n=7 Depth 0-3 m		Hole Depth	Z*, n=4 3-4.5 m	Probability (P)
	x	s.d.	×	s.d.	that x is same
Moisture (%)	8.0	3,5	22.4	4.2	P = 0.001
рн	2.77	0.2	4.0	0.4	P = 0.001
Log soluble solids (ppm)	3.9	0.6	4.0	0.3	P = 0.6
Log soluble copper (ppm)	1.0	0.8	2.3	0.9	P = 0.05
Log soluble iron (ppm)	2.3	1.4	(¹ / ₄)		
Log no. [¤] T. ferrooxidans	4.6	0.4	5.1	0.1	P = 0.05
Log no. S oxidisers pH4.8	2.8 (³ / ₇)	0.7	3.5	1.1	P = 0.3
Log no. S oxidisers pH6.2	ND		1.6 (² / ₄)	0.8	
og no. Acidophilic heterotrophs	ND		2.8	0.6	

Log no. bacteria = log organisms/g dry weight, or /mL; n = number of samples; \bar{x} = mean; s.d. = standard deviation; (p/q) = p out of q samples positive; ND = not detected.

TABLE 16
BASE OF INTERMEDIATE DUMP: COMPARISON OF
EARLY MAY 1979 AND LATE JUNE 1978 TO A
MAXIMUM DEPTH OF 4.5 m

			1	Base			
	May 1979		June	Probability (P)			
	Hole V, n=9		Hole Z, n=7 Depth: 0-3.0 m	Hole Z, n=7 Hole Z*, n=4 epth: 0-3.0 m Depth 3.0-4.5 m		that x is same	
	Depth: 0 Col. 1 X	s.d.	Col. 2	Col. 3 x	Cols. 1 & 2	Cols. 2 & 3	
Moisture (%)	14.8	7.6	8.0	22.4	P=0.05	P=0.1	
На	3.16	0.3	2.77	3.98	P=0.02	P=0.001	
Log soluble solids (ppm)	4.2	0.2	3.9	4.0	P=0.1	P=0.4	
Log soluble copper (ppm)	(2/9)		1.0	2.3			
Log soluble iron (ppm)	1.9	0.5	2.3	(1/4)	P=0.5		
Log no. a	3.0	0.8	4.6	5.1	P=0.001	P=0.001	
T. ferrooxidans Log no. S oxidisers pH4.8	(2/9)		2.8 (³ / ₇)	3.5			
Log no. S oxidisers pH6.2	ND					- 0 0	
Log no. Acidophilic heterotrophs pH3.5	2.9 (³ / ₉)	0.9	ND	2.8		P=0.9	

Log no. bacteria = log organisms/g dry weight, or /mL; n = number of samples; \bar{x} = mean; s.d. = standard deviation; (p/q) = p out of q samples positive; ND = not detected.

TABLE 17
INTERMEDIATE DUMP: COMPARISON OF
EARLY MAY 1979 AND LATE JUNE 1978

	Тор		Middle	Level		Base	
	May June		May	June	May	Ju	ine
	0-3.5 m	0-3 m	0-3.5 m	0-4 m	0-3.5 m	0-3 m	3-4.5 m
Moisture (%)	13.1		14.	.6	14.8	8.0	22.4
рН	3.30		3,	.22	3.16	2.77	3.98
Log soluble solids (ppm)	4.9		4.0	3.7	4.2	3	. 9
Log soluble copper (ppm)	3.0		1.7	1.3	0.4	1.0	2.3
Log soluble iron (ppm)	2.6		1.4	0.3	2.	1	0.3
Log no. ^α T. ferrooxidans	2.8	4.9	2.6	4.8	3.0	4.6	5.1
	· 				· · · · · · · · · · · · · · · · · · ·		

 $^{^{\}infty}$ Log no. bacteria = log organisms/g dry weight, or /mL.

TABLE 18
INTERMEDIATE DUMP: LATE JUNE 1978
VERTICAL PROFILES OF TEMPERATURE AND
RELATIVE HUMIDITY WITHIN THE HOLES

Hole	Distance from Top of Hole	°с	Ambient C	% RH	Ambient % RH
z	1.5	22		96	
	3.0	22	•	5-7	i
z*	3.5	31	27	76	10
	4.0	31	27	90	10
Y	0.5	33.5	29	100	10
	2.0	37	26.5	100	8
	4.0	43	27.5	100	8
x	0	31.5	25	83	8
	1.5	41.5	25	100	8
	3.0	44	27	100	8
		<u> </u>	<u> </u>	<u> </u>	

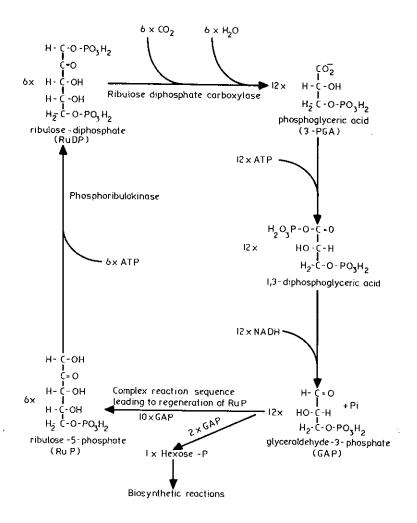
TABLE 19
INTERMEDIATE DUMP: EARLY MAY 1979
VERTICAL PROFILES OF TEMPERATURE AND
RELATIVE HUMIDITY WITHIN THE HOLES

Hole	Distance from Top of Hole		Ambient O _C	% RH	Ambient % RH
s	0.5	41	37	100	24
	1.0	45		100	
	1.5	44		100	
	2.0	45		100	
	2.5	45		100	
	3.0	47.5		100	
	3.5	47		100	
т	0.5	35	37.5	100	24
	1.0	37.5		100	
	1.5	37.5		100	
	2.0	37.5		100	
ļ	2.5	41		100	
	3.0	40		100	
	3.5	43		100	
v	0.5	36	40	100	15
	1.0	34		100	
	1.5	34		100	
	2.0	32.5		100	
	2.5	32		100	
	3.0	31		100	
	3.5	29		100	

TABLE 20
METAL TOLERANCE OF THIOBACILLI

	Maximum Concentrati	on Tolerated ($\mu g g^{-1}$)
Metal	$\frac{\text{T. ferrooxidans}}{\text{BJR-Kl}^1}^{\alpha}$	<u>T. thi</u> οοxidans ^α BJR-K01 ¹
As	500	500
Bi	1 000	1 000 ^β
Со	20 000	Υ
Cu	40 000	3 000
Pb	5 000	1 000
Mn	40 000	4 000
Мо	5	5
Hg	6	6
Ni	40 000	5 000
Zn	30 000 [€]	20 000

- α Isolated and characterised [Khalid 1978];
- β Turned black, possibly because of sulphide formation;
- Y Cobalt toxic to BJR-K01;
- 6 Medium became black, precipitation occurred;
- ε At $[Zn] = 40 000 \mu g g^{-1}$ growth occurred slowly;
 - T. ferrooxidans was grown on ferrous sulphate;
 - T. thiooxidans was grown on elemental sulphur;
- 1 Strain no. designations [Khalid 1978].



The following description is from Brock [1979]. The enzyme ribulose diphosphate carboxylase reacts one molecule of ${\rm CO}_2$ with one molecule of ribulose diphosphate (RuDP) to form two molecules of 3-phosphoglyceric acid (PGA), one of which contains the CO_2 molecule, the carbon atom being at the same oxidation level as free CO_2 . One molcule of $\mathrm{H}_2\mathrm{O}$ is also required during this step. PGA is then reduced to the oxidation level of carbohydrate and this step requires, for each PGA molecule, both one molecule of ATP and one of NADH to form one molecule of glyceraldehyde-3-phosphate. The regeneration of the RuDP used in the first step, requires three molecules of ATP and five molecules of glyceraldehyde phosphate to form three molecules of RuDP. The final step in the regeneration of RuDP, the phosphorylation of ribulose-5-phosphate (Ru-5-P) by ATP, is catalysed by the enzyme phosphoribulokinase. Both the enzymes ribulose diphosphate carboxylase and phosphoribulokinase are unique to autotrophic ${\rm CO}_2$ fixation. One carboxylation reaction requires one molecule each of ${\rm CO}_2$ and RuDP and produces two molecules of PGA which are converted to two molecules of glyceraldehyde phosphate. Since five molecules of glyceraldehyde phosphate are needed to complete one 'turn' of the cycle (i.e. to regenerate the RuDP), three carboxylations must take place for each turn of the cycle. Three carboxylations yield six glyceraldehyde phosphate molecules, of which five molecules are used (to regenerate the three molecules of RuDP) and therefore there is a net gain of one glyceraldehyde molecule for every three 60_2 molecules fixed. Therefore to convert three molecules of 60_2 to one of carbohydrate (i.e. glyceraldehyde phosphate molecule), nine ATP and six NADH are required. Thus, to produce one hexose sugar molecule, two glyceraldehyde molecules are necessary, raising the requirement to 18 ATP and 12 NADH molecules to convert six CO_2 molecules to one sugar molecule.

FIGURE 1. THE CALVIN CYCLE [Schlegel 1975; Brock 1979]

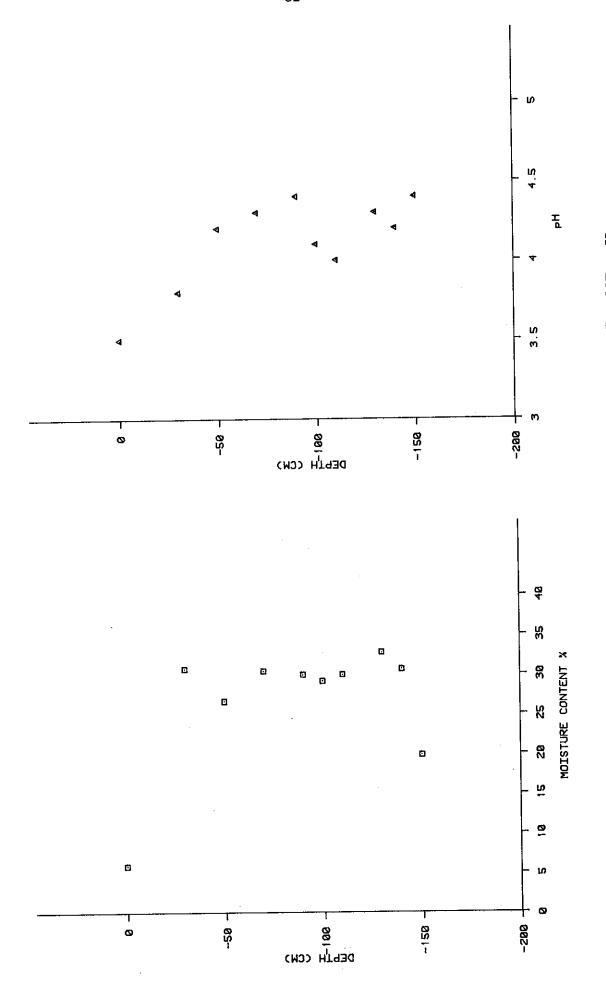


FIGURE 2. TAILINGS DAM, MOISTURE CONTENT AND PH

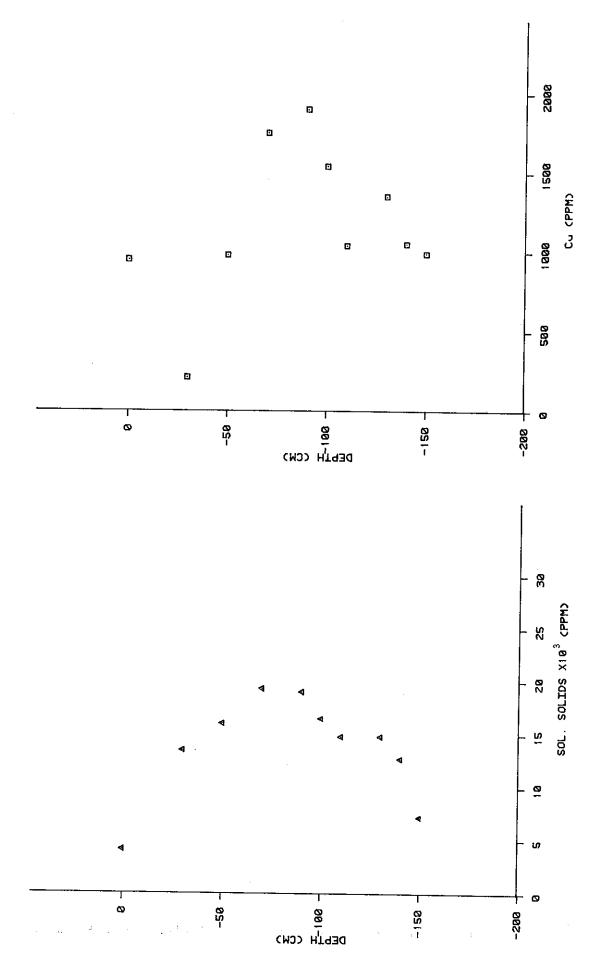
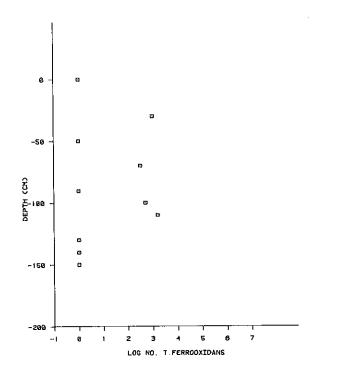
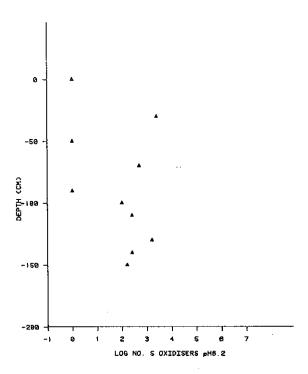


FIGURE 3. TAILINGS DAM, SOLUBLE SOLIDS AND COPPER CONTENT





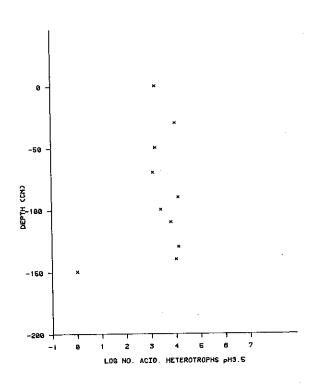
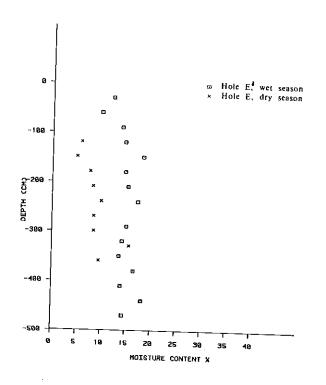
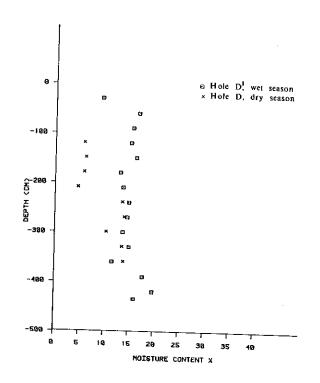


FIGURE 4. TAILINGS DAM, BACTERIAL POPULATIONS/g D.W.





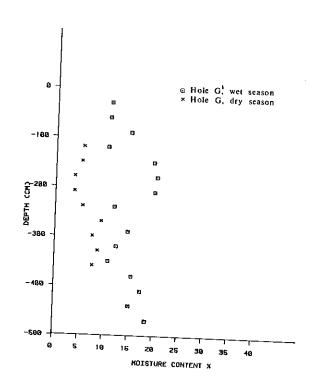
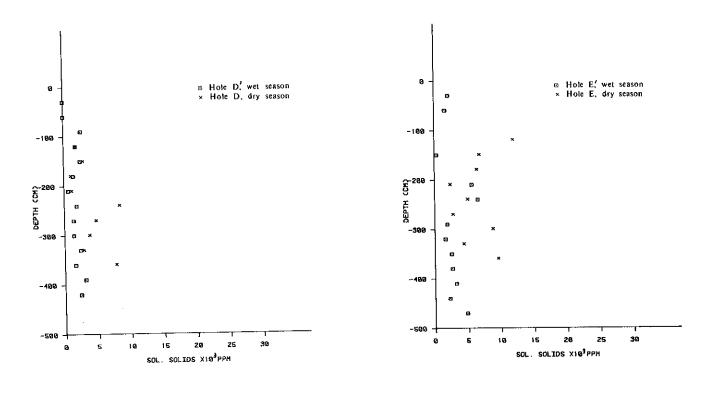


FIGURE 5. TOP OF WHITE'S DUMP, MOISTURE CONTENT



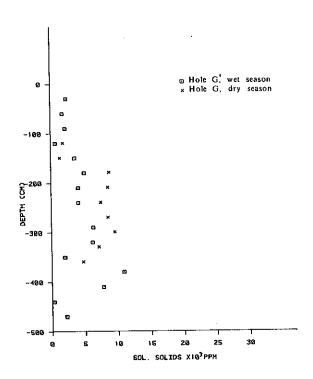


FIGURE 6. TOP OF WHITE'S DUMP, SOLUBLE SOLIDS CONTENT

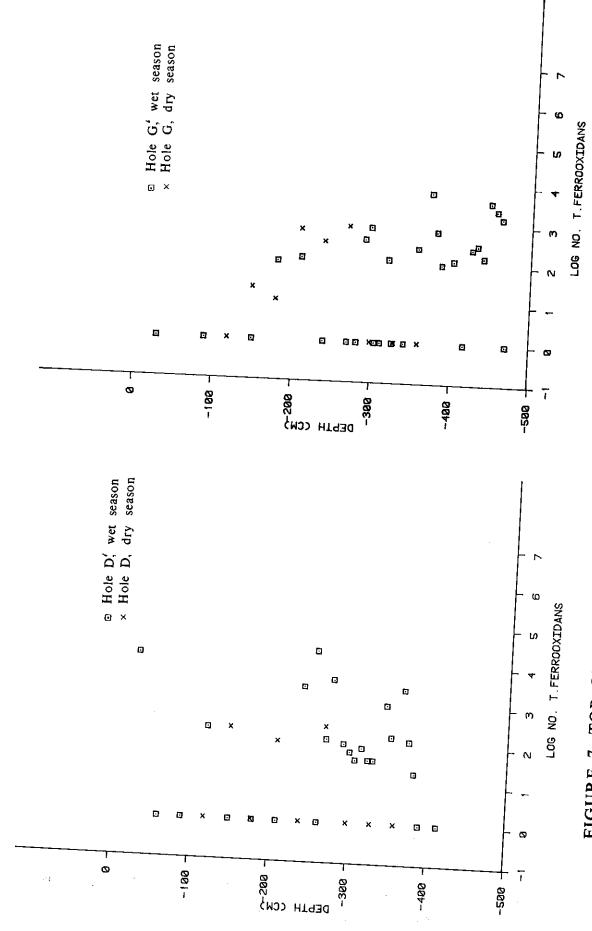
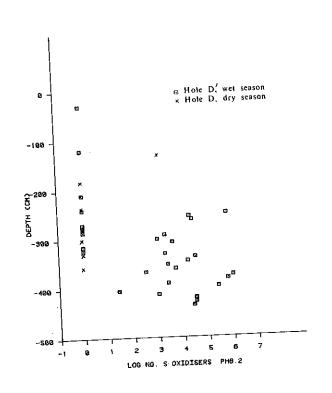
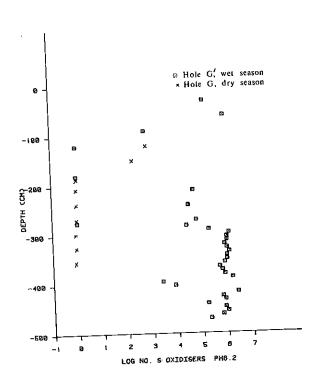


FIGURE 7. TOP OF WHITE'S DUMP, NUMBER OF T. FERROOXIDANS/g D.W.





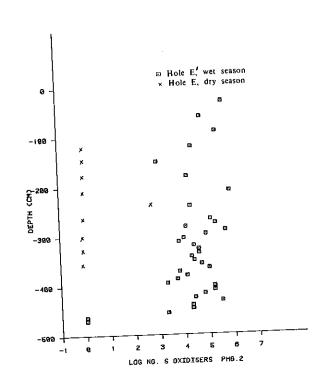
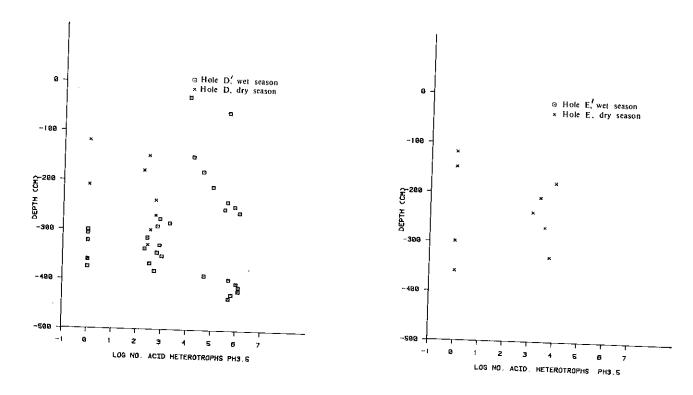


FIGURE 8. TOP OF WHITE'S DUMP, NUMBER OF S-OXIDISING BACTERIA (pH 6.2)/g D.W.



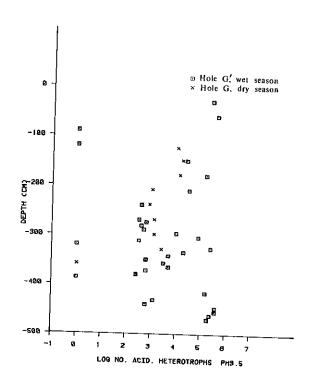


FIGURE 9. TOP OF WHITE'S DUMP, NUMBER OF ACIDOPHILIC HETEROTROPHS/g D.W.

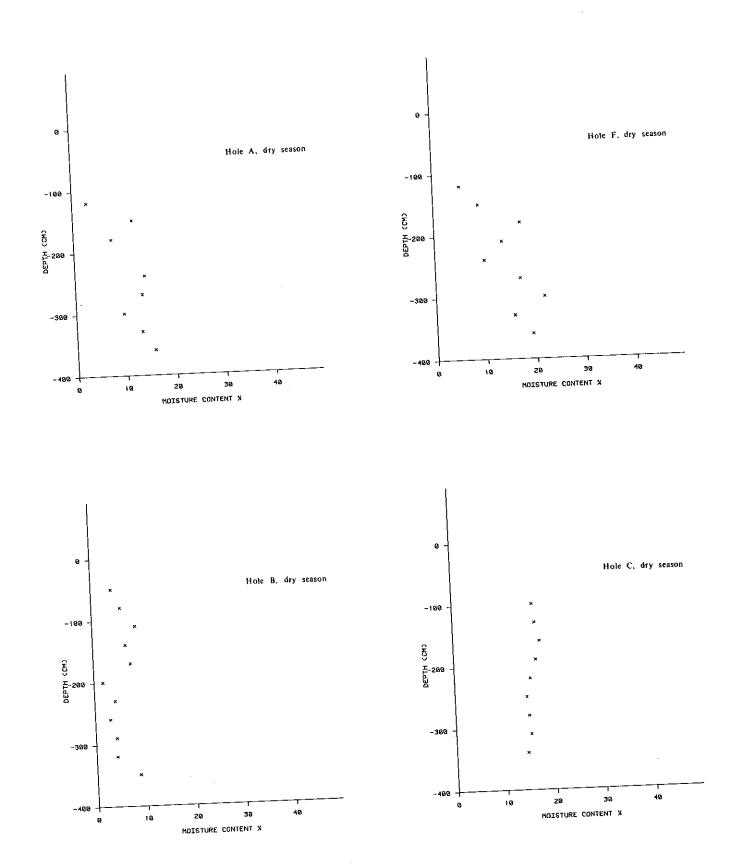
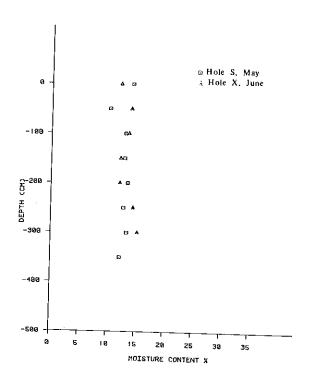
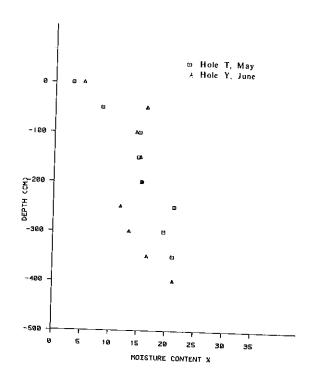


FIGURE 10. BASE OF WHITE'S DUMP, MOISTURE CONTENT





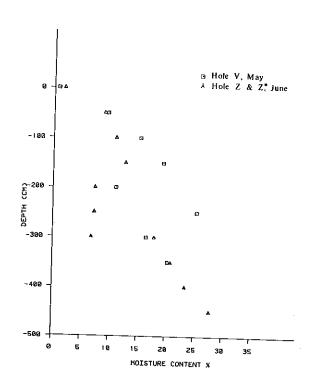
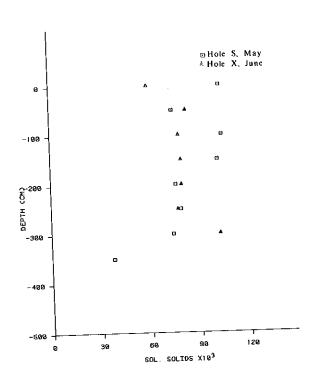
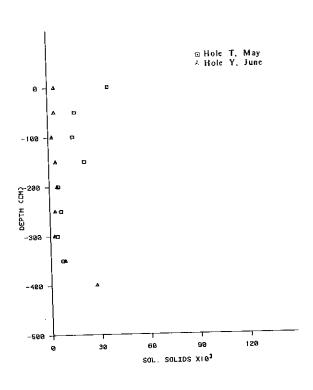


FIGURE 11. INTERMEDIATE DUMP, MOISTURE CONTENT





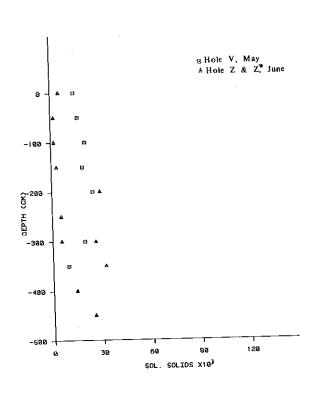
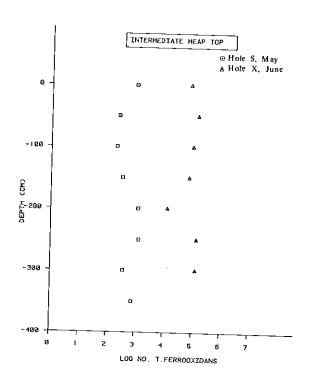
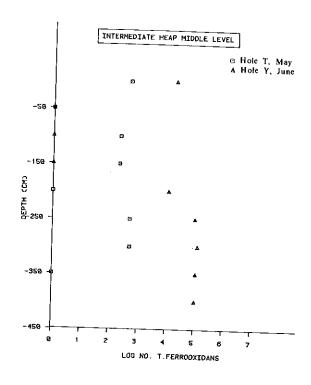


FIGURE 12. INTERMEDIATE DUMP, SOLUBLE SOLIDS CONTENT ppm





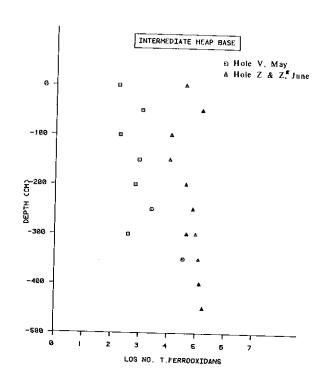
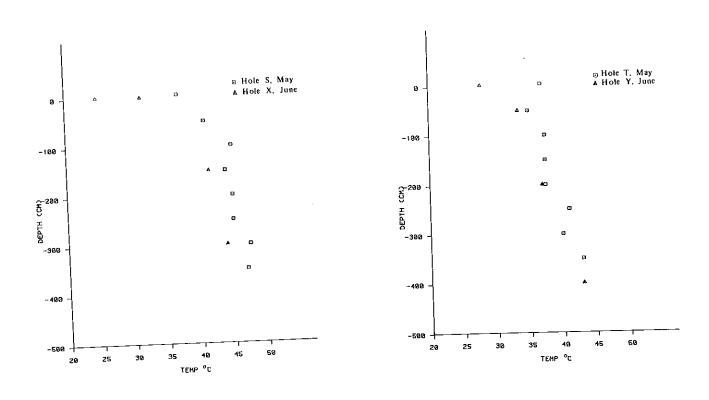


FIGURE 13. INTERMEDIATE DUMP, NUMBER OF T. FERROOXIDANS/g D.W.



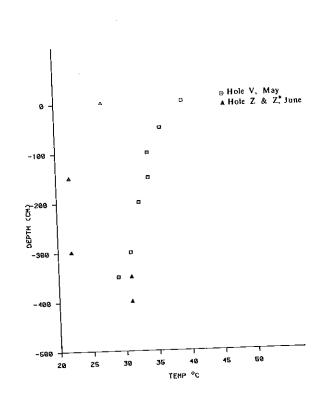


FIGURE 14. INTERMEDIATE DUMP, TEMPERATURE DISTRIBUTION

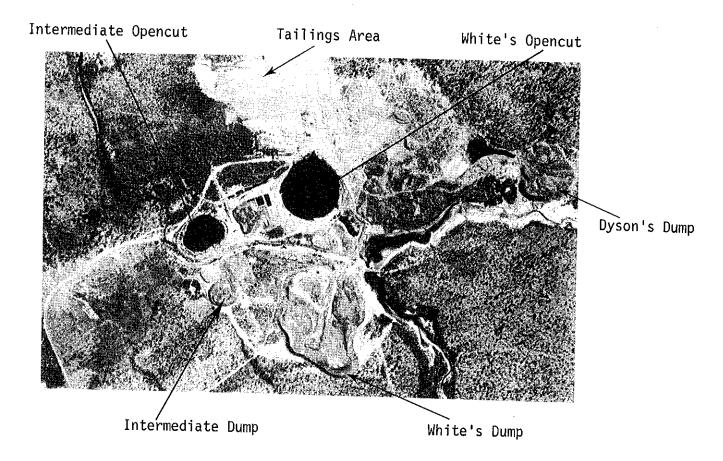


FIGURE 15. AERIAL VIEW OF RUM JUNGLE MINE SITE



FIGURE 16. AERIAL VIEW OF WHITE'S OVERBURDEN DUMP IN WET SEASON



FIGURE 17. SAMPLING HOLE G ON WHITE'S DUMP

APPENDIX A

SAMPLING LOCATIONS, AND PHYSICOCHEMICAL AND MICROBIOLOGICAL DATA

SULPHIDE AND OXIDE HEAPS 1975-1976

Note: The coordinates listed refer to the coordinates given in Map 3

Code: RJ no.	Coordinates of sample location	Description, Field Observations
	Tocacton	To Tructons
11	037~460	Material from top of Sulphide Heap - 15 cm deep. Pyrite and malachite abundant.
12	039-461	Mixed rock fines and sulphur - top of Sulphide Heap.
13	033-469	Material from top of Sulphide Heap - 20 cm deep.
14	045-465	Material from side of channel on top of Oxide Pile -
15	045-467	Material from a similar location on side of another channel on top of Oxide Heap.
16	047-465	Material from side of Oxide Heap, 4 metres up from base, 15 cm deep. Slightly damp. Temperature in sampling hole 40°C (ambient temp. 34°C).
<u>1</u> 7	040-470	Material from side of Sulphide Heap, 6 metres up from base, 15 cm deep. Some pale yellow efflorescent material. Slightly damp. Temperature in sampling hole, 38°C. (ambient temp. 34°C).
8	039-472	Similar sampling point to 17. Some pale yellow efflorescent material. Temperature in sampling hole 39°C (ambient temperature 34°C).

PHYSICOCHEMICAL AND MICROBIOLOGICAL AND OXIDE HEAP OXIDE HEAP;															
LOCATIO	: NC		12	13	17		18				14	1	5	16	
Sample no.		11	0.5	20	15	-	15		-		20	0.	5	15	
from Surface (Moisture	cm)	15 11.3	3.3	8.3	9.3		8.6	 	+		10,4	٩	9.9	13.9	
Content (%) Water solub	oles	64	127	92	4.0	,	5.0		-		18		19	21	
(x10 ³ p p n	n)	2.5	1.6	1.8	3.2	2	2.6				2.7	2	.9	2.4	
 	Fe	280	1280	4860	n		50				80		15	210	
Metal Content			620	300	64	.0	530				1050	<u> </u>		1270	, <u> </u>
(2.5)	Zn	40	n	n	r	n	n				n	_	n	n	\downarrow
<u>T. ferroox</u> (x10 ³)	idans pH2.5	0.1	ŊG	NG	0	.7	NG				0.4	4	0.8	1.2	
Low pH S oxidise	ers	No	; NG	, NG		ŊG	NG				N	G	NG	NG	;
(x10 ³) Intermed S oxidis	ers	Н	-												
(x10 ³) High pH S oxidis (x10 ³)	pH4. sers pH6.	N	iG N	G N	G	0.4	0.	.8			1	ŊG	NG	; N	IG
Acidoph: Heteroti (x10 ³)	ilic rophs pH3	.5					\ -\						-	 	
Nutrien Agar (x10 ³)	pH7	,	NG	NG 1	NG	NG		NG			_	ŊĠ	N	G i	NG
Desulfo	<u>Desulfovibrio</u>		tion of							waigh		<u>. </u>			

Enumeration of organisms as no. per ml or gram dry weight $n = \text{negligible (less than l p p m)} \quad NG = No Growth \quad W = Water Sample Blank space indicates sample was not tested.$

		SAMPLES COLLECTED MARCH 1976
Code RJ no.	of sampl	e Decemination no
84	038-467	From fresh sampling hole on top of Sulphide Heap. About 6 metres back from edge of heap. Temperature
85		28°C. Ambient temperature 24°C. At 5 cm depth. Same sampling hole, at 25 cm depth.
86		Same sampling hole, at 40 cm depth.
87		Same sampling hole. Seepage water which collected rapidly when depth of 60 cm reached. pH 2.3. Temperature 28°C. Ambient temperature 24°C.
88	046-470	Second sampling hole on top of Sulphide Heap. At 5 cm depth. Wet shaly material.
89		Same sampling hole. At 30 cm depth. Shale underlain by clayey ferruginous material at this depth.
90		Same sampling hole. at 60 cm depth. Clayey ferruginous material, which continued down to at least 70 cm depth.
91	032-468	Third sample hole on top of Sulphide Heap. Dark serpentine-like material, with some yellow efflorescence.
92		Same sampling hole. At 25 cm depth.
93	·	Same sampling hole. At 50 cm depth. Black serpentine-like
94		Same sample hole. At 80 cm depth. Clay admixed with black, serpentine-like material.
95	045-465	Sample hole on middle levee of Oxide Heap. At 5 cm.
96		Same sample hole at 25-30 cm depth. Wet.
97		Seepage from sample hole. pH 3. Temperature 28.2°C.
98		Water sample from pond at south end of top of Oxide Heap. pH 4.5. Water temperature 26°C.
9		Water sample from pond at north end of top of Oxide Heap. pH 4.5. Temperature 26°C.
00	034-462	Pond in middle of top of Sulphide Heap. Lumps of crude elemental sulphur lying nearby. pH 2.9. Temperature 28°C.

			102
			SAMPLES COLLECTED MARCH 1976
Cod RJ no		Coordinates of sample location	Description, Field Observations
10)3	049-462	Water sample from spring at base of Oxide Heap. pH 2.2. Temperature 30°C.
10	04	048-462	Rock sample from 5 metres up Oxide Heap from spring. 30 cm depth. Temperature same as ambient, 25 ⁰ C.
1	05	045-469	Rock sample from 5 metres up from base of Sulphide Heap. 30 cm depth. Temperature 28°C.
1	.06	045-470	Water sample from spring at base of Sulphide Heap. Deep green colour. pH 2.3 Temperature 31°C.
,	L07	043-470	Water sample from spring at base of Sulphide Heap. Deep green colour. pH 2.4. Temperature 29°C.
	108	043-469	Rock sample from 5 metres up heap from spring. 30 cm depth. Temperature same as ambient.
	109	041-469	Water sample from spring at base of Sulphide Heap. pH 2.3. Temperature 30°C.
	110	041-468	Rock sample from 5 metres up heap from spring. 30 cm depth. Temperature same as ambient.
	112	031-470	Water sample from spring at base of Sulphide Heap. Yellowish-green colour. pH 2.3. Temperature 38°C. Ambient temperature 25°C.
	113	031-469	Rock sample from 5 metres up heap from spring. 30 cm depth. Temperature 28.5°C.
	114	028-457	Water sample from spring at base of Sulphide Heap. Pale, yellowish-green colour. pH 2.4. Temperature 33°C.
	116	029-458	Rock sample from 5 metres up heap from spring. 30 cm depth. Temperature same as ambient.
	117	030-456	Water sample from spring at base of Sulphide Heap. Pale, yellow colour. pH 2.3. Temperature 30°C.
	118	031-456	Rock sample from 5 metres up heap from spring. 30 cm depth. Temperature same as ambient.

MARCH 1976

	<u>I</u>	OCATION:	. SI	ULPHID	E HEAP			11101	.0151	OTOG	ICAL	DAT	A			M	ARCH	1 1
	Sample 1	no.	84	4 8	5 {	86	81	7	88	T 8	39	90		91	T	2		
	Distance from Surfac		5	25	5 4	10	60	,	 5	3	_		-	<u></u>	9	2	93	
	Moisture Content	(%)	15.	2 15.	1 15	.6		16	 5.7	 	\dashv	60	+-	5	2!	5	50	4
	Water so: (xl0 ³ p p	lubles	11	22	_	7	3,7	+				17.5	+	5.3	14	1.1	9.5	5
	рН	<u>, /</u>	2.7	2.5		-+	2.4	2.		6.		11		8.4		21	17	
	Soluble	Fe	744	1005	1270	+		 	{	2.6 	-	3.4	2.	.5	2.	9	3.7	
	Metal Content (ppm)	Cu	580	634	1179	+	380	128	-+	601	- 2	101	44	18	92	0	126	
	, P P /	Zn	n	n	721	-	165 n	38	3	n 	+-	66	36	0	173	1 2	383	
ŀ	T. ferrooxi	dans			+			"	_	n 	1	n	n		n		n	
-	(×10 ³)	рН2.5	0.27	0.38	0.3	5 1	L.1	0.14	0	.17	0	.04	0.45	5 C),27	N	īG	
	Low pH S oxidisers (x10 ³) p	H3.5	0.99	3	0.5]	L	54	12	1,	0.08	I	ig	0.23	+	NG	N	G	
	Intermediat S oxidisers (x10 ³) pl	ерн Н4.8				 			+					-		-		
	High pH S oxidisers (x10 ³) pH	16.2	NG	0.24	NG	NO	3	NG	N	'G	N	G	NG	N		NO		
F	Acidophilic Meterotrophs (x10 ³) pH	3.5										+-					-	
A	hutrient gar x10 ³) pH7	, 0	.19	0.72	NG	0.	3	3.2	0.	.04	NG		NG	No.	G	0.1	4	
<u>D</u> e	esulfovibrio									+		+			+		-	
	Εì	numerati	on of							ł		J	1				ı	

Enumeration of organisms as no. per ml or gram dry weight $n = \text{negligible (less than 1 p p m)} \quad NG = No Growth \quad W = Water Sample Blank space indicates sample was not tested.$

-					ICOCIIL			n)													
I	LOCATIO	ON:	SULE	HIDE	HEAL) (CO	M.T	Т						Τ-		T_		T		7	
Sample	e no.		94		100	112	1	.13	11	4	11	6	117	\ <u> </u>	118	\ \		 		\ -\	
Distan			80	,				30			3	10		_ 	30_	+		+	<u></u>	4	
Moist	ace (ure ent (%)		11.	. 3	W	W		13	\ \	W	1.5	5.8	W	- -	14.1	-		+		-	
Water	Water solubles (x10 ³ ppm)		1.	4	0.6	5.0		23	1	17		9.0	2	1	9.7	7 - -	— —	+		-	
	рН	<u>"-/</u>	3	.7	2.8	2.9	,	2.5	2	2.6	2	.7	2.	3	2.0	6		-		\dashv	
Solu	ıble	Fe	 	77	26	n		635	1:	100	-	n	14	70	n	-		-		4	
Cont	Metal Content (p p m)		3:	389	26	n		1473	\ -\	750		n 	9	20	r	\ 				_	
		Zn		n	n	5	,	1	\ -\-	7	<u> </u>	n 	-	6	,	n 				_	
Ţ.ţ.	erroox	idans pH2.	5 C).11	0.2	25 2	.1	0.35	,	1.1		0.2	1	2	0.	2			_		
Sc	w pH oxidise	ers pH3.	5	NG	0.	0.53 NG		2.0	б	ИС	3	3		NG	0.	.37	ļ		-		
In	ntermed oxidis	iate p	oH								_		- - -						-		-
Hi S	igh pH oxidis x10 ³)	sers pH6	5.2	ŊG	No	3	NG	NO	3	0.	33	N	g \	NG	-	ng —	\ \ -		 		 -
H	Acidophilic Heterotrophs (x10 ³) pH3.5									\ \ \		\ \					+-		+		
P	Nutrier Agar (x10 ³)		17	0.2	22 1	1G	0.2	3 0.	.59	0	.2	0.	.31	MG	;	NG	1		+		
!	Desulf	ovibri	<u>io</u>											l aight							

Enumeration of organisms as no. per ml or gram dry weight $n = \text{negligible (less than l p p m)} \quad NG = No Growth \quad W = Water Sample Blank space indicates sample was not tested.$

MARCH 1976

	TO	'ATION:	OX	DE HE	AP						rie.	IRCH 19	76
	Sample no	o .	95	96	97	98	99	103	3 10	4 105	10	6 10	— 7
	Distance from Surface	(cm)	5	30	30			1	,	0 30	-		
	Moisture Content (' ዩ) ———	20.2	23.3	3 W	W	W	W	16	18	W	W	
	Water sol (x10 ³ p p		1.5	2.9	3.4	0.2	0.3	6.8	1.1	0.3	3 22	23	 }
	рН	.	3.7	3.7	3.1	3,3	3.1	2.6	3.4	3.0	2.4	2.4	
	Soluble Metal	Fe	2	n	n	1	3	360	n	12	925	1010	-
	Content (ppm)	Cu	48	205	335	13	21	880	14	46	3000	2875	1
-		Zn	n	n	n	n	n	n	n	n	n	20	1
	T. ferrooxi (x10 ³)	<u>dans</u> pH2.5	NG	0.9	0.25	NG	NG	0.55	0.13	0.09	0.4	0.75	
	Low pH S oxidisers (x10 ³)	s pH3.5	NG	0.02	52	2.3	0.95	1.7	NG	NG	NG	NG	
-	Intermediat S oxidisers (x10 ³) p	се рн э эн4.8						 					-
	High pH S oxidisers (x10 ³) p	; Н6.2	NG	NG	NG	NG	NG	NG	NG	0.02	NG	NG	
	Acidophilic Heterotroph (x10 ³) p	s H3.5											
	Nutrient Agar (x10 ³) p	1 7	NG	NG	0.1	ŊĠ	0.7	0.1	0.25	0.07	NG	NG	
	Desulfovibri	<u>lo</u>											ı
											1		

Enumeration of organisms as no. per ml or gram dry weight n = negligible (less than l p p m) NG = No Growth W = Water Sample Blank space indicates sample was not tested.

LOCAT	ION:	OXID	Е НЕАР	(CONT	D)		—-т		_		τ-	— т		-		1
Sample no.		108	109	110						<u></u>	-					
Distance from Surface	(çm)	30		30				·	_					_		
Moisture Content (%)	,	15.1	W	17.2					_		_			$\frac{1}{1}$		$\frac{1}{1}$
Water soluk	oles m)	0.4	46	0.4										 		-
На		3.0	2.3	3.7				<u> </u> 			_ -		<u> </u>		-	1
Soluble Metal	Fe	7	2500	n		-					-		-			-
Content (ppm)	Cu	17	7450	50		_					 		<u> </u>		_ 	4
	Zn	17	16	n				_		ļ	_				<u></u>	
T.ferroox	idans pH2.5	0.04	0.51	0.14												
Low pH S oxidise (xl0 ³)	rs pH3.5	NG	NG	NG					<u>. </u>							
Intermedi S oxidise (x10 ³)	late pH ers pH4.8						<u> </u>								\ \ \	
High pH S oxidise (x10 ³)	ers pH6.2	NG	6.4	0.02			,								\ \	
Acidophi Heterotr (x10 ³)	lic ophs pH3.	5				_				<u> </u>						
Nutrient Agar (x10 ³)		NO	; NG	; NG		 		 		\ \ -\	_	<u> </u>				
Desulfor	vibrio									. night						

Enumeration of organisms as no. per ml or gram dry weight n = negligible (less than 1 p p m) NG = No Growth W = Water Sample Blank space indicates sample was not tested.

APPENDIX B
SAMPLING LOCATIONS, AND PHYSICOCHEMICAL AND MICROBIOLOGICAL DATA
TAILINGS DAM 1976

Note: The coordinates listed refer to the coordinates given in $$\operatorname{\mathsf{Map}}\ 3$$

		SAMPLES COLLECTED SEPTEMBER 1976
Code: RJ no.	Coordinates of sample location	Description, Field Observations
226	069-538	Hole H was dug manually in the tailings dam. The hole was 160 cm deep and very gluey, black deposit. Pick and shovel used were coated with copper layer. Sample was taken at basement of the hole.
227		Hole H: Sample was collected at 10 cm from the basement.
228		Hole H: Sample was collected at 20 cm from the basement.
230		Hole H: Sample collected at 40 cm from the basement.
231	ľ	Hole H: Sample collected at 50 cm from the basement.
232		Hole H: Sample collected at 60 cm from the basement.
233		Hole H: Sample collected at 80 cm from the basement.
34		Hole H: Sample collected at 100 cm from the basement.
:35		Hole H: Sample collected at 120 cm from the basement.
36		Hole H: Sample collected at 150 cm from the basement surface.
}		the basement surface.
	1	
		7

SEPT. 1976

TAILINGS DAM HOLE H

LOCAT	ON:	TAIL	INGS D	AM HO	<u>. — т</u>							
Sample no.		226	227	228	230	231	232	2	233	234	235	236
Distance from Surface(c	m)	160	150	140	120	110	100	- 8	30	60	40	10
Moisture Content (%)		19.8	30.6	32.7	29.9	29.1	29.9	:	30.4	26.6	30.7	5.9
Water solu		7.2	13	15	15	17	19		19	16	14	4.1
pH		4.4	4.2	4.3	4.0	4.1	4.4	4	.3	4.2	3.8	3.5
Soluble	Fe	78	11	50	25	39	45		21	14	11	15
Metal Content (ppm)	Cu	995	1050	1350	1040	1540	192	0 1	L 7 50	980	210	950
	Zn	n	n	n	n	n	n		n	n	n	n
T. ferroox	idans pH2.5	ŊG	NG	NG	1.5	0.5	NG	3	0.32	ŊG	0.9	NG
Low pH S oxidise (x10 ³)	rs pH3.5	NG	NG	NG	NG	NG	No	3	NG	NG	NG	NG
Intermedi S oxidise (x10 ³)	ate pH ers pH4.8									<u> </u>		
High pH S oxidise (x10 ³)	ers pH6.2	0.1	5 0.2	3 1.5	0.25	5 0.1	1 N	ıG	0.5	1 NG	2.5	NG
Acidophi Heterotro (x10 ³)	lic ophs pH3.	5 NG	; 11	11	9.	3 2.	5]	L2	1.3	1.6	11	1.5
Nutrient Agar (x10 ³)	рн7	NO	3 4.5	3.5	6.	5 0.	75	8.7	9.5	0.9	7.5	5 0.21
Desulfor	<u>vibrio</u>	N	G NO	g NO	; N	ig i	NG	ŊG	NO	B No	G N	G NG

Enumeration of organisms as no. per ml or gram dry weight n=negligible (less than l p p m) NG=No Growth W=W at a Sample Blank space indicates sample was not tested.

APPENDIX C
SAMPLING LOCATIONS, AND PHYSICOCHEMICAL AND MICROBIOLOGICAL DATA
WHITE'S OVERBURDEN HEAP 1975-1979

Note: The coordinates listed refer to the coordinates given in $\operatorname{\mathsf{Map}}\ 3$

		SAMPLES COLLECTED SEPTEMBER 1975
Code: RJ no.	Coordinat of sampl location	Description Field of
25	095-368	Sample from top of White's Overburden Heap, 15 cm deep, near AAEC sampling hole WP1. Dry.
26		Sample from wall of AAEC sampling hole WPl on White's Overburden Heap, 150 cm below surface, 10-15 cm in. Some whitish efflorescent material adjacent. Damp.
27	063-384	Sample from top of White's Overburden Heap, 15 cm deep, near AAEC sampling hole WP3. Dry.
28		Sample from wall of AAEC sampling hole on White's Overburden Heap, near bottom (c. 2 metres down), 10-15 cm in. Some whitish efflorescence. Damp.
29	056-404	Sample from top of White's Overburden Heap, west of Tyre Valley, 15 cm deep. Slightly damp.
30	063-394	Sample from pink deposit on top of White's Overburden Heap, south of Tyre Valley. Dry.
31		Sample from heap of weathered shale on top of White's Overburden Heap, 20 cm in.
32		Hand sample of rocks from top of White's Overburden Heap.
33	098-388	Sample from wall of AAEC sampling hole WP2 on White's Overburden Heap, 150 cm below surface, 10-15 cm in. Damp. Some efflorescent material adjacent.
34		Sample from top of White's Overburden Heap, near AAEC sampling hole WP2, 30 cm deep. Slightly damp.
35	076-404	Sample from side wall of south-east entrance to Tyre Valley, 15 cm in., 3 metres up slope.
36	084-403	Sample from principal run-off valley on White's Overburden Heap, 15 cm deep.
37		Hand samples of weathered ores in principal runoff valley, White's Overburden Heap.
38	093-402	Sample from eastern wall of principal runoff valley on White's Overburden Heap. 3 metres up; 15 cm deep. Moist.
39	104-407	Sample from top of White's Overburden Heap above springs on north-east side. From 35 cm depth.
0	104-404	From a small heap on top of White's Overburden Heap, north-east corner. From 15 cm depth.

		114
-		SAMPLES COLLECTED SEPTEMBER 1975
Code: RJ no.	Coordinates of sample location	Description, Field Observations
41	105-415	From north-east wall of White's Overburden Heap, above Spring No. 4. About 3½ metres above efflorescence layer. From 20 cm depth. Slightly damp.
42		From above Spring No. 4, White's Overburden Heap. About halfway up efflorescence layer (130 cm above spring). From 20 cm depth. Moist.
43	108-412	From damp soil immediately under (5 cm in) surface coating of efflorescent salts on side of White's Overburden Heap south of springs.
44	107-412	Sample from side of heap, about 4 metres above efflorescence layer (above sample 43).
45	104-380	Sample from moist soil about 5 cm under efflorescence layer - towards south-east corner of White's Overburden Heap.
46	103-380	Sample from side of White's Overburden Heap, from 3 metres up slope in very loose material. From 25 cm depth. Slightly moist.
47	7 055-385	Hand sample of highly degraded rock on southwest side of White's Overburden Heap. Plain below denuded of vegetation.
4	8	From side of White's Overburden Heap, about 5 metres up and 20 cm in. Above an efflorescent patch. Damp. Warm to touch.
4	9 090-355	From southern side of White's Overburden Heap, about 7 metres up, 20 cm in. Grass & bushes right up to foot of heap at this point.
٥	092-355	From southern side of White's Overburden Heap, about 6 metres up & 20 cm in. Damp.
	054-479	Water from White's Opencut. pH, 2.5; Cu, 10 ppm.; Fe, 100 ppm.
	15	Mud from edge of water, White's Opencut.
	16 108-415	face of White's Overburden host
	17	Same location; hole dug to about 30 cm depth and allowed to fill with seepage. pH, 2.7; Cu, 60 ppm.; Fe, 200 ppm. Ni, 25 ppm.
1		

SEPT, 1975

		CATION	1: WH	ITE'S	OVERB	URDE	N HE	AP			.01.11 6/	11W			SE	PT,	1975	5
	Sample n	o .	25	5 26	,	27	2:	8 :	29	3	0	31	3	3	3	4	35	
	Distance from Surface) 15	15	0]	L5	200) 15		0.2	2 0.	. 2	150)	30	,	15	
	Moisture Content		2.6	14.	6 4	1.4	10.7	2.	6	7.2	2 5.	0	8.4	_	3.0	,	10	
	Water sol	ubles m)	9.6	2	5 2	.4	9.7	5.	3	3.0	+-		7,8	-+	1.4	\dashv	13.0	_
	рН		4.1	2.0	6 3	.3	2.5	5.	 2	6.8			2.7		4.6	-+	5.:	_
	Soluble	Fe	n	83	3	 n	52	 						+		-	3.]	_
	Metal Content		27	14		-+	- ·			22	n	_	n		n		n	
	(ppm.)	Cu ——		1.4	*	n	88	n		n 	n		32		n		n	7
		Zn	n	n	r	1	n	n		n	n		n	1	n	+	n	1
	T. <u>ferrooxi</u> (x10 ³)	dans pH2.5	NG	8.2	NG	;	NG	0.0	7	0.0	5 0.5	- - ;	7.2	+-	NG	+	0.1	
	Low pH S oxidisers (x10 ³)	s oH3.5	ŊG	NG	NG		NG	NG	+	NG	NG		NG	+-	NG	+	 NG	
	Intermediat S oxidisers (x10 ³) p	се рн з							$\frac{1}{ \cdot }$			+		-				
	High pH S oxidisers (x10 ³) p	Н6.2	0.2	0.2	ŊG		0.3	0.2	-	0.6	NG	1	. 4	1	 NG	0.	.05	
	Acidophilic Heterotroph (x10 ³) pH	s 43.5				-			 -			+-						
1	Nutrient Agar (x10 ³) pH	17	0.04	0.08	0.05		NG	0.6	-	0.7	NG	1	NG	0.	4		NG	
<u> </u>	esulfovibri	<u>o</u>									 -	 		<u> </u>			+	
									<u> </u>				j		- 1		- [

Enumeration of organisms as no. per ml or gram dry weight n = negligible (less than l p p m) NG = No Growth W = Water Sample Blank space indicates sample was not tested.

WHITE'S OVERBURDEN HEAP (CONT'D)

		WHITE'	S OVE	RBU	RDEN	HEAP	(CC	1 ' TMC)) 					 -
LOCATIO	JN:	36	38		39	40		41	42	43	44		45	46
Distance from Surface (cm)	15	15		35	15		20	20	5	0.2	<u>-</u>	5	25
Moisture Content (%)		3.6	13.1		4.5	2.3	9	9.9	16.6	12.2	2.	2	9.5	1.8
Water solub	oles	2.8	2.2	2	0.6	0.3]	L.O	22	21	1.	5	13	1.1
(х10 ³ ррп	<u>)</u>	3.6	3.	В	4.1	6.8		3.3	3.2	4.0	5.	5	3.6	3.6
a.l.blo	Fe	n	n	-	n	716		n	n	n	r	1	n	n
Soluble Metal Content	Cu	n	r	- -	n	n		n	36	54	,	n	21	n
(ppm:)	Zn	n	1	1	n	n		n	n	54		n	69	n
т. <u>ferroox</u> ; (х10 ³)	idans pH2.5	0.0	7 0	.04	NG	NG		ŊĠ	NG	0.0)6 N	īG	0.1	NG
Low pH S oxidise (x10 ³)	ers pH3.5	5 N	IG	NG	ŊG	NG	;	ŊG	ŊG	No	G 1	ŊG	NG	NG
Intermedi S oxidise (x10 ³)	 iate pl	Н								_				
High pH	High pH S oxidisers		NG	NG	NG	0	.1	NG	3.	7 N	iG	1.1	NG	NG
Acidophi Heterotr (x10 ³)	ilic rophs pH3	.5			,			\ \ \						
Nutrien Agar (x10 ³)	t pH7	,	NG	NO	3 N	G (0.7	No	3 1	.4	NG	26	1.	7 NG
Desulfo	vibric									ry weigh				

Enumeration of organisms as no. per ml or gram dry weight $n = \text{negligible (less than l p p m)} \quad NG = No Growth \quad W = Water Sample Blank space indicates sample was not tested.$

LOC	ATION:	WHIT	E'S O	ERBURDI	EN HEA	P (CON	IT'D);	SPRINGS	;	OPI	ENCUT
Sample no	·	48	49	50			16	17		14	15
Distanc from Surface		20	20	20				30	 	_	 _ _
Moisture Content (%) 	7.6	5.2	5.8			W	W		W	W
Water solu (x10 ³ p p		1.9	1.5	1.6							
На		3.7	4.2	4.2			3.1	2.6		2.1	2.2
Soluble Metal	Fe	n	n	n			29	18		50	25
Content (ppm)	Cu	n,	n	n			13	75		43	51
	Zn	n	n	n			46	40		6	6
T.ferrooxi (x10 ³)	dans pH2.5	0.06	NG	NG			1.2	0.7		0.4	0.8
Low pH S oxidiser: (x10 ³)	s oH3.5	NG	NG	NG			81	NG		NG	45
Intermediat S oxidisers (x10 ³)	се рн з он4.8										
High pH S oxidisers (x10 ³) p	эн6.2	0.7	11	0.8			NG	NG		NG	0.1
Acidophilio Heterotroph (x10 ³) p	s H3.5										
Nutrient Agar (x10 ³) p	н7	2.5	8.9	1.2			29	1.5		1.6	28
<u>Desulfovibr</u>	<u>io</u>										
· · ·	Framove						<u> </u>				

Enumeration of organisms as no. per ml or gram dry weight n = negligible (less than 1 p p m) NG = No Growth W = Water Sample Blank space indicates sample was not tested.

		SAMPLES COLLECTED MARCH 1976						
Code: RJ no.	Coordinates of sample location	Description, Field Observations						
63	062-384	From fresh sampling hole on top of White's Overburden Heap (near AAEC WP3). At 5 cm depth, from pyritic shale layer.						
64		Same sampling hole, at 25 cm depth. Streaks of iron and copper salts.						
65		Same sampling hole, at 50 cm depth.						
66	095-368	From fresh sampling hole on top of White's Overburden Heap (near AAEC WP1). Near experimental re-vegetation plots. At 5 cm depth.						
67		Same sampling hole, at 25 cm depth.						
68		Same sampling hole, at 50 cm depth.						
69	098-387	From fresh sampling hole on top of White's Overburden Heap (near AAEC WP2). At 5 cm depth.						
70		Same sampling hole, at 25 cm depth.						
71		Same sampling hole, at 50 cm depth.						
72	108-415	From White's Overburden Heap, Spring No. 4. pH 2.7. Temperature 36.2°C. Ambient temperature 28.2°C.						
73		Composite algae sample, from runoff from principal White's Heap springs. pH of runoff 2.8.						
74		From sample hole about 4 metres up heap from White's Spring No. 4, at 30 cm depth. Temperature 30°C. Ambient temperature 28°C.						
75	108-413	From White's Heap, Spring No. 3. pH 2.7. Temperature 37°C.						
76	110-408	From White's Heap, Spring No. 1. pH 2.9. Temperature 37.5°C.						
77		From sample hole about 6 metres up heap from White's Spring No. 1, at 30 cm depth. About a metre from a well-established tree. pH 4.5. Temperature 30°C.						
78	107-369							
79	From sample hole about 8 metres up heap from spri 30 cm depth. Temperature 28°C.							
101	055-479	Water from White's Opencut. pH 2.3.						
102	056-477	Mud from water margin at White's Opencut.						

MARCH 1976

LOCATION:	WHITE'S	OVERBURDEN	HEAP

					7114 111123	<u> </u>					
Sample no	o .	63	64	65	66	67	68	69	70	71	72
Distance from Surface	(cm)	5	25	50	5	25	50	5	25	50	
Moisture Content ((%) 	13.1	12.4	7.	1 14.8	3 11.	8.	8 9.	9 5.9) W
Water sol (x10 ³ p p		3.2	29	11	1.5	3.3	3 4.	2 0.	3 0.3	3 0.2	2 22
рH		4.7	6.5	6.2	3.7	3,5	3.1	6 4.	1 4.5	4.4	2.9
Soluble Metal Content (p p m) Fe Cu		16	3	n	n	n	2	5	n	n	56
		93	179	26	n	n	n	n	n	n	90
	Zn	n	5	n	n	n	n	n	n	n	29
T. <u>ferrooxi</u> (x10 ³)	dans pH2.5	0.04	0.1	0.12	0.06	0.29	0.1	NG	0.2	0.03	0.14
Iow pH S oxidiser: (x10 ³)	s oH3.5	1.6	29	34	2.7	0.87	14	NG	0.03	0.02	0.13
Intermediat S oxidisers (x10 ³)	те рн в он4.8										
High pH S oxidisers (x10 ³) p	; Н6.2	NG	390	2.7	NG	0.63	0.05	0.21	0.14	0,58	NG
Acidophilic Heterotroph (x10 ³) pi	s H3.5										
Nutrient Agar (x10 ³) pA	1 7	12	11	3.6	0.24	15	5.8	5.9	3.2	6.9	6.3
Desulfovibri	0										
	Enumera	tion of	organism	ne ae na		L					

Enumeration of organisms as no. per ml or gram dry weight n = negligible (less than l p p m) NG = No Growth W = Water Sample Blank space indicates sample was not tested.

120

MARCH 1976

WHITE'S OPENCUT LOCATION: WHITE'S HEAP (CONT'D); 102 101 79 77 78 76 75 74 73 Sample no. 0.5 Distance 30 30 30 from surface (cm) 22.3 W W 16.1 7.7 ₩ Moisture W 19.8 W Content (%) 2.5 5.8 0.03 29 0.01 24 23 Water solubles 0.7 15 $(x10^3 ppm)$ 3.1 2.7 4.3 3.3 3.7 2.9 3.3 2.9 3.7 рΗ 30 110 8 n 6 39 53 Fe 4 220 Soluble Metal 13 46 Content 7 95 4 31 148 Cu 26 n (p. p m.) 4 n 24 n 50 n Zn 36 n n 0.11 0.65 0.07 T.ferrooxidans 0.23 NG 0.65 0.2 0.03 NG $(x10_3)$ рн2.5 Low pH 0.3 NG NG 15 S oxidisers NG 17 3.4 6.0 NG $(x10^3)$ рн3.5 Intermediate pH s oxidisers $(x10^3)$ pH4.8 ŊG NG High pH 3.1 2.1 NG NG 0.45 s oxidisers NG NG $(x10^3)$ pH6.2 Acidophilic Heterotrophs $(x10^3)$ pH3.5 Nutrient NG NG 1.2 1.3 0.97 1.4 0.2 Agar 0.54 NG $(x10^3)$ pH7 <u>Desulfovibrio</u>

Enumeration of organisms as no. per ml or gram dry weight $n = \text{negligible (less than 1 p p m)} \quad NG = No Growth \quad W = Water Sample Blank space indicates sample was not tested.}$

		SAMPLES COLLECTED SEPTEMBER 1976
Code: RJ no. 151 152 153 154 155 156 157 158 159	of sam	ole D
152	106-368	White's Overburden Heap, near spring at SE corner, Hole A cut at the base of the heap. Soil sample taken at 10 cm from the basement of heap; moist. Hole A: Soil sample taken at 40 cm from the basement; moist.
		Hole A: Soil sample taken at 70 cm from the basement.
		Hole A: Soil sample taken at 100 cm from the basement.
156		Hole A: Soil sample taken at 130 cm from the basement. Hole A: Soil sample taken at 160 cm 5
		Hole A: Soil sample taken at 160 cm from the basement. Hole A: Soil sample taken at 190 cm from the basement.
		Hole A: Soil sample taken at 220 cm from the basement.
160		Hole A: Soil sample taken at 250 cm from the basement. No sample taken.
161 162		Bluish mineral specimen collected near base of Hole A. Bluish mineral specimen collected at 70 cm up from the base of Hole A.
163		Bluish mineral specimen collected at 150 cm up from the base of Hole A.
164	075-364	Hole B was started on 14/9/77, but was left overnight, and on 15/9/77 it was further sampled: very loose and friable. Sample collected at 10 cm from the basement.
65 66		Sample collected at 40 cm from the basement.
57		Sample collected at 70 cm from the basement.
88		Sample collected at 100 cm from the basement. Hole B: Sample taken at 130 cm from the basement.
0		Hole B: Sample taken at 160 cm from the basement.
1		Hole B: Sample taken at 190 cm from the basement. Hole B: Sample taken at 220 cm from the basement.

			122
			SAMPLES COLLECTED SEPTEMBER 1976
Code: RJ no.	of	rdinates sample cation	Description, Field Observations
172 173 174 175 176 17 17 17 18 18 11	073 6 7 8 8 9 80 81 82	097-367	Hole B: Sample taken at 250 cm from the basement. Hole B: Sample taken at 280 cm from the basement. Hole B: Sample taken at 310 cm from the basement; approx. 1.5 m from the surface. Hole C: Saturated with dead vegetation and heavy salt in soil. Very wet hole, red clay; sample taken at 10 cm from the basement. Hole C: Sample taken at 40 cm from the basement. Hole C: Sample taken at 70 cm from the basement. Hole C: Sample collected at 100 cm from the basement. Hole C: Sample collected at 130 cm from the basement. Hole C: Sample collected at 160 cm from the basement. Hole C: Sample collected at 190 cm from the basement. Hole C: Sample collected at 250 cm from the basement. Hole C: Sample collected at 250 cm from the basement, approximately 1 m below the surface. Hole D: Dug at the top of White's near vegetation plot. Sample taken at 10 cm from the basement, wet. In this hole at about 2-2.5 m depth, bright undecomposed pyrite and chalcopyrite could be seen. Hole D: Sample taken at 40 cm from the basement. Temperature was 40°C, while ambient temperature was 35°. Also surface was encrusted with fine needle crystals. Hole D: Sample taken at 70 cm from the basement. Hole D: Sample taken at 100 cm from the basement. Hole D: Sample taken at 100 cm from the basement. Hole D: Sample taken at 100 cm from the basement. Hole D: Sample taken at 100 cm from the basement. Hole D: Sample taken at 100 cm from the basement. Hole D: Sample taken at 100 cm from the basement.
			Hole D: Sample taken at 220 cm from the basement. Hole D: Sample taken at 250 cm from the basement.

		123							
		SAMPLES COLLECTED SEPTEMBER 1976							
Code RJ no.	of sample								
193	064-385	Hole E: Dug at the top of the White's SE corner. Near hard ground. Sample collected at 10 cm from the basement.							
194		Hole E: Sample collected at 40 cm from the basement.							
195		Hole E: Sample collected at 70 cm from the basement.							
196		Hole E: Sample taken at 100 cm from the basement.							
197		Hole E: Sample taken at 130 cm from the basement.							
198		Hole E: Sample taken at 160 cm from the basement.							
199		Hole E: Sample taken at 190 cm from the basement.							
200		Hole E: Sample taken at 220 cm from the basement.							
201		Hole E: Sample taken at 250 cm from the basement.							
202	108-418	Hole F was cut at the NE end of the White's base near spring 4. Sample was collected at 10 cm from the basement.							
203		Hole F: Sample was collected at 40 cm from the basement.							
204		Hole F: Sample collected at 70 cm from the basement.							
205		Hole F: Sample collected at 100 cm from the basement.							
206		Hole F: Sample collected at 130 cm from the basement.							
207		Hole F: Sample collected at 160 cm from the basement.							
208		Hole F: Sample collected at 190 cm from the basement.							
209		Hole F: Sample collected at 220 cm from the basement.							
210		Hole F: Sample collected at 250 cm from basement.							
211	104-415	Hole G was dug on the top of the White's heap just above spring No. 4. Sample was taken at 10 cm from the basement.							
212	Hole G: Sample collected at 40 cm from the basement: mineral grains, stable and bright.								
213		Hole G: Sample collected at 70 cm from the basement.							
214		Hole G: Sample collected at 100 cm from the basement.							

		124
		SAMPLES COLLECTED SEPTEMBER 1976
Code: RJ no.	Coordinates of sample location	Description, Field Observations
215 216 217 218 219 220 221 222 223 224 225		Hole G: Sample collected at 130 cm from the basement. Hole G: Sample collected at 160 cm from the basement. Hole G: Sample collected at 190 cm from the basement. Hole G: Sample collected at 220 cm from the basement. Hole G: Sample collected at 250 cm from the basement. No sample. Hole F: Surface growth sample. Hole F: Surface growth sample. Rock specimen from Hole F. Mineral specimen from Hole G. Undecomposed mineral crystals were noted. Rock specimen from Hole G. Some material enlarged with brown bulbous structures.

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τ.α.	73 m = 4.	. 1.702											
10	CATION	i: WH.	TE'S C	VERBUR	DEN HE	AP	HOLE	Α;		_		HOL	ЕВ
Sample n	o .	151	. 15	2 15	3 15	4 15	5 1	56	157	158	159	164	4
Distance from Surface	e (cm	360	33	0 30	0 270	24	0 21	LO	180	150	120	360	 ,
Moisture Content	(%)	15.	6 13	.1 9	.5 13	.3 13	.9 17	7.4	7.5	11.	8 2.	8 8.	6
Water sol (x10 ³ p p	Water solubles (x10 ³ ppm)		8 2	.3 3	.6 4	4	.6 3	.6	2.3	2.	7 8	38	\dashv
рH		3.	0 2	.7 2	.7 2.	6 2.	.5 2	.7	2.9	2.	9 2.9	3.5	
Soluble Metal	Metal		3.5	5 15	n	10	5		10	3	3	16	7
Content (ppm.)	Cu	n	40	35	20	15	12	5	n	10	2	n	+
	Zn	n	15	25	10	27	n	_	3	15	n	n	-
T. ferrooxi	<u>dans</u> pH2.5	NG	NG	NG	1.2	0.3	0.:	29	1.2	0.3	1.9	NG	$\frac{1}{2}$
Low pH S oxidiser (x10 ³)	s pH3.5	NG	NG	NG	NG	NG	NG	+	NG	NG	NG	NG	
Intermediat S oxidisers (x10 ³)	te pH s oH4.8						 						4
High pH S oxidisers (x10 ³) p	; ж6.2	NG	NG	NG	NG	NG	NG	+	NG	NG	NG	NG	
Acidophilic Heterotroph (x10 ³) p	s H3.5	NG	NG	2.6	6.6	7.5	60		11	11	0.5	5.4	
Nutrient Agar (x10 ³) pH	H7	NG	NG	0.32	2.3	0.53	11	(0.53	0.53	NG	NG	
Desulfovibri	<u>lo</u>	550	350	200	NG	NG	NG	1	NG	NG	NG	250	
	En mo	ration -	 								ŀ		l

Enumeration of organisms as no. per ml or gram dry weight n = negligible (less than 1 p p m) NG = No Growth W = Water Sample Blank space indicates sample was not tested.

LOCAT	ION:	WHITE	'S HEA	₽	но	LE B	(Cont	'd.)			
Sample no.		165	166	167	168	169	170	171	172	173	174
Distance from Surface	(cm)	300	300	270	240	210	180	150	120	90	60
Moisture			4.1	2.9	4.0	1.7	7.4	6.5	8.6	5.7	4.0
Water solubles		4.9	5.2	5	5.9	4.5	7	7	7.6	6.5	5.3
(x10 ³ p p m)		3.5	3.9	4.1	4.3	5.0	4.0	3.5	3.7	3.7	4.0
Soluble	Fe	16	15	25	31	27	19	n	16	n	n
Metal Content (ppm)	Cu	n	115	90	110	95	140	n	n	27	18
(P.P. w.)	Zn	n	n	n	n	n	n	n	n	n	n
T. ferroox (x10 ³)	idans pH2.5	NG	NG	0.75	NG	0.65	NG	NG	0.11	NG	NG
Low pH S oxidise (x10 ³)	rs pH3.5	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
Intermedi S oxidise (x10 ³)		1									
High pH S oxidise (x10 ³)	ers pH6.2	NG	0.2	NG	0.6	5 NG	NG	NG	1.5	NG	NG
Acidophi Heterotr (x10 ³)	Acidophilic Heterotrophs		5 NG	NG	11	17	41	60	15	11	5.3
Nutrient Agar (x10 ³)		NG	12	6.5	3.5	NG	15	10	11	9.5	19
	(x10 ³) pH7 Desulfovibrio		0 NG	NG	NG	NG	NG	, NG	, NG	NG	NG

Enumeration of organisms as no. per ml or gram dry weight n = negligible (less than l p p m) NG = No Growth W = Water Sample Blank space indicates sample was not tested.

<u>L</u>	OCATION:	WI	IITE	'S H	EAP	ICA:	L AN	ND M	MICRO	BIC		ICAI OLE		ГА		SE	PT :	197	6
Sample :	no.	17	75	176		75			T		T	OLIE		-		 			
Distance		+-			- 	77 		78 	17	79 —_	1	80	18	1 1	82] 1	L83		
from Surfac		36	0	330	30	00	2	70	24	0	23	LO	180) 1.	50	1	.20-		
Moisture Content	(%)	14	. 3	15.1	14,	8	14.	. 4	15	.2	16	5.5	17.	4 16	5.5	16	.1		
Water so (x10 ³ p	lubles om)	3.	. 5	5.1	2.	5	3,	1	5	.2	3	.9	 5.	3 5	5.6	-	.1		
Hq		6.	5	6.5	4.	5	4.	8	4.	.5	6	.5	6.0						\dashv
Soluble	Fe	n		n	n	+	n		 .	+		+			.9	6.	9		_
Metal Content	Cu	n		n	 -	+		\dashv		-		1		1	n	r. 			
(mqq)	Zn		+-		n	+	12	_			18	3	16	1	n	n	1		
m c		n		n	n		n		n		n		n	r	ì	n	1		7
T. ferroxi (x10 ³)	dans pH2.5	NG	1	1 G	NG	0	.03		NG	\ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \	NG	N		0.	03	NG	+		1
Low pH S oxidisers (x10 ³)	э энз. 5	NG	N	IG	NG	 		+		+-		+-					+		$\frac{1}{2}$
Intermediat S oxidisers	ерн		-				NG 	+	NG	1	√G	N	G 	NG	\perp	NG			
(x10 ³) p	H4.8																		
High pH S oxidisers (x10 ³) pH	16.2	NG	No	G	0.57	1.	7	2.	. 6	3.	6	NG	 ;	13	+-	 12			1
Acidophilic Heterotrophs (x10 ³) pH		NG	NO	;	NG	N.	G J	N	iG	N(0.			-			 -	
Nutrient Agar				+-							- 		-	NG	I	1G			
(x10 ³) pH7	, <u> </u>	NG	NG		NG	1.6	5	0.	76	NG	,	NG		0.31	0.	1			
Desulfovibric		10	230		NG	NG		NO.		NTC			+			-		\dashv	
E	numerati	on of	Orga	- 1	1			140		NG		NG		NG	N	G			

Enumeration of organisms as no. per ml or gram dry weight n = negligible (less than l p p m) NG = No Growth W = Water Sample Blank space indicates sample was not tested.

WHITE'S HEAP

HOLE D

LOCAT	ION:	MUTIT	S HEAP	— т			- т	 -			
Sample no.		184	185	186	187	188	189	190	191	192	
Distance from Surface	(cm)	120	150	180	210	240	270	300	330	360	
Moisture Content (%)		5.7	6	5.7	4.5	13.5	13.9	10.3	13.5	13.8	
Water solu	oles	1.8	2.8	1	1.1	8.3	4.7	3.7	2.8	7.7	
pH	n <u>)</u>	4.0	3.9	3.7	3.7	3.0	3.4	3.4	3,3	3.8	
Soluble	Fe	n	n	n	n	n	n	n	n	n	
Metal Content (p p m.)	Cu	48	55	51	75	171	25	12	47	16	
\(\frac{1}{2}\)	Zn	n	n	n	n	n	n	n	n	n	
<u>T.ferroox</u> (x10 ³)	idans pH2.5	NG	NG	NG	0.25	NG	0.11	NG	0.2	NG	
Low pH S oxidise (x10 ³)	rs pH3.5	NG	NG	NG	NG	NG	NG	ŊG	NG	NG	
Intermedi S oxidise (x10 ³)	ate pH ers pH4.8										
High pH S oxidise (x10 ³)	ers		NG	NG	NG	NG	ŊG	NG	1.2	NG	
Acidophi Heterotro (x10 ³)	lic	NG	0.2	6 0.33	0.4	5 0.5	3 NG	0.14	0.2	3 NG	
Nutrient Agar (x10 ³)		NG	NG	NG	NG	NG	NO	3.3	3 NG	1.2	
Desulfor		NG	No	; NG	NG	, NG	; N	G NG	NO	G NG	

Enumeration of organisms as no. per ml or gram dry weight $n = \text{negligible (less than 1 p p m)} \quad NG = No Growth \quad W = Water Sample Blank space indicates sample was not tested.$

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OI	CATION	: WH	ITE'S 1	HEAP	0123 7111	<i>-</i> 11.	LCROE		DLE 1		ΓA		SEP	SEPT. 1976				
Sample n	Sample no.		3 194	1 195	5 19	6	19	7	198	3 19	9 2	200	20:			_		
Distance from Surface	Distance from Surface (cm)) 330	300	27	270		0	210	18	0 1	.50	120	-		_		
Moisture Content (15.5	8.3	8.3		9.8	3	8.1	7.1	+	.8	5.7	_				
Water sol (x10 ³ p p	er solubles		4.3	8.7	2.7	1	4.9	,	2.3	+		.7	12	+				
рН			6.5	6.0	4.3	_	3.7		3.7	3.8						_		
Soluble Metal	Fe	n	n	n	n	1	n	+	n	n		— 1	n			-		
Content (ppm)	Cu	n	18	15	n		10	+	11	n	13		15	+		\dashv		
	Zn	n	n	n	n		n		n	n	r		n	-		$\frac{1}{2}$		
T.ferrooxi	<u>đans</u> pH2.5	NG	NG	NG	NG		NG	+	0.53	NG	NG		NG			1		
Low pH S oxidisers (x10 ³)	s pH3.5	NG	NG	NG	NG	+	NG	 	NG	NG	NG	_	NG					
Intermediat S oxidisers (x10 ³) p	e pH					+		 - -						-				
High pH S oxidisers (x10 ³) p	Н6.2	NG	NG	NG	NG	0	.53	N	IG	NG	NG	_	NG					
Acidophilic Heterotroph (x10 ³) pH	s H3.5	NG	5.7	NG	3.8	1.	. 3	2.	3	11	NG		NG					
Nutrient Agar (x10 ³) pH	17	NG	NG	NG	1.0	0.	.54	0.	33	NG	NG		0.12					
<u>Desulfovibri</u>	<u>o</u>	NG	NG	NG	NG	Ŋ	IG	NO	G	NG	NG	+	NG					

Enumeration of organisms as no. per ml or gram dry weight $n = \text{negligible (less than 1 p p m)} \quad NG = No Growth \quad W = Water Sample Blank space indicates sample was not tested.}$

SEPT. 1976

LOCAT	ON:		'S HEA	P			HO)LE]	F				,	 -1
Sample no.		202	203	204	205	2	:06	207	2	80	209	210		
Distance from Surface	(cm)	360	330	300	270] 2	240	210)]	L80	150	120	<u> </u>	
Moisture Content (%)		19.2	15.6	21.7	16.9	9	9.8	13.4	4]	17.2	8.9	5.3	-	_
Water solul	oles	5	3.9	12	5.9		1.2	0.6	6	0.2	0.02	0.02	<u> </u>	
pH	<u></u>	3.6	3.6	3.0	3.0	,	2.7	2.	8	2.9	3.2	3.3	 	
Soluble	Fe	16	75	16	178		n	n		10	65	13		
Metal Content (ppm)	Cu	n	n	25	51		65	11	.5	108	10	68		
	Zn	n	n	n	n		n	r	n	n	n	n		
<u>T.ferroox</u> (x10 ³)	idans pH2.5	NG	NG	NG	1.	5	1.3	Nº	G	1.1	0.4	45 0.	5	·
Low pH S oxidise (x10 ³)	rs pH3.5	NG	NG	NG	N	G	0.3	2 N	ıG	NG	NG	NG		
Intermedi S oxidise (x10 ³)	ate plers													
High pH S oxidise (x10 ³)			, NG	NG	N	ıG	ŊG	,	0.52	No	0.3	32 NO	3	
Acidophi Heterotr (x10 ³)	lic ophs pH3.	5 NO	s NG	; NG	3.	. 5	1.5		2.8	3.8	3 1.	2 0.	3	
Nutrient Agar (x10 ³)		N	g NO	g NG	0	.75	0.6	5 C	.35	2.	7 1.	0 0.	21	
	(x10 ³) pH7 Desulfovibrio		0 31	o NG	;	ŊG	NO	}	NG	NG	NG	; \ \ \	1G	

Enumeration of organisms as no. per ml or gram dry weight $n = \text{negligible (less than 1 p p m)} \quad NG = No Growth \quad W = Water Sample Blank space indicates sample was not tested.$

SEPT. 1976

WHITE'S HEAP LOCATION: HOLE G Sample no. 211 212 213 214 215 216 217 218 219 Distance from 360 330 300 270 240 Surface (cm) 210 180 150 120 Moisture 7.6 8.6 7.5 Content (%) 9.2 5.4 3.7 3.7 5.1 5.4 Water solubles (x10³ ppm) 4.7 7.1 9.4 8.5 7.4 8.5 8.7 1.3 1.8 рΗ 2.8 2.8 3.0 2.4 2.6 3.4 3.0 3.5 3.5 Fe n n Soluble n n n 10 n 15 n Metal Content Cu (ppm) 30 36 21 16 24 11 18 19 21 Zn n n n n n n n n n T.ferrooxidans $(x10^3)$ pH2.5 NG NG NG 0.73 0.33 0.64 0.01 0.02 NG Low pH S oxidisers $(x10^3)$ NG NG NG pH3.5 NG NG NG NG NG NG Intermediate pH S oxidisers $(x10^3)$ pH4.8 High pH S oxidisers $(x10^3)$ NG pH6.2 NG NG NG ŊG NG NG 0.21 0.73 Acidophilic Heterotrophs NG $(x10^3)$ 2.5 1.2 1.3 0.73 pH3.5 0.98 12 14 10 Nutrient Agar $(x10^3)$ рН7 NG 0.03 0.11 NG 10 NG 5.7 NG 1.3 <u>Desulfovibrio</u> NG NG NG NG NG NG NG NG NG

Enumeration of organisms as no. per ml or gram dry weight n = negligible (less than l p p m) NG = No Growth W = Water Sample Blank space indicates sample was not tested.

		132
		SAMPLES COLLECTED MARCH 1977
Code: RJ no.	Coordinates of sample location	Description, Field Observations
251 - A	097-366	Hole D' Soil sample collected from the base of Hole D', moist, disintegrated soil - temperature ambient.
251		Soil sample collected from 50 cm above the basement of Hole D' on the top of White's overburden heap, moist.
252		Soil sample collected from 57.5 cm above the basement, disintegrated soil.
253		Soil sample collected from 65 cm above the basement.
254		Soil sample collected from 72.5 cm above the basement, reddish, moist, mud-like soil.
255		Reddish soil collected from 80.0 cm above the basement of the hole, temperature ambient.
256		Reddish soil collected from 87.5 cm above the basement, moist.
257		Reddish soil collected from 95.0 cm above the basement.
258		Reddish soil collected from 102.5 cm above the basement.
259		Soil sample collected from 110 cm above basement.
260		Reddish brown soil sample collected from 117.5 cm above the basement.
261		Reddish soil sample collected from 125 cm above the basement of the Hole D'.
262		Soil sample collected from 132.5 cm above the basement of the hole.
263	3	Soil sample collected from 140 cm above the basement of the hole.
264	1	Soil sample collected from 147.5 cm above the basement of Hole D', moist, temperature ambient.
26	5	Soil sample collected from 155 cm above the basement of the Hole D', moist.
26	6	Reddish soil sample collected from 162.5 cm above the basement of the Hole D'.
26	7	Soil sample collected from 170 cm above the basement.
26	58	Soil sample collected from 177.5 cm above the basement.

		SAMPLES COLLECTED MARCH 1977
Code RJ no.	of sample	Description, Field Observations
269		Rock sample chipped from a boulder from 185 cm above the basement.
270		Rock sample collected from 192.5 cm above the basement.
271		Soil sample collected from 200 cm above the basement, soil disintegrated into fine particles.
272		Soil sample collected from 207.5 cm above the basement of the Hole D'.
273		Soil sample collected from 215 cm above the basement of the Hole D'.
274		Soil sample collected from 222.5 cm above the basement of
275		Soil sample collected from 230 cm above the basement.
276		Soil sample collected from 237.5 cm above the basement, brownish black in appearance.
277		Soil sample collected from 245 cm above the basement.
278		Soil sample collected from 275 cm above the basement, finely divided soil particles.
279		Soil sample collected from 305 cm above the basement.
280 281		Soil sample collected from 335 cm above the basement.
282		Sample collected from 365 cm above the basement.
283		Sample collected from 395 cm above the basement.
84		Sample collected from 425 cm above the basement. Sample collected from 455 cm above the basement about 30 cm from the top of the heap.
85		Hole E' Soil sample collected from the basement of the Hole E',
36	1	Soil sample collected from 7.5 cm above the basement of
7		Soil sample collected from 15 cm above the basement.

		SAMPLES COLLECTED MARCH 1977
Code: RJ no.	Coordinates of sample location	Description, Field Observations
-	location	Soil sample collected from 22.5 cm above the basement. Soil sample collected from 30 cm above the basement. Soil sample collected from 37.5 cm above the basement. Soil sample collected from 45 cm above the basement. Rock samples collected from 60 cm above the basement. Rock sample collected from 67.5 cm above the basement of the Hole E'. Soil sample collected from 75 cm above the basement. Soil sample collected from 82.5 cm above the basement. Soil sample collected from 90 cm above the basement. Soil sample collected from 97.5 cm above the basement. Soil sample collected from 105 cm above the basement. Soil sample collected from 112.5 cm above the basement, finely divided particles, moist. Soil sample collected from 120 cm above the basement of the hole. Soil sample collected from 120 cm above the basement. Soil sample collected from 127.5 cm above the basement. Soil sample collected from 135 cm above the basement. Soil sample collected from 142.5 cm above the basement. Soil sample collected from 142.5 cm above the basement.
30	6	Soil sample collected from 157.5 cm above the basement. Soil sample collected from 165 cm above the basement, yellow efflorescent material.
30	\ \	Soil sample collected from 172.5 cm above the basement. Soil sample collected from 180 cm above the basement. Brownish-black soil sample collected from 187.5 cm above the basement.

	<u> </u>	SAMPLES COLLECTED MARCH 1977
Code: RJ no.	Coordinates of sample location	Description, Field Observations
311 312 313 314 315 316 317 318 319	: : :	Soil sample collected from 195 cm above the basement. Soil sample collected from 202.5 cm above the basement. Sample collected from 230 cm above the basement, efflorescent material. Sample collected from 260 cm above the basement, efflorescent material. Sample collected from 290 cm above the basement. Sample collected from 320 cm above the basement, soil band saturated with water. Soil sample collected from 350 cm above the basement. Soil sample collected from 380 cm above the basement of Hole E', finely divided particles. Sample collected from 410 cm above the basement, yellow efflorescent material.
	04-414 Ho	Sample collected from 440 cm above the basement, about to cm below the surface. Colle G' Colle Sample taken from the basement of Hole G', moist oil particles.
27	So: Hoj	il sample taken from 7.5 cm above the basement of il sample taken from 15 cm above the basement of le G'.
0	Soi Hol	11 sample taken from 22.5 cm above the basement of 1 sample taken from 30 cm above the basement of 2 e G'. 1 sample taken from 37.5
	Soil Hole	l sample taken from 37.5 cm above the basement of sample taken from 45 cm above the basement of G'. sample taken from 52.5 cm above the basement, yellowish rescent material.

		136
		SAMPLES COLLECTED MARCH 1977
Code: RJ no.	Coordinates of sample location	Description, Field Observations
3	1 2	Soil sample taken from 60 cm above the basement, black shale. Soil sample collected from 67.5 cm above the basement, yellowish efflorescent material. Soil sample collected from 75 cm above the basement; yellowish-brown efflorescence. Soil sample collected from 82.5 cm above the basement. Soil sample collected from 90 cm above the basement; efflorescence. Soil sample collected from 97.5 cm above the basement, efflorescent material present. Soil samples collected from 105 cm above the basement, finely divided particles. Soil sample from 112.5 cm above the basement. Soil sample collected from 120 cm above the basement. Soil sample collected from 135 cm above the basement. Soil sample collected from 135 cm above the basement; efflorescent material present. Soil sample collected from 142.5 cm above the basement; efflorescent. Soil sample collected from 150 cm above the basement, small particles of pyrite present. Soil sample collected from 157.5 cm above the basement. Soil sample collected from 165 cm above the basement. Soil sample collected from 172.5 cm above the basement. Soil sample collected from 172.5 cm above the basement. Soil sample collected from 180 cm above the basement.
	351 352	soil sample collected from pyrite particles present. Soil sample collected from 202.5 cm above the basement; yell sample collected from 202.5 cm above the basement; yell sample collected from 202.5 cm above the basement; yell sample collected from 202.5 cm above the basement; yell sample collected from 202.5 cm above the basement; yell sample collected from 202.5 cm above the basement; yell sample collected from 202.5 cm above the basement; yell sample collected from 202.5 cm above the basement; yell sample collected from 202.5 cm above the basement; yell sample collected from 202.5 cm above the basement; yell sample collected from 202.5 cm above the basement; yell sample collected from 202.5 cm above the basement; yell sample collected from 202.5 cm above the basement; yell sample collected from 202.5 cm above the basement; yell sample collected from 202.5 cm above the basement; yell sample collected from 202.5 cm above the basement; yell sample collected from 202.5 cm above the basement; yell sample collected from 202.5 cm above the basement from 202.5 cm above the basem

		13/
	<u> </u>	SAMPLES COLLECTED MARCH 1977
Code: RJ no.	Coordinates of sample location	Description, Field Observations
353		Soil sample collected from 230 cm above the basement; pyrite particles present.
354		Soil particles collected from 260 cm above the basement, wet.
355		Soil sample collected from 290 cm above the basement, yellow efflorescence present.
356		Soil sample collected from 320 cm above the basement, yellow efflorescent material.
357 358		Soil sample collected from 350 cm above the basement.
359		Soil sample collected from 380 cm above the basement;
360		Soil sample collected from 410 cm above the basement. Soil sample collected from 440 cm above the basement, approximately 30 cm below the surface.
		·

LOCATION: WHITE'S OVERBURDEN HEAP HOLE D'												
Sample no.	ON.	251	252	253	254	25	5	256	257	258	259	260
Distance from Surface	(cm)	435	427.5	420	412.5	40)5	39 7. 5	390	382.5	375	367.5
Moisture Content (%)		16	22	20	17]	19	14	18	13	16	11
Water soluk	oles	5.9	3.5	2.4	1.8	.8 2.2		1.8	3.1	1.4	3.0	1.3
рн	<u>"</u>	3.6	3.5	3.1	3.2	3	.2	3.2	3.7	3.9	3.9	3.9
Soluble	Fe	n	n	n	n	1	.0	n	n	n	n	n
Metal Content (ppm)	Cu	20	n	n	15	2	25	n	n	n	31	n
	Zn	n	n	n	3		3	n	n	n	10	n
<u>T. ferroox</u> (x10 ³)	T. ferrooxidans (x10 ³) pH2.5		NG	NG	NG		ng	NG	NG	0.02	0.13	2.6
Low pH S oxidise (x10 ³)	ers pH3.5	NG	NG	NG	NG		NG	NG	NG	NG	NG	NG
Intermedi S oxidise (x10 ³)	iate pH ers pH4.8	1									_	
High pH S oxidis (x10 ³)	ers pH6.	2 25	29	3,8	3 1.0	,	27	239	2.5	699	1000	0.35
Acidophilic Heterotrophs (x10 ³) pH3.5		49	8 587	114	0 13	38	956	467	50	0.	47 NG	0.35
Nutrient Agar (x10 ³) pH7			1.	5 8.	0 50	2	273	3 9.6	5	0.	7 1.	0 16
Desulfovibrio		1	NG N	iG N	ig N	1G	No	G N	G N	g h	IG 1	IG NG

Enumeration of organisms as no. per ml or gram dry weight n = negligible (less than 1 p p m) NG = No Growth W = Water Sample Blank space indicates sample was not tested.

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LOCATION: HOLE D' (Cont'd)

Sample no	э.	261	262	263	264	265	266	267	268	269	270
Distance from Surface		360	352.5	345	337.5	330	322.	5 315	307.5	300	292.5
Moisture Content	(용) ————	12	15	15	14	15	16	11	17	14	1
Water sol		1.6	0.1	1.1	2.3	2.4	2.2	n	0.6	1.4	1.1
рH	•	3.9	4.0	3.9	4.1	3.9	4.0	4.0	4.1	4.4	4.0
Soluble Metal	Fe	n	n	n	10	n	n	n	18	n	n
Content (ppm)	Cu	10	n	n	18	n	n	n	10	n	n
	Zn	7	n	n	n	n	37	n	3	n	n
T. ferroox: (x10 ³)	T. ferrooxidans (x10 ³) pH2.5		0.17	1.1	Fe ³⁺	0.04	0.04	0.08	0.04	0.06	0.1
Low pH S oxidisen (x10 ³)	s pH3.5	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
Intermedia S oxidiser (x10 ³)	nte pH s pH4.8										
High pH S oxidiser (x10 ³)	s pH6.2	5.1	2.3	16	32	1.8	NG	NG	4.1	1.1	2.1
Acidophilic Heterotrophs (x10 ³) pH3.5		NG	1.0	0.64	0.22	0.71	NG	0.27	NG	NG	0.62
Nutrient Agar (x10 ³)	Agar		0.33	0.96	NG			0.55		1.1	
<u>Desulfovib</u>	(x10°) pH7 Desulfovibrio		NG	NG	NG	NG	NG	NG	NG	NG	NG
	Emmo			 -							

Enumeration of organisms as no. per ml or gram dry weight n = negligible (less than l p p m) NG = No Growth W = Water Sample Blank space indicates sample was not tested.

LOCATION:	HOLE	D¹	(Cont'd)

LOCAT	ion: 1	HOLE D	' (Con	t'd)					— т	т	
Sample no.		271	272	273	274	2 7 5	276	277	278	279	280
Distance from Surface	(cm)	285	277.5	270	262.5	255	247.5	240	210	180	150
Moisture Content (%)		17	13	15	18	14	15	15	14	13	16
Water solubles (x10 ³ p p m)		1.1	2.0	1.4	1.7	1.2	n	1.9	0.6	1.4	2.5
pH		3.8	4.0	3.9	3.5	3.8	3.7	3.6	3.4	3.6	3.2
Soluble	Fe	n	n	11.	n	n	n	210	n	10	n
Metal Content (ppm)	Cu	n	75	10	n	n	n	n	n	31	n
фрмл	Zn	n	n	15	n	n	n	n	n	5	n
T. ferroox (x10 ³)	idans pH2.5	Fe ³⁺	1 .	0.14	NG	22	Fe ³⁺	1	NG	NG	NG
Low pH S oxidise (x10 ³)	rs pH3.5	NG	NG	NG	NG	NG	0.4]	NG	NG	NG	NG
Intermedi S oxidise (x10 ³)	ate pH ers pH4.8										
High pH S oxidise (x10 ³)	ers pH6.2	ŊG	NG	ŊG	27	22	654	NG	NG	NG	NG
Acidophi Heterotro (x10 ³)		1.8	0.74	1	1304	280	823	413	105	43	14
Nutrient Agar (x10 ³)	рН7				NG				<u> </u>		
Desulfov	(x10 ³) pH7 Desulfovibrio		NG	L			NG gram dry	NG	ŊG	NG	NG

Enumeration of organisms as no. per ml or gram dry weight n = negligible (less than 1 p p m) NG = No Growth W = Water Sample Blank space indicates sample was not tested.

MARCH 1977

	<u> IC</u>	CATION	ı: HC	LE D'	(Con	t'd)			-0110	D 10	LOG J	ICA.	L DA	TĄ				MAF	CH :	1971
	Sample n	10.	281	28	32 :	283	28	4										-	 - 	
	Distance from Surface		120	9	00	60	3(0							ļ		-			_
	Moisture Content	(%)	15	1	6	17	9.:	3					 -			<u> </u>	-			_
	Water sol	lubles om)	1.8	2.	6	n	n										-			\dashv
	рН		3.7	3.	7 3	.2	3.5	5		1										\dashv
	Soluble Metal	Fe	n	10	:	n	n	+	<u> </u>						_			-		\dashv
	Content (ppm)	Cu	n	18	_ 1	n	n									7		+		_
		Zn	n	n	r	1	n			\top		+		-		1	<u> </u>	+		-
	T. <u>ferrooxi</u> (x10 ³)	dans pH2.5	0.19	NG	NG	;	12			-		+		-				+		1
1	Low pH S oxidiser (x10 ³)	в рН3.5	NG	NG	NG		NG	+		-		-		+		+		+		
] S	Intermedia 5 oxidiser: (x10 ³) p	te pH s oH4.8						- 			- -	+		+			 .	-		
S	ligh`pH Oxidisers x10 ³) p	ы ЭН6.2	NG	NG	NG	_	NG	 		 		 	· <u> </u>	+		+		-		
] He	cidophilic eterotroph x10 ³) p	s H3.5			361	-	11	 				 				+			<u> </u>	
Ag	utrient gar (10 ³) p	H7				1			: _		_			-		-	, 	-		
<u>De</u>	sulfovibri	.0	NG	NG	NG	N	1G											 		

Enumeration of organisms as no. per ml or gram dry weight n = negligible (less than 1 p p m) NG = No Growth W = Water Sample Blank space indicates sample was not tested.

IOCAT'I	roni.		S HEAP			НО	LE E	7 			<u></u>	- 1 -		
Sample no.	J.	285	286	287	288	2	:89	290	25	91	292	2	93	294
Distance from Surface	(cm)	470	462.5	455	447.	5 4	140	432.5	4	25	417.5	5 4	10	402.5
Moisture Content (%)	Ì	14	16	1.3	14		18	13		17	13		14	15
Water solul		4.8	3.3	4.4	2.2		2.2	2.5	C	.3	0.2	- ; - -	3.2	3.5
рН		5.7	5.4	5.7	5.8		4.8	5.7		5.0	5.6	,	6.0	6.0
Soluble	Fe	110	50	67	78		n	n	<u> </u>	n	n		15	n
Metal. Content (p p m.)	Cu	10	20	165	1,0	,	10	n		n	n	_	29	10
	Zn	n	n	n	n		n	n		n	n	_	n	n
T. ferrooxidans (x10 ³) pH2.5		NG	NG	NG	N	G	0.2	4 NG		NG	NG	;	ng	NG
Low pH S oxidise (x10 ³)	ers pH3.5	NG	NG	NG	N	i G	NG	NG	; 	NG	No	3	NG	NG
Intermed: S oxidise (x10 ³)	iate pH ers pH4.8													
High pH S oxidis (x10 ³)	ers pH6.2	2 NO	g NG	2.	1	19	20	2	95	27	6	7	145	154
Acidophilic Heterotrophs (x10 ³) pH3.		5 N	G	N	G	ng 	NG	;	ŊĠ	ŊG	I I		NG	; NG
Nutrient Agar (x10 ³) pH7			3	6					3.2	1.	3	5.8	12	7.3
(x10°) pH7 Desulfovibrio		1	1G N	iG I	1G	NG	N	G	ŊG	No	G	ng ——	N	G NG

Enumeration of organisms as no. per ml or gram dry weight n = negligible (less than 1 p p m) NG = No Growth W = Water Sample Blank space indicates sample was not tested.

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LOCATION: HOLE E' (Cont'd)

	CATTON:											
Sample n	0.	295	296	297	298	299	300	301	302	303	304	
Distance from Surface		395	387.5	380	372.5	364.5	358	350	342.5	335	327.	 5
Moisture Content		14	12	17	15	16	14	14	12	13	 	
Water sol		1.5	3.5	2.6	5.5	8.2	3.9	2.5	1.7	0.6	1.6	_
рН	рН		5.9	6.5	6.3	6.2	6.6	6.5	5.3	6.4	4.9	_
Soluble Metal	Fe	n	n	n	120	1 5	19	n	n	85	17	1
Content (ppm)	Cu	45	n	n	43	n	91	n	n	17	n	1
	Zn	5	n	n	n	n	n	n	n	n	n	1
T. ferroox (x10 ³)	idans pH2.5	NG	NG	NG	ŊG	NG	NG	NG	NG	NG	NG	1
Low pH S oxidisen (x10 ³)	rs pH3.5	NG	NG	NG	NG	NG	NG	NG	3.8	NG	NG	
Intermedia S oxidiser (x10 ³)	ate pH cs pH4.8										 	
High pH S oxidiser (x10 ³)	rs pH6.2	2.2	5.0	12	6.9	104	46	25	22	37	38	1
Acidophili Heterotrop (x10 ³)	c hs pH3.5	0.43		NG	NG	NG	NG	NG	NG	NG	NG	
Nutrient Agar (x10 ³)	рН7	ŊG	1.9	5.8	1.5	6.1	9.9	23	1.0	4.4	0.43	
<u>Desulfovibrio</u>		NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	
	_								1			

Enumeration of organisms as no. per ml or gram dry weight n = negligible (less than l p p m) NG = No Growth W = Water Sample Blank space indicates sample was not tested.

LOCAT	ION: H	OLE E'	(Cont	'd)							
Sample no.		305	306	307	308	309	310	311	312	313	314
Distance from Surface	(cm)	320	312.5	305	297.5	290	282.5	275	267.5	240	210
Moisture Content (%)	·	14	8.1	16	12	15	12	15	13	17	15
Water solu		1.6	2.6	9.9	2.5	1.9	0.8	2.2	1.8	6.5	5.6
рН		4.7	5.3	5.1	5.7	6.2	5.1	6.5	6.5	6.5	6.5
Soluble	Fe	17	n	96	20	n	n	5	n	16	n
Metal Content (p.p.m.)	Cu	n	n	78	19	n	n	15	n	18	n
	Zn	n	n	n	n	n	n	n	n	n	n
T.ferroox	idans pH2.5	NG	NG	NG	NG	NG	NG	NG	ŊG	NG	ŊG
Low pH S oxidise (x10 ³)	rs pH3.5	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
Intermedi S oxidise (xl0 ³)											
High pH S oxidise (x10 ³)	ers pH6.2	26	5.6	1:1	73	449	12	206	116	20	845
Acidophil Heterotro (x10 ³)	lic ophs pH3.5	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
Nutrient Agar (x10 ³)	рн7	0.8	2	NG	NG	76	NG	3.5	2.1	NG	2.0
Desulfov	<u>ibrio</u>	NG	NG	NG	NG	NG	NG	NG	NO	NG	NG

Enumeration of organisms as no. per ml or gram dry weight n=negligible (less than l p p m) NG = No Growth W = Water Sample Blank space indicates sample was not tested.

LOCATIO	ON: HO	OLE E'	(Cont	d)			-101100	31CA	L DA	TA			MA	RCH	197	17
Sample no.	31	.5 31	6 3	17	318	319	9 3	20					T	 -	T-	
Distance from Surface(cm)	18	0 15	0 1	20 9	 90	60	3	o	-		-		+-		-	
Moisture Content (%)	1	5 18	8 :	15	14	9.8	3	12					-		-	
Water solubles (xl0 ³ p p m)	5 O.:	2 0.3	3 0.	.9 3	.3	1.6	2	.1							-	
рн	6.1	7 6.9	5.	1 5	.0	4.0	4.	2	<u></u>							
Soluble Metal	n	n	7	8 r	1	n	n			+						\dashv
Content (ppm) Cu	n	n	1	0 n	,	n	10	1		+		1	 -	-		\dashv
Zn	n	n	n	n		n	n	1		+		+		+	 _	\dashv
T. <u>ferrooxidans</u> (x10 ³) pH2.5	NG	NG	NG	NG		0.02	NG	+	-	+		+		+		-
Low pH S oxidisers (x10 ³) pH3.5	NG	NG	NG	NG		NG	NG	_}_		+	-	+		+		-
Intermediate pH S oxidisers (x10 ³) pH4.8								-				+		+		
High pH S oxidisers (x10 ³) pH6.2	15	1.0	23	228		51	518	+-		-		-		+	 -	
Acidophilic Heterotrophs (x10 ³) pH3.5	NG	NG	NG	NG	N	īG	NG	-		 		-		-		
Nutrient Agar (x10 ³) pH7	3.6	NG	0.3	0.21	N	G	31	-						-	<u>-</u>	
<u>Desulfovibrio</u>	NG	NG	ŊĠ	NG	No	G	NG	 			\dashv			-		!

Enumeration of organisms as no. per ml or gram dry weight n = negligible (less than 1 p p.m.) NG = No Growth W = Water Sample Blank space indicates sample was not tested.

MARCH 1977

		r ITIHW		IEAP	7170113	•		IOLI	E G'	'									٦
LOCATI	ON:	325	3.	26	327] 3	328	32	9	33	0	33	1	332	\ -\-	333	<u> </u>	334	\ \ -\
Distance from Surface(c		470	462	.5	455	4	47.5	44	10	432	2.5	42	5	417	.5	410		102.	5
Moisture Content (%)		19		18	15		16]	15]	16]	.7	20) 	17 ——	, 	16	4
Water solu	bles	2.2		2.6	5.3	4	1.4	0	.3	1	.2	1	.3	8.6	6 	7.8	3	13	-
(x10 ³ p p)	<u>m)</u>	3.6	- -	3.7	3.8		3.6	3	.5	3	.2	3	. 4	3.	4	3.	6	4.0)
Soluble	Fe	n		10	15		38		n		n	\ \ \	n	11	.0	n		n	_
Metal Content (p p m)	Cu	15		25	10		40		n 10		10		n	\ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \	25 	r	1	19	\dashv
(Zn			n	n		n		n		n		n	· ;	n 	\ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \	n 	n —	_
<u>T.ferroox</u> (x10 ³)	cidans pH2.5	NO	-	1.5	2.	5	3.8		0.1	.7	0.3	5	0.2	5 N	IG 	И	G	0.	13
Low pH S oxidis (x10 ³)	ers	5 N	3	NG	N	īG	NG		Nº	G \	NG	; -	N G	; 1	1G	1	1G	N	G
Intermed S oxidis (x10 ³)	 liate p	H										-\ \							
High pH s oxidi (x10 ³)			219	627	8	86	76	7	158	3	73	1	65	4	250	00	N G	8	.3
Acidoph Heterot	rophs	5	21	238	3	354	41	4	0.	68	1.	. 2	N	IG	1!	50		\ -\	
Nutries Agar (x10 ³)	nt		ŊG	4	8	ŊĠ	0.	.26	0.	45	1	NG 	1	NG	N 	G		_	
	ovibri	+	ŊG	N	īG	NG	;	NG]	NG 		NG	hpica	NG	ľ	1G	И	G	NG

Enumeration of organisms as no. per ml or gram dry weight $n = \text{negligible (less than l p p m)} \quad NG = No Growth \quad W = Water Sample Blank space indicates sample was not tested.$

MARCH 1977

	CATIO	N: HO	LE G'	(Cont'	đ)		01.02	5101	30 9 10	AL DA	T'A			ľ	MARC	H 19	∌ 7
Sample n	no.	33!	5 336	5 3	37	338	33	9	340	34	1	342	2	343	3 :	344	<u> </u>
Distance from Surfac	e e _(cm)	395	387.	.5 3:	80 3	72.5	36!	5	357.	5 35		342	_	225			
Moisture Content		14	13	16	5	16			15	1.	$\neg \dagger$	11		335	+	327. 16	5
Water so (x10 ³ p		10.	7 3.0	10.	.9	7.1		- -	3.9	2.0	\dashv	3.7	+	7.0		.6	\dashv
Hq		4.	1 4.0	3.	8	3.4	3.5	; 3	3.7	3.8	+	3.9	+	3.7	3		1
Soluble Metal	Fe	n	n	n		n		1	n	15	-	n	+	n		18	$\frac{1}{2}$
Content (ppm)	Cu	95	11.0	95	J	LO			n	20	+-	n	+	n	+-	 55	1
	Zn	n	n	n		n			 n	n		n	\dagger	n	r		1
T. <u>ferroox</u> (x10 ³)	idans pH2.5	NG	0.11	0.6	7 6.	4	NG	0	.24	NG	- I	 1G	 	 1G	0.	13	
Low pH S oxidiser (x10 ³)	pH3.5	NG	NG	NG	N	G	NG	N	1G	NG	N	IG	N	 iG	+	G	
Intermedia S oxidiser (x10 ³)	te pH s pH4.8					-		 -					-				
High pH S oxidiser (x10 ³)	s oH6.2	2.2	1420	804	637	7 5	518	7	87	990	8	90	12	40	108	30	
Acidophilic Heterotroph (x10 ³) r	ons OH3.5		NG	0.27	0.6	4	5.4	2	.8	0.25	4.	.5		 18	26	57	
Nutrient Agar (x10 ³) p	Н7		2.9	NG	NG		NG	1	NG	NG.	N	1G	<u> </u>	IG	NG		
 Desulfovibr	<u>io</u>	NG	NG	NG	NG		NG	N	1G	NG	N	IG	N	G	NG		
	T1-								[- 1		- 1			

Enumeration of organisms as no. per ml or gram dry weight n = negligible (less than l p p m) NG = No Growth W = Water Sample Blank space indicates sample was not tested.

LOCATION: HOLE G' (Cont'd)

LOCAT	ion: H	OTE C.	(Cont	u,							
Sample no.		345	346	347	348	349	350	351	352	353	354
Distance from Surface(c	m)	320	312.5	305	297.5	290	282.5	275	267.5	240	210
Moisture Content (%)		12	14	16	12,	15	17	13	13	12	20
Water solul		6.2	3.5	3.6	4.3	6.3	4.1	3.0	3.9	4.1	4.1
pH .	<u></u>	3.6	3.7	3.8	3.3	3.6	3.7	3.8	3.5	2.9	2.8
Soluble	Fe	17	n	n	n	17	19	n	n	n	n
Metal Content (ppm.)	Cu .	191	n	18	25	95	18	17	n	n	17
	Zn	n	n	n	n	n	n	n	n	n	5
<u>T, ferroox</u> (x10 ³)	idans pH2.5	NG	NG	NG	0.74	0.36	NG	NG	NG	NG	0.12
Low pH S oxidise (x10 ³)	rs pH3.5	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
Intermedi S oxidise (x10 ³)	ate pH ers pH4.8										
High pH S oxidise (x10 ³)	ers pH6.2	739	952	995	1110	184	26	NG	58	31	49
Acidophi Heterotro (x10 ³)	lic ophs pH3.5	ŊG	0.3	2 73	9.6	0.4	5 0.4	12 0.62	0.3	0.36	29
Nutrient Agar (x10 ³)	рН7	1.0	0.3	NG	0.3	7 2.	7 2.	1 4.1	ŊG	NG	NG
Desulfor	<u>ibrio</u>	ИС	3 NG	NG	ŊG	NG	N	G NO	g NG	; NG	NG

Enumeration of organisms as no. per ml or gram dry weight n = negligible (less than 1 p p m) NG = No Growth W = Water Sample Blank space indicates sample was not tested.

MARCH 1977

LOCATION: HOLE G' (Cont'd)

r		CATION	HOLL		nic uj								
	Sample r	ю.	355	356	357	358	359	360					 .
	Distance from Surfac	e (cm)	180	150	120	90	60	30	+			-	
	Moisture Content		20	20	10	1,5	11	11					
	Water so		5.0	3.6	0.7	2.2	1.8	2.4		 		_	
	Нд		2.5	3.0	2.9	3.5	3.5	3.1			 		
	Soluble Metal	Fe	n	16	n	n	n	n	 				_
	Content (p.p.m.)	Cu	n	29	n	10	11	5	 				_
		Zn	n	n	n	n	n	n					-
	T. <u>ferro</u> ox (x10 ³)	<u>idans</u> pH2.5	0.1	NG	NG	ŊG	NG	NG					_
	Low pH S oxidise (x10 ³)	rs pH3.5	NG	NG	NG	NG	NG	NG				-	
ן נ	Intermedia S oxidiser (x10 ³)	ate pH cs pH4.8										 	
8	High pH 5 oxidiser (x10 ³)	s pH6.2	NG	NG	NG	0.69	976	175					
Н	cidophili (eterotrop ×10 ³)	c hs pH3.5	157	24	ŊĠ	ŊĠ	424	265				 	-
A	utrient gar x10 ³) _]	рН7	ŊĠ		NG	0.46	NG	NG		<u> </u>			
De	esulfoviba	<u>rio</u>	NG	NG	NG	NG	NG	NG					
		Enimo	cation of]	1

Enumeration of organisms as no. per ml or gram dry weight n = negligible (less than 1 p p m.) NG = No Growth W = Water Sample Blank space indicates sample was not tested.

		SAMPLES COLLECTED JUNE 1978
Code: RJ no.	Coordinates of sample location	Description, Field Observations
397		White's Overburden Heap: Hole J cut into side about 4 m from base. Material very dry, crumbly, loose and collapsible. Difficult to maintain balance when sampling. Soil sample taken at top of hole.
398		Soil sample taken at 0.75 m from top.
399		Soil sample taken at 1.5 m from top.
466		White's Spring No.4 water sample, pH 3.4.
1.		

PHYSICOCHEMICAL AND MICROBIOLOGICAL DATA WHITE'S HEAD

	<u> </u>	CATION	WHI HOL	TE'S H	EAP	CAL ANI	MICE	ROB:	IOLOGI	CAL DAT			JUI	JE 1	1978	
	Sample r	10.	397	398	399					SPRING 466			-	\top	 -	
	Distance from Surface		0	75	150							-	-	+		-
	Moisture Content		5.0	5.5	5.2				 	W		+		+	-	-
	Water sol (x10 ³ p p	lubles om)	n	1.6	3.4					18	+			+		$\frac{1}{1}$
-	рН		4.9	5.1	5.1					3.4		-		+		1
	Soluble Metal Content	Fe	n	n	n					125		-		+-		
	(ppm)	Cu	308	66	369					95	+			 		
L		Zn	n	n	n					23		+		 		
	T. <u>ferrooxi</u> (x10 ³)	dans pH2.5	67	44	7.4					0.06		+-				
	Low pH S oxidisers (x10 ³)	s OH3.5	NG	NG	NG		 	1		NG		-			_	
٤	ntermediat oxidisers x10 ³) p	ерн з	2.8	0.86	0.3	ļ .		+		0.41					-	

NG

0.3

NG

Enumeration of organisms as no. per ml or gram dry weight n = negligible (less than 1 p.p.m.) NG = No Growth W = Water Sample Blank space indicates sample was not tested.

pH6.2

pH3.5

pH7

0.14

NG

NG

0.43

NG

NG

0.06

NG

NG

NG

High pH S oxidisers

Acidophilic Heterotrophs

Desulfovibrio

 $(x10^3)$

 $(x10^3)$

Nutrient Agar

 $(x10^3)$

		152	7
		SAMPLES COLLECTED MAY 1979	_
Code: RJ no.	Coordinates of sample location	Description, Field Observations	_
404 405 406 407	109-414 112-413 107-414 118-417	Base White's Heap Sloppy mud sample. Surface mud sample, dark brown, very wet. Water sample at Spring No.4. Algae green and fresh. Soil sample; white, crusty efflorescent material, layer about 3 mm thick.	

LOC	ATION:	BAS	E WHITE	'S HEAT	P AT S	SPRIM	IG 1	10.4					
Sample no) .	404	405							400	5		
Distance from Surface	(cm)							 					
Moisture Content (%) -—	61.2	38.	31.8				-		W			
Water solu (x10 ³ p p	ubles m.)	69	36	20						30			_
рН		3.0	3.2	3.8						3.4			
Soluble Metal	Fe	492	46	185						700	+	 	
Content (ppm)	Cu	103	26	120						170		1	
	Zn	43	9	109						8		- 	
T. ferrooxid (x10 ³)	dans pH2.5	45	11	NG						0.75	5		
Low pH S oxidisers (x10 ³)	5 0H3.5	NG	NG	NG						NG		+	
Intermediat S oxidisers (x10 ³) p	се рн Б	24	12	NG					 	3.7			
High pH S oxidisers (x10 ³) p	H6.2	NG	1.2	NG				<u> </u>		6.4			
Acidophilic Heterotroph (x10 ³) pl	s H3.5	53	27	NG						NG			
Nutrient Agar (x10 ³) pH	H7	NG	NG	NG		_				6.3			
Desulfovibri	<u>io</u>	NG	NG							NG			

Enumeration of organisms as no. per ml or gram dry weight $n = \text{negligible (less than 1 p p m)} \quad NG = No Growth \quad W = Water Sample Blank space indicates sample was not tested.$

APPENDIX D
SAMPLING LOCATIONS, AND PHYSICOCHEMICAL AND MICROBIOLOGICAL DATA
INTERMEDIATE OVERBURDEN HEAP 1978-1979

Note: The coordinates listed refer to the coordinates given in $\operatorname{\mathsf{Map}}$ 3

		SAMPLES COLLECTED JUNE 1978
Code RJ no.	Coordinates of sample location	Description, Field Observations
370	007-417	Intermediate Overburden Heap: Hole Z cut at base of heap. Material on surface, and along edges of cut hole very loose. Soil sample taken at top of hole.
371		Soil sample taken 0.5 m from top of hole.
372		Soil sample taken 1.0 m from top of hole.
373 374 375		Soil sample taken 1.5 m from top of hole. Rocks dark. " " 2.0 m " " " " " Soil sample taken 2.5 m from top.
376		Soil sample taken 3.0 m from top. Hole Z very open - could feel draughts coming out of hole. Could not dig into very base at this point because of large rocks and ground not hard nor dry enough to support backhoe.
377	006-416	Hole Z* shifted about 3 metres to the right of Hole Z and dug from the last level at Hole Z (i.e. 3 m from top of hole) to base. Soil sample taken 3 m from top.
378		Soil sample taken 3.5 m from top.
379		Soil sample taken 4.0 m from top.
380		Soil sample taken 4.5 m from top.
381	012-414	Hole Y cut at the middle level of Intermediate Heap, into the side of the top layer, directly in line above Hole Z. Soil sample taken at top of hole.
382		Soil sample taken at 0.5 m from top of hole.
383		Soil sample taken at 1.0 m from top of hole.
384		Soil sample taken at 1.5 m from top of hole.
385		Soil sample taken at 2.0 m from top of hole.
386		Soil sample taken at 2.5 m from top of hole.
387		Soil sample taken at 3.0 m from top of hole.
388		Soil sample taken at 3.5 m from top of hole.
89		Soil sample taken at 4.0 m from top of hole, i.e. at base

		130
		SAMPLES COLLECTED JUNE 1978
Code: RJ no.	Coordinates of sample location	Description, Field Observations
390	016-412	Hole X cut down into the top of Intermediate Heap in line with Holes Z and Y. About 4 m deep. Rocks exposed along the sides of the hole appeared to be coated with a blue deposit. Soil sample taken at top of hole.
391		Soil sample taken at 0.5 m from top.
392		Soil sample taken at 1.0 m from top.
393		Soil sample taken at 1.5 m from top.
394		Soil sample taken at 2.0 m from top.
395		Soil sample taken at 2.5 m from top.
396		Soil sample taken at 3.0 m from top, i.e. at base of hole.
ļ		

JUNE 1978

LOCA	MOLTA	INTER	MEDIATE	НЕАР	НС	DLE Z				
Sample no		370	371	372	373	374	375	376		
Distance from Surface			50	100	150	200	250	300		
Moisture Content (%)		1.8	8.9	11	12.7	7.4	7.3	6.8		
Water solu (x10 ³ p.p		5.1	1.9	1.8	3.1	29	26	32		
рН			3.1	2.8	3.0	2.5	2.6	2.6		
Soluble Metal	Fe	142	n	96	24	5728	3212	3501		
Content (ppm)	Cu	56	6	n	n	135	21	12		
	Zn	n	n	n	n	54 .	27	129		
1 2	T.ferrooxidans (x10 ³) pH2.5		144	12	11	42	74	46		
Low pH S oxidiser (x10 ³)	s pH3.5	NG	NG	NG	NG	NG	NG	NG		
Intermedia S oxidiser (x10 ³)	te pH s pH4.8	NG	NG	NG	0.2	3.6	0.32	NG		
High pH S oxidiser (x10 ³)	s pH6.2	NG	NG	NG	NG	NG	NG	NG		
Acidophili Heterotrop (x10 ³)	c hs pH3.5	NG	NG	NG	NG	NG	NG	NG		
Nutrient Agar (x10 ³)	рн7	NG	NG	NG	NG	0.47	0.13	0.08		
Desulfovib	r <u>io</u>							NG		

Enumeration of organisms as no. per ml or gram dry weight n=negligible (less than 1 p p m) NG = No Growth W = Water Sample Blank space indicates sample was not tested.

LOCA	TION:	INTER	4EDIATE	HEAP	HOI	E Z*			
Sample no.		377	378	379	380				
Distance from Surface	(am)	300	350	400	450				
Moisture Content (%)		18.0	20.5	23.4	27.8				
	Water solubles (x10 ³ p p m)		5.4	14	25				
рН		3.5	4.1	4.4	3.9				
Soluble Metal	Fe	16	n	n	n				
Content (ppm)	Cu	24	44	837	1248			-	
	Zn	60	64	122	177				
T.ferrooxi	dans pH2.5	94	126	136	177				
Low pH S oxidiser (x10 ³)	s pH3.5	NG	NG	NG	NG				
Intermedia S oxidiser (x10 ³)	nte pH s pH4.8	0.19	0.68	27	18				
High pH S oxidiser (x10 ³)	s pH6.2	0.01	NG	NG	NG				
Heterotrop	Acidophilic Heterotrophs (x10 ³) pH3.5		0.29	0.14	3.0				
Nutrient Agar (x10 ³)	рН7	NG	NG	NG	NG				
<u>Desulfovik</u>	r <u>io</u>				NG				

Enumeration of organisms as no. per ml or gram dry weight n = negligible (less than l p p m) NG = No Growth W = Water Sample Blank space indicates sample was not tested.

JUNE 1978

LOCATION:		INTERM	EDIATE	HEAP	HOL	ΕY					
Sample no.		381	382	383	384	385	386	387	388	389	
Distance from Surface (cm)		0	50	100	150	200	250	300	350	400	
Moisture Content (%)		5.0	16.2	14.4	15.2	15.5	11.8	13.4	16.6	21.2	
Water solu (x10 ³ p p		4.0	3.8	2.2	4.0	4.6	3.1	2.5	8.7	27	
рН		3.6	3.3	3.2	3.5	3.3	3.2	3.4	3.1	3.1	
Soluble Metal	Fe	n	n	n	n	n	n	n	20	28	
Content (ppm)	Cu	15	7	14	31	16	8	11	20	333	
;	Zn	n	n	n	n	n	n	n	n	21	
1	T.ferroxidans (x10 ³) pH2.5		NG	NG	NG	12	105	133	114	108	
Low pH S oxidiser (x10 ³)	rs pH3.5	NG	NG	NG	NG	NG	NG	NG	NG	NG	
Intermedia S oxidiser (x10 ³)	ate pH cs pH4.8	NG	NG	NG	NG	0.16	1.0	1.5	NG	0.79	
High pH S oxidiser (x10 ³)	rs pH6.2	NG	NG	NG	NG	NG	NG	0.27	NG	NG	
Acidophili Heterotrop (x10 ³)		NG	NG	NG	NG	NG	1.1	0.11	0.16	1.1	
Nutrient Agar (x10 ³)	рН7	NG	NG	NG	NG	NG	NG	NG	0.74	NG	
Desulfovil	or <u>io</u>									NG	

Enumeration of organisms as no. per ml or gram dry weight n=negligible (less than l p p m) NG = No Growth W = Water Sample Blank space indicates sample was not tested.

LOCA	TION:	INTERN	ŒDIATE	OVERBU	JRDEN H	EAP	HOLE	X			
Sample no.		390	391	392	393	394	395	396	į		
Distance from Surface ((am)	0	50	100	150	200	250	300			
Moisture Content (%	s)	12.1	14.0	13.6	12.2	12.1	14.5	15.3			
Water solu (x10 ³ p p		60	83	78	79	79	77	102	-		
рH		2.9	2.8	3.2	3.4	3.5	3.5	3.5			
Soluble	Fe	1295	1318	701	480	436	174	328			
Metal Content (ppm)	Cu	712	1014	136	186	2993	2261	3063			
	Zn	n	n	n	n	n	n	n		,	
T, ferrooxi	i <u>dans</u> pH2.5	77	140	97	72	13	139	130			
Low pH S oxidiser (x10 ³)	rs pH3.5	NG	NG	NG	NG	NG	NG	NG			
Intermedia S oxidiser (x10 ³)		0.03	NG	NG	NG	NG	NG	NG			
High pH S oxidiser (x10 ³)	rs pH6.2	0.11	NG	NG	NG	NG	NG	NG			
Heterotrop	Acidophilic Heterotrophs (x10 ³) pH3.5		NG	NG	NG	NG	NG	NG			·
Nutrient Agar (x10 ³)	рН7	NG	NG	3.5	NG	NG	NG	NG			
Desulfovil	orio							NG			

Enumeration of organisms as no. per ml or gram dry weight n = negligible (less than 1 p p m) NG = No Growth W = Water Sample Blank space indicates sample was not tested.

		SAMPLES COLLECTED MAY 1979
Code: RJ no.	Coordinates of sample location	Description, Field Observations
		INTERMEDIATE OVERBURDEN HEAP:
	015-412	HOLE S: Hole dug at top of Intermediate Heap, about 13 m to right of Hole X (1978). Pyrite exposed, soil brown and moist. Exposed rocks have blue faces, strong sulphur smell in hole.
412		Soil sample at surface; grey with yellow particles, damp.
413		Soil sample 0.5 m from surface; brown and yellow particles, material loose.
414		Soil sample 1.0 m from surface; exposed pyrite, moist.
415		Soil sample 1.5 m from surface; exposed pyrite, moist.
416		Soil sample 2.0 m from surface; brown with red and yellow particles, moist.
417		Soil sample 2.5 m from surface; dark brown material, moist.
418		Soil sample 3.0 m from surface; dark brown material, moist.
419		Soil sample 3.5 m from surface, at base of hole; dark grey material with red particles, moist.
	011-413	HOLE T: Hole dug at middle level of Intermediate Heap, about 20 m to right of Hole Y (1978). Material drier than Hole S and has many large rocks. Base of hole is about 0.7 m below surface of road.
420		Soil sample 3.5 m from surface of hole, at base; grey and yellow material, very damp, 'clayey'.
421		Soil sample 3.0 m from surface; dark grey, yellow and brown material, moist.
422		Soil sample 2.5 m from surface; dark grey, yellow and brown material, moist.
423		Soil sample 2.0 m from surface; many large rocks with ferric oxides.

		SAMPLES COLLECTED MAY 1979
Code: RJ	Coordinates of sample location	Description, Field Observations
424		Soil sample 1.5 m from surface; dark grey with some yellow particles, moist.
425		Soil sample 1.0 m from surface; brown, loose material, moist.
426		Soil sample 0.5 m from surface; brown loose material, fairly dry.
427		Soil sample taken at surface of hole; grey, loose material, very dry.
:	007-415	HOLE V: Hole dug at base of Intermediate Heap, in line with Holes S and T. Material loose, mainly large rocks, very porous with water seeping out making hole very wet.
428		Soil sample taken at surface of hole; very dry, large stones and rocks.
429		Soil sample 0.5 m from surface; grey-brown, loose material, fairly dry.
430		Soil sample 1.0 m from surface; grey loose material, fairly dry.
431		Soil sample 1.5 m from surface; brown material, moist.
432		Soil sample 2.0 m from surface; dark brown material, slightly moist.
433		Soil sample 2.5 m from surface; yellow material, very moist, 'clayey'.
434		Soil sample 3.0 m from surface; brown material, moist.
435		Soil sample 3.5 m from surface, at base; grey material, moist
436		Water sample collected at base of hole from free-flowing water seepage.

LOCA	ATION:	INTER	MEDIATE	OVERB	URDEN 1	ΉΕΑΡ	HOL	ES		
Sample no	•	412	413	414	415	416	417	418	419	
Distance from Surface	(cm)	0	50	100	150	200	250	300	350	<u> </u>
Moisture Content (9	≹) ————————————————————————————————————	14.2	10.2	13.0	12.9	13.5	12.8	13.4	12.2	
Water solu (x10 ³ p.p		103	74	104	101	76	78	74	38	
рН		2.6	3.4	3.0	3.6	3.6	3.4	3.5	3.5	
Soluble Metal	Fe	4343	171	1532	285	123	264	172	211	
Content (ppm)	Cu	300	684	3153	4057	2003	565	1902	1470	
	Zn	n	n	n	n	n	n	63	n	
<u>T.ferrooxi</u> (x10 ³)	dans pH2.5	0.97	0.24	0.2	0.33	1.2	1.3	0.37	0.78	
Low pH S oxidiser (x10 ³)	s pH3.5	NG	NG	NG	NG	NG	NG	NG	NG	
Intermedia S oxidiser (x10 ³)	te pH s pH4.8	NG	NG	NG	NG	NG	NG	NG	NG	
High pH S oxidiser (x10 ³)	s рН6.2	NG	NG	NG	NG	NG	NG	NG	NG	
Acidophilio Heterotroph (x10 ³) I	c hs oH3.5	NG	NG	NG	NG	NG	NG	NG	NG	
Nutrient Agar (x10 ³) p	рН7	NG	NG	NG	0.1	0.15	0.1	0.1	NG	
Desulfovib	rio								NG	

Enumeration of organisms as no. per ml or gram dry weight n = negligible (less than l p p m) NG = No Growth W = Water Sample Blank space indicates sample was not tested.

LOCAT	YON:	INTERM	DIATE :	HEAP	ноі	ET					
Sample no.		420	421	422	423	424	425	426	427		
Distance from Surface (cm)	350	300	250	200	150	100	50	0		
Moisture Content (%		21.1	19.5	21.3	15.5	14.8	15.0	8.3	3.1		
Water solu		7.0	4.3	6.7	5.3	21	15	16	37		
рн		3.0	2.8	2.9	3.1	3.4	3.3	3.4	3.2		
Soluble	Fe	1.36	68	76	27	17	38	26	n	· · · ·	
Metal. Content (p.p.m.)	Cu	78	41	59	36	43	70	48	65		
	Zn	n	n	59	n	n	n	n	n		
<u>T.ferroox</u> : (x10 ³)	idans pH2.5	NG	0.54	0.52	NG	0.22	0.23	NG	0.5		
Low pH S oxidise (x10 ³)	rs pH3.5	NG	NG	NG	NG	NG	NG	NG	NG		
Intermedi S oxidise (x10 ³)	ate pH ers pH4.8	NG	0.22	NG	NG	NG	NG	NG	NG		
High pH S oxidise (x10 ³)	ers pH6.2	NG	NG	NG	NG	NG	NG	NG	NG		
Acidophil Heterotro (x10 ³)	lic ophs pH3.5	NG	NG	NG	NG	NG	NG	NG	NG		
Nutrient Agar (x10 ³)	рН7	0.37	0.31	. NG	NG	0.21	1.1	NG	0.78		
Desulfov	<u>ibrio</u>	NG		!							

Enumeration of organisms as no. per ml or gram dry weight n=negligible (less than l p p m) NG = No Growth W = Water Sample Blank space indicates sample was not tested.

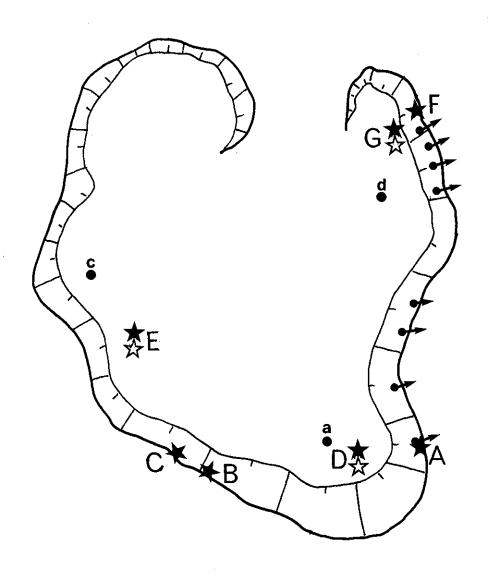
MAY 1979

LOC	ATION:	INTE	RMEDIA:	ге неар)	HOLE	<u> v</u>				
Sample no	•	428	429	430	431	432	433	434	435	436	
Distance from Surface			50	100	150	200	250	300	350	350	
Moisture Content (8)	0.8	9.5	15.3	19.4	11.1	25.4	16.5	20.3	W	
Water solu (x10 ³ p p		14	16	20	19	25	10	19	9.4	38	
рН		3.1	3.1	3.0	2.8	3.2	3.3	3.7	3.1	2.7	
Soluble Metal	Fe	46	25	26	59	477	125	34	108	650	
Content (ppm)	Cu	n	n	n	n	'n	n	n	23	73	
	Zn	n	n	n	n	n	n	n	11	11	
T.ferrooxi (x10 ³)	dans pH2.5	0.16	1.1	0.78	0.89	0.68	2.6	0.4	35	2.6	
Low pH S oxidiser (x10 ³)	s pH3.5	NG	NG	NG	NG	NG	NG	NG	NG	NG	
Intermedia S oxidiser (x10 ³)		NG	NG	NG	NG	NG	22.	NG	1.7	NG	
High pH S oxidiser: (x10 ³)		NG	NG	NG	NG	NG	NG	NG	NG	NG	
Acidophilio Heterotroph (x10 ³) I	c ns oH3.5	NG	NG	NG	NG	NG	0.1	5.1	NH	1.3	
Nutrient Agar (x10 ³) p	эн7	0.22	NG	NG	NG	NG	NG	NG	NG	NG	
Desulfovibr	Framo								NG	NG	

Enumeration of organisms as no. per ml or gram dry weight $n = \text{negligible (less than } 1 \text{ p p m)} \quad \text{NG} = \text{No Growth} \quad \text{W} = \text{Water Sample Blank space indicates sample was not tested.}$

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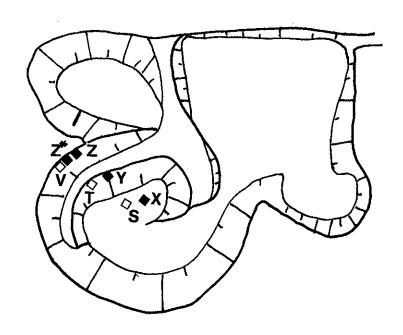
Dry Season, September 1976, sampling trench

Wet Season, March 1977, sampling trench

• AAEC temperature probe holes

Springs

MAP 1. WHITE'S OVERBURDEN DUMP SHOWING AAEC PROBE HOLES AND SAMPLING TRENCHES



- June 1978, sampling trench
- ☐ May 1979, sampling trench

MAP 2. INTERMEDIATE OVERBURDEN DUMP SHOWING SAMPLING TRENCHES