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THE WEATHERING OF PLATINUM FROM NUGGETS AND P
IMMOBILISATION BY CUPRIAVIDUS METALLIDURANS

(Spine title: WEATHERING AND IMMOBILISATION OF PLATIN

(Thesis format: Integrated Article)

by

Sean Gordon Campbell

Graduate Program in Earth Sciences

A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science

The School of Graduate and Postdoctoral Studies
The University of Western Ontario
London, Ontario, Canada

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THE UNIVERSITY OF WESTERN ONTARIO
School of Graduate and Postdoctoral Studies

CERTIFICATE OF EXAMINATION

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The thesis by

Sean Gordon Campbell

entitled:

The Weathering of Platinum from Nuggets and P
Immobilisation by *Cupriavidus metallidurans*

is accepted in partial fulfillment of the
requirements for the degree of
Master of Science

______________________	______________________________________
Date	Chair of the Thesis Examination Boa

Abstract

Two studies were conducted to investigate the biogeochemistry of
the first project, platinum nuggets were examined to evaluate mine
precipitation processes occurring at the nugget-soil solution interface. The pr
dissolution pits, cavities, striations, as well as acicular, iron ox
grain surface evidence that this interface is an importa
chemical weathering. Element mapping revealed that struc
can be linked to morphological features on the grain surface. In the s
soil bacterium *Cupriavidus metallidurans* was reacted with aqueous platinu
and rapidly immobilised platinum. XANES/EXAFS analysis
demonstrated that platinum chloride bound to the bacteria and platinum
from chloride to carboxyl functional groups. TEM revealed *C. metallidurans* was
able to precipitate nano-sized colloidal platinum. Both of these stu
importance of nano-phase and organo-platinum compounds in natural syste

Keywords: platinum, surficial weathering, Fifield Platinum Pro
Cupriavidus metallidurans, immobilisation, secondary biomineralisation

Co-Authorship Statement

Chapter 2, entitled Surficial weathering of platinum grains,
S.G. Campbell, R. Gordon, F. Reith and G. Southam has been prepared as
manuscript for submission to Geology for publication. Campbell perfor
microscopy and analyses, drafted the manuscript and prepared the figures
provided technical support at Argonne National Laborat
platinum grains and Reith and Southam provided the fund
and advised on all aspects of this study.

Chapter 3, entitled Immobilisation of platinum by Cupriavidus metallidurans,
was authored by S.G. Campbell, L. Maclean, D. Brewe, F.
has been prepared as a manuscript for submission to Langmuir for publication.
Campbell designed and conducted the experiments, microscopy and an
the manuscript and prepared the tables and figures. Macl
synchrotron data, Brewe provided technical support at Ar
and Reith and Southam provided funding to support this research and
aspects of this study.

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No man is an island[1], nor is a thesis one man. I have to admit, I words& I hope I didn t miss anyone, but in case I did, than

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[1] Donne, J., 1624, in Meditation XVII.

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List of Abbreviations and Symbols

°C — Celsius

µg — Microgram

µL — Microlitre

µm — Micron or micrometre

µM — Micromolar

µXRD — Micro-X-Ray Diffraction

aq — Aqueous

ATP — Adenosine triphosphate

CFU — Colony forming unit

DDI water — Distilled, deionised water

EDS — Energy dispersive spectroscopy

EMS — Electron Microscopy Sciences

eV — Electron volt

EXAFS — Extended X-ray Absorption Fine Structure

FEG — Field emission gun

hr — Hour

ICP-AES — Inductively coupled plasma atomic emission spectroscop

keV — Kilo-electron volt

km — Kilometre

L — Litre

M — Molarity

mg — Milligram

min	Minutes
mL	Millilitre
mm	Millimetre
mM	Millimolar
nm	Nanometre
g	Gram
PGE	Platinum group element
PGM	Platinum group mineral
pH	$-\log[H^+]$
ppb	Parts per billion
ppm	Parts per million
SEM	Scanning electron microscope
SRB	Sulphate-reducing bacteria
t	Time
TEM	Transmission electron microscope
XANES	X-ray Absorption Near Edge Structure
XAS	X-ray Absorption Spectroscopy

Chapter 1

Introduction

Platinum, one of the six platinum group elements (PG
osmium, iridium, palladium, ruthenium, and rhodium), is highly sought after beca
its physical and chemical properties (Macdonald 1987; Naldrett 1991; N
Platinum is resistant to oxidation and corrosion and it is
and industrial processes (Kathib 2011). Platinum-based chemotherapy drugs like
cisplatin are also used in the treatment of cancers (Alderden et al. 2006). The
price of this metal has increased over time because the s
pace with growing demand. Platinum is among the rares
concentration in the lithosphere typically 0.4-5 ppb. To be mined
profitably, platinum deposits require 1,000-10,000 fold enrichment over ba
level concentrations (Mungall and Naldrett 2008; Kesler 2010). There has been
increase in platinum deposit exploration and research over 0 years to better
understand the natural ore-forming processes to discover new deposits, but the genesis
of many platinum deposits remains unclear (Maier 2005).

1.1 Platinum enrichment in the crust

Principal PGE ore deposits are formed by magmatic, hydrothermal, and alluvial
processes (Macdonald 1987). Igneous bodies host most of the world's economic
deposits with an average grade of 5-20 g/t (5-20 ppm) (Nixon and Hammack
1991). The genesis of platinum deposits in mafic-ultramafic complexes, including the

primary deposits that give rise to alluvial placers, is inextricably tied to sul
chemistry and the processes that transport platinum and s
crust (Macdonald 1987; Mungall 2005; Mungall and Naldrett 2008).

Platinum-rich deposits are generated when sulphur concentrat
the available platinum from the silicate melt (Maier 2005; Mungall and
Naldrett 2008). During a metallogenic event, platinum-rich sulphu
ascends from the mantle to the crust where it becomes trapped. O
sulphur saturation in the magma occurs and an immiscibl
accumulates at the bottom of the magma chamber where it separates the highly
chalcophile platinum into the sulphide horizon (Maier 2005; Mungal
Naldrett 2008; Koek et al. 2010). Platinum sulphides form during crystallization
step (Mungall and Naldrett 2008). These magmatic processes often for
platinum-bearing reefs within large mafic-ultramafic intrusions (Ma
2005). Although poorly understood, hydrothermal brines can cause
redistribution of PGE (Mungall and Naldrett 2008).

Alluvial PGE placers are weathered from concentrically zoned ma
ultramafic "Alaskan-type" or "Ural-type" (and to a lesser extent "ophiolitic-type")
primary magmatic intrusions that were formed by processe
above (Macdonald 1987; Nixon and Hammack 1991; Tolstykh et al. 2005; Koek et al.
2010). Alaskan- and Ural-type complexes intrude into folded belts, continental mo
crato-platform environments and transitional oceanic-continental (Nixon and
Hammack 1991; Tolstykh et al. 2005).

1.2. Platinum placer deposit formation

An important source of platinum is from placer deposits (Cabri and Basset 2005). There are several types of deposits but alluvial placers provide the majority of placer platinum (Mertie 1969). The formation of an alluvial platinum pla begins with the extraction of platinum bearing minerals from source rocks at expos or near Earth's surface. Koek et al. (2010) describes a process where minerals from primary magmatic deposits (hypogene platinum minerals) within supergene and lateritic profiles (sites of secondary enrichment) experience chemical and physical weathering. Over time, the platiniferous material is physically and chemically liberated from the source depos and washed into streams where it is concentrated, deposited and tra gravitational sorting and the action of water. Alternatively, in some cases, platinum-bearing material is weathered and lighter mineral fractions are p eroded away. Although Alaskan-Ural type magmatic intrusions are the primary sources of placer platinum, other types of magmatic intrusions can generate pl Platinum minerals have been documented in the alluvial sedi reefs of South Africa's Bushveld Igneous Complex where the world's largest reserves of PGEs are hosted (Melcher et al. 2005).

1.3 Platinum in supergene systems

In alluvial placers and other surficial weathering profiles the proper conditions for platinum mobilization exist. Platinum can be remob from exposed primary magmatic deposits and form secondary

enrichment (Bowles 1986; Arzate et al. 2001; Hanley 2005). Supergene
and laterization are intense chemical weathering processes that enrich primary ore
deposits containing subeconomic sulphide concentrations (Guilbert 198
These regions commonly occur in tropical and semiarid clim
temperatures and humidity promote chemical oxidation (
waters above the water table are able to leach a large vo
redeposit them into smaller volumes of high grade mate
(Guilbert 1986).

Pyrite (FeS_2) is the most common hypogene sulphide an
enrichment that takes place in the supergene zone starts with
metal sulphides (Guilbert 1986). When exposed to oxygen, water and iron/sulphur
oxidising bacteria above the water table, pyrite forms aqueous sulphuric acid (H_2SO_4)
and ferric sulphate ($Fe_2(SO_4)_3$), which in turn act as solvents for o
sulphides, including copper, zinc, silver (Mann 1984; Bowles 1986; Guilbert
1986; Enders 2000). Ferric sulphate is reduced to ferrous ($FeSO_4$) during the
oxidation of other metallic sulphides. The dissolved metals will either be ca
from the site of oxidation by meteoric waters or will perc
(Anderson 1982; Enders 2000). Because platinum and gold have similar chemical
properties, it is reasonable to conclude that processes responsible for the mobilisation of
gold are comparable for the dissolution and migration of platinum. In fact, platinum is
more easily oxidised and more soluble than gold, since conditions
favourable for gold dissolution and mobility should also be favourable fo
platinum solubility (Bowles 1986; Cabral et al. 2007; Reith et al. 2007).

Acidified and oxidised waters descend and leave behind a leached cap that lacks the target metal. An insoluble mixture of hydrous **iron**-sulphates including jarosite ($KFe_3(OH)_6(SO_4)_2$), iron-oxyhydroxides such as goethite (and hematite (Fe_2O_3) are also formed during the oxidation of pyrite in th leached cap, giving it the characteristic red-orange appearance (Anderson 1982; E 2000; Suárez et al. 2008). As evidence of this oxidising pro oxyhydroxide coatings have been found on Ottermann platinum n Augusthihus 1967; Bowles 1986; Suárez et al. 2008).

Descending surficial waters carry oxidised metal below the water t where redox conditions favour reduction and precipitatio mediates deposition of the ores as it readily transfers its other metals. These metals usually have a stronger affin strip pyrite of its sulphur. Much metal is leached at the surface fo however, they are not removed from the supergene zone. The acidic wa neutralised when they come into contact with carbonates This process is cyclical and ongoing. Fluctuations in the water table shift the physical location of the redox boundary. Over time, thick blankets of metal can form and be of economic value (Anderson 1982; Enders 2000).

1.4. Aqueous platinum geochemistry

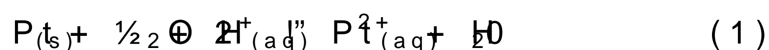
Platinum is mobile under supergene conditions (Fuchs and Rose 1974; Bowles 1986; Azaroual et al. 2001). The platinum content of natural waters is 3 to 6 ord magnitude less concentrated than in the lithosphere;

concentrations are dependent on a range of factors (Barefoot 1997; Mungall and Naldrett 2008; Kolek et al. 2010; Kubrakova et al. 2011). Surficial water flowing through areas with elevated levels of primary and/or secondary platinum deposits and/or their mine tailings, would presumably have more waters draining areas lacking platinum enrichment; however, the concentration of platinum is also controlled by its solubility, the redox properties of the aqueous medium as well as platinum's ability to form complexes with ligands commonly found in surficial waters, its dissolution, transportation and sorption (Bowles 1986; Colombo et al. 2008; Kubrakova et al. 2011). Furthermore, hydrodynamic characteristics will also influence the migration of dissolved platinum (Kubrakova et al. 2011).

At standard surface water temperatures (25 °C), platinum cannot be transported as simple aqueous ions except at extremely oxidizing, acidic conditions and a must complex with other dissolved ligands to be stable Wood 1988). Complexation is an important process by whi cations bind to one or more negatively charged anions complexing species, concentrations of simple metal ions solutions would remain too low to form economic deposits. C complexed to anions in solution, however, can be concent generate profitable grades of metal (Hanley 2005).

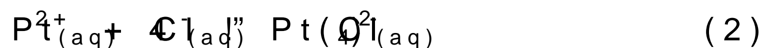
The literature lacks reliable experimental data on the stability platinum species and complexes in surficial waters (Mountain and Wo 1988; Colombo et al. 2008). The predicted oxidation state for pla

Pt^{2+} as shown in Equation 1 (Hanley 2005). At very oxidising conditions, platinum ca
exist as Pt^{4+} ion, but Pt^{2+} is the more common aqueous species. A
experimental data relating to the thermodynamic stabilit
systems relating to this particular oxidation state is limited (Hanley 2005; Colombo et al. 2008).
Platinum (II) prefers to form covalent interactions with anions such as chlorine,
cyanide, hydroxides, bisulphides and thiosulphate (Mountain and Wood 19
Vlassopoulos et al. 1990). Complexation, however, depends on
platinum's preference for various ligands; it ultimately de
the transporting fluids and the availability of ligands in
Rose 1974).

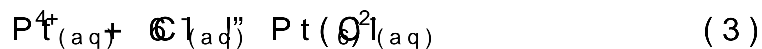


$$Pt_{(s)} + ½O_2 + 2H^+_{(aq)} \rightarrow Pt^{2+}_{(aq)} + H_2O \quad (1)$$

In oxidising waters that are highly acidic, common chloride anions
stable complexes with Pt^{2+} and Pt^{4+} (Fuchs and Rose 1974; Bowles 1986;
Wood 1988). These reactions are given in Equations 2 and (Hanley 2005; Colombo et
al. 2008). The aqueous Pt^{2+} compounds $PtCl^+$, $PtCl_2$, $PtCl_3^-$ are known to exist, bu
$PtCl_4^{2-}$ is the predominant anion at 25°C (Azaroual et al. and Mountain and Woo
2001).



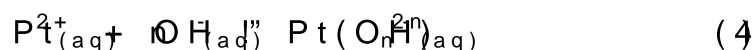
$$Pt^{2+}_{(aq)} + 4Cl^-_{(aq)} \rightarrow Pt(Cl)_4^{2-}{}_{(aq)} \quad (2)$$



$$Pt^{4+}_{(aq)} + 6Cl^-_{(aq)} \rightarrow Pt(Cl)_6^{2-}{}_{(aq)} \quad (3)$$

In oxidising environments with more basic (less acidic)
complexation with hydroxide is possible (Azaroual et al. 2001; Colombo et al. 2008).
Equation 4 shows a simplified reaction (Wood 1991). Hydroxyl ions are always pr
in water, especially in neutral and basic waters, and plati

predominate over platinum chloride complexes when chloride is limiting (Mountain and Wood 1988; Wood et al. 1992; Azaroual et al. 2001). Aqueous Pt^{2+} forms hydroxide compounds, including $PtOH^+$, $Pt(OH)_2$ and $Pt(OH)_4^{2-}$. As pH increases, the predominance of these complexes moves to more hydroxylated species (Moun
Wood 1988; Wood 1991; Azaroual et al. 2001). Mixed chloro-hydroxy complexes are considered to be unimportant for platinum mobility (Mounta

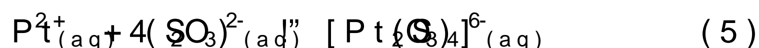


$$Pt^{2+}_{(aq)} + nOH^-_{(aq)} \rightarrow Pt(OH)_n^{2-n}{}_{(aq)} \qquad (4)$$

Depending on the concentration of other anions that
Pt^{2+} cations, aqueous organic carboxylic acids can mobilise p
(Wood 1990; Hanley 2005). Humic substances are components of
normally found in soil and water. Carboxyl functional groups in humic acid
abundant oxygen and are dissolved in neutral waters, the carboxylic acids
become negatively charged and react with platinum to form soluble platinum
organo complexes or stable platinum-organic colloids (Vlassopoulos et al. 1990; Wood
1990; Wood et al. 1992; Kubrakova et al. 2011). Humic substances have also been
shown to increase the stability of amorphous $Pt(OH)_2$ (Wood 1990; Hanley 200
Despite the fact that ammonia and cyanides form stable complexes with plat
not abundant in natural waters. Furthermore, their solubility with platin
in surface waters by hydroxides (Fuchs and Rose 1974; Mountain and Wood 198

Bisulphide would be expected to form a significant portion of
complexes in sulphur-rich supergene environments, however platin
when conditions are favourable for bisulphide formation (Mountain and Wood
There are, however, metastable platinum(II) thiosulphate complexes tha

form in oxidised and acid sulphate environments (Anthony and Williams 199
Thiosulphate complexation appears to be important during the weat
bearing sulphide ores, therefore, any platinum sulphides in alluvial
sediments draining the Bushveld complex could be a source of platinu
mobilisation within these deposits (Goldhaber 1983; Wood et al. 1992; Anthony and
Williams 1994; Mallen et al. 2005). Equation 5 depicts this process (A
Williams 1994).



$$Pt^{2+}_{(aq)} + 4(S_2O_3)^{2-}_{(aq)} \rightarrow [Pt(S_2O_3)_4]^{6-}_{(aq)} \quad (5)$$

These aqueous platinum complexes are transported by surface water until the
solution encounters an environment that favours chemica
precipitation. Subsequent deposition or precipitation then occurs (Bowles 1986;
Wood 1990). Chemical transformation involves the formation
metastable dissolved platinum complex, e.g. a platinum chloride compound
transformed to a platinum hydroxide species when in acidic, m
basic lakes, rivers or ground water (Wood 1990). If humic
aqueous system, an organic matter protected platinum col
1990; Wood et al. 1992). If dissolved platinum is unstable in the new regime, it ma
chemically reduced to metallic platinum and precipitated out of solution (Bowles
1986). It is worth noting that while a number of aqueous
possible, the kinetics of the reactions are generally slow, i.e. require geologic time, to occ
unless catalysed (Westland 1981; Wood 1990).

15. Formation of secondary platinum and platinum grains

Placer platinum commonly occurs as platinum-metal alloy, e.g., platinum grains from Alaskan-Ural type complexes and laterite weathering profiles comm occur as primary platinum (Bowles 1986; Cabri et al. 1996; Tolstykh et al. 2005). These grains are often found in deposits where no nuggets of that s in the ultramafic source rock. PGE grains in host rocks are commonly < 5 but placer grains are up to several centimetres (Bowles 1986; Melcher et al. 2005). This increase in platinum nugget size from the source to evidence that platinum nuggets grew as "secondary" mineraliza (Cousins and Kinloch 1976; Bowles 1986; Bowles et al. 1994; Cabri et al. 1996). It is thought that elemental and alloyed platinum nanoparticles grow through the continual accretion of other metals to form platin metal alloy grains (Bowles 1986; Melcher et al. 2005; Cabral et al. 2011). As iron is freely available in the lateritic zone, platinum could form Pt-Fe alloys (Bowles 1986). It may be fair to speculate that dissolved copper could in laterit combine with Pt-Fe alloys to form the Pt-Fe-Cu grains found in placers (Cab et al. 1996). Primary high temperature magmatic and hydrotherm alteration, however, cannot be ruled out as the source of the detrital platinum grains (Slansky et al. 1991; Cabri et al. 1996, Tolstykh et al. 2002; Melcher et al. 2005).

Mineralogical and petrologic studies of platinum-metal alloys have shown accretionary zones of growth on the periphery of some nuggets. Stumpfl and Tarkian (1976) noted the presence of platinum growth halos aroun proof that this process occurs. Likewise, Bowles (1986) also cites

overgrowth of PGE crystal faces on older, weathered grai[ns] et al. (2011) finds that iron-oxyhydroxides formed in supergene environments from the iron sulphides could preferentially scavenge inorganic PG[E] or adsorb them to their positively charged oxyhydroxide surfaces. Mi[xed] platinum species could be transported as colloids in the en[...] sediments.

On the other hand, palladium-platinum alloy grains show altera[tion] suggesting that the nuggets are "shrinking" and are undergo[ing] ... particular grains, the periphery has experienced selectiv[e] ... platinum. In essence, the palladium-platinum grains are becoming enriche[d] ... as they are altered and weathered (Cabral et al. 2007; ... Suárez et al. 2008). Suárez et al. (2008) described highly irregular and fractured shapes of s[...] supergene platinum nuggets as evidence of in situ alteration in weatherin[g] profiles.

The alteration of placer platinum, i.e., dissolution, precipitation and growth of grains, is the subject of ongoing controversy in the lite[rature] (Traoré et al. 2008). Further characterisation of the mineral dissolution-precipitation processes occu[rring at] the nugget-soil solution interface needs to be undertaken to fully understand the geochemical cycling of platiniferous material in surficial w[...]

1.6 Geomicrobiology of platinum

Changing chemical conditions within surficial weathering con[...] placers or supergene/lateritic deposits) in conjunction with significant environmental facto[rs]

promote the abiotic dissolution and precipitation of compl
role the biosphere plays in platinum mobility. Platinum reactivity
with bacteria is largely unknown as few experiments have been con
study this interaction. As such, any future work to elucidate platin
biogeochemistry should be done in relation to other metal-bacteria
reaction systems.

Microbes surviving in environments with high metal concentrations
have the ability to either use these metal compounds as part of their metabolis
sources of nutrition or energy) or they are able to mitigate toxic effects (Reith et
al 2009). Metal resistance is genetically encoded and involves
detoxification mechanisms (Silver 1996; Reith et al. 2009). To have any toxic effe
metal cations must first be internalised in the cell. They can pass passively if dr
a chemiosmotic gradient or actively if the energy molecule adenosin
(ATP) is expended during transport. Once internalised, th
necessary for normal cell activity or compete with ot
physiological activity (Nies 1999). Mitigation requires that the metals are neutralised ar
and/or expelled from the bacteria. Neutralisation requires that the toxic
chemically reduced and/or complexed to other ligands at t
Commonly, these products are toxic in themselves so subsequ
may still be effluxed. Efflux systems pump out ions at the expense
cation/proton antiporter proteins within the cell envelope
energy gradient that exists across the membrane to pump out metal cations and pump
in protons (Silver 1996; Nies 1999; Reith et al. 2009)

The ability to metabolise or detoxify metallic compounds allows […] participate in a number of reactions. For example, […] known to catalyze reactions in supergene systems (Singer and Stumm 1970; Enders et al. 2006). Acidithiobacillus thiooxidans, a sulphur oxidising bacterium, and Acidithiobacillus ferrooxidans, an iron and sulphur oxidiser, have been shown to contribute to the formation of acid mine drainage by gener[ating acid and] oxidising pyrite faster than the abiotic rate (Singer and [Stumm 1970;] Suzuki 1989; Enders et al. 2006). Similar aerobic, chemolithoautotro[phic] bacteria have also contributed to the supergene enrichment of copper in the Moren[ci] Porphyry Copper Deposit in Arizona, USA. Calculations suggest a few kilograms of bacteria active in 100 m^3 of weathering rock within the deposit could mobilise between 0.13 and 0.47 moles of copper each year as part of their metabolism (Enders et al. 2006). Anaerobic sulphate reducing bacteria found in acid mine drainage sit[es reduce] sulphate compounds as part of their metabolism, forming metal sulph[ides] (Donald and Southam 1999; Enders et al. 2006). Enders et al. (2006) calculated tha[t] approximately 1.2% of all copper leached (both abiotically and biotically) from the Morenci deposit could be fixed by sulphate reducing bacteria. Thus microbes coupled with inorganic processes contribute to supergene copper enrichment.

Microorganisms are involved in the biogeochemical c[ycling of] metals, such as gold, at surface conditions (Reith et al. 2009; Southam et al. 2009). The formation of gold in Australia, Alaska and the famous Witwatersrand in South Africa have been attributed to bact[eria (Enders] et al. 2006 and references). Because gold and platinum share a similar reactivity in aqueou[s]

systems, understanding the interactions between bacteria and gold could guide
work in platinum-microbe interactions (Bowles et al. 1986, 1990; Vlassopo
Cabral et al. 2007).

Numerous laboratory-based experiments have shown that bacteria are
precipitate gold from gold solutions (Southam and Beveridge 1994, 1996; Reith et
al. 2006; Lengke et al. 2006; Lengke and Southam 2007). Southam and Beveridg
(1994) related the formation of octahedral gold by bacteria to octahed
gold found in placer deposits of Western Australia and the Witwatersrand
1984; Mineev et al. 1993). Filamentous cyanobacteria have been used to precip
gold and the morphology of the end product mimics the shape of gold grain
found in the Witwatersrand basin (Mossman and Dyer 1985; Lengke et al. 2006). This
morphology is bacterioform and scanning electron microscopy (SEM) of small
nuggets from gold deposits have revealed grains coated
particles that resemble gold-encrusted bacteria (Reith et al. 2006; Southam et al.
2009). Sulphate reducing bacteria have been shown chemically to reduce aqueous
to elemental gold nanoparticles. Further diagenesis of the
scale octahedral gold (Lengke and Southam 2007). These experimental
studies provide evidence that bacteria can participate in the biogeo
gold and could contribute to the formation of secondary g
(Reith et al. 2006; Southam et al. 2009).

Research on bacteria-mediated platinum immobilization and
formation is in its infancy. Pioneering laboratory studies by Lengke et al. (2006)
showed that upon exposure to aqueous platinum chloride species,

platinum, producing spherical platinum (II-) organics which were later reduced to crystalline elemental platinum. A recent finding by Cabral et al. (2011) supports experimental evidence that bacteria-mediated precipitation is a comp[onent of] platinum nugget formation. Backscattered electron mode scanning electron microscopy (BSE-SEM) of small alluvial platinum grains revealed one which has morphologies that resemble mineralised bacteria. This is some of the first evidence of bacterioform platinum in the literature. The authors propose that n[ugget] growth started with bioreduction of platinum onto a microbe covered decayi[ng] root. As the nugget grew and microbial cells were entombed, v[...] microbial activity concentrated biotic precipitation on the nugget. Dissolved platinum and subsequent electrochemical accretion of elemental [platinum to the] growing grain likely worked in conjunction with bioreduct[ion] and microbe-mediated precipitation were not mutually exclusive. These mechanisms were complementary and both contributed to platinum grain formation in placer deposits.

1.7 Hypothesis and study objectives

In order to improve our understanding of the biogeoch[emistry ...] studies were conducted as part of this thesis. As platinum is mobile in the supergen[e] environment, signs of chemical and physical weathering should be identifiable on the surface of placer grains. As microorganisms have proven successful [in] immobilising aqueous gold, they should be able to play a role in the bioaccumulation [of] weathered placer platinum material. For example, Cupriavidus metallidurans

negative, gold-precipitating β-proteobacterium found to be living on the
grains obtained from auriferous soils in northern Queensl
Gold Mine and the Hit or Miss Gold Mine), was cultured
platinum-chloride species (Reith et al. 2006; 2009)

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Chapter 2

Surficial weathering of platinum grains

2.1. Introduction

Platinum, one of the six platinum group elements (PGEs), is
resistance to oxidation and corrosion has made it an ideal
and automobile catalytic converters; yet, platinum is not
1987, Nixon and Hammack 1991). Platinum is mobile under s
environments. It can be oxidised, dissolved, complexed
ligands, transported, precipitated and deposited (Fuchs a
Azaroual et al. 2001; Hanley 2005). Proper conditions for platinum re
commonly occur in alluvial systems, lateritic and supergene environments (Bo
1986; Azaroual et al. 2001; Freyssinet et al. 2005; Hanley 2005)

Alluvial platinum grains typically occur as platinum-iron alloys and are
associated with Alaskan-type primary magmatic intrusions (B
Macdonald 1987; Nixon and Hammack 1991; Tolstykh et al. 2005;
Koek et al. 2010). These circular mafic/ultramafic complexes are usually < 10 km
in diameter and are often found near volcanic arc sutures (Nixon
Hammack 1991; Teluk et al. 2001; Tolstykh 2005). When platinum bearing h
weathered, platiniferous material is physically and chemi
and transported in fluvial gravel deposition centres. Alternatively, in some
cases, platinum-bearing material is weathered in situ and lighter host rock fractio
eroded away, leaving a residual deposit of PGEs (Koek et al. 2010).

Favourable chemical and biological conditions for meta[l] weathering commonly prevail in semi-arid to tropical climates (Anderson 1... Sillitoe 2005). Mobilisation can occur when the chemical proc[esses of] enrichment and alteration extract metal from source rock expo[sed at the] surface. Pyrite (FeS_2) is a common mineral in supergene environme[nts]... exposed to oxygen, water, iron and sulphur oxidising bacteria, it ca[n]... acid (H_2SO_4) and ferric sulphate ($Fe_2(SO_4)_3$), the latter of which mobilises o[ther] sulphides including copper, silver and gold (Mann 1984; B... Enders 2000; Sillitoe 2005). Surficial waters dissolve su[lphide] metal and carry it below the water table where it is subsequently re-deposited in economic concentrations (Guilbert 1986).

The acidic and oxidising supergene waters that mobili[se]... could presumably solubilise platinum because oxidised palladium is more soluble than gold (Bowles 1986; Cabral et al. 2007). To date, however, mineralogical and petrologic analyses of platinum gr[ains]... explained the alteration processes affecting placer platin[um]... commonly larger than the source rock they are derived from, argued that fr... platinum grains grow and undergo secondary mineralisation (Cou... 1976; Stumpfl and Tarkian 1976; Bowles 1986; Cabral et al. 1994; Bowles 1996). Conversely, other studies indicate that platinum is susceptible to dissolution and weathering in environments (Cabral et al. 2007; Tertré et al. 2008).

In order to fully understand the crystal chemistry of sulfur-bearing minerals in weathering environments, further characterization of mineral precipitation processes occurring at the weathering interface needs to chemical evidence of these processes should be identifiable and detectable using electron microscopy and x-ray spectroscopy.

2.2. Material and Methods

Platinum nuggets, collected by gravity separation from platiniferous an
auriferous soils near Fifield, New South Wales, Australia were examined to
characterise surface weathering processes. Upon collection, grains were imm
submerged in 2% glutaraldehyde$_{(aq)}$ to chemically fix any biological

Description of Fifield, New South Wales, Australia

The Fifield Platinum Province (Figure A1) is an area of past and curren
exploration and excavation located 380 km WNW of Syd
Australia (Johan et al. 1989; Andrew et al. 1995, Teluk 2001). Alluvial and
platinum and gold are presumed to be genetically linked to a 500
Devonian ultramafic Alaska/Ural-type intrusions that intrude Ordovician-Camb
metasedimentary rocks. An Ordovician paleosuture reopen
result of crustal extension in the Lachlan Orogeny, allowing platinum-bearing
Alaska/Ural dunites and peridotites were emplaced (Johan et al. 1989; Andrew et al.
1995; Teluk 2001; Gray and Foster 2004; Kosek et al. 2010). Subsequent perio
eluvial weathering, lateritization (Figure A2) and fluvial erosion have rew
platiniferous material throughout the Cenozoic (Teluk 200 are
thought to be primary, having been eroded directly fro
However, a primary source of mineralisation for these grains has not been f
(Slansky et al. 1991; Teluk 2001).

Scanning electron microscopy

Platinum grains were dehydrated using 25%$_{(aq)}$, 50%$_{(aq)}$, 75%$_{(aq)}$ and 100%$_{(aq)}$ ethanol series for 15 minutes at each step. Grains were dried using a Tousimis® Samdri®-PVT-3B drier and placed onto 12 mm carbon (Electron Microscopy Sciences [EMS]). Samples were osmi 5 nm with a Filgen® OPC80T osmium plasma coater. Osmium coati reduce sample charging. A LEO 1530 field emission scanning electron microscope (FEG-SEM) (University of Western Ontario, Western Facility), a Zeiss 1540-XB FEG-SEM (University of Western Ontario, Nanofabrication Facility) and a Hitachi SU 6600 Analytical FEG-SEM (University of Western Ontario, Department of Earth Sciences ZAPLab) were used to examine platinum grains and produce high-resolution micrographs. Several platinum grains, representing the diversity of morphological features observed in SEM, were selected for further characterization. An Oxford Instruments INCA sight energy dispersive spectrophotometer (EDS) on the Z Xmax Silicon Drift Detector EDS on the Hitachi SU 6600 SEM were used for element identification.

X-ray emission spectroscopy and X-ray Absorption Near Edge Structure (spectroscopy data collection and analysis

Two platinum grains screened by SEM-EDS were selected for further analysis at the Pacific Northwest Consortium/X-ray Science Division (PNC/XSD) Sector-20 Insertion Device beamline at the Advanced Photon Source, Ar National Laboratory, Argonne, Illinois, USA. X-ray emission spectroscopy

conducted to map the elemental distribution of X-ray emission. X
energy reference values from Kortright and Thompson (20
elements present in the scan. X-ray Absorption Near Edge Structure (XAN
measurements were conducted at spots to determine the oxidatio
iron. Triplicate XANES energy measurements of the Fe K-edge were collected fro
each spot and were compared to the inflection of an iron
(Williams 2001). Beam energy was set to a maximum of 7100 eV so that os
would not be excited.

Element maps and X-ray emission spectra were analysed u
Instruments LabVIEW™ 2-D Scan Plot v. 4 and National Instruments LabVIEW™ 2-
D Scan Plot v. 3. XANES data was processed by ATHENA software (Ravel
and Newville 2005). The Fe K-edge of the samples was compared to the K-edge of th
the reference foil. Sample edge energies higher than that of the foil (7112
eV) indicated oxidised iron

Micro X-Ray Diffraction (µXRD)

Micro X-ray diffraction (µXRD) data was collected on the platinum grains using
the Bruker AXS D8 Discover microdiffractometer in the De
at the University of Western Ontario. This instrument uses a Co Kα radiation source.
An omega scan was performed from 7.5 to 45 degrees, with
and a beam size of 300 µm. Data was collected using a HI-STAR 2-D detector and a
associated General Area Detector Diffraction System (GADDS) software and
using Bruker's DIFFRACplus EVA software in tact for analys

2.3. Results and discussion

Examination of bulk nugget morphology and weathering textur around the periphery of the platinum grains (see Figure 2.1-2.3) was generally in good agreement with Slansky et al. (1991), who concluded that platinum grains from Fifield are primary (magmatic) in nature.

The overall morphology of the nuggets varied considerably. This heteroge suggests that the grains were subjected to differing degrees of chem weathering. A number of fluvial and lateritic events affected the Fifield Plati Province throughout the Cenozoic era and the characteristic morpho this and previous work is partly indicative of those conditions (Telu grains in Figure 2.1A and Figure 2.2A exhibit more rounding and higher while the grain in Figure 2.1C is rounded but has low sphericity. Ro projections dominate the shape of the grain in Figure 2.2A. **Figure 2.3A** shows a sub-angular/rounded grain with low sphericity, and the **arrow** denotes relict crystal face or a plane of weakness. The arrows in Figure 2.1C and Figure 2.2A indicate impressions of 10s of micrometre-scale crystals that eroded away from the gra

Pitting, cavities and striations are common on the s demonstrate that the soil-nugget interface is an important site of mecha and chemical weathering. Textures indicate dissolution pits and cavities observed infilled with organics and clays from the environment (Figures 2.1D and 2.3A). Long parallel scars on the grain in Figure 2.3 may relate to

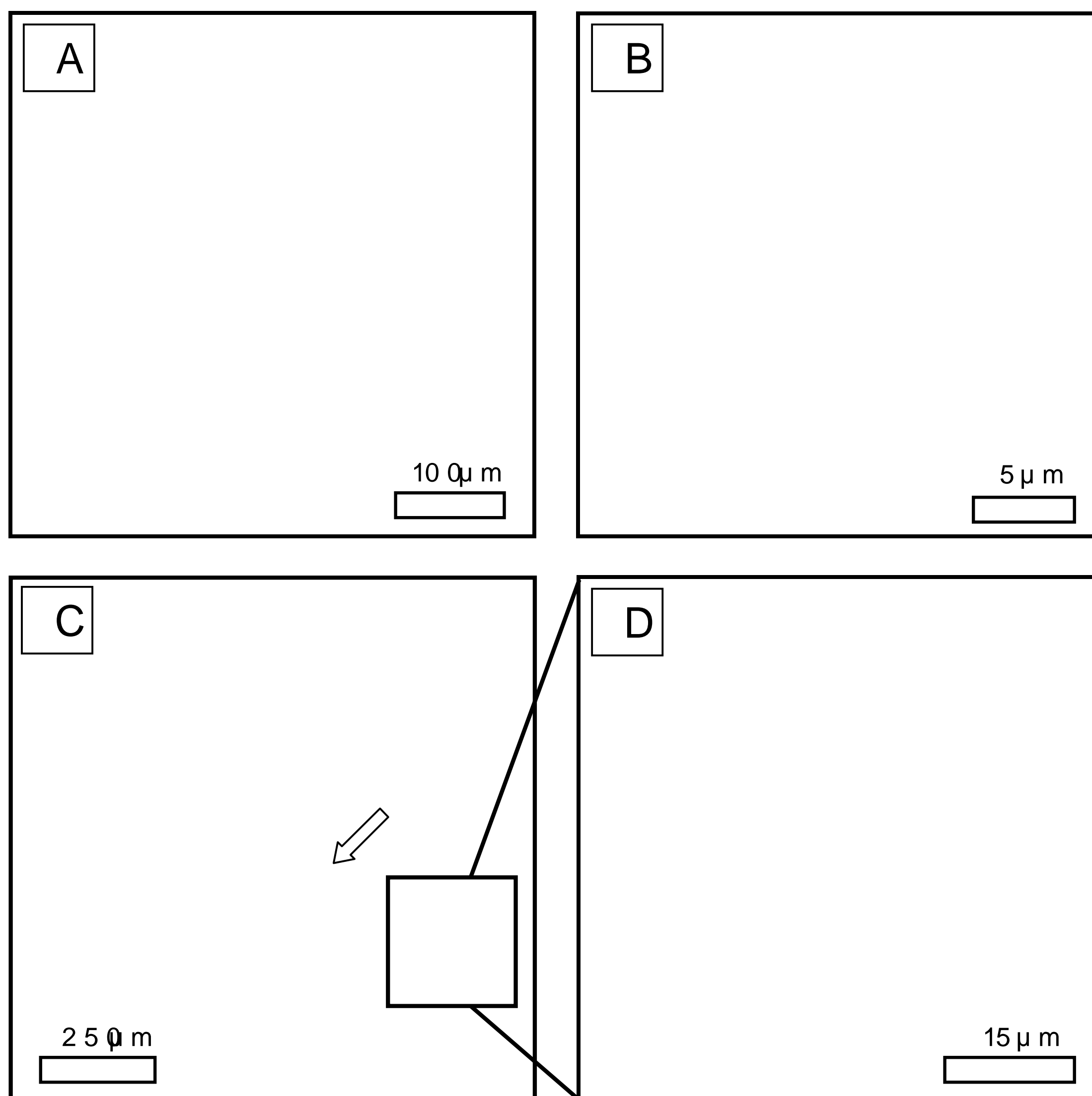

Figure 21. A: SEM micrograph of sub-rounded platinum nugget; B: Note dented and scratched surface of nugget in A, filled with clay and organics (darker
C: SEM micrograph of a deeply pitted sub-rounded platinum nugget; D: Higher magnification SEM micrograph of a region from within th
relatively deep circular pits filled with secondary mineral grains from

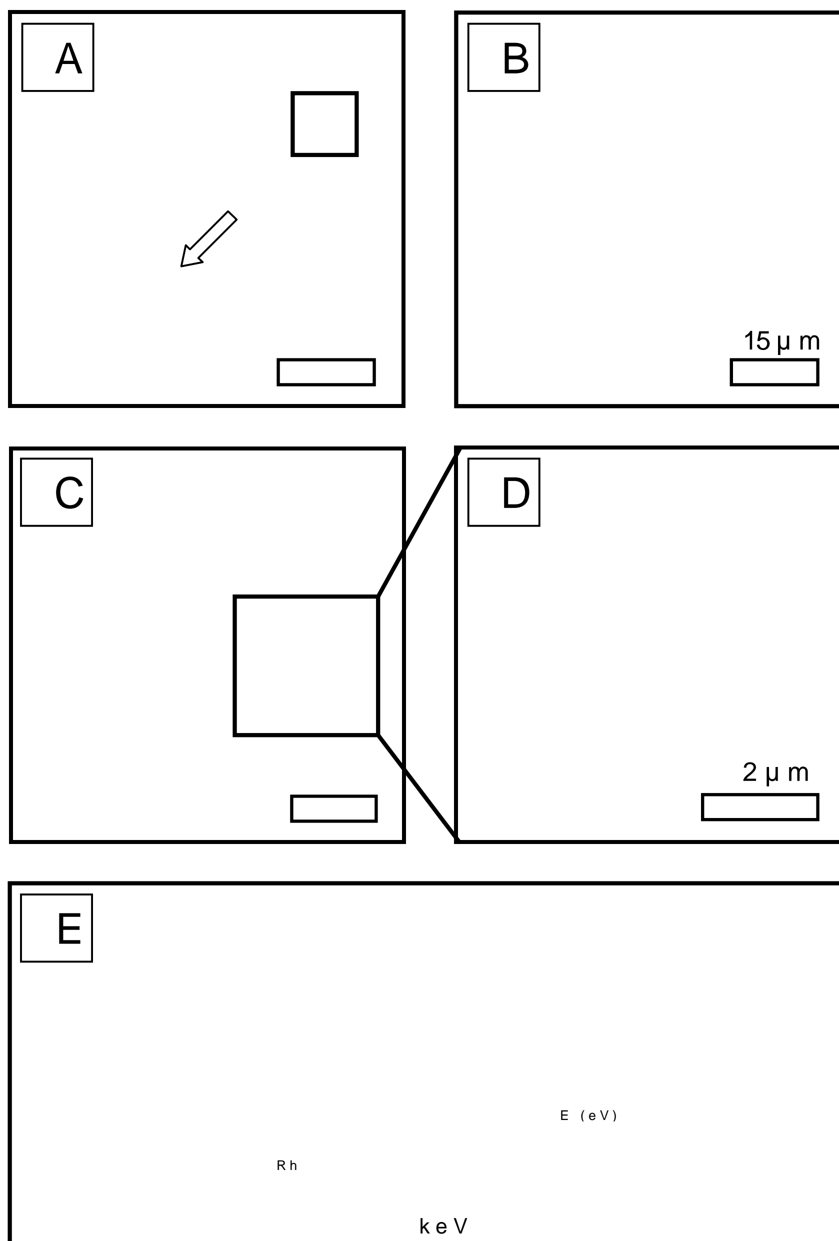

Figure 2. A: SEM micrograph of a heavily weathered platinu[m] B: SEM micrograph of alloyed grain. Note the absence of deep e[...] oxides and cubic minerals; C: SEM micrograph of squared area in A. A[...] acicular, iron oxides coats the surface of the grain; D: SEM micrograph of area not[...] C. The spectrum of the acicular, iron oxide is shown in E. Chromite is present in th[...] samples. The inset is the XANES spectra of Fe^{3+}. The presence of oxidised [...] indicates this nugget was in an aerobic weathering enviro[n...]

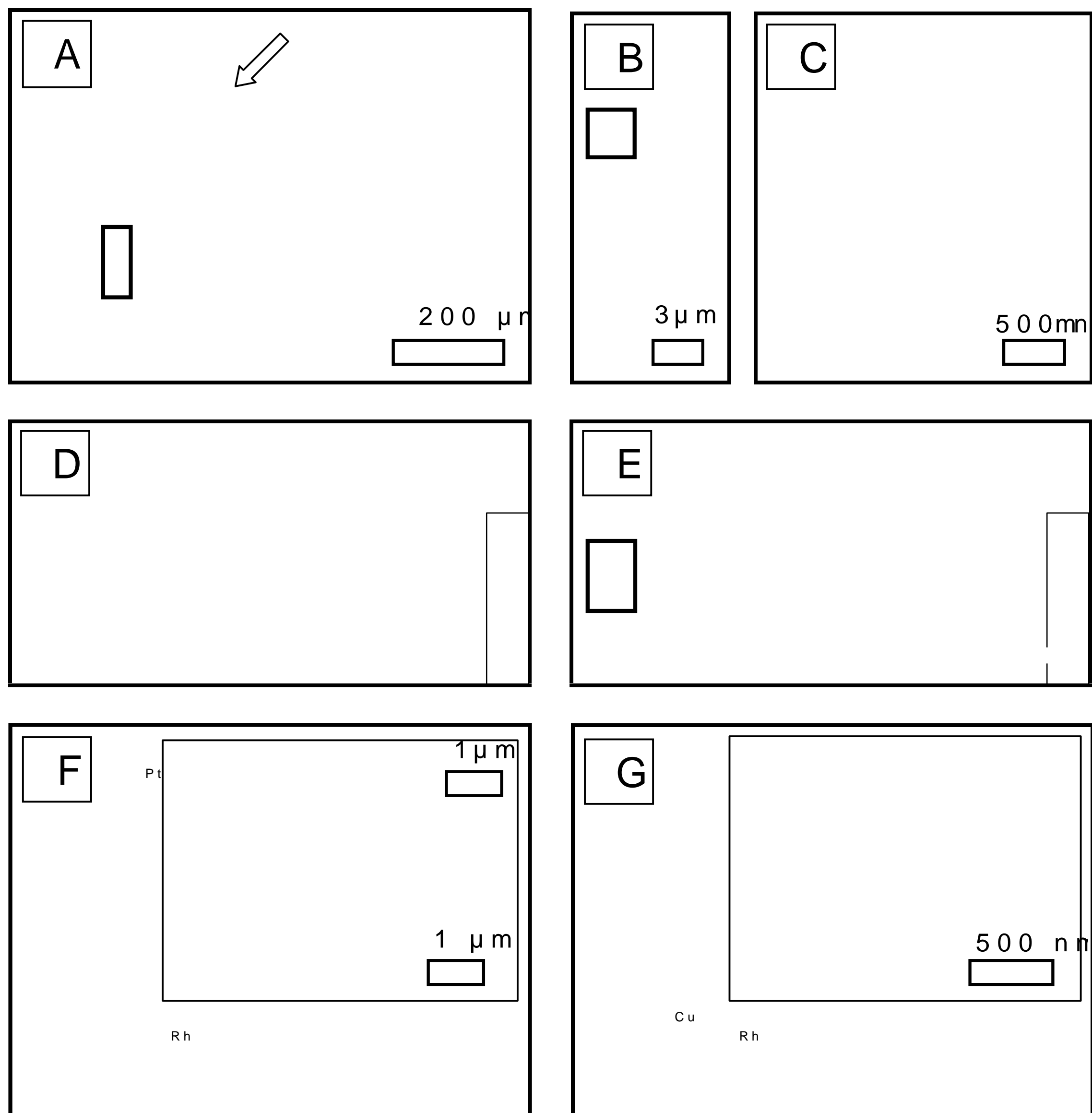

Figure 23. A: SEM micrograph of a weathered platinum grain; B: SEM micrograph of area noted in A; C: SEM micrograph of squared area in B. Note minerals; D and E: Iron and copper element map of grain in A, re rectangular area in E, which shows a streak of copper th rich cubic minerals in B; F: SEM micrograph of cubic pits and E comparing the elemental composition of the bulk grain ar The bulk nugget is a Pt-Fe alloy (with trace Cu, Ni, and Rh) while t show elevated copper; G: SEM and EDS of the acicular, iron oxides. of Ce. Scale bars in D and E show relative element co upward from low concentration (black) to high concentratio

the preferential weathering of osmiridium from the exsolution of osmiridium isoferroplatinum, as observed by Slansky et al. (1991). Figures 2.3B and C show 50 nm-sized cubic minerals embedded within the surface of the grain. The presence of these cubic minerals extending out from the surface of the platinum with similar sized holes in the surface (Figure 2.3F), suggests that they likely out from the grain, releasing Cu and Pt to the environment. Ultimately, removal of these individual nanophase materials would occur over greater distances than grains and may contribute to the development of a larger from these grains

Red-orange coatings found on the periphery of the grains were determined to be iron. Using SEM, acicular iron oxides were primarily found within the clay patches and directly affixed to the grain surface (Figures 2.2C, 2.2D and 2.3G). The morphology of the iron oxides was slightly different between grains, suggesting that an array of insoluble iron oxides possessing various hydration s these placer nuggets. Limonite, goethite and other iron oxides and found on platinum nuggets (Ottemann and Augustithis 19 al 2008). Energy dispersive spectroscopy (Figures 2.2E and 2.3G) and XANES spectra (inset Figure 2.2E) confirmed that iron existed in an oxidised Fe^{3+} state and was likely bound to oxygen. The K-edge occurred approximately 13 eV up en from the Fe^{0} reference standard, indicative of Fe^{3+} species (O'Day et al. 2004). The oxidation of iron promotes the formation of acid through t could contribute to platinum solubilisation, in particular (Gilbert 1986; the precipitation of acicular, iron oxides immediately on

produced a local acidic interface that could have cont
dissolution and the release of additional iron from the gr
below) (Guilbert 1986).

The placer grains were determined to be Fe-platinum alloys (Figure 2.3F) with lesser amounts of copper (Figure 2.3F). µXRD was performed on the gr
in Figure 2.3A in order to determine the structure of the cubic minerals, howev
bulk nugget was sampled and found to be isoferroplatinum (Pt_3Fe), which is
consistent with Slansky et al. (1991) and Cabri et al. (1996). Trace amounts of nickel (not
shown) and rhodium were also detected. Tetraferroplatinum was noted by Slansk
al (1991) as being the most common mineral in the Fifie
mapping of the grain shown in Figure 2.3A was conducted. The Fe map in Figure 2.3D
and the Cu map in Figure 2.3E clearly resemble the grain shown in the SE
micrograph. Element mapping of the grain shows that a
linked to morphological features on grains. The Cu map, i
copper highs (squared area) that correspond to cubic minerals seen in
Figures 2.3B and 2.3C. These cubic minerals have a higher coppe
bulk grain (Figure 2.3F) and their exposure at the nugget surface s
more resistant than the bulk grain and so not dissolved. Nickel, not detected by EDS
was found in small quantities using synchrotron radiatio
found by EDS but not detected using synchrotron energy.
these elements cannot be definitively quantified due to the heterogeneity and 3
dimensional nature of the sample surface, which could have affected electron s
and detection. Nickel and copper, however, are known to r

iron in reducing environments (Melchert 2005). Levels of osmium have been detected in Fifield placer grains (Slansky et al. 1991). Trace amounts of t earth element, cerium, were found to be associated with t reported by Andrew et al. (1995) as being present in some of the intrusive complexes.

Platinum-sulphide minerals were not observed in these gr consistent with prior studies (Slansky et al. 1991). The original magma m likely sulphide poor and any sulphur in the primarily PGM s replaced during post metasomatism could have been weathered aw surface conditions (Johan et al. 1989; Andrew et al. 1995; Freyssinet et al. 2005; Sillitoe 2005). Hydrothermal alteration of primary platinum mi not explain why the platinum hosted in the Fifield source rock (undiscovered to date) micron sized while alluvial placers are measured in millimetres or fractions thereo (Johan et al. 1989; Slansky et al. 1991; Teluk 2001). This 1000-fold increase has been attributed to the dissolution, precipitation and subsequent accretion of pl alluvial and lateritic regimes by several groups, e.g., Cousins and Kinloch (19 Bowles (1986) and Bowles et al. (1994). However, the structural and chemical make-up of these grains does not support the growth of platinu process (Slansky et al. 1991). Only when the hard rock source of F is found will the diagenesis be better understood. For now, no this study and similar studies clearly show signs of chemi at the nugget-solution interface. The grains are dissolving from the weathering products are being mobilised into the en where they can participate in a variety of abiotic, and potentially biotic Understanding

these reactions is key to improving our ability to recover and track its movement in nature, leadrinegstofnthwis unrdecisoou metal.

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Chapter 3

Immobilisation of platinum by *Cupriavidus metallidurans*

3.1 Introduction

Microorganisms are able to thrive in a variety of extreme co
including strongly acidified soils and metal-rich mineralised zones (Ehrlich 2006; Anders
Reith et al. 2009). Some metal-tolerant bacteria in these environments
out a niche existence by being able to use metal-containing compounds a
sources of nutrition and energy (Reith et al. 2009). Alternatively, some bact
invoke genetically encoded detoxification mechanisms to
metal concentrations. These involve metal reduction, comple
precipitation, efflux or a combination thereof (Silver 19
2009).

The biogeochemical cycling of metals and the format
deposits can be attributed to microbial activity (Cloud 1968; Cloud
Mossman and Dyer 1985; Southam and Beveridge 1996; Reith et al. 2007). Convincing
evidence from in vitro studies has shown that bacteria are able to precip
gold-rich solutions (Southam and Beveridge 1994, 1996; Reith et al. 2009;
Lengke et al. 2006c, d; Lengke and Southam 2007). The morpho
precipitates so closely resemble the gold found in place
South Africa's Witwatersrand basin that a biogenic origin of these auriferous deposits
is now suspected (Wilson 1984; Mossman and Dyer 1985; Mossman et al. 1998;

and Beveridge 1994; Reith et al. 2006, 2007). The similar geochemical be platinum and gold suggests that a comparable cycle for pla could exist.

Platinum, one of the six platinum group elements, is a 1987; Nixon and Hammack 1991). Its concentration in the can reach economic concentrations in primary and seconda Naldrett 2008; et al. 2010). Under surficial weathering conditi mobile as Pt^{2+} or Pt^{4+}. It commonly forms complexes with chlorides thiosulphates, but amorphous platinum-organic and -hydroxide colloids occur (Mountain and Wood 1988; et al. 1990; Wood 1990; Anthony Williams 1994; Azaroual et al. 2001; Hanley 2005; Colombo et al. 2008). Aqueous platinum is transported in the surficial environment until transformation or chemical precipitation. Subsequent migration and deposition platiniferous material may occur (Bowles 1986; Wood 1990) assumed primarily abiotic, bacteria-mediated platinum immobilisation contribute to the formation of platinum placer grains (Reith et al. 2009).

Lengke et al. (2006b) demonstrated that cyanobacteria are able to aqueous Pt^{4+} species in a stepwise reaction that first produ extracellular spherical platinum(II)-organic colloids. The amorphous p (II) colloids experienced further diagenesis to produce cr These experimental findings were recently supported by the discovery and characterisation of small alluvial platinum grains with morphologies characteri platinum-encrusted bacteria. Microbes attached to decaying organic plant root) precipitated platinum from solution onto their

thought that continued bioreduction and subsequent electrochemical acc
platiniferous nanoparticles likely contributed to the forma
grains (Cabral et al. 2011).

Further investigation is still needed to understand t
platinum biomineralisation. For this work, biogeochemically dive
proven ability to immobilise gold should be reacted with
characterise their response to platinum to determine wheth
biogeochemical cycle of Pt exists. This study will employ the use of *Cupriavidus*
metallidurans CH34, formerly *Wautersia metallidurans*, *Ralstonia metallidurans*,
Ralstonia eutropha and *Alcaligenes eutrophus*. *C. metallidurans* is an aerobic, gram
negative, facultative chemolithoautotrophic bacterium known for
being resistant to toxic concentrations of a number of metal ions, e.g., Cu, Pb, Zn, Cd,
Ag and Au (Mergeay et al. 1985, 2003; Reith et al. 2006, 2007 and Monchy et al. 2007).
Resistance is primarily facilitated by metal transport ATPase efflux proteins in the
cell envelope and cation reduction mechanisms, which have been shown to confer metal immobilisation (Nies 1999; Reith et al. 2005; Ledrich et al.
2007). This bacterium was found living on the surface of gold grains
from auriferous soils in northern Australia (Reith et al. 2006, 2009). Reith cultured
bacterium and reacted it with aqueous gold chloride. *C. metallidurans* rapidly
precipitated gold within hours. This bacterium's capability to accumulate gold
suggests that a multidisciplinary study of its reactivity with chloride
species found in natural systems should produce comparable reactivi
platinum.

3.2. Materials and methods

Cupriavidus metallidurans

Cupriavidus metallidurans strain CH34 (ATCC® 43123™) acquired from the American Type Culture Collection in Manassas, Virginia
ATCC® prescribed liquid medium containing 5 g/L peptone and 3 g/L beef extract
(ingredients of Bacto® Nutrient Broth [Difco Laboratories; Detroit,
Before experimentation, the culture was transferred (~10
stationary/early death phase) to 13 x 100 mm borosilicate
(capped with plastic push caps to allow for gas exchange and to prevent
evaporation and contamination) and grown to early station
maximise the amount of metabolically active biomass. The
incubated at room temperature (21-23°C) under ambient CO_2 conditions.

After incubation, separate batches in 50 mL Falcon tubes
thoroughly vortexed (VWR Analog Vortex Mixer) to ensure a consis
For the platinum immobilisation experiments, 1 mL aliquots of the bacterial suspension
were transferred to microcentrifuge tubes and harvested by centrifugation at 12,000 × g
for 5 min using a VWR Galaxy 16 microcentrifuge. After
supernatant was decanted and the cells were washed by resuspension in filter-
sterilised distilled, deionised (DDI) water. The cells were thoroughly vor
remove any remaining culture medium. After vortexing, the bacterial suspension
centrifuged again at 12,000 × g for 5 min. The supernatant was discarded and the
bacterial pellet was resuspended in the experimental platinum solutions. The
number of bacteria in a washed sample was determined by the direct count

using a Petroff Hausser Counting Chamber and a phase contrast light microscope (Zeiss
Fluorescent Z1 microscope).

C. metallidurans aqueous platinum experiments

The bacterial experiments were conducted to examine C. metallidurans the bio-immobilisation of platinum from aqueous solutions of platin
(II) chloride (K_2PtCl_4, Premion®, 99.99+% [metal basis], Pt 46.4% min; Alfa Aesar®,
Ward Hill, Massachusetts USA) and platinum (IV) chloride ($PtCl_4$, Premion®, 99.99+%
[metal basis], Pt 57% min; Alfa Aesar®). Platinum salts were dissolved in DDI at
16.2 MΩcm obtained from a Millipore system.

Washed C. metallidurans samples were suspended in 1 mL aqueous p
solutions (from stock solutions of 0.5 µM [0.1 µg/mL], 5
µg/mL], 500 µM [100 µg/mL] and 5000 µM [1000 µg/mL] final Pt^{2+} or Pt^{4+}
concentrations) at room temperature (23 °C) for 1 min, 1 h, 1 day, 2 weeks and
weeks. Experiments were maintained in the dark because
when exposed to light (Lengke 2006). Microcentrifuge tubes contain
bacteria-platinum mixture were only removed from darkness
during longer exposure times. Reactions were performed i

Following exposure, reaction tubes were centrifuged (X g force, 5 min)
and the supernatant was removed for chemical analysis. The
remaining bacterial pellets were washed 1 time by resuspension in filter-sterilised DD
water to remove unreacted aqueous platinum and then centrifug
5 min. Excess water was decanted and the pellet resuspended in 1 mL wa

filter-sterilised DDI water in preparation for whole mount

microscopy examination of bacteria. Bacterial cells that were

thin-sectioned were suspended in 2% glutaraldehyde rather than w

C. metallidurans aqueous Pt^{2+}-chloride and Pt^{4+}-chloride kill curves

The effect of aqueous Pt^{2+}-chloride and Pt^{4+}-chloride on bacteria viability w determined using the spread plate method. Washed cells were resuspended in 1 mL of water and used in serial dilutions with filter-sterilised DDI water. Each dilution was plated in duplicate on beef extract-peptone agar plates that contain (in g/L) peptone, 5; beef extract, 3; agar, 15 (Difco Laborator for 4 days at room temperature (21-23 °C) under ambient atmospheric conditions. Colony-forming units (CFU) were counted with the aid of a New Brunswick C-110 Colony Counter with digital counter and probe.

Chemical analyses

Platinum concentrations were measured over the cou using a Perkin-Elmer Optima 3000 DV System Inductively Coupled Pl Emission Spectrometer (ICP-AES). Instrumental uncertainty for measured platinum was ≤5%, with a detection limit of 0.05 µM (0.01 µg/mL). Samples for ICP-AES were diluted as necessary with filter-sterilised DDI water (Table 3.1). Platinu calibration were prepared with the aqueous Pt^{2+} and Pt^{4+} stock solutions used in the laboratory-based *C. metallidurans* aqueous experiments. These calibr standards were diluted by the same factor as the corresp

(Table 3.1). The pH of the stock solutions was measured using an Instrument Basic pH meter. The electrodes were calibrated in buffer Analytical uncertainty of pH measurements is ±0.01 unit solutions after exposure to bacteria was monitored using the ColorpHast® Non-bleeding Indicator strips.

Transmission electron microscopy (TEM)

Unstained whole sample mounts and thin sections (prior to T = 0 h) of reaction with the Pt solutions and controls were examined using Phillips CM-10 transmission electron microscope (TEM) operate ultra-thin sections). The whole mounts were prepared by flo Sciences (EMS) Formvar-coated 100 mesh copper grids on a drop of c several minutes to allow the bacteria to adsorb to the grid. Grids were then washed by gentle dipping into a drop of sterile DDI water. The grids were completely air dry prior to microscopy.

Samples for ultrathin sectioning were fixed overnight in 2% glutaraldehyde$_{(aq)}$ (EMS; Hatfield, Pennsylvania USA). Fixed cultures were s 14,000 X g for 1 min. The supernatant was discarded and was enrobed in 2% (weight/volume) Noble Agar (Difco Laboratories) and dehydr using a 25%, 50% 75% and 3 X 100% acetone series (incu The acetone was slowly replaced with an EMS epoxy resin (cultures were incubated at 1 hr intervals in a 50%:50% [v/v], 25%:75% [v/v] a acetone:epoxy resin series). Epoxy resin contained a 2.5:2 Embedded

Table 3.1. Dilution factors of Pt^{2+} and Pt^{4+} supernatants and standards. Dil
required because submitted samples exceed a concentration of 100 ¼M (20
¼g/mL), i.e., the linear portion of the standard curve.

Initial concentr of platinum	Dilution facto for ICP-AES
5000 ¼M or 1000 ¼	100x
500 ¼M or 100 ¼g/	10x
50 ¼M or 10 ¼g/m	10x
5 ¼M or 1 ¼g/mL	10x

812, DDSA (Dodecenyl Succinic Anhydride) and NMA (Na
Samples were incubated overnight in 100% epoxy resin ar
with fresh epoxy resin containing the accelerator -30 (2,4,6-
tri(dimethylaminoethyl)phenol, EMS). Ratios by volume were 2.5:2:1:0
were cured in a 60°C oven (Blue M Electric Company Bac
Gravity Convection) for 48 h, until hard. Embedded samples were thin-sectioned ult
using a Reichert-Jung Ultracut E ultramicrotome equipped with a Diatome!" diamond
knife to a thickness of 70 nm and collected on 100-mesh Formvar copper ca
grids.

X-ray Absorption Spectroscopy (XAS) data collection

XAS energy measurements from the platinum-bacteria reactions, including XANES (X-ray Absorption Near Edge Structure) and EXAFS (X-ray Absorption Fine Structure), were conducted at the Pacific Northwest Consortium/X-ray Science division (PNC/XSD) sector 20-BM beamline at the Advanced Photo
Argonne National Laboratory, Argonne, Illinois, USA. The oxidation state of plat
can be determined by XANES while information regarding
local coordination of platinum is provided by EXAFS data. D
state and binding partners of platinum will shed light on how aqu
bacterial cells (Kelly et al. 2006). XAS energy measurements of the L_3-edge were
collected from each sample and were calibrated to the in
11564 eV (Williams 2001).

Prior to any XAS measurements, C. metallidurans were reacted following the previously described "Cupriavidus metallidurans and aqueous platinum experiments". For XAS, replicate reactions were harvested, washed by centrifugation and re-suspended in 200 µL water in order to concentrate the sample for detection. The reacted bacterial samples were then placed in a teflon fluid cell with teflon-coated kapton film window or pipetted directly onto Whatman® 42 Ashless Filter Papers. XAS measurements on the conducted in fluorescence mode.

Reference compounds were needed to determine the platinum in these reactions. Standards were selected to represent the most likely oxidation states and bonding environments of platinum expected in the reacted bacterial samples. The XAS spectra for model compounds inc (IV) chloride ($PtCl_4$), platinum (IV) cyanide ($K_2Pt(CN)_6$), platinum (II) chloride (K_2PtCl_4), platinum (II) cyanide ($K_2Pt(CN)_4$) and platinum (II) tetraammine nitrate ($(NH_3)_4Pt(NO_3)_2$) were measured in aqueous form. Liquid stocks (up to 65 mM) were prepared from solid compounds without pH XAS spectra for platinum (IV) oxide hydrate ($PtO_2 \cdot xH_2O$), platinum-cis-(II) diamminedichloride (cisplatin; $Pt(NH_3)_2Cl_2$), platinum (II) sulphide (PtS) and platin foil (Pt) were measured in solid form due to their insoluble. With the exception of the foil and platinum (II) sulphide, the standards were teflon fluid cells with kapton film windows. Platinum (II) sulphide was precipit and was concentrated onto Millipore™ 0.45 µm, 13 mm membrane filters. compounds were commercially available from Alfa Aesar® and Sigma-Aldrich® (with

exception of platinum(II)-sulphide, which was synthesized in the laborato
equal molar solutions of aqueous platinum(II)-chloride (K_2PtCl_4) with aqueous sodium
sulphide 9-hydrate [Na_2S"$9H_2O$] (J.T. Baker®, Phillipsburg, New Jersey US
produce a black (PtS) precipitate (confirmed by EDS). The reference spectrum o
platinum foil was simultaneously collected during measur
reacted, bacterial samples. Incident energy from the beam did not al
the standards or samples.

XAS data analysis

ATHENA Analysis software was used to process XAS data (Ravel and Newville
2005). Energy scans from samples and standards were aligned to the calib
standard platinum foil reference energy. Multiple scans w
and were averaged to produce a single spectrum represen
and complexation state. XANES spectra were analyzed by
comparing the energy position of the platinum edge in the
the edge energy location from the standards. Linear combination
mathematically identified which standard(s) aligned with
immobilized platinum. Energy position alignment correspon
the immobilized platinum (Ledingham 2006). EXAFS data analysis was
in ARTEMIS, a XAS analysis software program complimen
and Newville 2005). Mathematical derivatives of post edge en
bound platinum in the bacterial samples were fitted to pos

platinum in the standard alignment, normalized to platinum ch

indicated potential binding partners of the immobilized platinum (

3.3 Results

Laboratory-based Cd metallic and aqueous platinum experiments

The addition of K_2PtCl_4 and $PtCl_4$ to filter-sterilised, DDI water promot
hydrolysis of salts, as indicated by a drop in the pH of the overall
K_2PtCl_4 solutions was: 5000 µM (1000 µg/mL; pH 4.2), 500 µM (100 µg/mL; pH 5.2),
50 µM (10 µg/mL; pH 5.8), and 5 µM (1 µg/mL; pH 6.1). The pH of $PtCl_4$ at 5000 µM
(1000 µg/mL; pH 2.2), 500 µM (100 µg/mL; pH 3.0), 50 µM (10 µg/mL; pH 4.1), and 5
µM (1 µg/mL; pH 5.3). The pH of the solution after reaction with b
not change significantly from the pH of the solution. The addition of 5000 µM
K_2PtCl_4 turned the pellet a grey-brown colour at 1 min, 1 hr, 1 day, 2 we
exposure times. Similarly, the addition of 5000 µM $PtCl_4$ to the washed bacterial p
immediately turned the white pellet yellow at all exposure
were much fainter or not identifiable at lower concentrat
provided macroscopic evidence that platinum was binding to the cells.

Table 3.2 and Table 3.3 were prepared by comparing
count to the unreacted count for the appropriate exposure
to a standard CFU count (5 X 10^8 CFU/mL) to allow comparison between exposur
times and treatments. The experiments demonstrated that
upon exposure of bacteria to 5000 µM Pt^{2+} and Pt^{4+}, no colonies grew. Platin
toxicity was directly proportional to concentration and Pt^{2+}
solutions being slightly less toxic than Pt^{4+} solutions for a given parti
concentration

Table 3.2. Toxicity study where *C. metallidurans* was reacted with Pt^{2+} aqueous solutions. Increased exposure time and increased concent viable cells, as indicated by a decrease in CFU/mL.

Pt^{2+} concentrat	CFU/mL after 1 min expo	CFU/mL af 1 hr expos	CFU/mL af 1 day expo
0 µM or 0 µg/mL	5.0×10^8	5.0×10^8	5.0×10^8
0.5 µM or 0.1 µg/m	4.9×10^8	2.7×10^8	4.6×10^8
5 µM or 1 µg/mL	3.7×10^8	3.2×10^8	4.3×10^6
50 µM or 10 µg/mL	4.1×10^8	1.3×10^7	2.1×10^3
500 µM or 100 µg/m	9.9×10^6	0	0
5000 µM or 1000 µg/m	0	0	0

Table 3.3. Toxicity study where *C. metallidurans* was reacted with Pt^{4+} aqueous solutions. Increased exposure time and increased concentration resu viable cells, as indicated by a decrease in CFU/mL.

Pt^{4+} concentrat	CFU/mL after 1 min expo	CFU/mL af 1 hr expos	CFU/mL af 1 day expo
0 µM or 0 µg/mL	5.0×10^8	5.0×10^8	5.0×10^8
0.5 µM or 0.1 µg/m	4.5×10^8	3.2×10^8	3.3×10^8
5 µM or 1 µg/mL	4.9×10^8	2.5×10^8	7.6×10^7
50 µM or 10 µg/mL	1.1×10^8	6.4×10^6	4.8×10^3
500 µM or 100 µg/m	6.6×10^2	0	0
5000 µM or 1000 µg/m	0	0	0

The amount of soluble platinum remaining in-solution

AES. These results were then subtracted from starting concentrations, normalized to 1 mg dry weight of C. metallidurans and averaged to determine the amount of platinum immobilized. The mass of C. metallidurans was calculated using the mass of Escherichia coli (2.95 X 10^{-10} mg dry weight per cell), a gram-negative rod shaped bacterium approximately ~23% larger than C. metallidurans (Neidhardt et al. 1990). Volume estimates for C. metallidurans based on diameter and measurements of bacteria shown in the TEM micrographs (Figures 3.1, 3.2, 3.3, 3.5, and 3.6). As rod shaped bacteria can be modeled as a cylinder bounded by two half spheres, the volume can be calculate Bankston 1988).

$$V = 4/3(\pi r^3) + \pi r^2 r \qquad (1)$$

ICP-AES results are shown in Tables 3.4 and 3.5. I immediately upon exposure to platinum. As exposure time incr concentration, the amount immobilized also increased; how at these longer exposure times the rate of immobilization did not match. The at 1 min effective concentration of platinum associated with cells, based on cell vo exceed the T = 0 solution concentration in all reaction sys C. metallidurans. Cell saturation was not reached after 1 day exposure time bacteria in 5000 µM Pt^{2+} solutions.

Table 3.4. Immobilisation study with *C. metallidurans* exposed to Pt^{2+} solutions. Measurements are effective concentrations within the bacteria and µg Pt immobilised per mg dry cell weight. Increased exposure time resulted in increased immobilisation.

Pt^{2+} concentration	Immobilisation after 1 min exposure (mM; µg/mg)	Immobilisation after 1 h exposure (mM; µg/mg)	Immobilisation after 1 day exposure (mM; µg/mg)
5 µM or 1 mg/L	1.8; 0.44	1.6; 0.40	15; 3.5
50 µM or 10 mg/L	51; 13	44; 11	67; 16
500 µM or 100 mg/L	120; 28	120; 29	410; 100
5000 µM or 1000 mg/L	850; 210	800; 200	850; 210

Table 3.5. Immobilisation study with *C. metallidurans* exposed to Pt^{4+} solutions. Measurements are effective concentration within the bacteria and µg Pt immobilised per mg dry weight. Increased exposure time resulted in increased immobilisation.

Pt^{4+} concentration	Immobilisation after 1 min exposure (mM; µg/mg)	Immobilisation after 1 h exposure (mM; µg/mg)	Immobilisation after 1 day exposure (mM; µg/mg)
5 µM or 1 mg/L	1.6; 0.40	2.6; 0.66	9.6; 2.4
50 µM or 10 mg/L	11; 2.7	13; 3.2	40; 9.8
500 µM or 100 mg/L	45; 11	160; 40	210; 51
5000 µM or 1000 mg/L	310; 75	350; 84	870; 210

TEM

TEM micrographs of C. metallidurans exposed to platinum solution

presented in Figures 3.2 to 3.6. Micrographs of the cont

shown in Figure 3.1. Note that the cytoplasmic contents

3.1A. Furthermore, internal detail of the cell is lacking in thin-section as shown in

Figure 3.1B. The cell envelope is visible as a white halo

staining was applied to these bacteria; therefore, any dif

following micrographs are attributed to aqueous platinum.

Figure 3.2A demonstrates that bacterial staining oc

exposure to 500 μM Pt^{2+}, 1 h. However, not all bacteria were stained

platinum. Generally, longer exposure times revealed more staining, especially a

poles (Figure 3.2B). Some bacteria produced nano-scale platinum colloids a

day (Figures 3.2C and D). Nanoparticles were not observed at short

Figure 3.3A shows that at 1 min bacterial cells did not bind platinum from

5000 μM Pt^{2+} solution. The majority of cells were stained wi

exposure time point. All bacteria were observed to be cons

after 24 hours. Platinum colloid formation was observed at 1 h and 1 day exposure

times (Figures 3.3C, E and F). Figure 3.3C shows a partially lysed

platinum nanoparticles bound in cytoplasmic material. Cell lysis may be a response t

platinum toxicity and/or the pH of the solution. TEM micrographs o

bacteria exposed to 5000 μM Pt^{2+} for 1 h are shown in Figure 3.4. Platinum nanop

along the periphery of the cell wall, within the cytoplas

vesicles. C. metallidurans likely released membrane vesicles as a protective measure

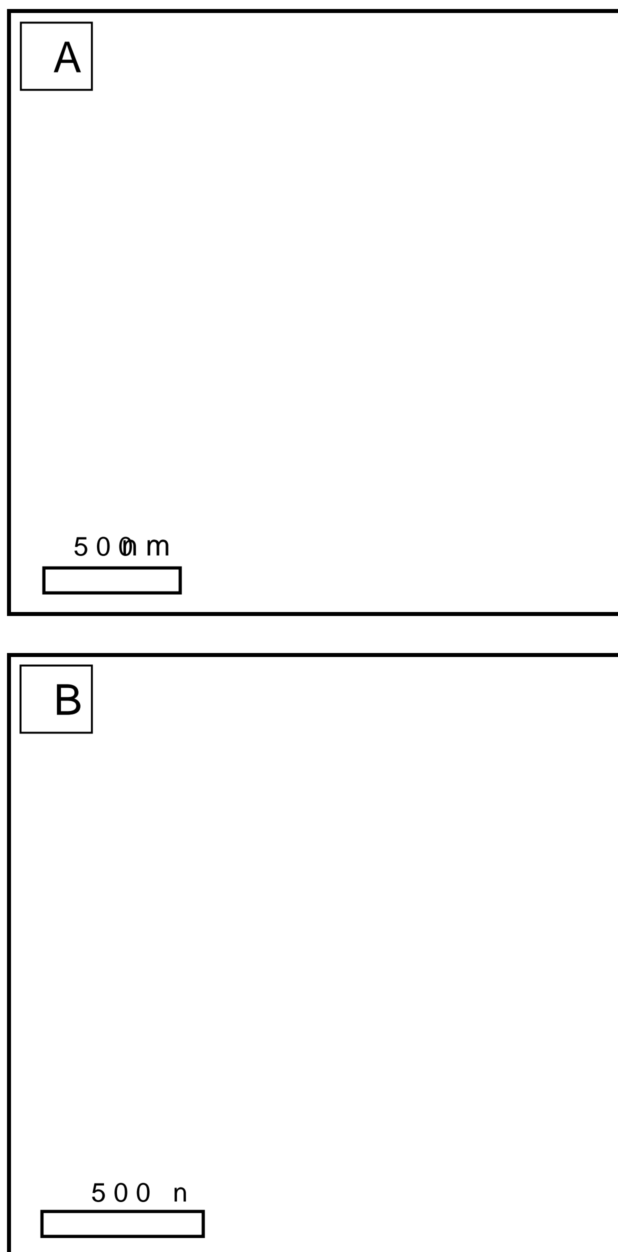

Figure 3.1 A: Whole mount TEM micrograph of an unreacted C. metallidurans bacterium. Phosphate bodies within the cell are clearly v generally electron transparent. B: Unstained thin section TEM microgra unreacted C. metallidurans. Note the internal detail of cell is lackin was applied.

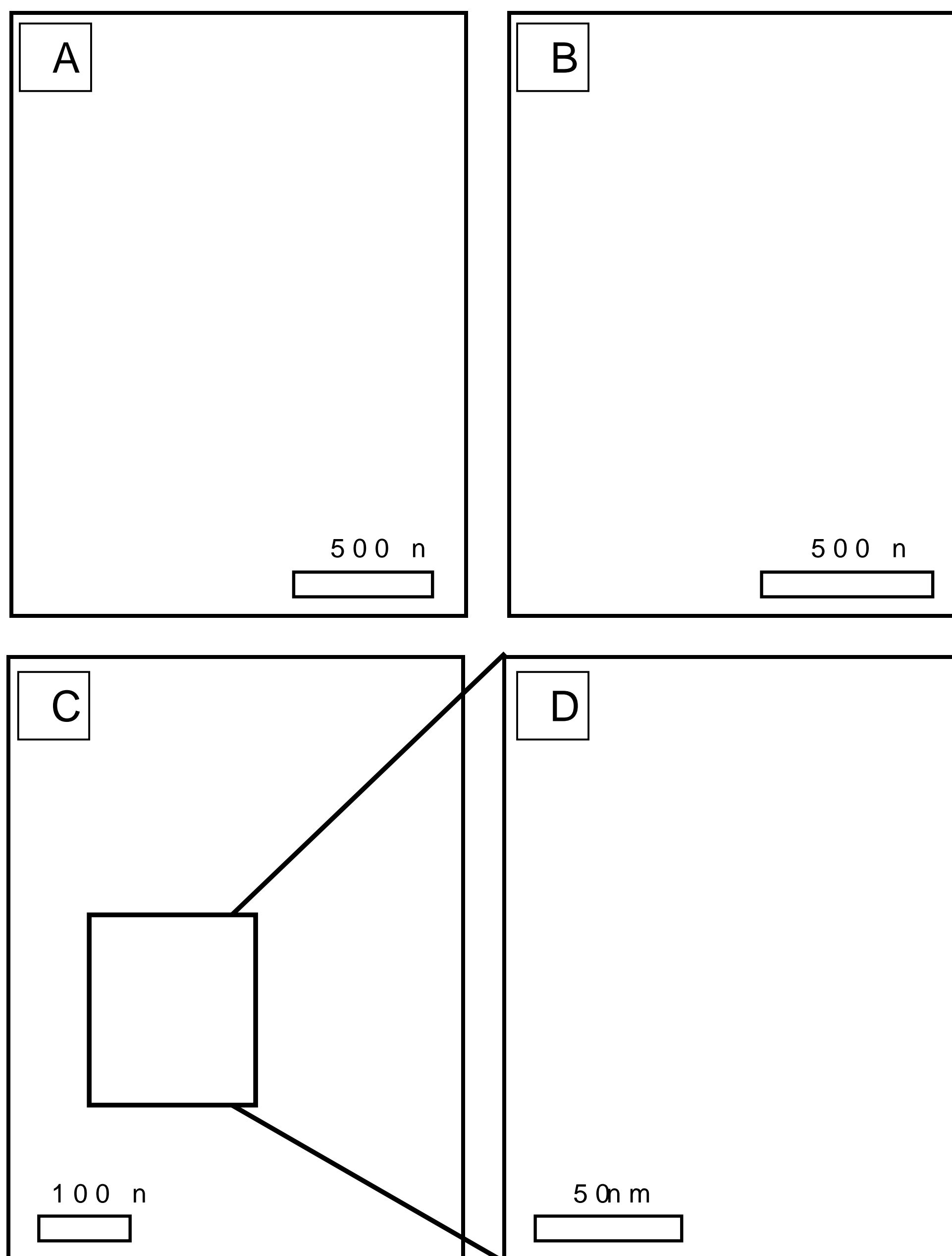

Figure 3.2. Whole mount TEM micrographs of *C. metallidurans* incubated with 500 µM Pt^{2+} (100 µg/mL) for A: 1 min, B & C: 1 day. D: Micrograph of squared area in C. At 1 day, all cells were stained (B) but some cells produced nanoparticles (C and D).

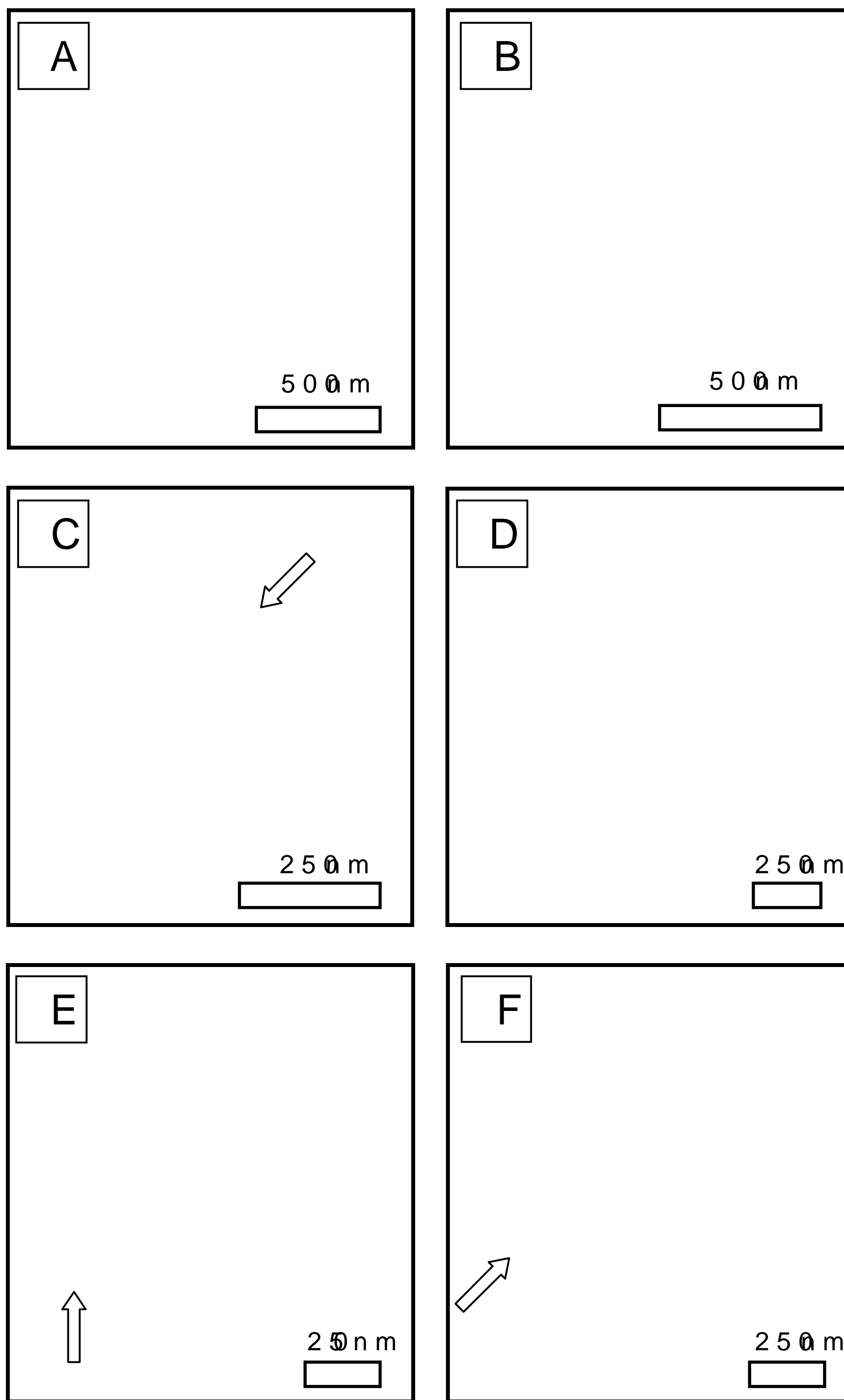

Figure 3.3 Whole mount TEM micrographs of C. metallidurans exposed to 5000 µM Pt^{2+} (1000 µg/mL) for: A: 1 min. Binding of Pt is minimal as cell re
electron transparent; B & C: 1 hr; D, E and F: 1 day. At longer incubation times (B-F),
cell staining is apparent in all cells. Some cells show nan
indicated by arrows.

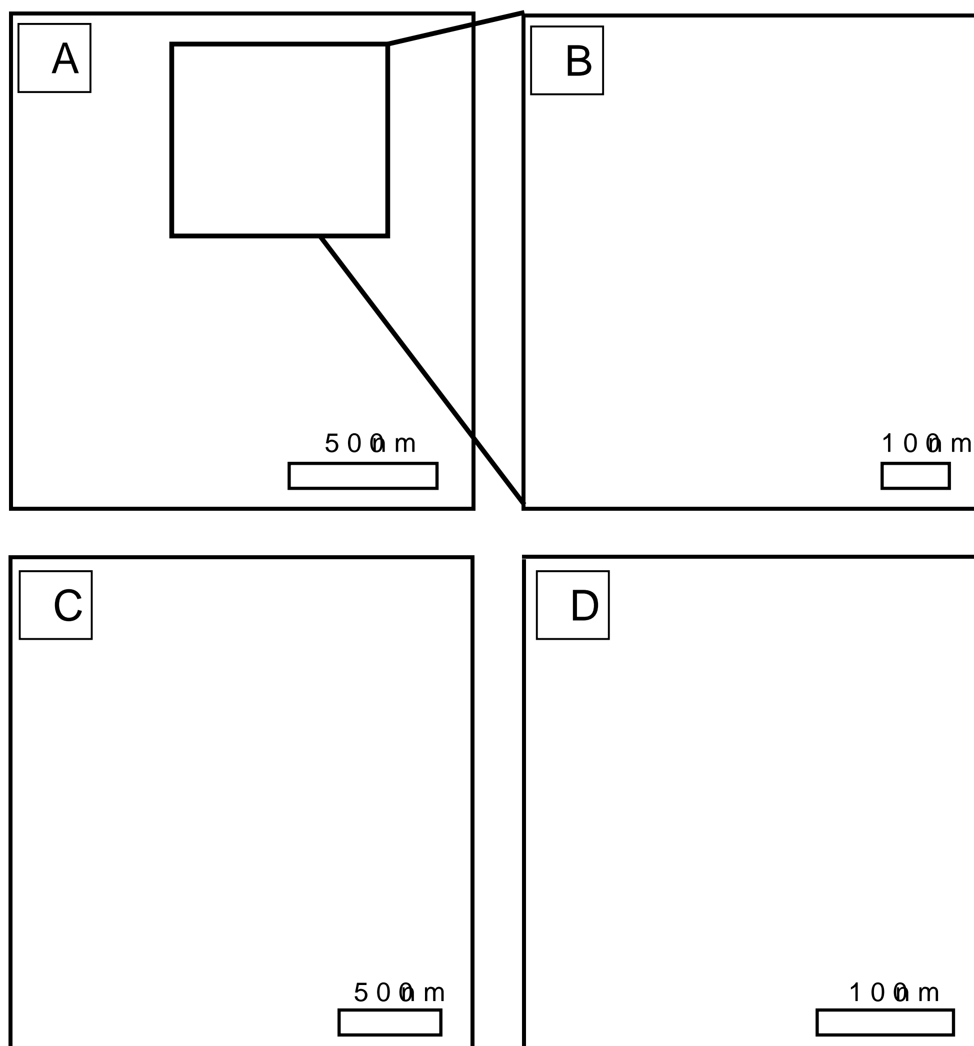

Figure 3.14: Ultra-thin section TEM micrographs of C. metallidurans exposed to 5000 µM Pt^{2+} for 1 hr. A: Nanoparticle Pt along cell envelope. B: Micrograph of square area noted in A. C: Micrograph of immobilised Pt along cell enve[lope] cell. D: Nanoparticle Pt immobilisation along the membrane periphery [of] vesicle.

to keep toxic material away from the cell (Reith et al., 2006; Lengke et al., 2007).

As indicated by the micrographs in Figure 3.5 and *C. metallidurans* responded to Pt^{4+} solution much like it did to Pt^{2+} solutions. Cell staining appeared to b immediate but not complete at 1 min (Figure 3.5A). Longer exposure time produced a darker stain and occurred preferentially at the bacterial cells pole nanoparticles were observed to precipitate along the cell wall (Figures 3.6A and B). Cell lysis was observed at 5000 µM Pt^{4+} concentrations. Released cytoplasmic material bound platinum-nanoparticles (Figure [illegible]). As with 5000 µM Pt^{2+} solutions, cell lysis could have been a response of the chlorides, the low plat pH of the solution, or a combination of both

It was noted that cells adhered to each other upon solution, perhaps as a result of the neutralization of anionic charge gro envelope by platinum and hydronium, allowing hydrophobicity to aggregation (Goldberg et al., 1990). This response could protect some interior by shielding them from the platinum complexes. immediate platinum binding by all cells was observed.

XAS Spectra

XAS spectra in Figures 3.7-3.9 show the speciation and binding p platinum immobilized by bacteria. Figure 3.7 is the XANES with 5000 µM (1000 µg/mL) Pt^{2+} and Pt^{4+} for up to 4 weeks. The peak edge bin energy of Pt^{2+} is slightly less than the Pt^{4+} peak, indicating that electrons more tightly in the more oxidized platinum species. There

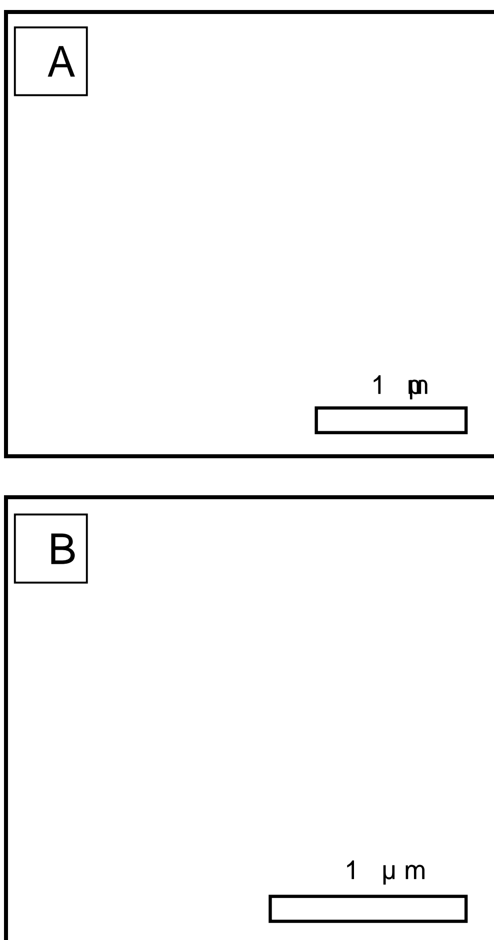

Figure 3.5 Whole mount TEM micrographs of C. metallidurans treated with 500 μM Pt^{4+} (100 μg/mL) for: A: 1 min; and C: 1 day. As incubation time increase
became more pronounced. Colloidal platinum was not obse

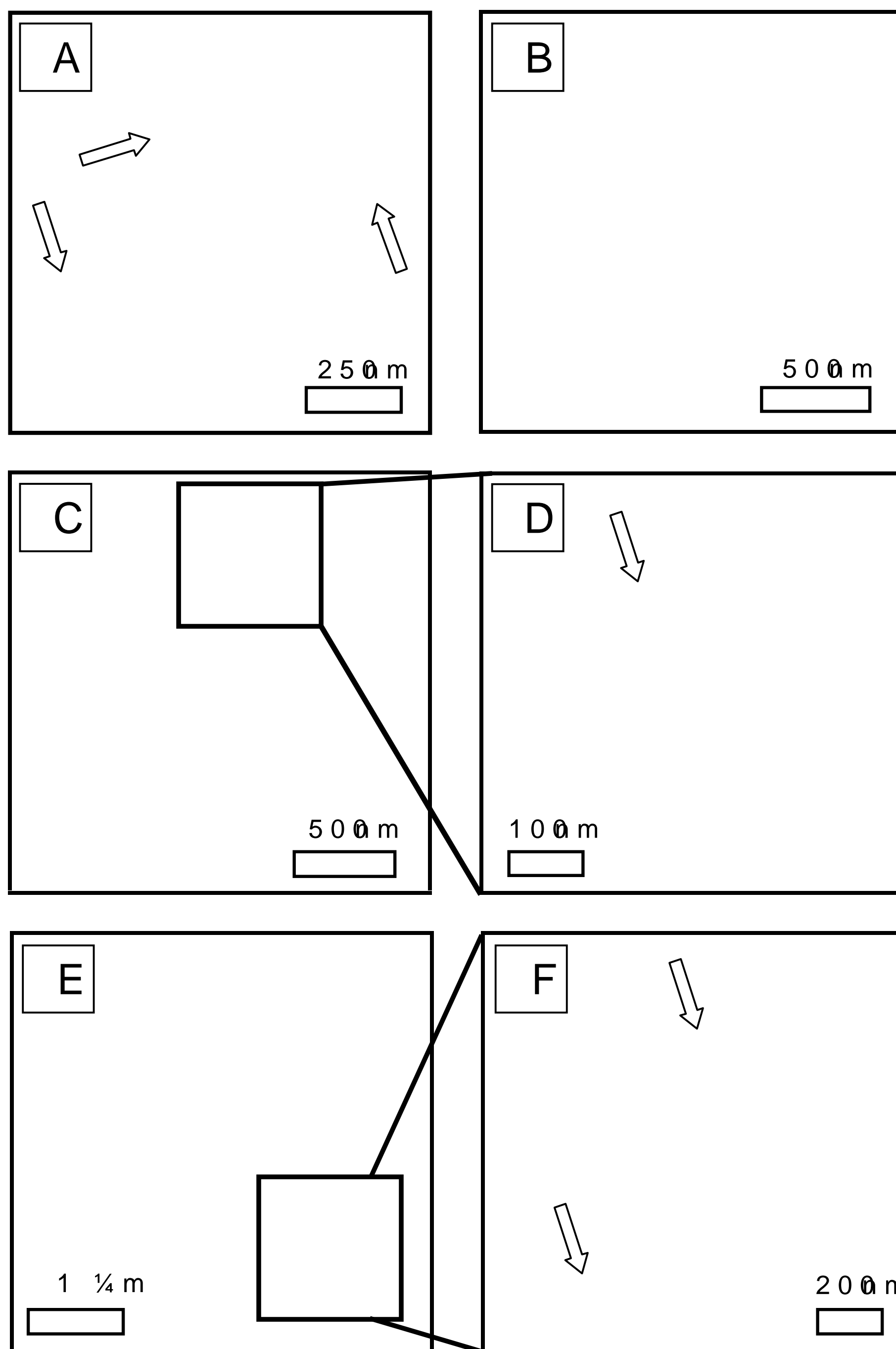

Figure 3.6. TEM micrographs of Chlorella cells reacted with 5000 µM (1000 µg/mL) Pt^{4+}. A: 1 min (whole mount); B: 1 hr (thin section). Pt has been immobilised at the cell envelope. C & D: 1 hr (whole mount); E & F: 1 day (whole mount). Cells have lysed and cytoplasmic m possesses colloidal Pt. Arrows indicate immobilised Pt n becomes more pronounced at longer incubation times (C-F).

Figure 3.7: XANES Spectra of bacteria reacted with 50 and 100 μM Pt^{2+} to Pt^{4+} (1 to 100 mg/ml) days. The line corresponds to the Pt^{2+} energy. Note that the peak energy of Pt^{4+} is slightly higher energy than the Pt^{2+} peak due to the higher oxidation state. There is no downward shift of Pt^{2+} or Pt^{4+} over the 28 days, indicating that synchrotron energy did not

energy for either immobilised platinum species, indicatin
did not detect a change in platinum oxidation over the experiment. It is
important to note that at least 10% of the immobilised pla
synchrotron radiation would detect the change in speciati
are representative of the XANES spectra obtained at low concentrations (data not
shown).

Figures 3.8 and 3.9 show the XANES and EXAFS spe
with 5000 µM (1000 µg/mL) Pt^{2+} for 4 weeks. Similar spectra were o
reactions with 5000 µM (1000 µg/mL) Pt^{4+} as well as with 500 µM Pt^{2+} and Pt^{4+}
solutions (data not shown). Examination of the EXAFS
demonstrated that the Pt-Cl fingerprint decreased in intensity over
that the chlorine bound to Pt^{2+} was replaced by another binding partn
indicates that the replacement was immediate, as the short
between bonds occurred within 1 min; however, gradual i
Pt^{2+} with substitution of chlorine continued over the 4 wee
replacing chlorine is best matched to oxygen on a carboxyl grou
indicative of Pt-O bond on carboxyl groups is ~2.06Å, which com
2.07 Å and 2.08 Å bond distances (Andersson and Ackland 1983; Ahmed 2000; Moret et
al 2009)

Figure 3.8. XANES and EXAFS spectra of bacteria reacted with Pt^{2+} solutions (1000 µg/mL) for 1
days. The arrow notes the Pt-Cl fingerprint, which decreases over time. There i
Pt^{2+} over the 28 days, indicating that synchrotron energy did not detec

Figure 3.9: EXAFS spectra of bacteria reacted with 500 µM (100 µg/mL) Pt^{2+} solution for up to 28 h. The arrows show the first shell binding partners of Pt: Pt-Cl and Pt-C. Note that Pt

3.4. Discussion

The interaction of *C. metallidurans* with $PtCl_4$ and K_2PtCl_4 resulted in a variety of bacterial responses: cell death and cytolysis, plat nanoparticle formation, which were directly proportional to p time (Tables 3.2-3.5).

The literature lacks consistent experimental data on Pt^{2+} and Pt^{4+} complexation. Potential Pt^{2+} complexes present in the reaction system include $Pt(OH)_n^{2-n}$ and $PtCl_n^{2-n}$. Likely Pt^{4+} compounds include $Pt(OH)_n^{4-n}$ and $PtCl_n^{4-n}$, but very little data exists for these species (Bowles et al. 1986, Mountain and Wood 1988; Wood et al. 1992; Azaroual et al. 2001; Colombo et al. 2008). EXAFS confirmed chloride complexes to be the dominant aqueous species prior to reaction with ... Observed uptake of these Pt^{2+} and Pt^{4+} complexes from solution is consistent with the literature described uptake of other metals, i.e., by immobilisation on the cell envelope a within the cytoplasm (Beveridge and Murray 1980; Nies 19

Platinum accumulation occurred throughout the cell and nanoparticle formation and immobilisation occurred at and within the cell. Cell surface reactivit controlled by the proton exchange capacity of carboxyl (R-COOH), phosphoryl (R-OPO$_3$H$_2$ and (R$_2$-PO(OH))), amine (R-NH$_3^+$) and hydroxyl (R-OH) functional groups on the membrane. In all but the 500 µM Pt^{4+} reactions, 5000 µM Pt most carbox groups would have been deprotonated, negatively charged and avail reaction with oxidised platinum; phosphoryl and hydroxyl groups would have remained mostly protonated and positively charged. The pH conditions consiste the acidity of platinum aqueous solutions used in this experiment (Beveridge and

Murray 1980; Fein et al. 1997; Guiné et al. 2006). XANES and EXAFS spectra cl...
show the replacement of chlorine for oxygen on carboxyl ...
binding mechanism. It is worth noting that immobilised platinum occur...
platinum-organo complexes at the cell surface and in the cy...
organo-platinum complexes may be preferred to inorganic com...
present. This process has been described in natural systems where carboxyl function...
groups on soluble organic acids are able to react with pla...
organo complexes (Vlassopoulos et al. 1990; Wood 1990; Wood et al. 1992;
Kubrakova et al. 2011).

As suggested by Beveridge and Murray (1980), platinum bindin...
envelope likely proceeded first as a stoichiometric event...
platinum and then as a nucleation event, where nanopart...
non-stoichiometric formation, which continued at a slower pace. The ... occur...
progressive staining of the cell and the formation of nanoparticles at inc...
times and concentrations indicated that this process may ...
system. Platinum immobilisation was also observed in the cytoplasm. The rapid uptake ...
of platinum may have occurred ... membrane transporter proteins ...
regulate the entry of these particular compounds, i.e. all complexes freely enter...
cell driven by the chemical gradient that existed across the en... (Nies 1999)
Passive diffusion would have ceased once platinum ... instanta...
concentrations within the cell equal the platinum concentration within th...
Aqueous platinum that entered ... neutral pH of the cytoplasm...

bound to deprotonated functional groups, with carboxyl and complexed functional groups (Fein et al. 1997; Guine et al. 2006).

Bacterial cells that remained viable following exposur platinum would have presumably initiated a biological resp an attempt to survive. Internalised metals inhibit the function of importa bind to important functional groups, or compete with other important physi cations. Detoxification responses include chemical reduction or co metal to a less toxic state and/or efflux out of the cell. These the ce mitigation responses drain the cell of energy and cause oxidati continued cell death at longer exposure times (Silver 1996; Nies 1999). XANES did not indicate platinum reduction, however, the appearance nanometre-scale platinum colloids in the cytoplasm (and at the cell e evidence that some platinum reduction occurred.

The relationship between toxicity and immobilization comparison of Tables 3.4 and Tables 3.5. For a particular platinu species, immobilisation of platinum generally correlated with concomitant deat of cells, e.g. the more platinum that was immobilised, the mo Resistance of C. metallidurans to pH 4.1, 50 µM Pt^{4+} solution demonstrates tha toxicity experienced by the cells in the 2500 µM Pt^{2+} solution (pH 4.0) was primarily due to a platinum effect. Similarly, cell death was immedia µM and 5000 µM Pt^{4+} solutions, but when C. metallidurans was exposed to pH comparable solutions of HCl, corresponding cell death was not observed (data not shown). Intuitively, acidic solutions will induce some

results demonstrate that platinum immobilization trigger toxicity. It is important to note that dead cells cannot actively, in turn, bind pl immobilisation occurred long after cell death, e.g. the 5000 µM Pt^{4+} solution killed all bacteria within 1 minute and platinum immobilization continued. Dead cells no longer metabolise and the proton motive force that fuels efflux pumps does not operate; consequently, there is less competition for platinum groups (Urrutia et al. 1992). The reactivity of dead C. metallidurans cells is known to be higher than the reactivity of live cells (Guiné et al. 2007). In this situation, the appearance of new reactive sites from denatured c cytoplasmic contents likely contributed to the continued even when cell death was instantaneous.

Further examination and comparison of Tables 3.2 to 3.5 revea differences between the Pt^{2+} reaction systems and Pt^{4+} systems. First, Pt^{2+} reactions were slightly less toxic than Pt^{4+}-chloride solutions. While Pt^{2+}-chloride solutions were not as acidic as Pt^{4+}-chloride solutions, bacteria that remained viable following exp have been able to neutralise and pump out Pt^{2+} cations but not Pt^{4+}. C. metallidurans has divalent cation efflux ATPases ([illegible] 2005). Instead, if the bacterium could not efflux the highly oxidised platinum, then its attempt to neu it internally would have brought about a fatal oxidative stress (N 2009). It is also important to note that bacteria were able to immobilize more platinum from Pt^{2+} solutions than from Pt^{4+} solutions. From a concentration and c character of platinum vs. hydronium, platinum systems exceeded h by approximately 2 orders of magnitude and generally

concentration in Pt^{4+} systems. Therefore, Pt^{2+} would have only competed
hydronium for carboxyl binding sites whereas Pt^{4+} would have more compe
and therefore less opportunity to be bound and immobilise

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Chapter 4

Conclusions

The traditional notion that platinum is inert has bee
biogeochemical paradigm (Anthony and Williams 1994).
transport of platinum from the mantle to the crust and subsequent weat
of PGM-bearing host rocks is an incomplete metallogeny of
Subsequent-occurring dissolution, transportation and precip
biological and abiotic processes, potentially geological, are now cons
be key components in placer platinum formation (Fuchs and Rose 1974; Bowles
Azaroual et al. 2001; Hanley 2005; Reith et al. 2009). The structural and c
examination of platinum grains, revealing features, combined with
biogeochemistry of candidate platinum compounds have de
more to learn about the mobility of platinum in natural sy
has highlighted the importance of organo-platinum compounds i
platinum exploration, which could lead to applications for

With respect to the current controversy regarding
precipitation and growth of platinum grains (Traore et al. 2008), this highlighted
work on Australian platinum grains, which provides clea
platinum found in natural systems ultimately comes from
material, supports the dissolution-growth concept. The superficial feat
highlight the importance of examining platinum grains i
secondary imaging before, more classic, cross polishing to

mineral grains. Deep weathering scars, pits and cavities of platinum grains are important sites of mechanical and weathering. SEM micrographs show delicate, 150 nm cubic copper minerals that could be lost by polishing and wouldn t be as obviou imaging, which does not achieve the same resolution as dissolution of the bulk nugget around these minerals demo homogenous, reflecting the geological conditions of the minerals may be left in the regolith or the alluvium. The pre oxides provides evidence that acidic conditions occur a presents a possible mechanism for chemical dissolution (Guilbert 1986; Sole 2005).

For future work on this system, the next step in chara from Fifield requires that they be polished and examined using the same tec as was used to study their surfaces. Any possible weathering link b features and the bulk , interior of the nugget needs to heterogeneity, from the periphery of platinum grains to preferential weathering of the bulk grain (Cabral et al. 2007; the cubic Traoré et al. 2008). Synchrotron element mapping of polished ... should also be interior morphological features noted in microscopy wit Element mapping would likely require micro-XRF, ideally a spot size of 10 detect the chemical gradient from surface to interior and and precipitation processes affecting platinum grains.

A large number of microorganism have been implicate[d in the] cycling of metals at near-surface conditions (Southam and [...] Reith et al. 2009; Southam et al. 2009). The precipitation of secondary [...] bacteria has been well documented (Southam and Beverid[ge] [...] 2006, 2009; Lengke et al. 2006b, c, Lengke and Southam 2007). A c[...] microbe-mediated immobilisation of platinum was hypothesize[d] [...] chemical similarity of gold and platinum, but did not occur [...] et al. 1990; Cabral et al. 2007). Cupriavidus metallidurans, a known metal resistant soil bacterium that has been implicated in the biogeochemical cycling of [...] (Reith et al. 2006; 2009), immobilised appreciable amounts, i.e., % of pla[tinum] [...] quantities by mass (see Tables 3.4 and 3.5). The platinum transformation i[...] appreciable amounts of secondary platinum. TEM clearly d[...] immobilised at the cell envelope and within the cytoplasm [...] immobilisation was determined by synchrotron radiation species [...] (existing as platinum chloride) preferentially bound to exposed carboxyl oxyge[n] groups on the cell wall and within the cytoplasm (Beverid[ge] [...] Bowles 1986, Mountain and Wood 1988; Fein et al. 1997; Wood et al. [...] Azaroual et al. 2001; Guiné et al. 2006). Although platinum reduction was no[t] [...] synchrotron methods, the reduction of at least some of the [...] 2 nm particles of elemental platinum was observed. Due to the number [...] ubiquitous nature of bacteria in near-surface environments and the h[...] both Pt^{2+} and Pt^{4+} for organically derived carboxyl functional g[roups] [...] suggests that platinum [...] must be important in natural systems

bearing materials are exposed to weathering conditions, platinum in association with organic acids and the biosphe

Work into understanding the biosphere's influence, i.e., the biogeochemical cycle of platinum, needs to continue. Recommendatio experiment include exposing C. metallidurans to aqueous platinum species fo periods of time (months) to promote and examine platinum et al. (2006a) exposed cyanobacteria to aqueous platinum for up to a mo reported sub-micron platinum particles. Given that consortia of other microbes should be tested for their ability to immob Likely, many bacteria are able to passively precipitate platinu combined presence in weathering profiles may provid transformation. Also, platinum-bacteria interactions are likely not re immobilisation of aqueous platinum species, but also catalyse the weath platinum-bearing material in primary ore deposits. The sulph Acidithiobacillus thiooxidans and iron and sulphur oxidiser Acidithiobacillus ferrooxidans contribute to the supergene leaching of metal sulphides via me oxidation and sulphuric acid production (Singer and Stumm 1970; Lizama a 1989; Sillitoe 2005; Enders et al. 2006). As platinum-sulphides are the main PG most of the world's platinum deposits, including the Merensky Reef of South Africa's Bushveld Igneous Complex, bacteria may be able to enhan the host rocks (Macdonald 1987; Teluk 2001).

The current value of platinum has nearly matched all-time gold highs over the last number of years. Continued exploration and min

these prices and companies will lo[illegible] processing techniques to boost the[illegible] once inacce[illegible]solved in mine waste rock piles could i[illegible] efficiency for mining companies. Further[illegible] and precious metals, microbes may one day be used as bi[illegible] platinum enrichment (Corbisier et al., 1997; Reith 2009).

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Appendix to Chapter 2

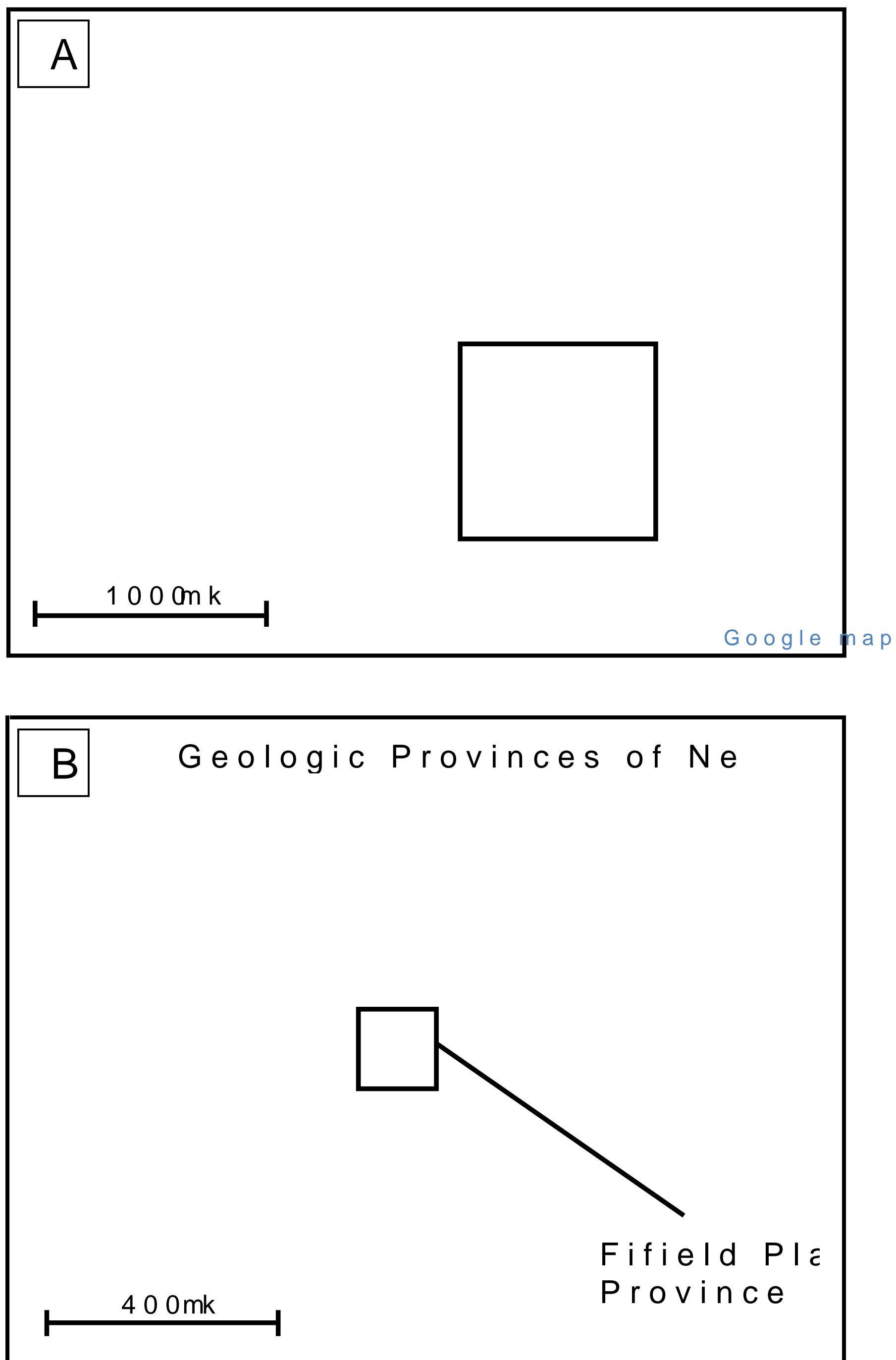

Figure A.1 A: Map of Australia with state of New South Wales; B: Geologic provinces of New South Wales and squared area noting th
(Xie et al. 2009)

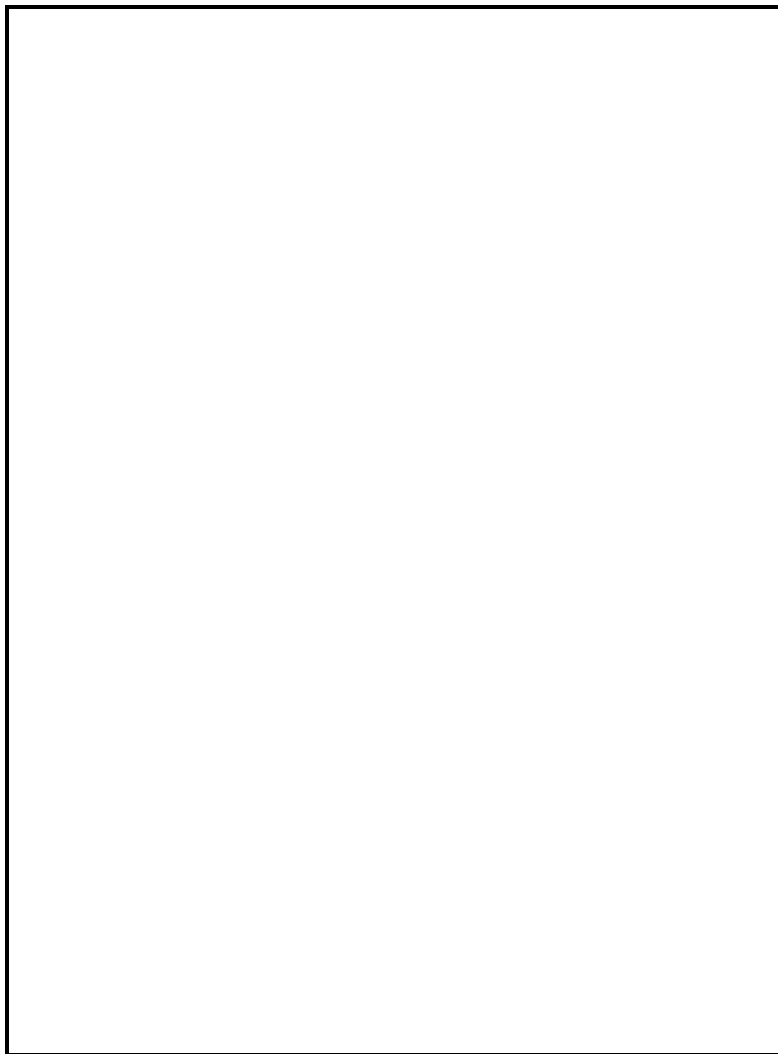

Figure A.2. Schematic profile of Fifield Platinum Province la
Platinum is found in high grade goethite zone.

Figure A3. µXRD of platinum grain in Figure 2.3F. The alloy is identified as (Pt_3Fe).

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