An Investigation into the Biological Treatment of Platinum Refinery Effluent

THESIS
Submitted in partial fulfilment of the requirements for the Degree of

MASTER OF SCIENCE

At Rhodes University

by
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January 2002

Dedicated to my son
Clarke Roland Smith 12 May 1980 to 23 May 2001

Abstract

This Review and project will discuss and demonstrate the use made of Biotechnology in the production and reduction of metals. It will look at how and why metal binding takes place, known platinum group metal speciation will be included. Examples of how to improve metal binding efficiency will be discussed by stimulating ligand activity by polarisation.

Various biotechnical options available, with emphasis placed on the use of the aquatic fern and algae will be given as examples of biological treatment of heavy metals in particular the aquatic fern Azolla.

The method of standard preparation and the use of Inductively Coupled Plasma Emission Spectrophotometer (ICP) used for analytical analysis will be included so that consideration can be given to the collection of analytical data in the provision of evidence to support or provide a conclusion.

The outcome of the test work utilising the aquatic plant Azolla has proven that it can be used to remediate platinum refinery effluent.

This process can offer an alternative to the classical chemical method normally used, which is economically viable and environmentally friendly in comparison to the common methods of refinery effluent treatment.

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Pg

LIST OF ABBREVIATIONS

mV (Milli – Volt) Unit of measurement of Redox Potential.
Redox Potential State of Reduction or Oxidation.
pH Measurement of Alkalinity or Acidity
Effluent Liquid Waste
Dams Evaporation ponds for storing liquid waste.
Pgm Platinum group metals.
Liquor Solution containing dissolved pgm or base metals.
WPR Western Platinum Refinery.
NEMA National Environmental Management Act, 107 of 1998
Pt Platinum
Pd Palladium
Au Gold
Rh Rhodium
Ru Ruthenium
Ir Iridium
Cu Copper
Ni Nickel
Pb Lead
Ag Silver
Se Selenium
As Arsenic
Te Tellurium
Zn Zinc
Al Aluminium
Fe Iron

ACKNOWLEDGEMENTS

Grateful thanks are due to:

- Prof. P Rose, Dr. K Whittington Jones for their supervisory role and support.
- Prof. J Duncan, Rob van Hille and Paula Atunes, for their project guidance.
- Mr. A Keeley, General manager, Western Platinum Refinery for the support and giving me the opportunity to enter the Masters programme.
- My wife Margaret and my family for their love, support and belief in me.
- To Mr. V Marran for his support throughout the programme.

CHAPTER ONE: INTRODUCTION

1.1 Introduction

This review will be used to support a research project for the biological treatment of final effluent emanating from a Precious Metals Refinery responsible for producing platinum group metals (pgm), often referred to as noble metals due to their resistance to corrosive conditions.

The examples given in this review will be utilised to fashion and guide that project with the ultimate objective being to provide a biological treatment option for waste effluent that is financially viable and environmentally sustainable.

If this is the goal, then the aim of this review and test is to look at an available biological option, one must also know the extent of the problem and the prevailing conditions that already exist in order to secure a successful outcome.

Those conditions and the extent of the problem will be explained in some detail in the next section; (1.2 Background).

1.2 Background

Western Platinum Refinery is owned by Lonmin and controlled through its board of directors in London and is the third largest producer of pgm in the world. The refinery receives platinum rich concentrate from its mines situated in the Rustenburg area. Then through the utilisation of classical chemistry extracts the precious metals with a minimum purity of 99.95 ppm, which is then sold on the world commodity markets.

The highest single financial loss and most significant environmental threat towards a negative impact on the environment occurs through the disposal of final effluent. This study and later research project is to attempt to provide a solution for this scenario.

1.3 Environmental Impact

Significant quantities of alkaline and acid effluent are generated each month, which pose a serious threat to the environment. The main threat is that of ground water contamination through seepage taking place at on-site evaporation dams. Though the practise of returning effluent to these dams has ceased recently, two dams still remain. Not only has the quality of groundwater in the surrounding area deteriorated, but there is an identified contaminated plume spreading along a main aquifer, which requires remedial action. (Appendix A)

However, the refinery is taking action to reduce effluent volume at source and does not dispose of effluent at end of pipe. It now utilises the services of a

permitted hazardous waste site, however it can not relinquish ownership of effluent generated because of the fundamental principals of "cradle to grave" and the "polluter pays" contained within South African environmental legislation:

Every person who causes, has caused or may cause significant pollution or degradation of the environment must take reasonable measures to prevent such pollution or degradation from occurring, continuing or reoccurring, or, in so far as such harm to the environment is authorised by law or cannot reasonably be avoided or stopped to minimise and rectify such pollution or degradation of the environment.

(National Environmental Management Act, 1998. Chapter 7 section, 28 .1)

It is quite clearly defined in the National Environmental Management Act (NEMA),

What the legal requirements are and who and what reasonable actions should be taken in the prevention of pollution. The complete definition described in all of the remainder of section 28 completes the scenario of the action that will be taken in the case of failure of person/s or an organisation to comply with prescribed legislation. (Appendix B)

1.4 Financial Impact

The present effluent streams contain traces of pgm (<10ppm), that are considered economically not viable to process further due to their low concentrations. However these small concentrations amount to a significant pgm loss over a financial reporting period of twelve months and relate to an extraordinary high revenue loss for the site (table 1.1).

Added to this loss is the average monthly cost of R600.000 for safe disposal at a hazardous waste site. Due to the unavailability of alternative class HH hazardous waste sites in Gauteng, the refinery is vulnerable to inflated disposal costs, which increase year after year.

Table 1.1 Provides data from WPR metal accounting system. Note that the Rand / Dollar exchange rate is the average for WPR financial year Oct 2000 to Sept 2001

TOTAL EFFLUENT DISCHARGED							
	TOTAL PGM's (Kgs)	Pt (KGS)	Pd (KGS)	Au (KGS)	Rh (KGS)	Ru (KGS)	Ir (KGS)
YEAR ENDED SEP 97	294.413	65.371	28.036	20.050	45.068	44.671	91.217
YEAR ENDED SEP 98	147.020	27.855	19.805	16.485	28.700	27.760	26.415
YEAR ENDED SEP 99	209.635	26.320	17.745	19.120	43.480	45.425	57.545
YEAR ENDED SEP 00	490.888	85.975	35.517	22.769	114.181	113.988	118.458
YEAR ENDED SEP 01	347.114	73.623	28.284	24.376	67.042	88.889	64.900
TOTAL Kgs	1 489.070	279.144	129.387	102.800	298.471	320.733	358.535
TOTAL Troz	47 874.688	8 974.683	4 159.887	3 305.095	9 596.061	10 311.800	11 527.162
$ PER Troz		425	330	281	750	100	415
$ VALUE	19,127,732	3 814 240	1 372 763	928 732	7 197 045	1 031 180	4 783 772
ZAR / $		9.594	9.594	9.594	9.594	9.594	9.594
TOTAL VALUE ZAR	183,503,813	36 592 297	13 169 735	8 909 881	69 045 575	9 892 729	45 893 597

CHAPTER TWO: EFFLUENT CHARACTERISTICS

2.1 Effluent

The final effluent contains various quantities of those elements found in the typical concentrate feedstock received by the refinery (table 2.1).

However those elements contained in the initial feedstock are no longer in the same chemical state. The entire processing of platinum group metals is based on a change in their metallurgical structure, essentially through the controlled change of the rate of oxidation or reduction by chemical addition.

Table 2.1 This table provides data of the average percentage elemental breakdown of concentrate (feedstock). Data was gathered from monthly composite October 2001 (Appendix C). Analytical values were completed by ICP analysis.

Element	Percentage
Platinum	32.465
Palladium	14.886
Gold	0.792
Rhodium	5.126
Ruthenium	8.856
Iridium	1.952
Copper	6.275
Nickel	3.318
Iron	2.335
Lead	1.321
Silver	0.905
Selenium	0.496
Arsenic	1.818
Tellurium	0.786
Zinc	0.001
Calcium	1.032
Chromium	0.624
Titanium	0.054
Cobalt	0.144

2.2 Metal Species

To overlook these state of changes would be a gross oversight and could be detrimental to any intended project. Most metal ions, when present at sufficient concentrations, have the potential for being toxic to biological systems. Such metals may be essential at low concentrations (e.g. copper, zinc) or have no known biological function (e.g. cadmium, mercury) and compete with a functional metal. Metal speciation is a crucial (table 2.2), but frequently overlooked, factor in determining metal toxicity in both laboratory and environmental situations. Although the influence of organic chelating agents (which include most common medium constituents) in moderating metal toxicity is generally appreciated, pH, water hardness and buffers can influence observed toxicity's (Hughes and Poole, 1989,Metals and Micro-organisms).

From the above statement by Hughes and Poole (1989) it would seem that identification of the correct metal species is critical for any successful outcome involving metal separation be it by a biological or chemical method. Thus, metals must be oxidised in order to produce soluble species. As already mentioned, the species in solution are almost entirely complexes of metals. Manipulation of the chemistry of the soluble complexes is carried out in order to achieve separation and purification and, finally, pure metal is regenerated from complexes by reduction.

(E W Stern, Aqueous Chemistry of Precious Metals Paper 1981).

Successful separation and the chosen method of manipulation are dependent on the metal species type and the complexes they have formed.

Also, the indication in particular of the effects pH, oxidisation and chelating agents may have on the biological recovery of pgm from effluent.

These effects have been discussed in more detail under Metal Binding in Biological Molecules (chapter 3.1).

Table 2.2This table provides the typical species to be found for pgm. Included is the reaction that takes place and the redox potentials associated with that reaction(*)

Element	Species	Reaction	Redox Potential
Platinum	Pt^{2+}	$Pt^{2+} + 2e^- \Rightarrow Pt$	1,188
	Pt^{4+}	$Pt^{4+} + 4e^- \Rightarrow Pt$	1.115
	$PtCl_4^{2-}$	$PtCl_4^{2-} + 2e^- \Rightarrow Pt + 4Cl^-$	0,758
	$PtCl_4^{2-}$	$PtCl_4^{2-} + 4e^- \Rightarrow Pt + 4Cl^-$	0,744
	$PtCL_6^{2-}$	$PtCl_6^{2-} + 2e \Rightarrow PtCl_4^{2-} + 2Cl^-$	0,726
	$Pt(NH_3)_4^{2+}$	$Pt(NH_3)_4^{2+} + 2e^- \Rightarrow Pt + 4NH_3$	0,277
Palladium	Pd^{2+}	$Pd^{2+} + 2e = Pd$	0,915
	$PdCl_4^{2-}$	$PdCl_4^{2-} + 2e = Pd + 4Cl^-$	0,62
	$PdCl_6^{2-}$	$PdCl_6^{2-} + 2e = PdCl_4^{2-} + 2Cl^-$	1,470
	$Pd(NH_3)_4^{2+}$	$Pd(NH_3)_4^{2+} + 2e = Pd + 4NH_3$	ca 0,0
Gold	Au^+	$Au^+ + e = Au$	(1,83)
	Au^{3+}	$Au^{3+} + 3e = Au$	1,52
	$AuCl_2^-$	$AuCl_2^- + e = Au + Cl^-$	1,154
	$AuCl_4^-$	$AuCl_4^- + 3e = Au + 4Cl^-$	1,002
	$AuCl_4^-$	$AuCl_4^- + 2e = AuCl_2^- + 2Cl^-$	0,926
	$Au(NH_3)_2^+$	$Au(NH_3)_2^+ + e = Au + 2NH_3$	0,56
Rhodium	Rh^{3+}	$Rh^{3+} + 3e = Rh$	0,76
	$RhCl_6^{3-}$	$RhCl_6^{3-} + 3e = Rh + 6Cl^-$	0,50
Ruthenium	Ru^{3+}	$Ru^{3+} + e = Ru^{2+}$	0,249
	$RuO_4(aq)$	$RuO_4(aq) + e = RuO_4^-$	0,99
	RuO_4^-	$RuO_4^- + 4H^+ 3e = RuO_2 + 2H_2O$	1,533
Iridium	Ir^{3+}	$Ir^{3+} + 3e = Ir$	(1,16)
	$IrCl_6^{3-}$	$IrCl_6^{3-} + 3e = Ir + 6Cl^-$	0,86
	$IrCl_6^{2-}$	$IrCl_6^{2-} + 4e = Ir + 6Cl^-$	0,86
	$IrCl_6^{2-}$	$IrCl_6^{2-} + e = IrCl_6^{3-}$	0,867
	$IrCl_5(H_20)^-$	$IrCl_5(H_2O)^- + e = IrCl_5(H_2O)^{2-}$	1,00
	$IrCl_4(H_2O)_2$	$IrCl_4(H_2O)_2 + e = IrCl_4(H_2O)_2^-$	1,22
	$IrCl_3(H_2O)_3^+$	$IrCl_3(H_2O)_3^+ + e = IrCl_3(H_2O)_3$	1,30

The compilation of(*Bard *et al, 1985*)(Standard Potentials in Aqueous Solution)

CHAPTER THREE: METAL BINDING

3.1 Metal Binding in Biological Molecules

It is important to understand how the different metals are extracted or separated from each other and what is happening biologically that may help to enhance that separation.

The biological function of metals is intimately linked to the nature of the ligands present in the coordination sphere. In some cases, the coordination positions around the metal may be fully filled by donor atoms from one multidentate molecule such as a protein. In others, one or more coordination positions may be available for the binding of a substrate molecule, either by expansion of the coordination number or, more usually, by having readily-displaceable water molecules coordinated to the metal centre.

(Hughes and Poole, 1989, Metals and Micro-organisms)

A ligand involves the binding of a donor atom to a metal with one single ligand attachment known as a monodentate.

A multidentate is a multitude of attachments forming an even stronger bond. This may seem to be the ideal situation for the process of metal separation. However consideration should also be given of the process of final metal removal and how one would remove it from the binding.

In the classical refining process this is done by reagent washes or in the case of treating a solid mass, smelting is normally utilised.

3.2 Metal Binding Groups

It is stated that there are three major types of binding groups to which metal ions will bind, they are biopolymers, proteins, nucleic acids and poly-saccharides.

The ligand groups available include negatively charged groups such as carboxylate, thiolate or phosphate, and groups such as the amine function, which coordinate to the metal centre through lone pairs of electrons. Metal ions are also bound by specialised macrocyclic ligands such as porphyrins, while transition metals, notably iron, are often present in enzymes in dimes or clusters with bridging sulphide or oxide ligands.

(Hughes and Poole, 1989, Metals and Micro-organisms).

Hughes and Poole (1989) go further to explain in greater detail the ligand groups and structure.

Transition of metal ions is dependant upon their charge ratio (polarisation) and a cation with an high positive charge density can be more efficient in metal binding because the high positive charge encourages strong ligand interaction.

Ionic size decreases across the Periodic Table, from left to right and that alkali metals can be resistant to metal binding due to poor ligand interaction.

CHAPTER FOUR: METAL ATTRACTION

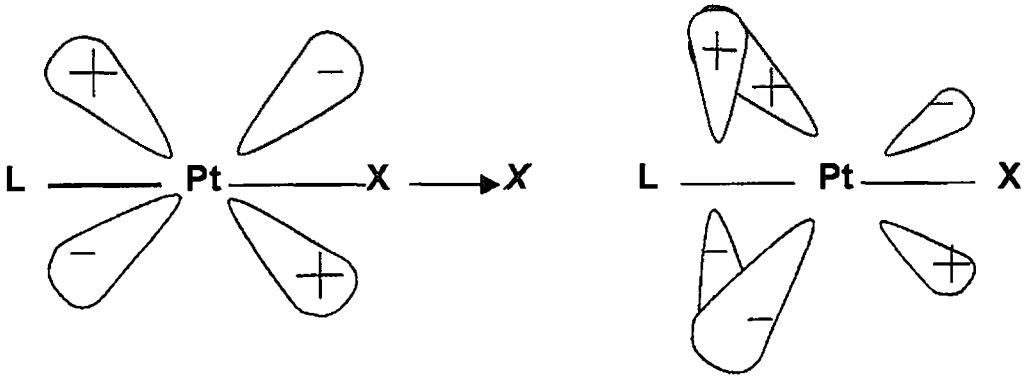
4.1 Polarisation or trans-effect

It now seems apparent that metal binding efficiency relies on successful ligand interaction and to obtain strong ligand bonding also requires high charge ratios, known as polarisation.

Polarisation can be stimulated kinetically and the effects are well documented in classical pgm chemistry. The polarisation or σ-trans-effect theory considers the trans–effect to be principally electrostatic in origin and transmitted through σ-bonds (electron donors). Thus in a complex with four identical ligands the polarisation of each of the ligands by the metal ion will be the same and a dipole will not result. However, if one ligand (L) is more polarisable than the others, then an induced dipole will result and the distribution of the electron density will move through the σ-bond towards the ligand (X) trans to L. It has often been stated that as a result of this the Pt-X bond (Platinum) becomes weakened and lengthened. Although sometimes true, this is not always so, since the kinetic trans-effect may arise simply because the trans –group (L) owns rather more of the empty p_σ orbital of the metal in the transition state than in the ground state, thus reducing the energy difference between the ground state and the transition state. The stronger the σ-donor ability of the trans-group the lower becomes the energy difference between the two states and, hence, the greater the trans effect of that group. Thus a very polarisable trans-ligand (L), of which the hydride ion is a good example, should exhibit a strong trans-effect. Also, since the polarisability of the metal ion is very

important, the trans-effect of a ligand should be less in palladium (II) than platinum (II) because the covalency of palladium (II) complexes is less than that of platinum (II) complexes.

(F R Hartley, 1973, The Chemistry of Platinum and Palladium)



L does not form π-bonds L forms π-bonds

Figure 4.1 Schematic representation of the bonding mechanism for the trans effect.
(F R Hartley, 1973, The Chemistry of Platinum and Palladium)

4.2 Metal Toxicity and pH

The process of metal binding has been discussed (and the importance of recognition of the metal species present) but of equal or even greater importance is how those metals will react with the micro-organisms. They may stimulate growth or prove to be lethal.

There are several reports that Mg and Zn alleviate toxicity of Ni to micro-organisms (Babich and Stotzky, 1982), possibly by competition between these cations, of similar non-hydrated ionic radii, for common sites on the cell surface. Calcium and magnesium alleviate the toxicity of cadium to *A. niger*. Other metal combinations are synergistic in their toxic effects. For example, Ni and Cd were synergistic towards growth and of *K. pneumoniae* (Ainsworth, *et*

al, 1980) but antagonistic towards growth, photosynthesis and N_2 fixation by *Anabaena inequalis* (Stratton and Corke, 1979).

It has also been stated that changes to pH of an environment can alter levels of toxicity.

Alkaline pH 8.5 eliminates the toxicity of nickel to various fungi (Babich and Stotzky, 1982). The observed, strong pH-dependence of the absorption of trace metals is largely due to the pH-dependant variation of metal species (Stumm and Bilinski, 1972).

Though it is recognised that certain metals or alteration of their state may prove to be detrimental to microorganisms the cause may not be so easily defined. Caution needs to be exercised in attributing toxicity to the metal *per se*, as since toxicity can result quiet indirectly. The apparent toxicity of lead to fungi and algae, for example, has been attributed to the depletion of phosphate by precipitation as $Pb_3 (PO_4)_2$
(Ross, 1975; Schulze and Brand, 1978).

The next chapter will provide examples of where microbiological processes or studies using algae or aquatic ferns have been used to separate or bind metals.

CHAPTER 5: BIOREMEDIATION OF METAL CONSTRAINTS; CASE STUDIES

5.1 Applications of Recombinant Algae for the Treatment of Heavy Metal Contamination in Aqueous solution

The Department of Biochemistry & Molecular Biology, Ohio State, USA, principal investigator R T Sayre, under took this study between 1994 and 1997, the objective was to:

- Characterise the heavy metal binding sites on unicellular algae.
- To generate transgenic algae expressing high affinity heavy metal binding proteins.
- To compare and contrast heavy metal binding capacity of wild type and transformed algae.
- To determine the effectiveness of transgenic algae expressing heavy metal binding proteins in the removal of heavy metals from water.
- To develop harvesting techniques for the removal of algae from water.

(http://www.sg.ohio-state.edu/Project/Projectrept.cfm?ProjectNumber=R/BT-3)

This study was not only of interest because of the obvious treatment of heavy metals by algae but because of the proposed enhanced recovery and a comparison made between wild and transformed algae.

The rationale behind the project was that, heavy metals including cadmium, lead and cobalt are major contaminants at many sites around the world. These metals are toxic to humans and animals. The unicellular algae

Chalmydomonas however, is tolerant of high levels of heavy metals. We propose to express the heavy metal binding protein metallothionein in *Chalmydomonas* to increase its heavy metal binding capacity. These algae will then be removed from the water to decontaminate them. Acid treatment of the harvested algae will release the heavy metals so that they can be recycled.

(http://www.sg.ohio-state.edu/Project/Projectrept.cfm?ProjectNumber=R/BT-3)

It is interesting in that the intent is to increase binding capacity and previously we saw that increased ligand activity achieved by polarisation can increases binding. The increase in capacity for binding was to be achieved by protein stimulation. Also interesting is that the heavy metals would then be removed by acid washing and this is frequently the scenario in metal recovery from resin or ion exchange.

The study strategy outlined the following:

- Characterise the heavy metal binding fractions in the green unicellular algae, Chlamydomonas reinhardtii using a combination of biochemical fractionation procedures, inhibitor studies and biophysical techniques.
- Generate transgenic algae expressing heavy metal binding proteins (MT-II) with high binding affinities.
- Compare and contrast the chemistry of heavy metal binding sites in the wild type and transgenic algae expressing metallothioneins.
- Determine the effectiveness of using transgenic algae expressing metallothioneins for the treatment of heavy metal contaminated waste water.

Using the single celled, genetically transformable alga *Chlamydomonas reinhardtii* we will characterise the heavy metal (Cd, Co, Pb) binding sites as a function of heavy metal concentration and using the fluorescent transition metal probe, Europium. Biomolecules that bind heavy metals will be identified using standard biochemical fractionation techniques. Subsequently, genes encoding metallothioneins will be transferred into *Chlamydomonas*. Their expression and heavy metal binding traits will be characterised by gel electrophoresis autoradiography and atomic absorption. We will also develop procedures to isolate heavy metal-containing algae based on phototaxis, centrifugation, or filtration technologies.

It was demonstrated that (a foreign metallothionein-II (MT-II) gene) could be effectively expressed in *Chalmydomonas*. At low cadmium concentrations that cells expressing metallothionein bound twice as much cadmium per cell. At high cadmium concentrations that induce phytochelatin synthesis we demonstrated that cells expressing MT-II bound the same amount of cadmium per cell as wild type cells. The cells expressing MT-II, however, were able to grow to two-fold higher densities than wild type cells at toxic (LD = 50) cadmium concentrations. We also demonstrated that wild type cells have cadmium specific binding sites on their cell surface that do not bind other competing metals. These cadmium sites are pH sensitive and bind cadmium at neutral or alkaline pH. At low pH the cadmium is released. This pH dependent cadmium binding and released can be carried out multiple times with no loss in binding capacity. In addition, we characterised selenium and chromium binding sites. These metal binding sites also were pH sensitive.

Titrations and spectroscopic analyses (IR and EXAFS) indicated that the heavy metal binding sites are likely to be carboxylate, amino and sulphate groups.

(http://www.sg.ohio-state.edu/Project/Projectrept.cfm?ProjectNumber=R/BT-3)

Summary

It would be fair to say that there could be reliable conclusions made from this study in that:

- That binding took place through a mixture of adsorption and absorption.
- That with protein stimulation (metallothionien) the cadmium uptake was increased two fold at an LD=50.
- That with protein stimulation (metallothionien) increased growth of the algae.
- That binding was pH dependent.
- That when the cadmium was released from the algae due to low pH levels there was no detrimental effect on the algae and it could be still used with the same efficiency
- That metal binding can be site specific.

It is quiet evident from the outcome of the study that algae can be utilised to recover heavy metals and that algae can be engineered to increase metal recovery.

5.2 Biocomplexation of Heavy Metals by Engineered Algae heavy metal

Department of Biochemistry & Molecular Biology, Ohio State, USA, principal investigator R T Sayre, under took a follow up study between 1998 and 2000. The objective of the study was to develop a renewable biological system to selectively recover toxic heavy metals from contaminated sites and waste streams. Traditional methods of sequestering trace elements have involved chemical engineering approaches in which the elements are precipitated or sorbed from the medium. These methods are rarely selective and result in large volumes of waste. In contrast, living organisms have been shown to selectively sequester heavy metals by a variety of methods including: charged groups (carboxylic acid, amino and sulphate groups) and heavy metal binding proteins (e.g., metallothioneins). The algae have many features, which make them ideal candidates for the selective removal and concentration of heavy metals.

(http://www.sg.ohio-state.edu/Project/Projectrept.cfm?ProjectNumber=R/BT-4)

Sayre argues that biological systems are more selective in metal recovery than the traditional chemical processes and produce less waste thus a viable recovery option. He further supports this argument, a biologically renewable heavy metal recovery system will provide a less expensive and more selective alternative to the chemical based precipitation or ion exchange systems which are currently in use.

(http://www.sg.ohio-state.edu/Project/Projectrept.cfm?ProjectNumber=R/BT-4)

He again used the algae, *Chalmydomonas reinhardtii.* Which he knew was metal selective and robust to levels of toxicity that would be lethal to humans. However this time he also intended to utilise engineered and dried algae to increase binding.

His experimental strategy was:

- To further characterise the chemistry of the heavy metal binding sites of the algae
- Introduce genes and gene fusion's encoding heavy metal binding domains into the model transformable alga, *Chlamydomonas reinhardtii,* and
- Chemically cross-link and stabilise dried algal powders with enhanced heavy metal binding properties.

Ultimately, these "heavy metal sponges" will be used to bioremediate heavy metal contaminated sub-surface, surface, and waste stream sites, as well as recover heavy metals from aqueous waste effluents, basis, trace metal pollution is among the most pervasive and serious environmental problem facing the biosphere.

(http://www.sg.ohio-state.edu/Project/Projectrept.cfm?ProjectNumber=R/BT-4)

During Sayre first study the assumption could be made that metal uptake would take place through live algae and therefore application would be limited to effluent streams, contaminated water sources etc.

However with his new proposal "heavy metal sponges", the assumption can be made that this new biological treatment method could be more multipurpose

in its application and utilised in-situ or ex-situ and not limited to a water source.

Again Sayre points out why the biological route is more appropriate, living organisms have been shown to selectively sequester heavy metals by a variety of methods including: charged groups (carboxylic acid, amino and sulphate groups) and heavy metal binding proteins (e.g., metallothioneis). The algae have many features, which make them ideal candidates for the selective removal and concentration of heavy metals. Some of these features include:

- Tolerance to heavy metals [100 µM]
- Many can grow autotrophically as well as heterotrophically
- Large surface area/volume ratios
- Phototaxy (a useful feature for harvesting)
- Expression of phytochelatins (heavy metal binding peptides)
- High non-specific metal binding capacities
- They have the potential for genetic manipulation

(http://www.sg.ohio-state.edu/Project/Projectrept.cfm?ProjectNumber=R/BT-4)

The outcome of the study was that Satre demonstrated that he:

- Identified binding sites for cadmium, selenium, uranium, copper and chromate.
- It also was demonstrated that cadmium binding was reversible as a function of the pH.
- That the algae could be used numerous times (16 cycles of metal binding).

- That transformed algae are resistant to 5-times the lethal cadmium concentration.
- The cells expressing higher levels of cysteine bind 50% more metal at cadmium concentration that induce phytochelatin synthesis.
- We have characterised the chemistry of cadmium binding sites including bond distances and ligand identity.

Summary

In this study, though not fully complete Satre again proved that recovery of metals by a biological process could be a viable alternative than that of chemical separation.

He also backed up his initial study that engineered algae could be kind of a "super breed" that was more efficient in metal binding and could survive at higher levels of toxicity.

It also seems now that the pH may be critical to effect binding, however could metal release be due to the state of the metal (cadmium)in that it is soluble at low pH and will go into solution. Maybe a question to be asked does the algae excrete a chemical substance that could effect pH?

Also again there is evidence that ligand and the increased ligand activity is key to metal binding, was this increased ligand activity stimulated by the additional protein in the gene?

Also interesting dried algae could actually be more effective.

5.3 Phytoremediation of heavy metals using plant cells and organs

At the 14th Australasian Biotechnology conference in Adelaide Professor Pauline Doran gives more evaluation and support to why use plants to recovery heavy metals.

She indicates that certain plants in fact thrive on heavy metals and high levels of toxicity and therefore should be used to solve various pollution problems caused by mobile heavy metal.

What is of interest that she advocates harvesting these plants for disposal or recovery, this could have an application in the refining industry that the harvested metal could be directed to the smelter process .

Professor Doran writes; pollution of water and soil by heavy metals deposited in mining and industrial operations is an environmental problem in many areas around the world. Although most plant species cannot tolerate high concentrations of heavy metals, certain plants thrive under these conditions. Of those species that actively accumulate metals, some called "hyper-accumulators" store metals in their tissues at concentrations far exceeding those in the environment.

Hyper-accumulators of Ni, Co, Cu, Zn, Mn, Pb, Cd, Cr and Se have been described, and several hyper-accumulating species of Pb, Co and Ni are native to Australia.

Concentrations of metals found in these plants generally range from 1 to 5% measured on a dry weight basis; however levels above 10% have been found in particular organs of some species. Hyper-accumulator plants have

enormous potential for phytoremediation of contaminated land, being capable of translocating metal ions from the soil to the leaves, which can then be periodically harvested for disposal or metal recovery.
(http:www.biotech.unsw.edu.au/research3.htm)

Summary

This project funded by the Australian Research Council, where hyper-accumulating plant species are being studied to assess their potential to recover metal. Of note is the interest however in the kinetics of metal ion uptake under a range of environmental conditions, which they are also busy assessing.
Again one can see in this third case study that plants, be they land or aquatic variety have the potential to recover metal, however none of the aforementioned provided sites of practical application.

5.4 Binding Heavy Metals to Delicate Plants

At the Hebrew University's Faculty of Agriculture, Professor Elisha Tel-Or and a team of botanists have successfully utilised the aquatic Azolla ferns and water lilies to absorb heavy metals.
Both species are native to South Africa and can be found in the Highveld region and are therefore available for biological processing in that region. Even more interesting is the success of using Azolla to polish precious metal streams of effluent.

Professor Elisha Tel-Or again gives credibility to the endurance of plants in toxic conditions to recover heavy metals.

Azolla plants have a long life and will absorb copper, cadmium, zinc, chromium, and nickel at 500 times their concentration in common effluents. Professor Tel-Or found that because the heavy metals bind to the cell walls of the plant, Azolla can be equally effective when dried and pressed. This attribute makes it possible to transform the Azolla into a product – a biofilter – rather than use it as a living plant that must be nurtured and attended. Biofilters can also be "planted" anywhere, especially close to the source of a potential pollutant. This reduces the amount of effluents that must be treated to a minimum and optimises the efficiency of the biofilter, which can be targeted to treat one kind of metal, instead of a complex "chemical soup" of various compounds in a larger body of water.

(http://www.israel-embassy.org.uk/web/pages/greenfil.htm)

Again we see the ability to bind heavy metals is not removed by drying but it can be made more user friendly, therefore can be adapted to a variety of industrial applications.

Such biofilters are being tested in a number of industrial settings in Israel, including a nickel cadmium battery plant, a lead car battery factory and a plating facility using chromium. The same filters also proved effective in reclaiming gold in a jewellery-making establishment. Not only can the fern prevent pollution but when the Azolla is burned, its "naturally-enriched ash" contains six percent gold and four percent platinum, which can be retrieved.

(http://www.israel-embassy.org.uk/web/pages/greenfil.htm)

Of real interest that during the polishing of gold waste streams, platinum also attached to the Azolla that was probably present, has a trace element. This trace element could then be concentrated unfortunately there is no mention of point of saturation.

Professor Tel-Or also gave another example of the durability and success of Azolla.

Another potential use for Azolla biofilters is to strain out radioactive materials. Tests on radioactive uranium tracings in solutions originating in a nuclear research facility were 99% purified after being passed through an Azolla filter. The process is expected to significantly reduce the volume of radioactive wastes that must be stored. Moreover, radioactive isotopes can be recovered and "recycled" in this manner.

(http://www.israel-embassy.org.uk/web/pages/greenfil.htm)

Summary

From the information provided we can see the high potential of the fern Azolla to recover heavy metal in many industrial applications.

The durability is again proven and that there is no detrimental effect of binding when the Azolla is dried. In fact when the Azolla is dried it is in a form that can be easily managed i.e. Biofilters.

If platinum followed the gold during the process could other metals follow platinum if it was the main element or, would there be metal competition that would prevent binding?

The test work would verify if Azolla was metal specific or if it was capable of the extraction of a combination of pgm elements.

In the context of the need to research a biological means of recovering metals from platinum effluent streams, the following aims and objectives were formulated:

- To evaluate the weed Azolla as a biological agent to bind heavy metals.
- To evaluate changes in pH in affecting metal recovery.
- To evaluate changes in redox potential in affecting metal recovery.
- To compare sun dried Azolla and Azolla dried at 50^0C.
- To provide analytical data that would support conclusions made.

CHAPTER SIX: METHODS AND MATERIALS

6.1 Test Rig

A test rig was designed and built, which consisted of a 100 litre glass storage tank, which then allowed effluent to gravity feed through a 20 mm PVC ball valve into a 20 litre glass Feed tank. All fed lines were constructed of 20mm PVC diameter piping. Effluent was then again gravity feed directly to the pump (Make EHEIM type 3250 (220v), flow rate 23 litre per minute). The effluent was then pumped through a 20mm glass screw valve (used to control flow rate) installed at the bottom of a 400m x 25mm glass column, which was used to hold the Azolla, glass wool was packed at the end of each column to prevent solid by-pass of Azolla. A 10mm plastic hose was at the top of the Azolla column and the circulated effluent was discharged into glass measuring beakers (Appendix G). Samples were than taken at the relevant intervals.

The up-flow was to avoid channelling of liquor through the Azolla and to ensure maximum surface contact.

The initial construction was later changed after the second test run to incorporate a second column. 20mm PVC was used to construct the link from the first column to the base of the second column again to ensure up-flow. (Figure 6.1 and 6.2)

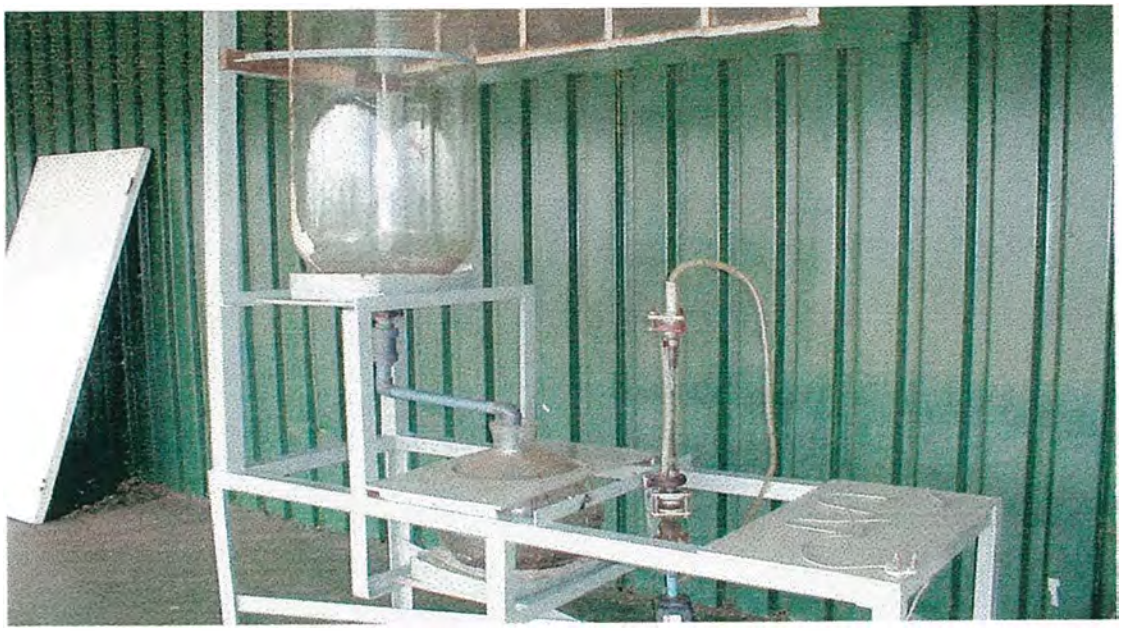


Figure 6.1 Single column construction utilised during Runs 1 and 2.

Figure 6.2 Double column construction utilised for Runs 3 to 7.

6.2 Inductively Coupled Plasma Emission Spectrophotometer (ICP)

Measurement of any project and the ability to provide analytical data that is reliable are essential. Without reliable data there can be no reasoned conclusion, one of the most efficient instruments utilised in the pgm industry is the ICP.

Elemental concentrations can be measured to ppb (parts per billion) levels using the Inductively Coupled Plasma Emission Spectrophotometer (ICP). The ICP spectrophotometer utilises plasma to excite elemental electrons, which produce photons unique to each element. An example of this procedure is one used for a whole rock analysis. In this procedure a lithium meta-borate flux is generally used to digest the specimen. Once digested, the solution is introduced to the plasma allowing elemental concentration comparisons to known concentration curves. Using stoichiometric techniques elemental concentrations can be converted into molecular weight percentages. An inductively coupled plasma source atomises and excites even the most refractory elements with high efficiency. With this ICP, several elements can be determined simultaneously without the need for repeated aspirations, adjustment of instrument parameters and tracking of the samples.

(http://www.imp.mtu.edu/matchar/Induc_Coup_Plas.html)

The ability to provide reliable results of numerous elements from a single solution with a quick turn around time is essential in pgm refining, thus the ICP is one of the most appropriate instruments.

6.3 Initial preparation of Azolla

Azolla was first recovered from a local water source (Blesbokspruit). It was then added into a tank containing normal municipal water and placed in direct sunlight to encourage growth.

Quantities were then recovered and separated to provide (A) Sun dried Azolla, which was first washed with de-mineralised water to remove any foreign objects and (B) Azolla tried at 50^0C, which was then screened to remove any husks and large particles.

During this stage test were undertaken to assess moisture content and relative density of the "Blesbokspruit" Azolla (Appendix D).

6.4 Azolla standard preparation by Microwave Technology

Azolla being an organic product, complete dissolution by conventional analytical techniques is difficult.

Most techniques require the removal of organic material by ashing at elevated temperatures in an oxidising atmosphere using a muffle or by " wet ashing" using mixtures of perchloric acid, sulphuric acid and nitric acid. This normally results in losses of certain analytes by volatilisation and the formation of insoluble compounds.

Wet ashing, which is preferable for organic samples, is an extremely dangerous technique, which requires expertise and knowledge of the reactions taking place. Perchlorates being highly explosive if not carefully controlled.

The introduction of microwave technology has proved to be highly successful in the field of cationic analysis of organic material (coal, dairy products, plastics, flora analysis etc.).

The advantages of sample preparation by microwave technology are:

1 short dissolving period (4 to 6 orders quicker)

2 complete destruction of organic matter

3 retention of analytes of interest (volatiles)

4 avoids contamination

5 simplifies matrix requirements for instrumental analysis

6 sealed system allows for attainment of high temperatures (200 degree centigrade)

7 No acid fumes to contend with

8 sealed system allows for achieving elevated pressures (30 bar) therefore achieving temperatures higher than atmospheric boiling points

9 small amounts of reagents used

However one problem is the limited sample size for microwave preparation.

The method developed for the dissolution of Azolla for evaluation by ICP techniques are:

1 Samples received were transferred to a 200-ml porcelain-evaporating dish and allowed to dry at 50 degree Centigrade in an oven. (Mass loss in the region of 75 %). This step acts as a pre-concentration method for lowering the limits of quantitative determination by ICP.

2 The dried samples were crushed in a mortar and pestle and passed through a 600 um screen for analytical fines and homogeneity.

3 Samples were weighed out in duplicate (0.3 gm each) and transferred to PTFE pressure vessels, 1.5-ml de-mineralised water added as a wetting agent, followed by 10-ml conc. Nitric acid and 5ml of 30 % hydrogen peroxide.

4 Pressure vessels were sealed, positioned into the rotor body and transferred to the microwave for treatment.

5 The program selected and commenced were:

2 minutes at 300 watts

2 minutes at 0 watts

6 minutes at 400 watts

2 minutes at 0 watts

5 minutes at 600 watts

3 minutes at 0 watts

4 minutes at 800 watts

10 minutes venting

6 The cooled pressure vessels were removed and carefully opened.

7 The duplicate samples were combined in a 100-ml squat beaker and carefully evaporated down to approximately 30-ml. and cooled.

8 50-ml volumetric flasks were prepared with 2.5-ml of 5 gm / litre Indium solution.

9 The sample contained in the 100-ml squat beaker were transferred to the volumetric flask and made up to volume with de-mineralised water for ICP evaluation.

Six standards were prepared for ICP using the microwave technique and Azolla as the matrix.

A stock solution containing Al, As, Cu, Au, Ir, Fe, Pb, Ni, Pd, Ag, Pt, Rh, Ru, Se, Te, and Zn were made up and aliquots spiked into the Azolla prior to microwaving (at concentrations of 50ppm and 200ppm).

The microwave parameters used were the same as for the sample preparation.

(R Breckenridge, Chief Research Scientist, Western Platinum Refinery, Pers. Comm)

6.5 ICP standard preparation for analysis

Quantitative analysis associates the emitted energy with the number of atoms in the sample. Atomic emission is not an absolute method, because the relationship between the emitted intensity from a line and the concentration of the associated elements must be calculated, and this determines the lines calibration curve.

The advantage of ICP atomic emission spectrometry is that in most cases these calibration curves will be linear over several orders of concentration. Great care is taken in the construction of calibration curves, as these will determine the accuracy of the analysis. The curves are constructed by the measurement of standards.

These standards are samples whose matrix is close to that of the samples to be analysed and the concentrations in elements to be analysed are known. The selection of concentration in standards is essential to obtain accurate results. All the concentrations of the elements to be analysed from unknown samples should be within the concentration range of the standards used.

(Appendix E). At the end of the phase of measurement of standards (calibration) linear regression calculations is performed for each element as shown also in (Appendix E). An intensity vs. concentration analytical curve is determined. The points of the calibration for the calibration of the regression are, for each element, the concentration of the standards given by the user and the intensities measured.

A curve of the first order is determined as follows:

Concentration = a x intensity + b

If there is a sufficient number of standards (at least three), a curve of the second order can be calculated:

Concentration = a x $(\text{intensity})^2$ + b x intensity + c

The internal standard used (Indium) to improve the precision of the results. An internal standard is an element that is added in equal amounts in the calibration solutions and in the analytical samples.

Fluctuations due to temperature variations, generator efficiency, gasses sample take-up, etc. may produce variations in the intensity of elements measured, but will do so to the same degree for the internal standard. Consequently, the ratio of intensities will remain relatively constant.

The results were obtained by using the Jobin Yvon ICP. The optical system of JY employs a holographic grating in its monochromator. The length of the monochromator is one meter thereby providing unrivalled resolution. It is the

biggest grating of all spectrometers available. The quality of the analytical results together with the background equivalent concentration and the limit of detection are directly dependent on the amount of light that falls on the detector. This light throughput is a direct function of grating size. The grating of the JY spectrometer is thermally controlled thereby having negligible thermal expansion and zero distortion on the surface.

6.6 Sampling Procedure and Analytical Reporting

All effluent utilised in tests were first filtered through 0.5-micron filter cartridge to remove any suspended solids. This was done to ensure that samples taken would be free of any solids that could affect analysis of liquor and in determining the binding effects of Azolla.

A Head sample was taken before each test (run) to determine value.

All samples were taken in a new and clean sample bottle to prevent contamination.

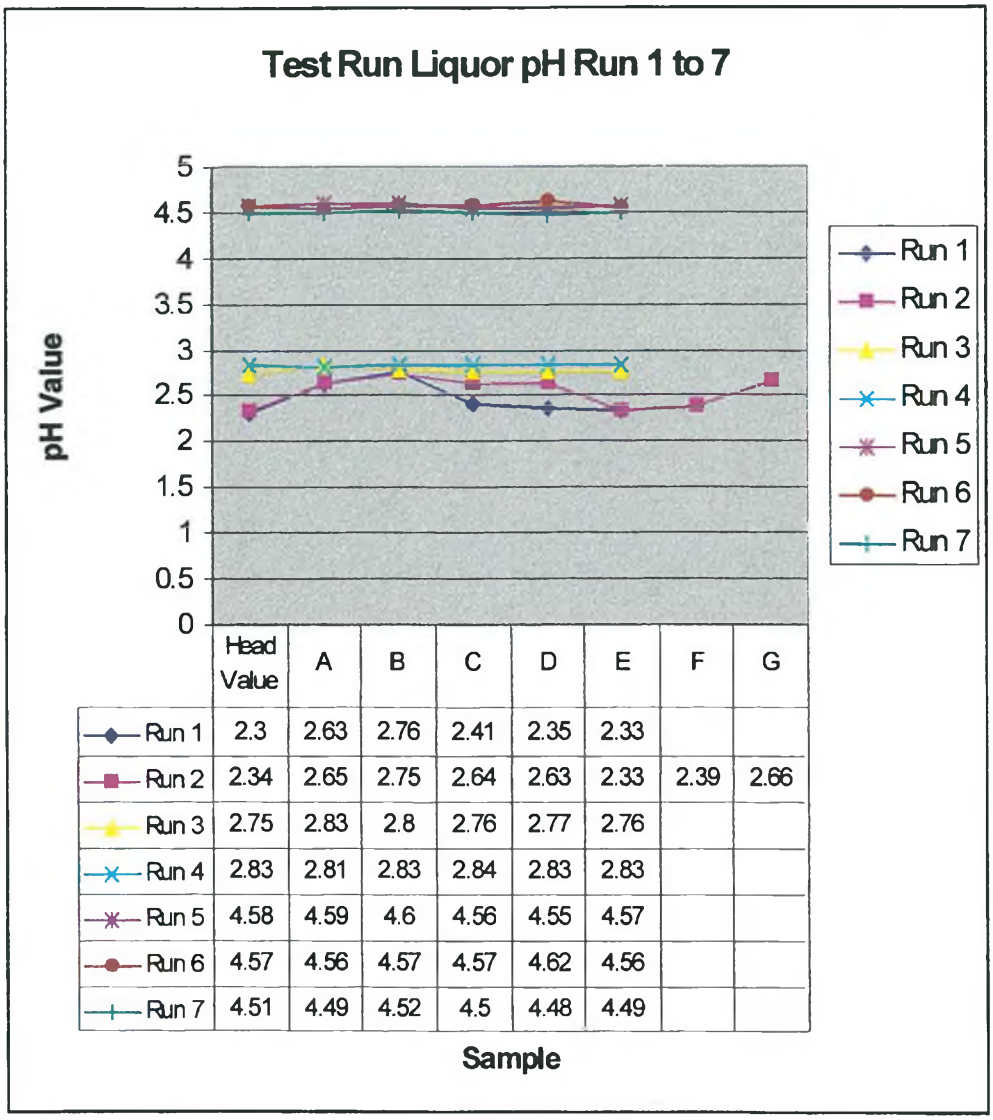
All analytical results for all runs are in graphical presentation and observations are made for each individual run. The elements reported have been separated due to value differences and scale. Fe is on separate graph due to scale where relevant. The term head value was used to describe initial metal values before treatment.

Redox potential and pH were taken of all samples. (Figure 6.3, 6.4)

Measurements were taken at all sample points and have been tabulated to make comparisons.

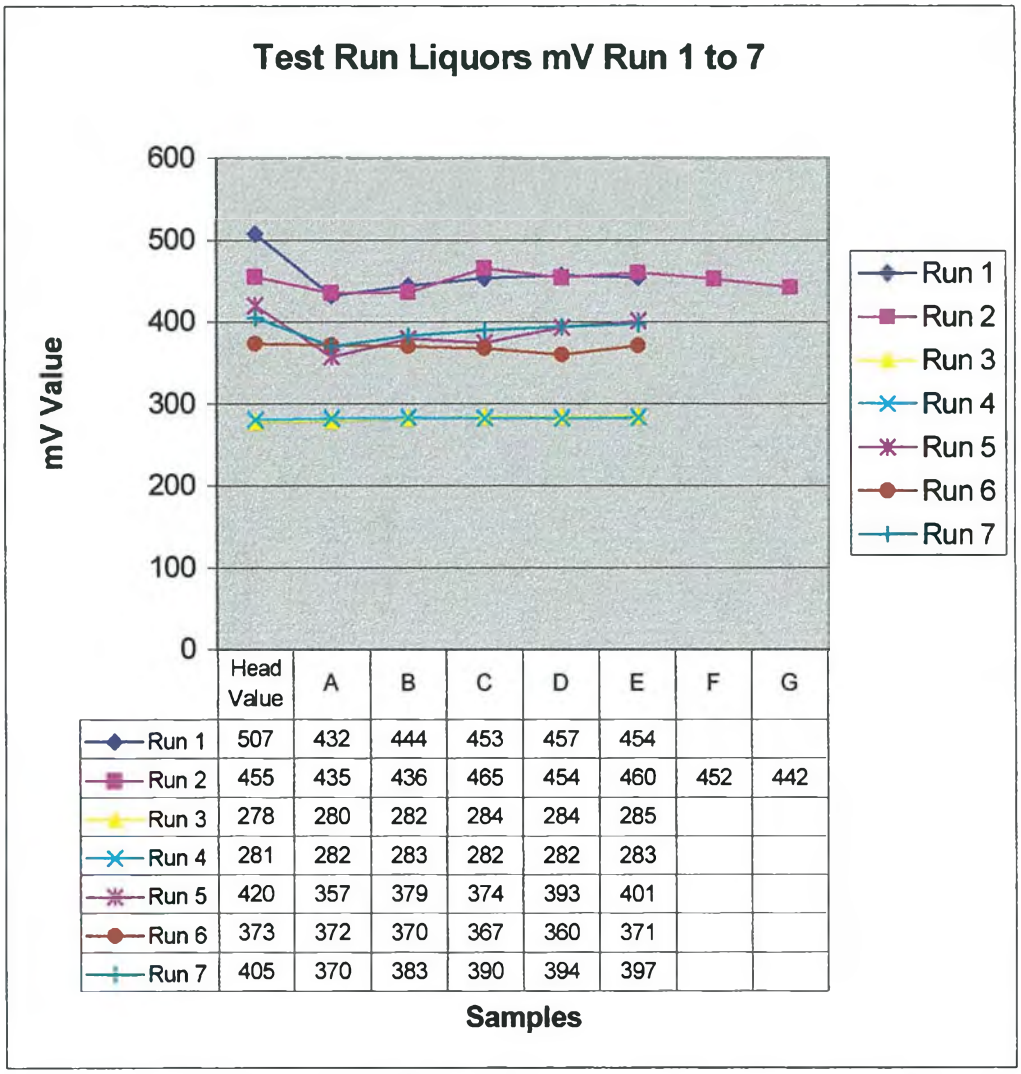
The relevance of fluctuations is discussed in final conclusion.

All test results including pH and redox potentials have been tabulated for each test run (Appendix F).



	Head Value	A	B	C	D	E	F	G
Run 1	2.3	2.63	2.76	2.41	2.35	2.33		
Run 2	2.34	2.65	2.75	2.64	2.63	2.33	2.39	2.66
Run 3	2.75	2.83	2.8	2.76	2.77	2.76		
Run 4	2.83	2.81	2.83	2.84	2.83	2.83		
Run 5	4.58	4.59	4.6	4.56	4.55	4.57		
Run 6	4.57	4.56	4.57	4.57	4.62	4.56		
Run 7	4.51	4.49	4.52	4.5	4.48	4.49		

Figure 6.3 Most of the runs appear fairly stable the biggest fluctuations appear in run one and two.



	Head Value	A	B	C	D	E	F	G
Run 1	507	432	444	453	457	454		
Run 2	455	435	436	465	454	460	452	442
Run 3	278	280	282	284	284	285		
Run 4	281	282	283	282	282	283		
Run 5	420	357	379	374	393	401		
Run 6	373	372	370	367	360	371		
Run 7	405	370	383	390	394	397		

Figure 6.4 Changes in redox potential can be observed during most runs with runs 3 and 4 being the least effected.

CHAPTER SEVEN: TEST RUN 1. RESULTS

7.1 Test Run 1 using single column and 50^0 C dried Azolla

Ten grams of Azolla were loaded into the column on commencing the test.

A sample was taken to determine the head value of liquor before commencing.

A sample of Azolla was also taken to provide a head value prior to commencing the run.

The initial flow rate was 500ml per hour, which then slowed to 300ml per hour.

The restriction in the flow rate resulted in stopping the test, opening the column in an attempt to loosen the Azolla.

The test run then continued with significantly better results and the test work flow rate was completed at 1800ml per hour.

During the run, five liquor samples were taken every 500ml and analysed. (Figure 7.1, 7.2, 7.3)

The Azolla was then unloaded from the column, this was then sent for analysis. (Figure 7.4, 7.5,7.6)

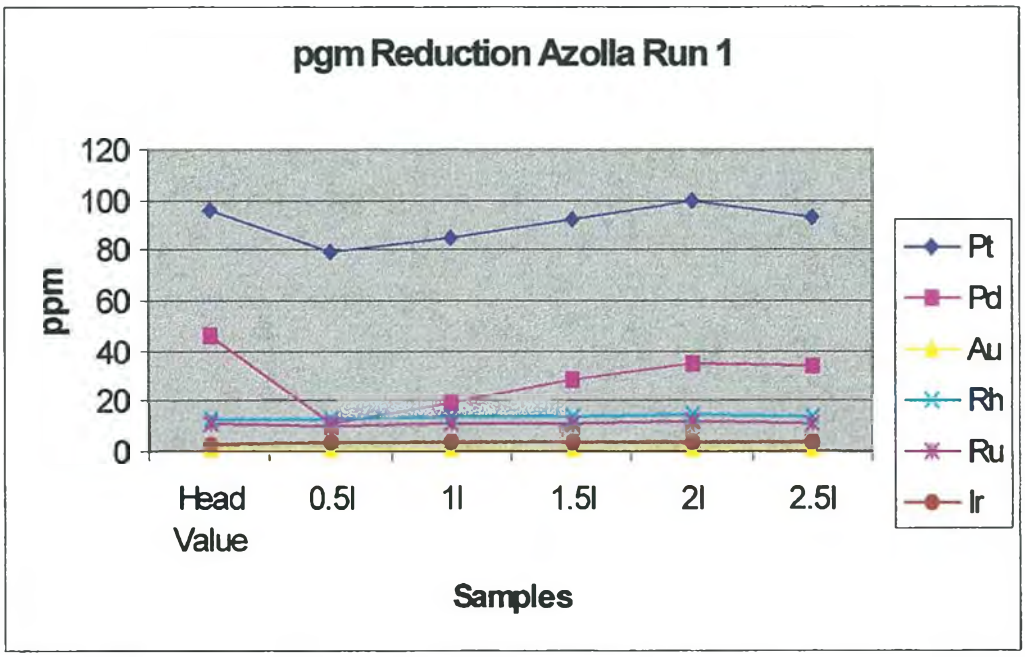


Figure 7.1 Showing initial effluent head value for pgm resulting in an reduction of Pt and Pd from effluent at first sample, thereafter a re-release.

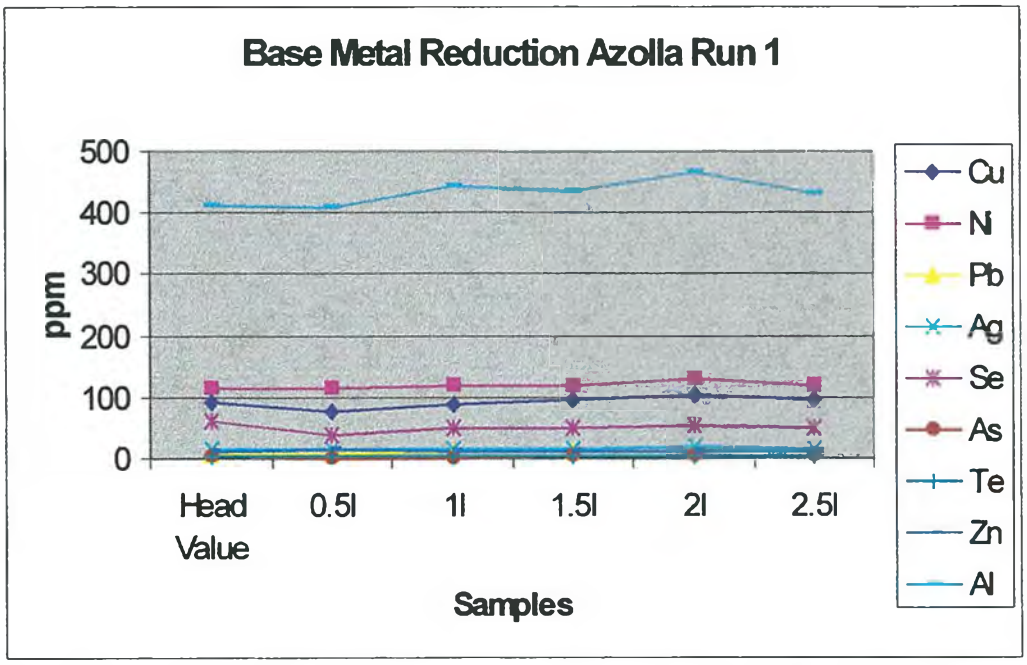


Figure 7.2 Showing effluent head value for base metal resulting in an initial reduction of Cu and Se from effluent at first sample, thereafter a re-release.

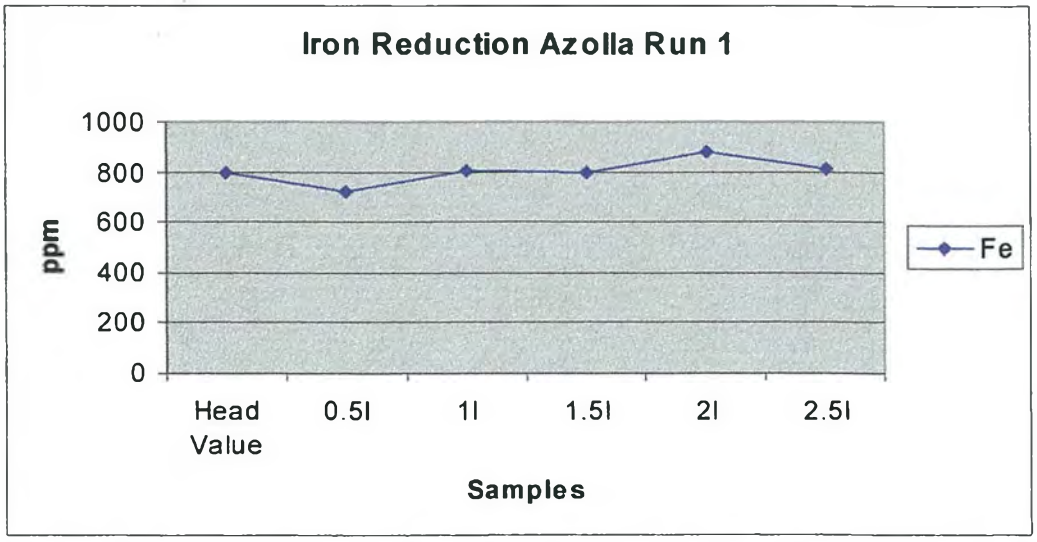
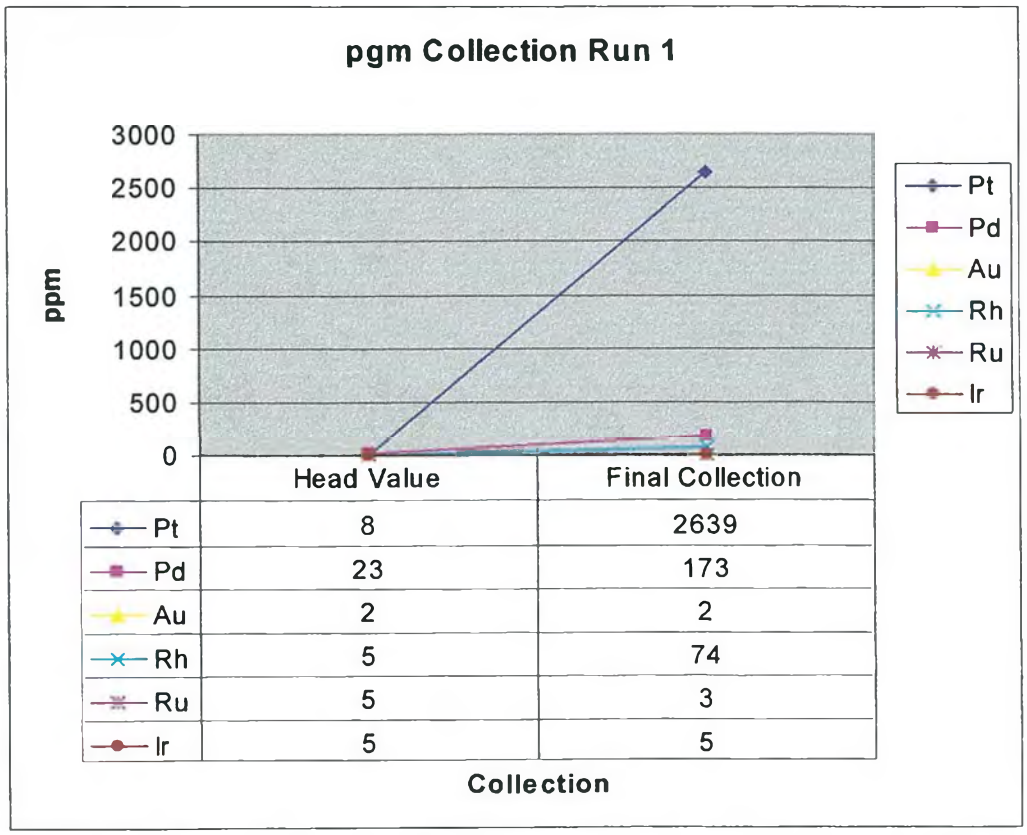


Figure 7.3 Showing initial effluent head value for Fe resulting in an initial reduction of Fe from effluent at first sample, then higher concentrations due to possible Azolla saturation.



	Head Value	Final Collection
Pt	8	2639
Pd	23	173
Au	2	2
Rh	5	74
Ru	5	3
Ir	5	5

Figure 7.4 Showing significant collection of Pt from levels detected at analysis of head value.

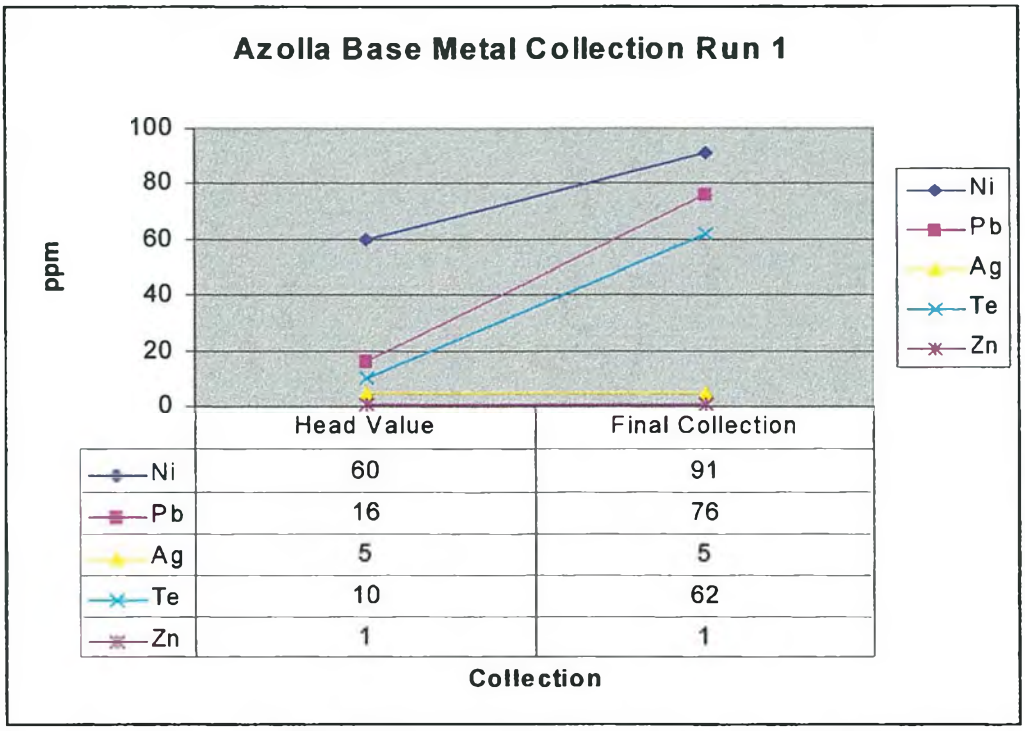


Figure 7.5 Showing Base Metal head value and final metal collection.

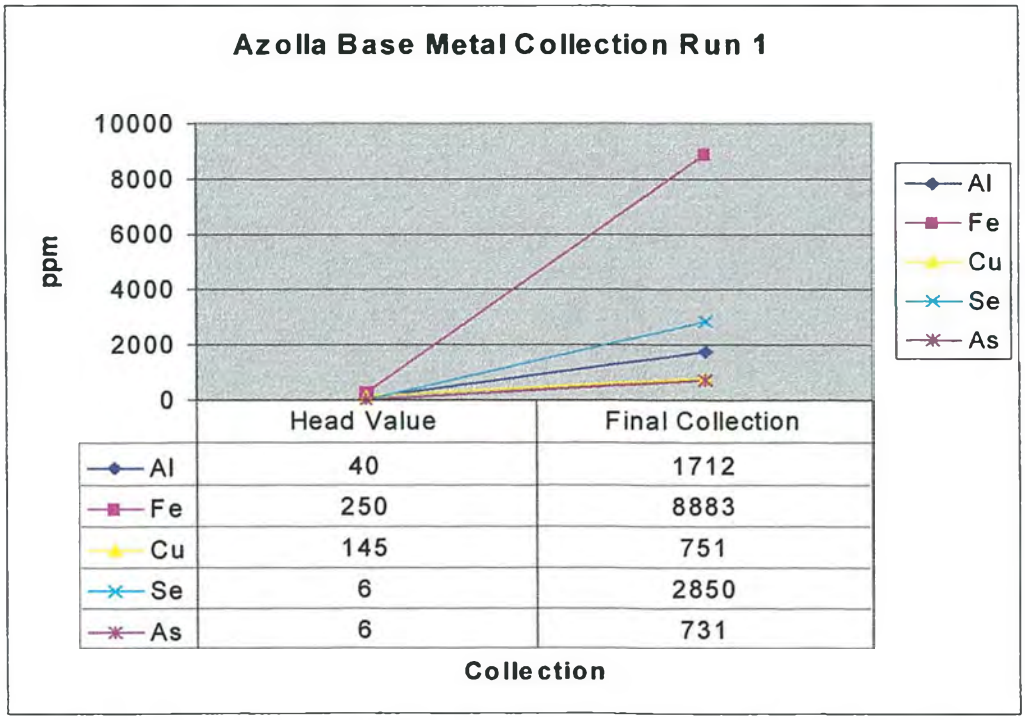


Figure 7.6 Showing initial value of Azolla and significant final Fe and Se collection.

CHAPTER EIGHT: TEST RUN 2. RESULTS

8.1 Test Run 2 using single column and 50^0 C dried Azolla with de-mineralised water addition

On run 1 ten grams of Azolla were loaded into the column on commencing the test. However due to problems experienced during test run 1 it was decided to add de-mineralised water to the Azolla prior to the loading to allow for expansion.

A decision was also taken to take a sample based on time not volume due to problems experienced previously with the flow rate (15minutes intervals).

During this tests seven samples were taken (Figure 8.1, 8.2, 8.3).

This was done to see if saturation of the Azolla was time dependent.

The flow rate of this run was maintained at 1.5 litres per hour.

A sample was taken to determine the head value of liquor before commencing.

The same Azolla was utilised as that in run 1 therefore the head value is the same as run 1. (Figure 8.4, 8.5, 8.6).

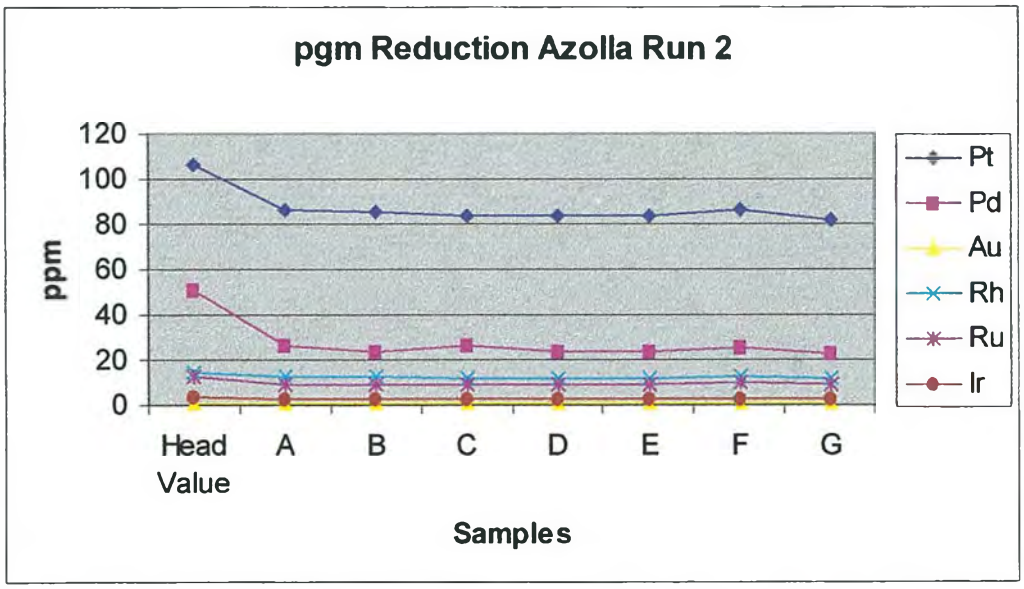


Figure 8.1 Showing liquor head value for pgm resulting in significant reduction of Pt and Pd and thereafter an almost constant level of removal. (Pt 106ppm to 82ppm, Pd 51ppm to 30ppm).

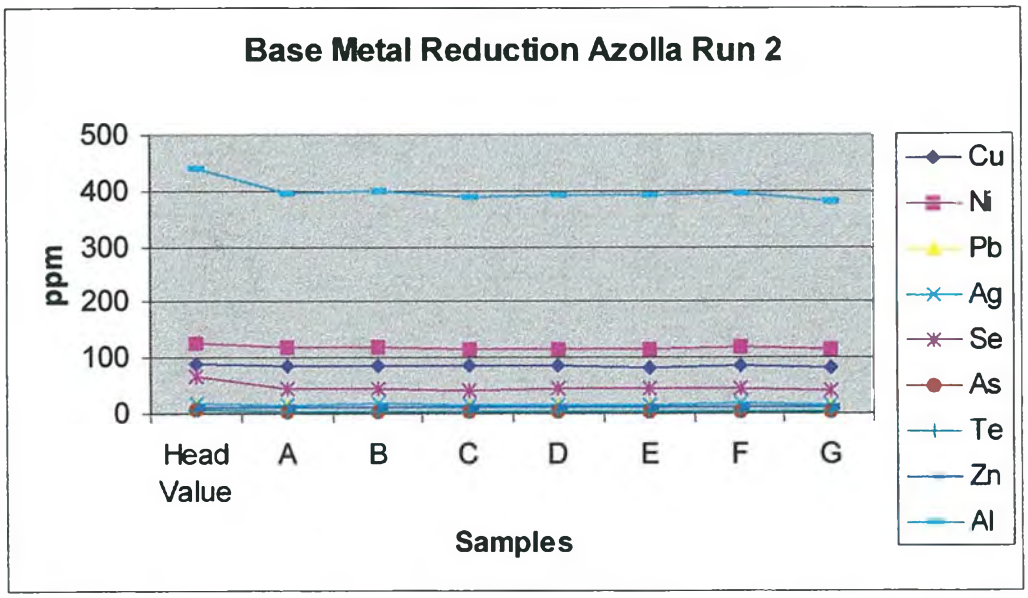


Figure 8.2 Showing liquor head value for base metal resulting in good reduction of Al (441ppm to 380ppm), Se (69ppm to 46ppm) and Te (6ppm to 2ppm).

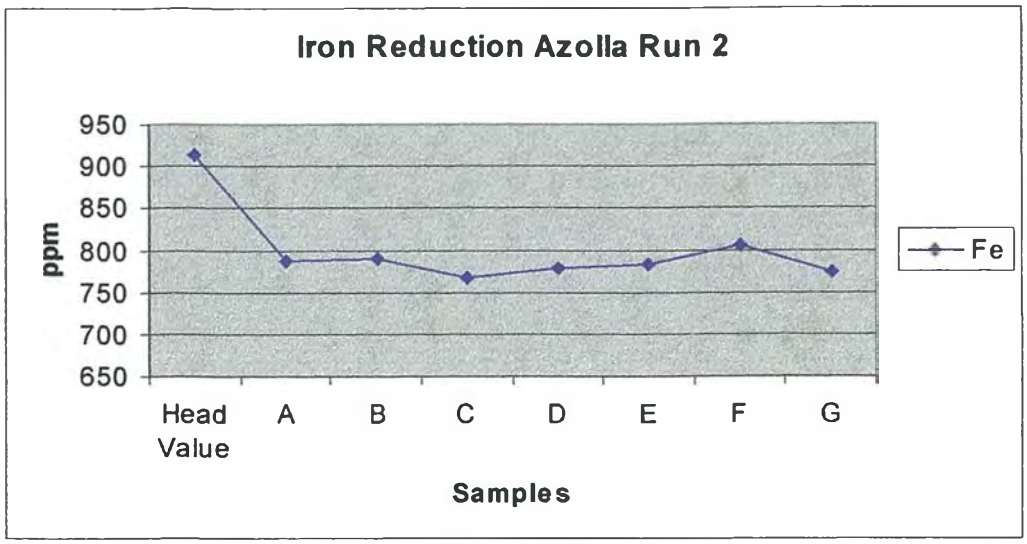
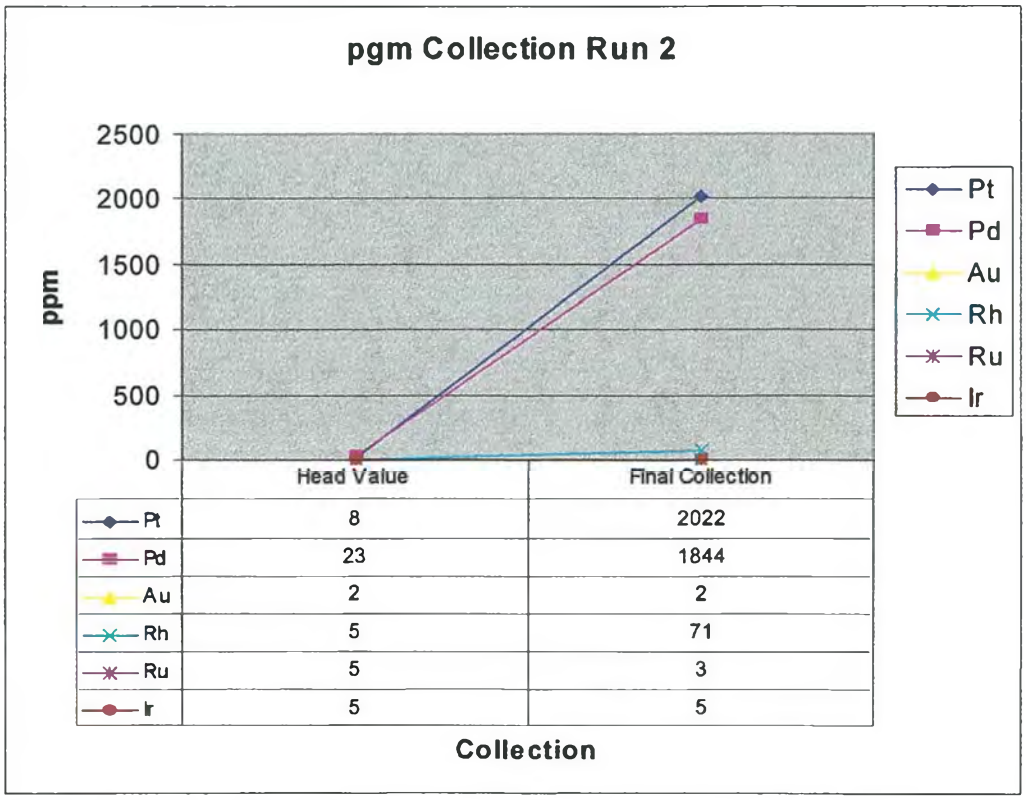


Figure 8.3 Showing liquor head value for Fe resulting in good reduction of Fe from effluent, which shows an almost constant removal.



	Head Value	Final Collection
Pt	8	2022
Pd	23	1844
Au	2	2
Rh	5	71
Ru	5	3
Ir	5	5

Figure 8.4 Showing Azolla head value and significant collection of Pt, Pd and Rh, verifies the almost constant level of pgm removal seen in Figure 9.1.

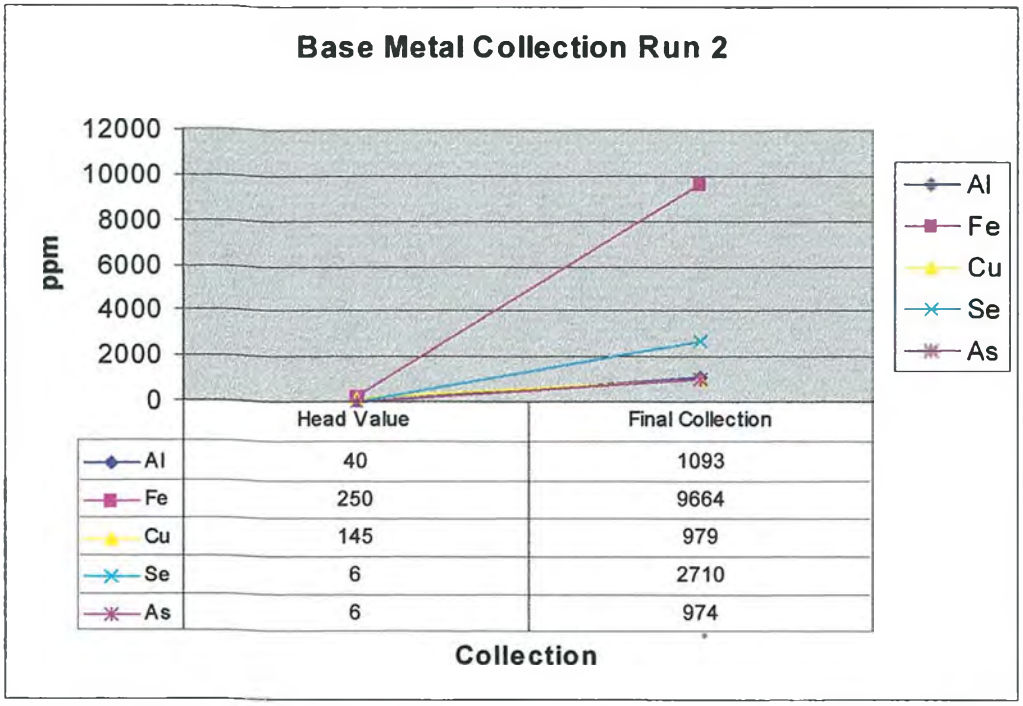


Figure 8.5 Showing Azolla head value and showing excellent collection of Fe, Se and Al.

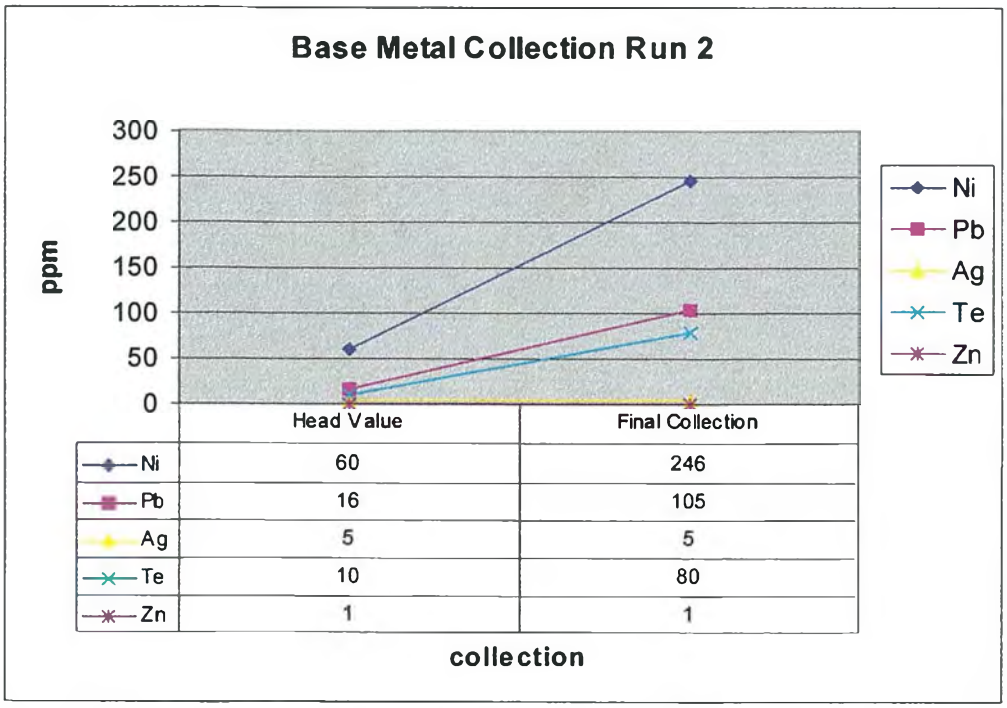


Figure 8.6 Showing Azolla head value and showing excellent collection of base metal excluding Ag.

CHAPTER NINE: TEST RUN 3. RESULTS

9.1 Test Run 3 using double column and Sun dried Azolla

Before commencing run 3, ten grams of Azolla were loaded into each column. Five samples were taken during this test run at an interval of a litre, the flow rate was increased to that of previous runs and was set at approximately one litre per ten minutes (100ml per minute). This flow rate proved difficult to control again due to channelling the actual flow rate varied between samples B to E.

A sample was taken to determine the head value of liquor before commencing. (Figure 9.1, 9.2, 9.3).

Low pgm value liquor was utilised in this test (Pt 17ppm).

A sample of the sun dried Azolla was taken to determine the head value. (Figure 9.4, 9.5, 9.6).

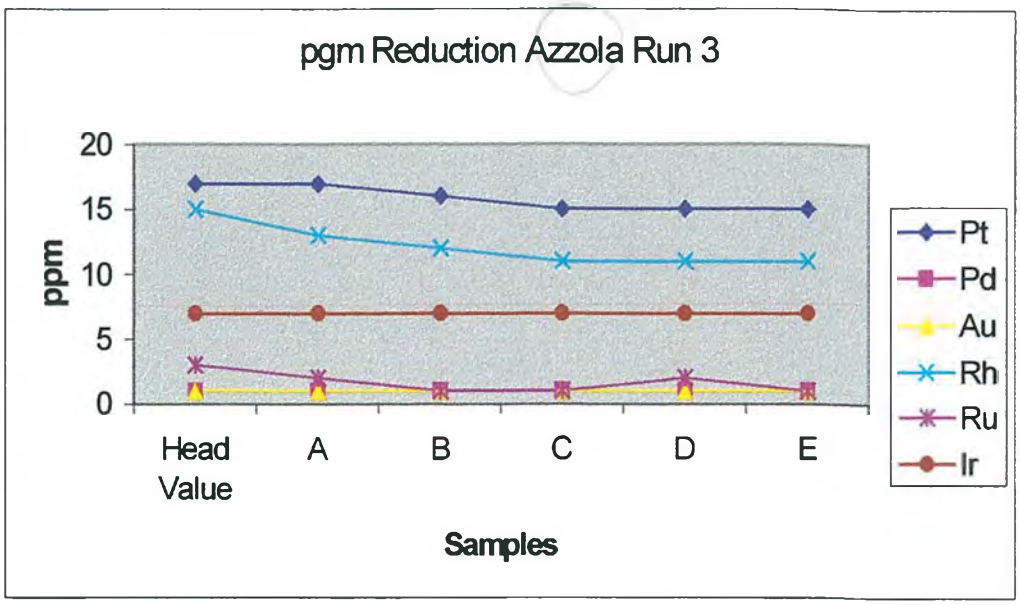


Figure 9.1 Showing liquor head value for pgm resulting in good removal in particular for Rh (15ppm to 11ppm) and Ru (3ppm to 1ppm).

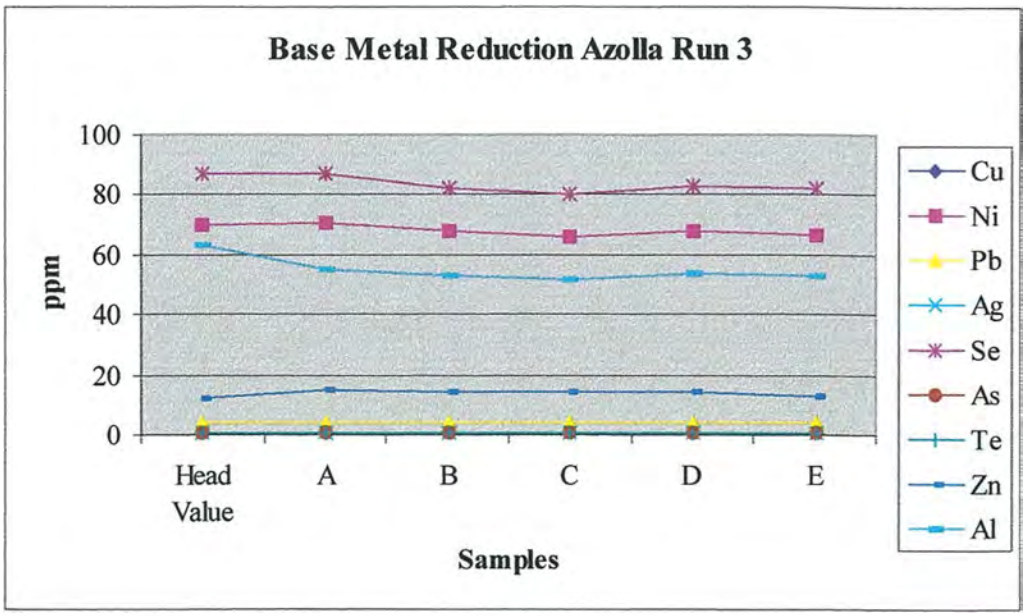


Figure 9.2 Showing liquor head value for base metal resulting in a reduction of base metal value however not so significant. The highest reduction was that of Al (63ppm to 50ppm).

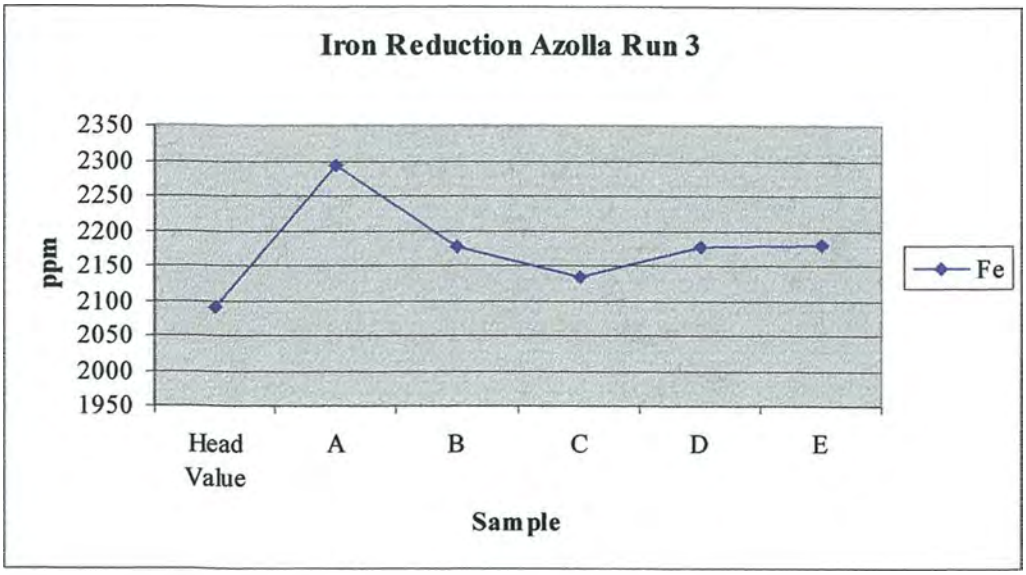
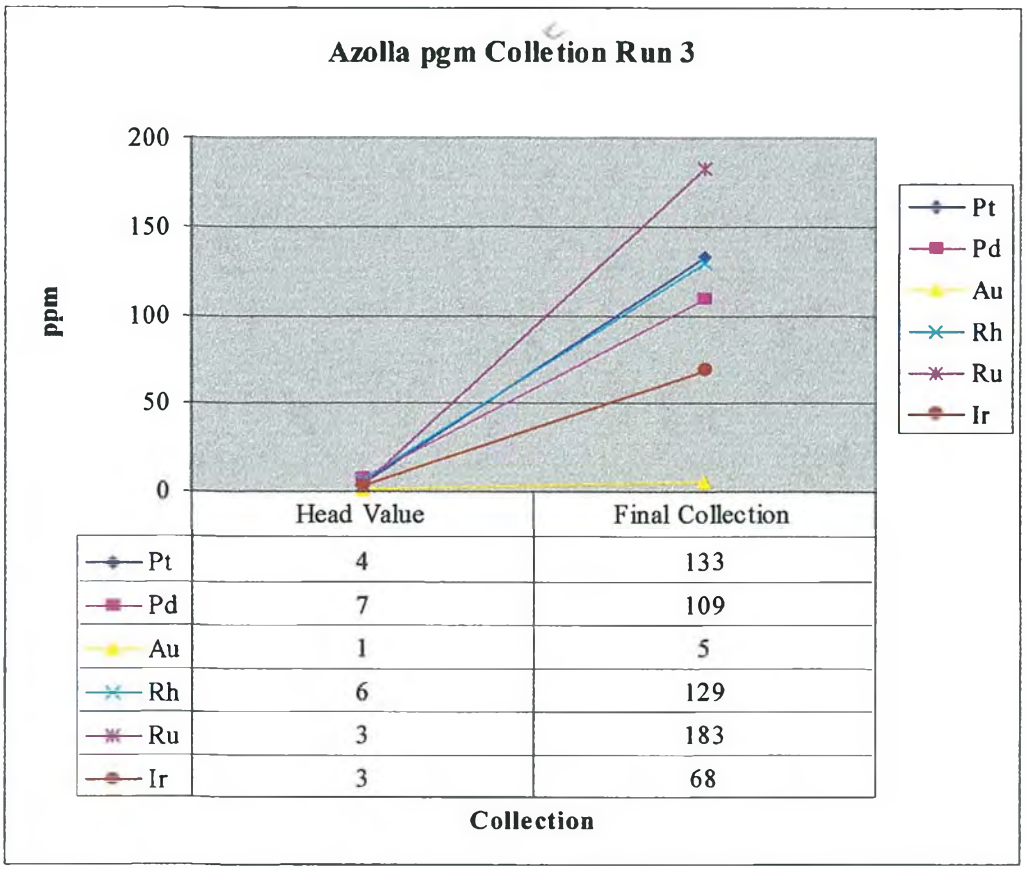


Figure 9.3 Showing liquor head value for Fe resulting in a significant Fe increase in value, which may be the result of leaching of Fe contained in the Azolla.



	Head Value	Final Collection
Pt	4	133
Pd	7	109
Au	1	5
Rh	6	129
Ru	3	183
Ir	3	68

Figure 9.4 Showing Azolla head value and significant collection of all pgm's.

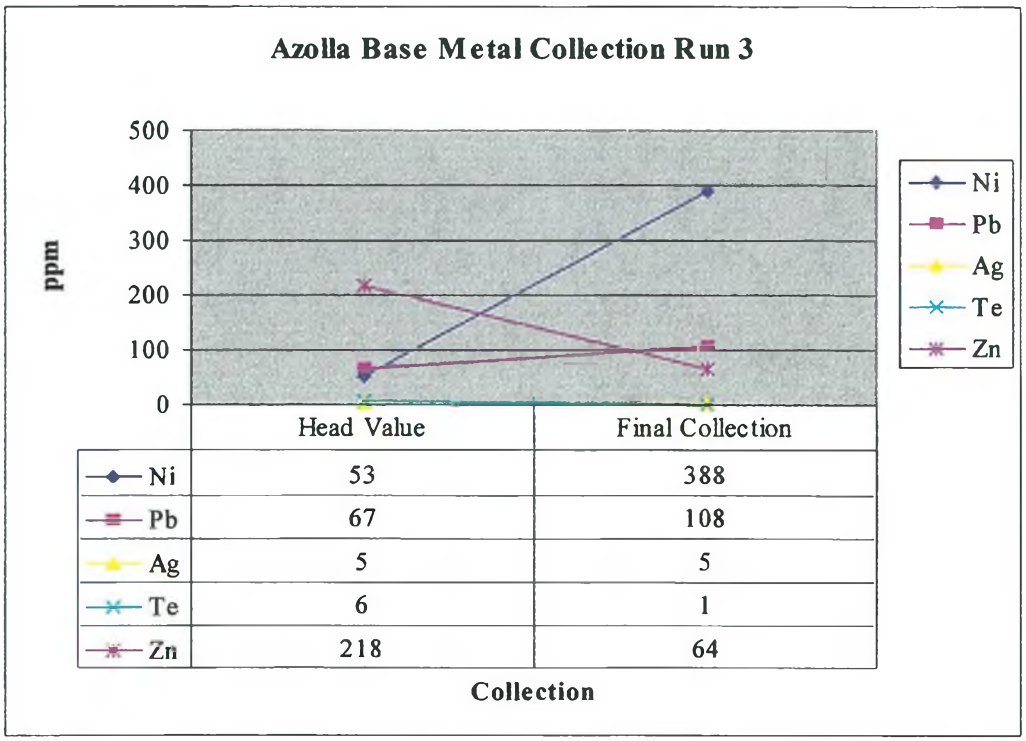


Figure 9.5 Showing Azolla head value and showing poor collection of Te and Al during this run.

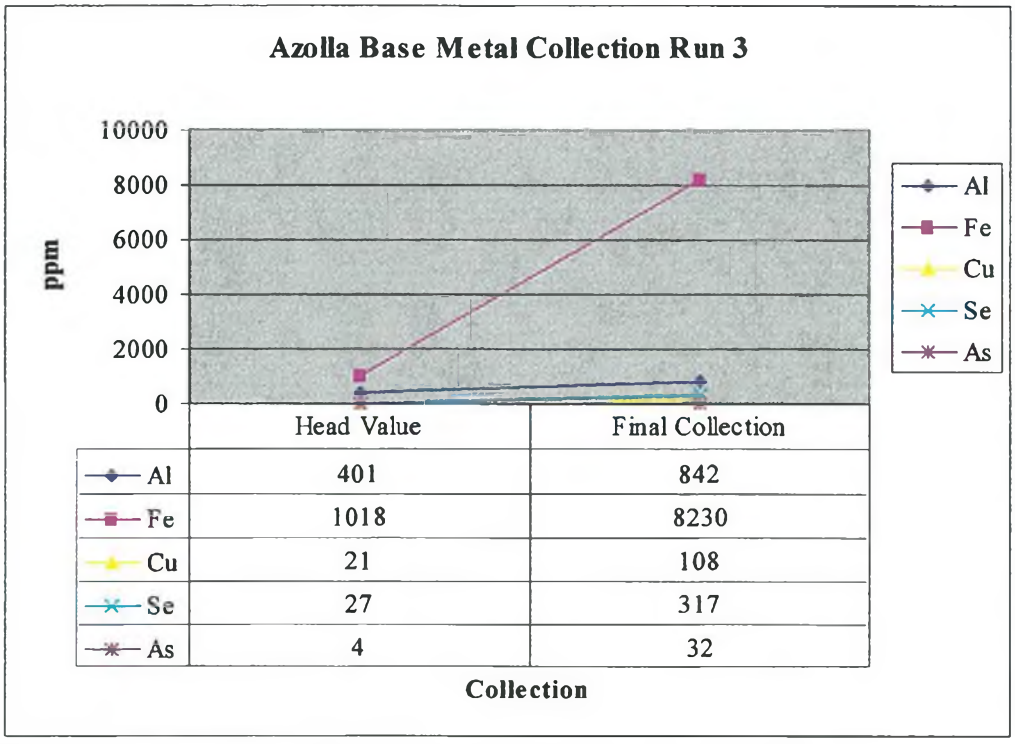


Figure 9.6 Showing Azolla head value and excellent Fe collection.

CHAPTER TEN: TEST RUN 4. RESULTS

10.1 Test Run 4 using double column and Sun dried Azolla

Same as test run 3, ten grams of sun dried Azolla was loaded into each column. Five samples were again taken during this test run at an interval of a litre, the flow rate on this occasion was reduced to one litre per twenty minutes (50ml per minute).

The same liquor that was circulated through test run 3 with now slightly less head value was utilised in this test to determine if the new Azolla would effect the liquor further. (Figure 10.1, 10.2, 10.3).

The head value for Azolla was the same used in test run 3. (Figure 10.4, 10.5, 10.6).

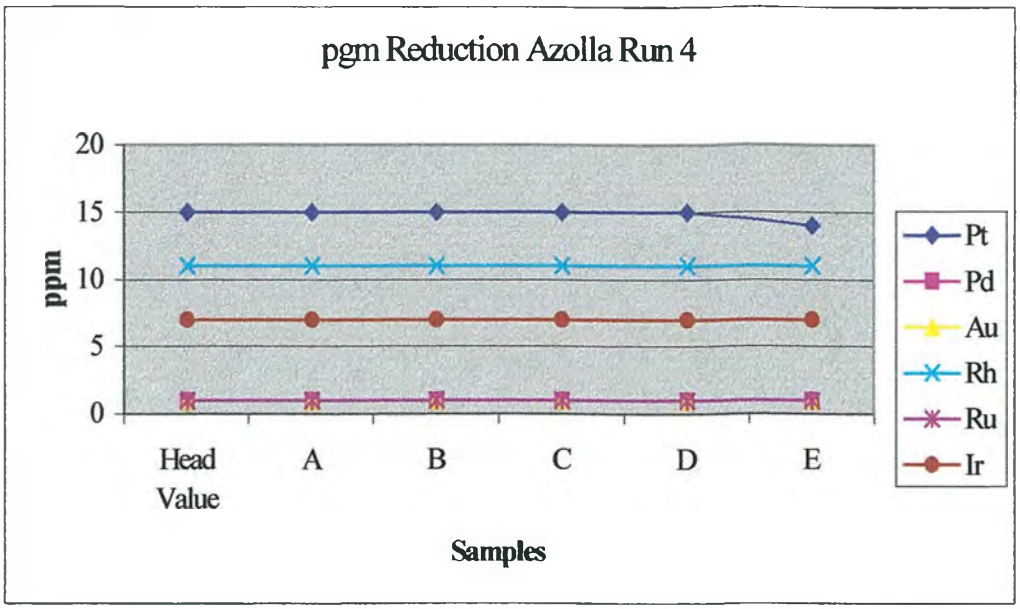


Figure 10.1 Showing liquor head value for pgm resulting almost zero extraction other than 1ppm removal of platinum.

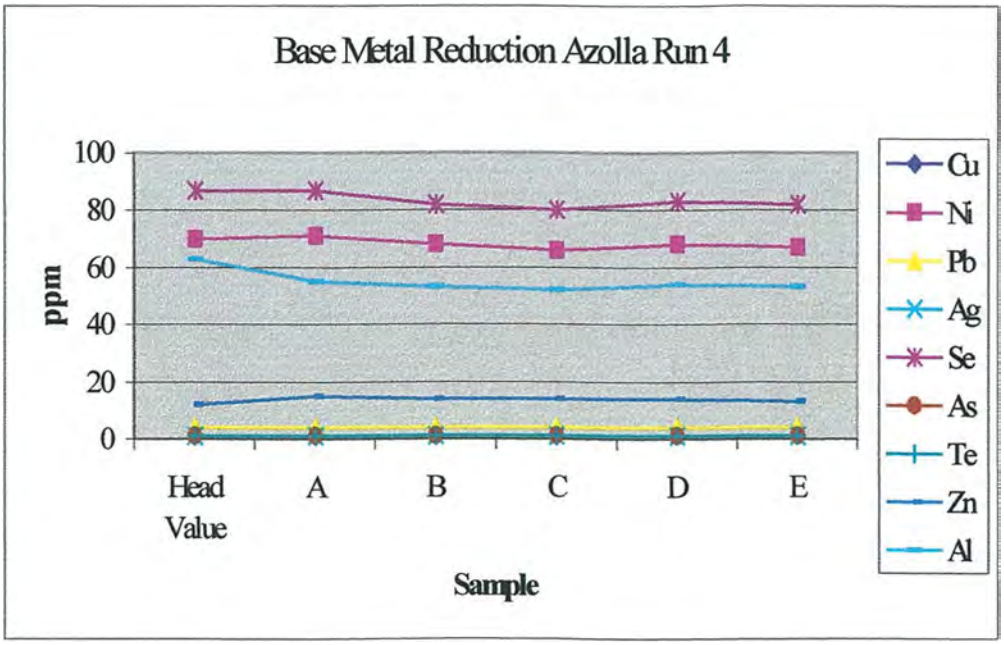


Figure 10.2 Showing liquor head value for base metal and we witness the opposite of the pgm scenario, base metal collection continued.

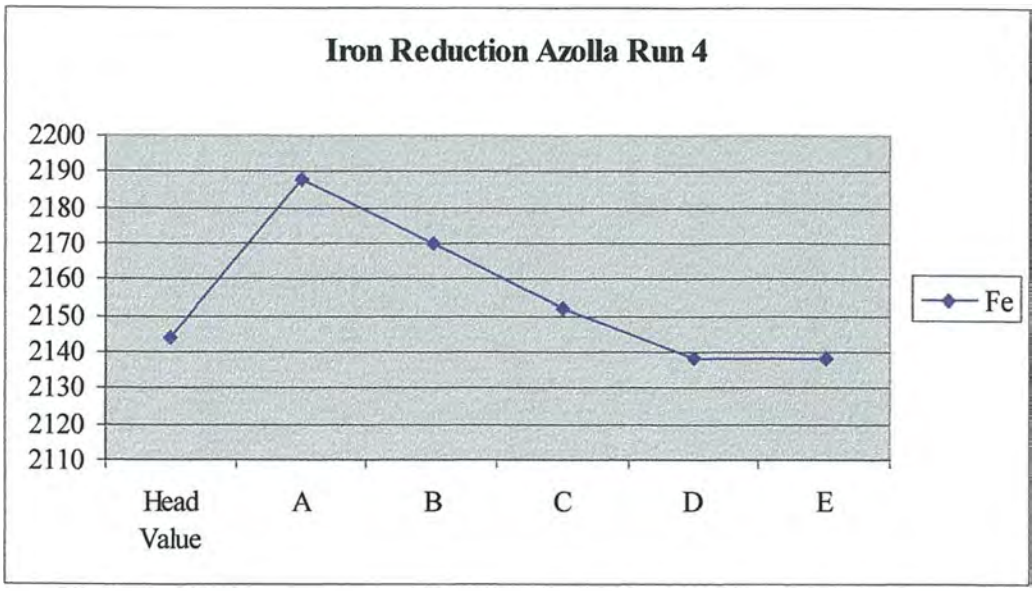
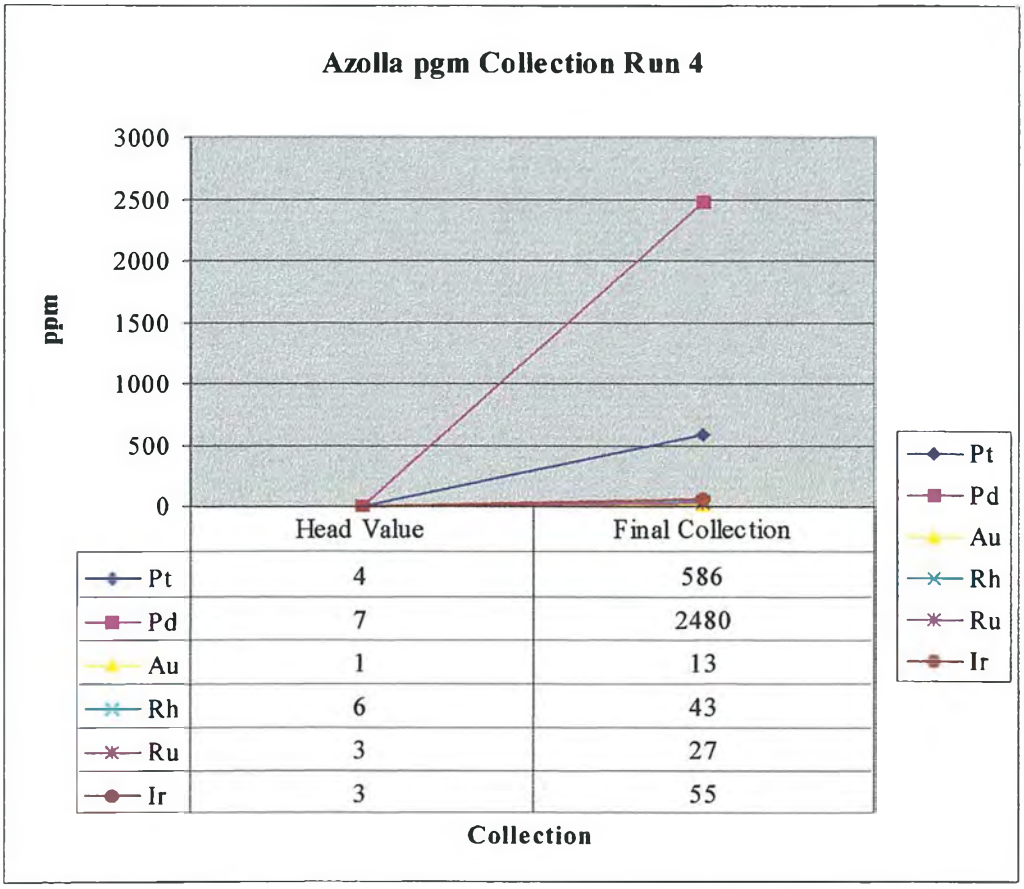


Figure 10.3 Showing liquor head value for Fe resulting in an initial release of Fe, then an almost linear uptake.



	Head Value	Final Collection
Pt	4	586
Pd	7	2480
Au	1	13
Rh	6	43
Ru	3	27
Ir	3	55

Figure 10.4 Showing Azolla head value and significant collection of Pd.

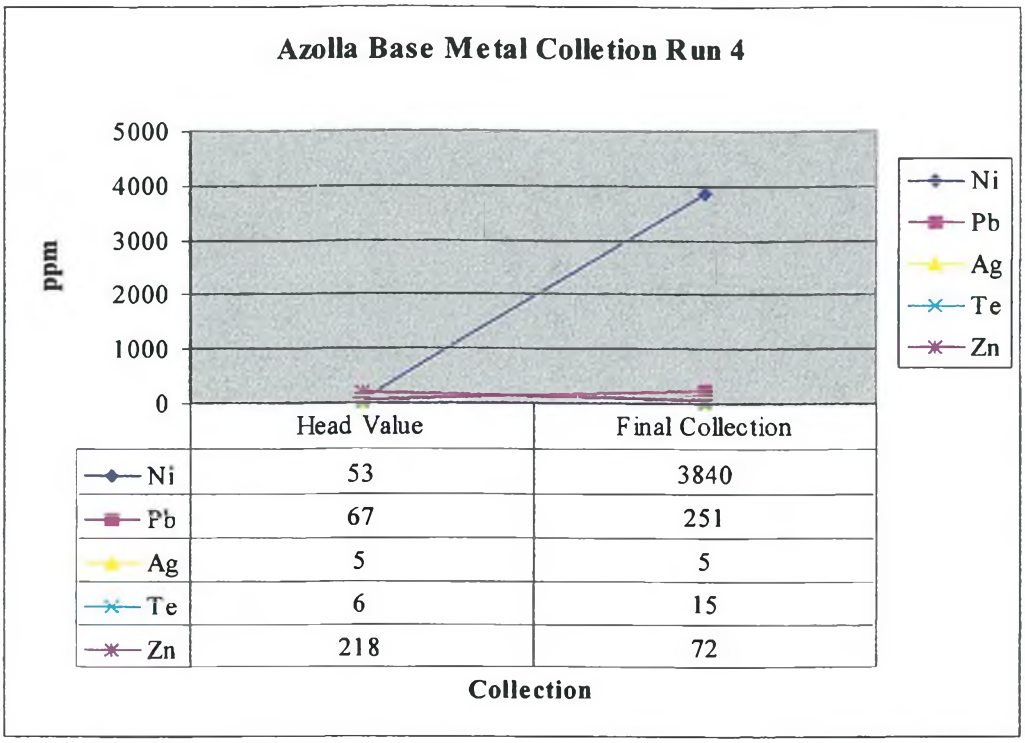


Figure 10.5 Showing Azolla head value and showing excellent Ni collection during this run.

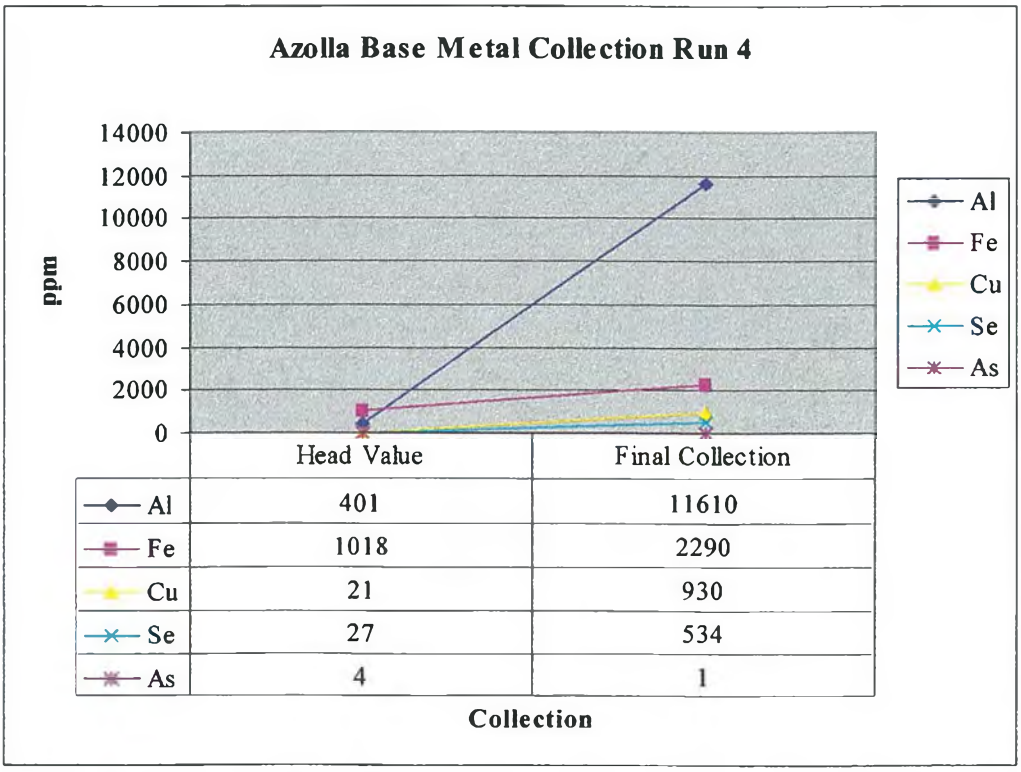


Figure 10.6 Showing Azolla head value and showing excellent base metal collection.

CHAPTER ELEVEN: TEST RUN 5 and 6 RESULTS

11.1 Test Run 5 and 6 using double column and Sun dried Azolla

Same as test run 3 and 4, ten grams of sun dried Azolla was loaded into each column five samples were again taken during this test run at an interval of a litre, the flow rate on this occasion was reduced further to approximately one litre per hour.

New liquor was circulated through the test with high head values (Pt 190ppm) A sample of the liquor was taken to determine head value. (Figure 11.1, 11.2, 11.3).

The head value for Azolla was the same used in test run 3 and 4. (Figure 1.4, 11.5, 11.6, 11.7).

It was decided not to remove the Azolla from the columns after this run and that we would re-circulate run 5 liquor in run 6 to determine the effect.

Note

Fe was not detected in head value of liquor.

The Azolla was only removed after run 6 and the results given are a reflection of two test runs of the same liquor.

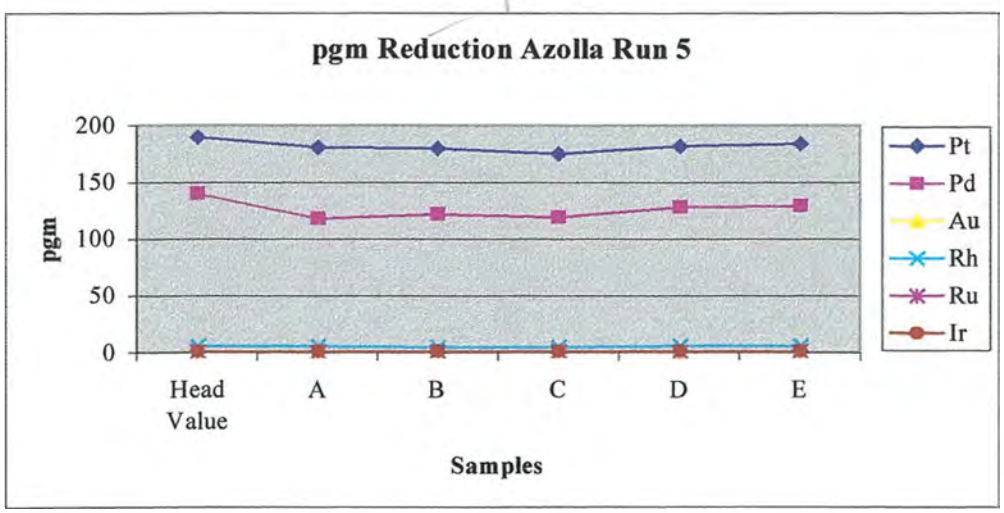


Figure 11.1 Showing liquor head value for pgm initially resulting in good Pt and Pd removal until point C then re-releases.

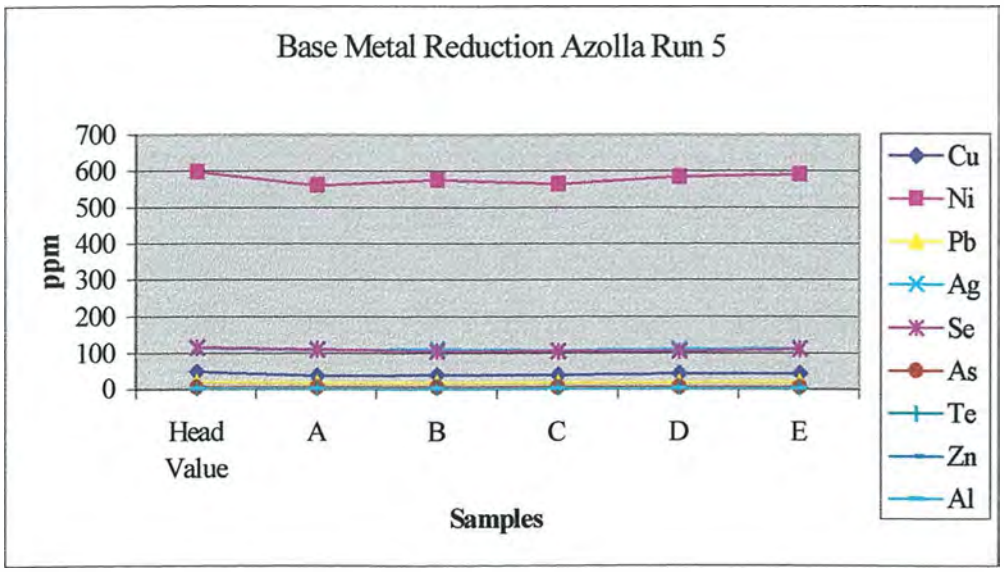


Figure 11.2 Showing liquor head value for base metal. A similar observation can be made to that of pgm scenario, initially good uptake then re-release at point C.

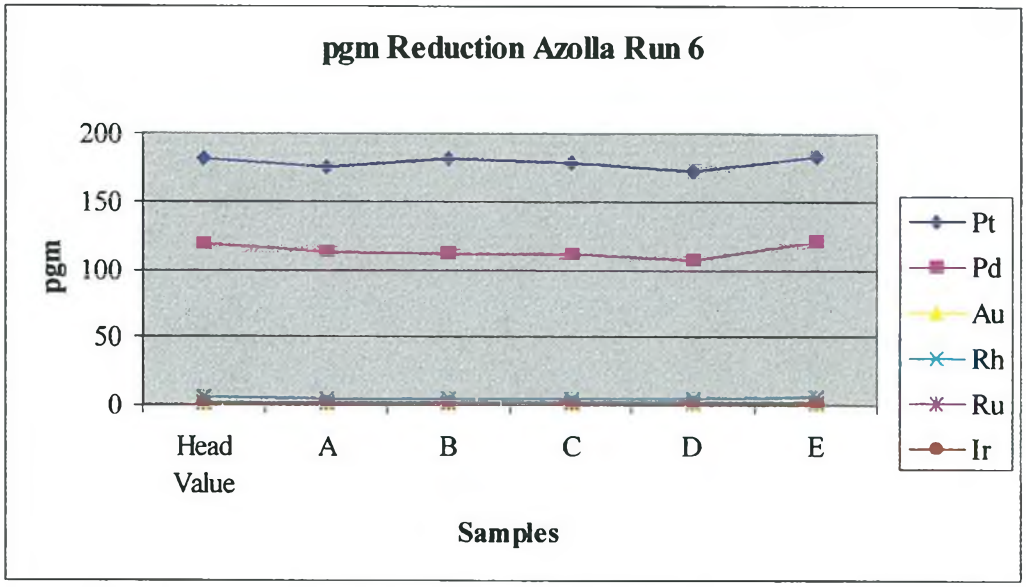


Figure 11.3 Showing liquor head value for pgm, again good Pt and Pd removal until point D then re-release. However the Azolla was still collecting on the second run, Pd at point D was 107ppm from a head value of 119ppm.

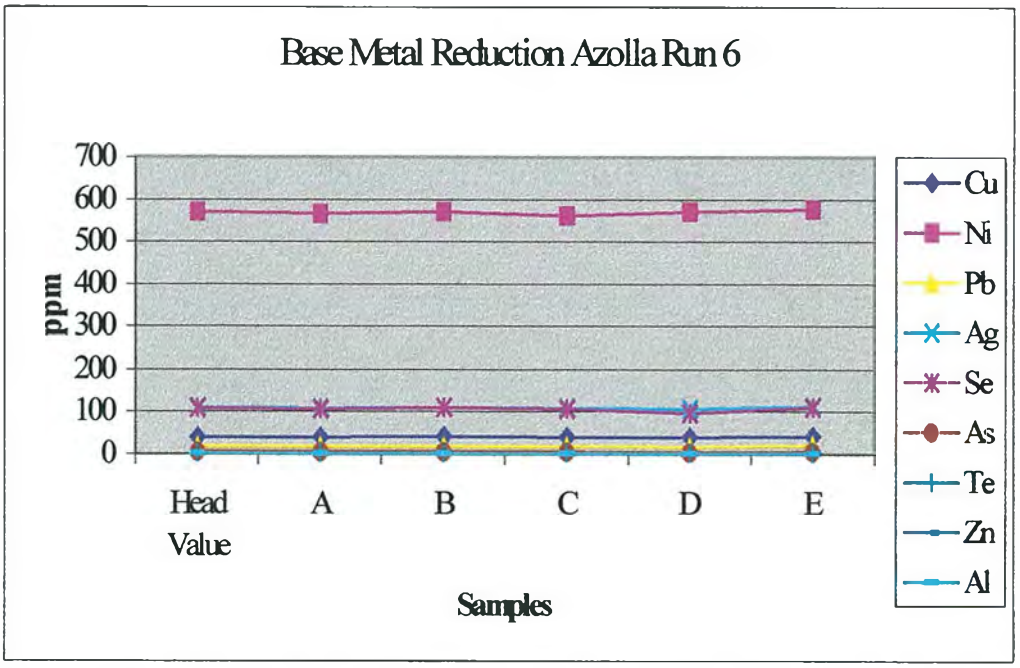
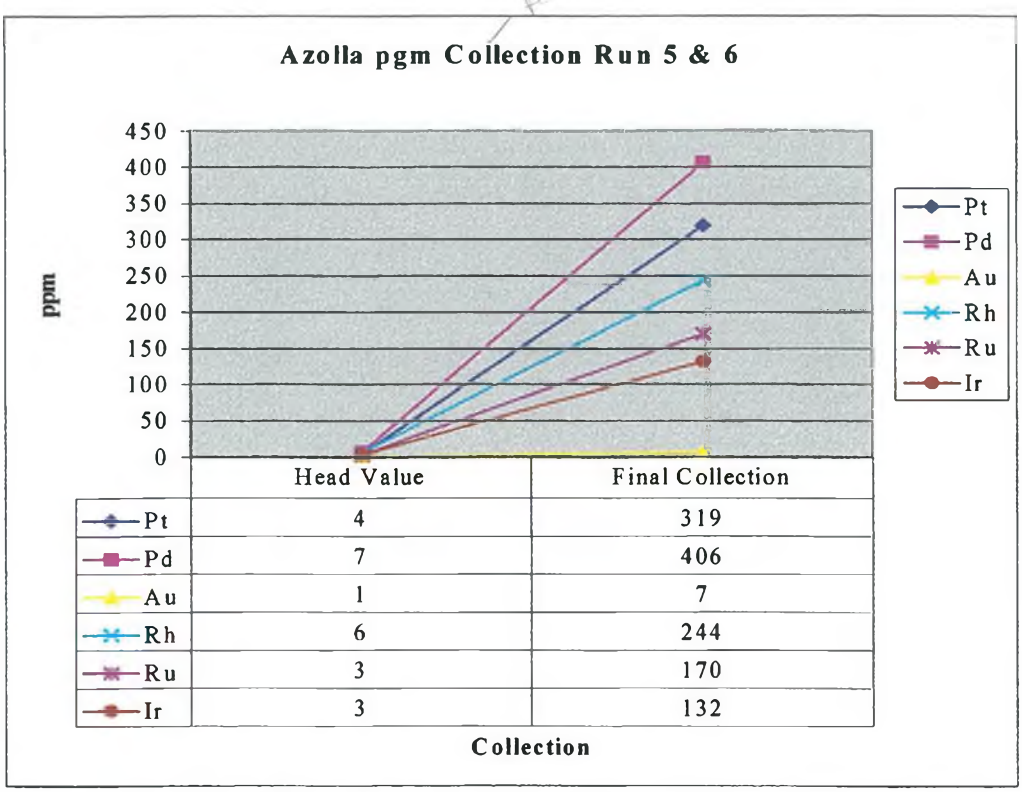
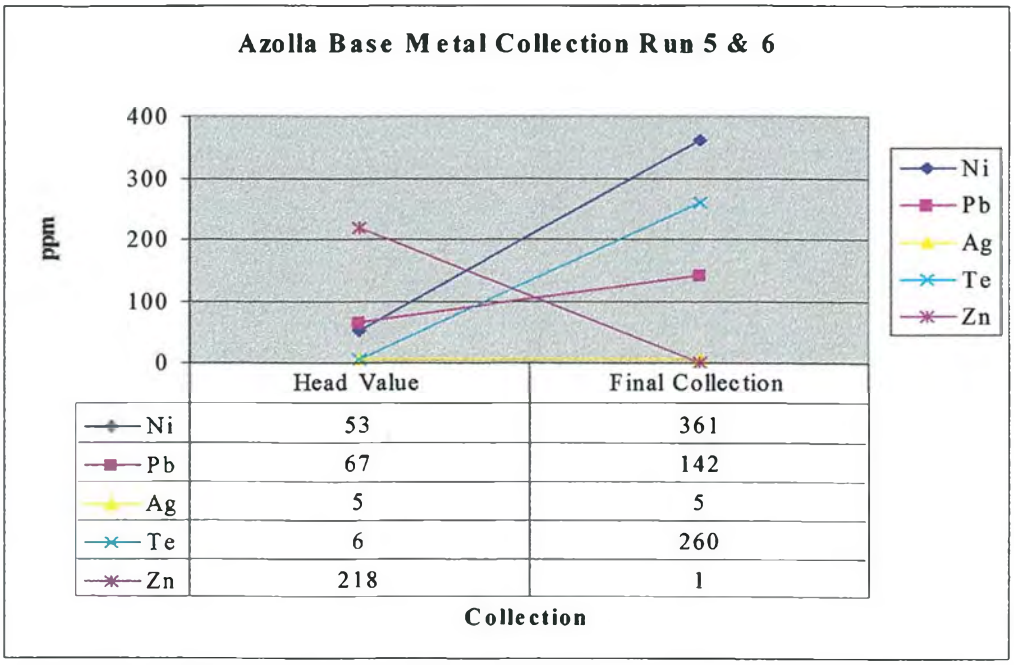


Figure 11.4 Showing liquor head value for base metal. Very little base metal activity can be observed other than Se until re-release.



	Head Value	Final Collection
Pt	4	319
Pd	7	406
Au	1	7
Rh	6	244
Ru	3	170
Ir	3	132

Figure 11.5 Showing Azolla head value. Collection of all pgm elements can be observed but not in high values as seen during previous runs.



	Head Value	Final Collection
Ni	53	361
Pb	67	142
Ag	5	5
Te	6	260
Zn	218	1

Figure 11.6 Showing Azolla head value showing good Te and Ni collection and possible analytical error with head value of Zn.

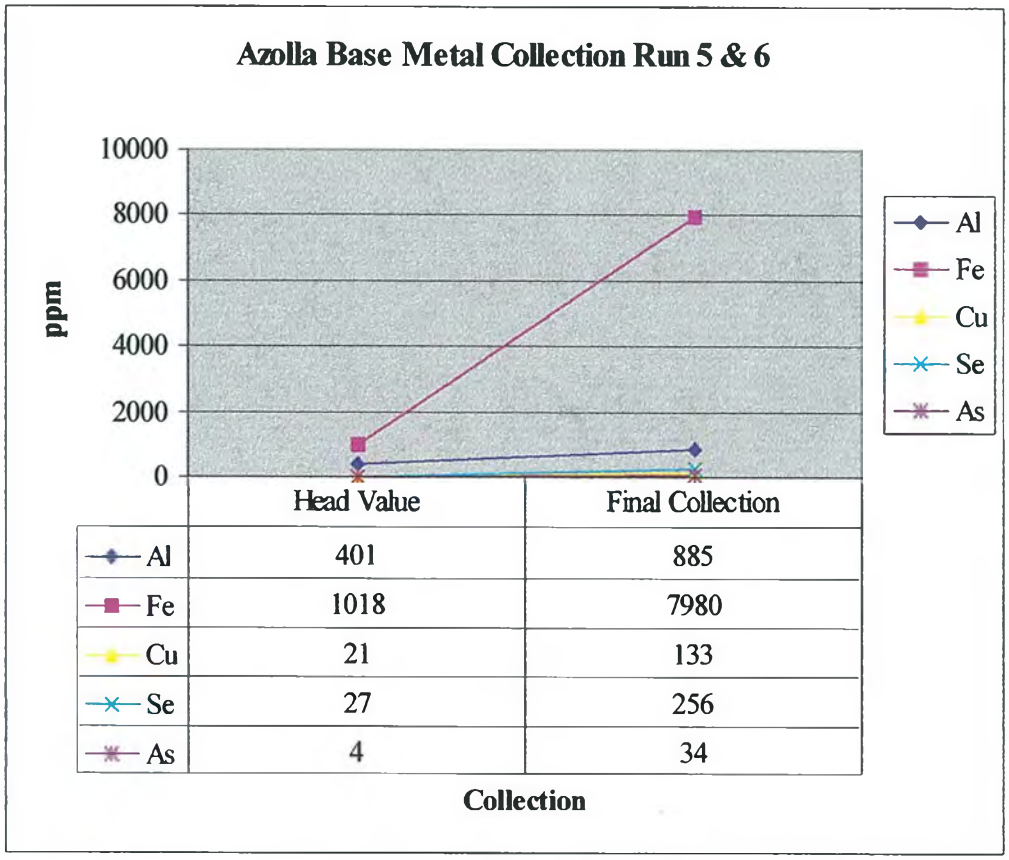


Figure 11.7 Showing Azolla head value and excellent base metal collection is again apparent.

CHAPTER TWELVE: TEST RUN 7. RESULTS

12.1 Test Run 7 using double column and 50^0 C dried Azolla

Ten grams of Azolla were loaded into each column before commencing the test. Five samples were again taken during this test run at an interval of a litre. The flow rate on this occasion was again one litre per hour.

New liquor was circulated through the test with even higher head values (Pt 218ppm) the highest used thus far.

A sample of the liquor was taken to determine head value. (Figure 12.1, 12.2).

The head value for Azolla was the same used in test run 1 and 2. (Figure 12.3, 12.4, 12.5).

Note

Fe was not detected in head value of liquor.

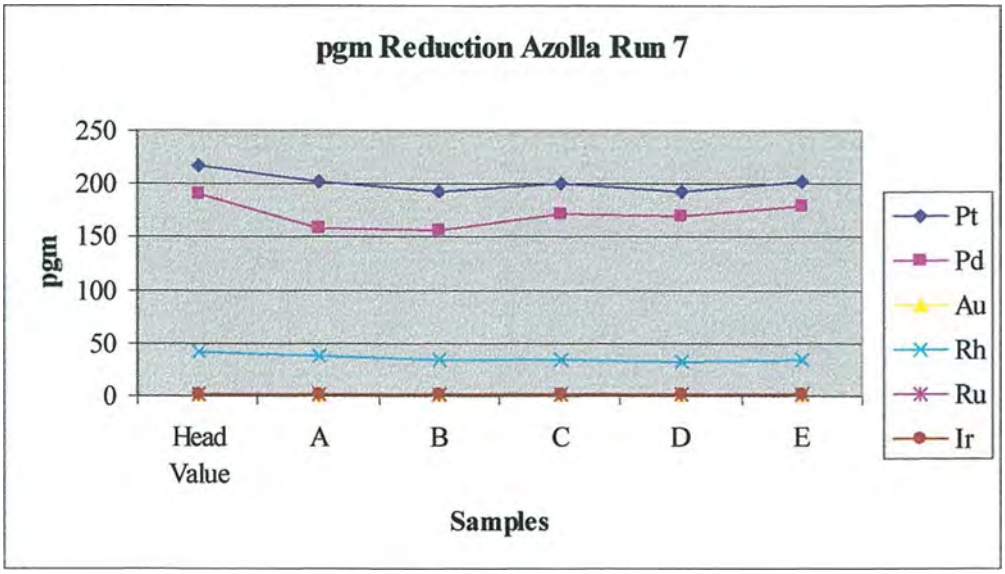


Figure 12.1 Showing liquor head value for pgm. Good Pt and Pd removal again is observed until release. At point B Pt was 192ppm from 218ppm and Pd was 157ppm from 218 ppm.

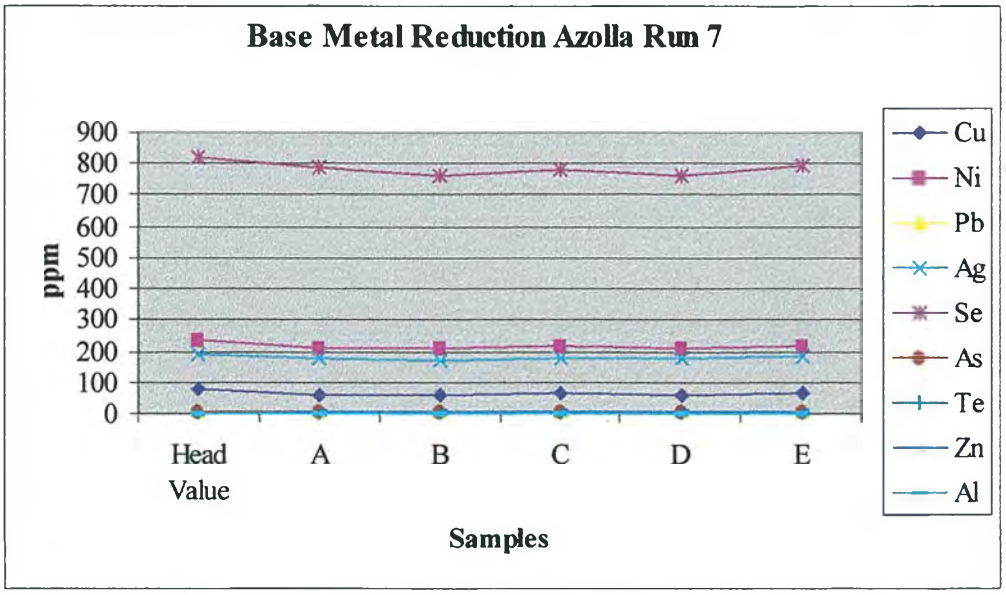


Figure 12.2 Showing liquor head value for base metal. Good Se removal can be observed and it should be noted that Se removal is normally associated in pgm refining with Au or Pd removal, which was the scenario during this run.

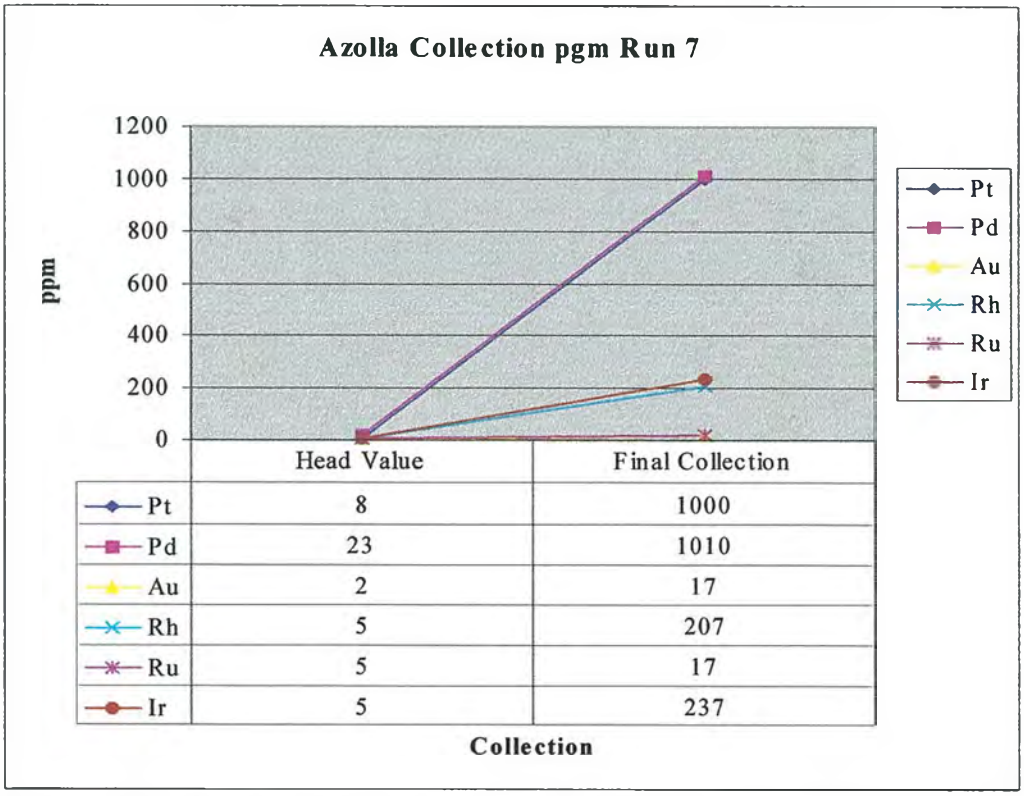


Figure 12.3 Showing Azolla head value. Excellent collection of all pgm elements can be observed especially Pt and Pd.

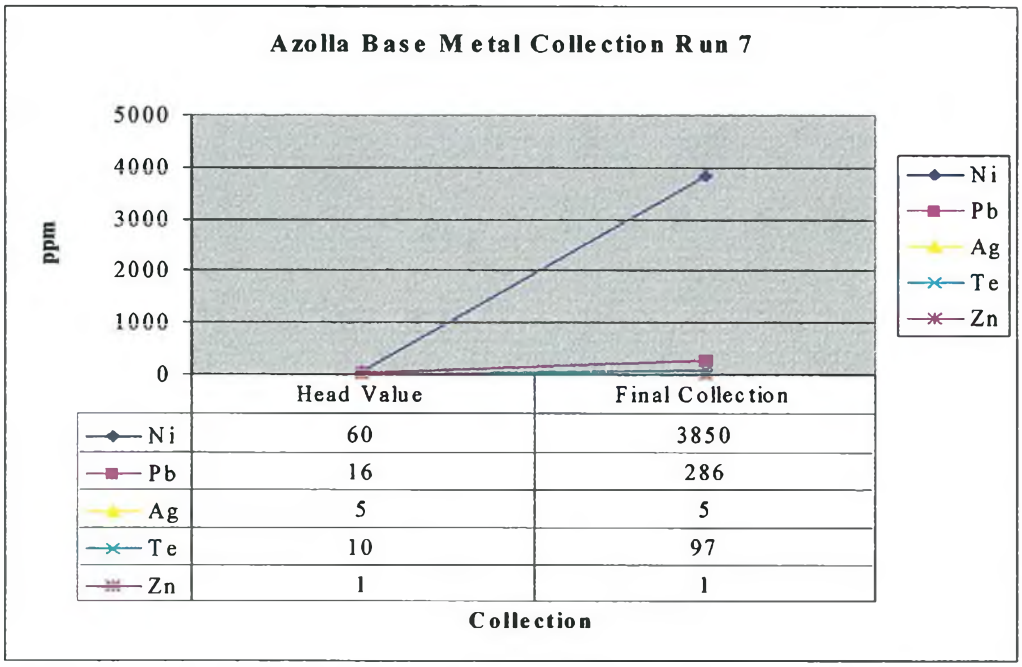
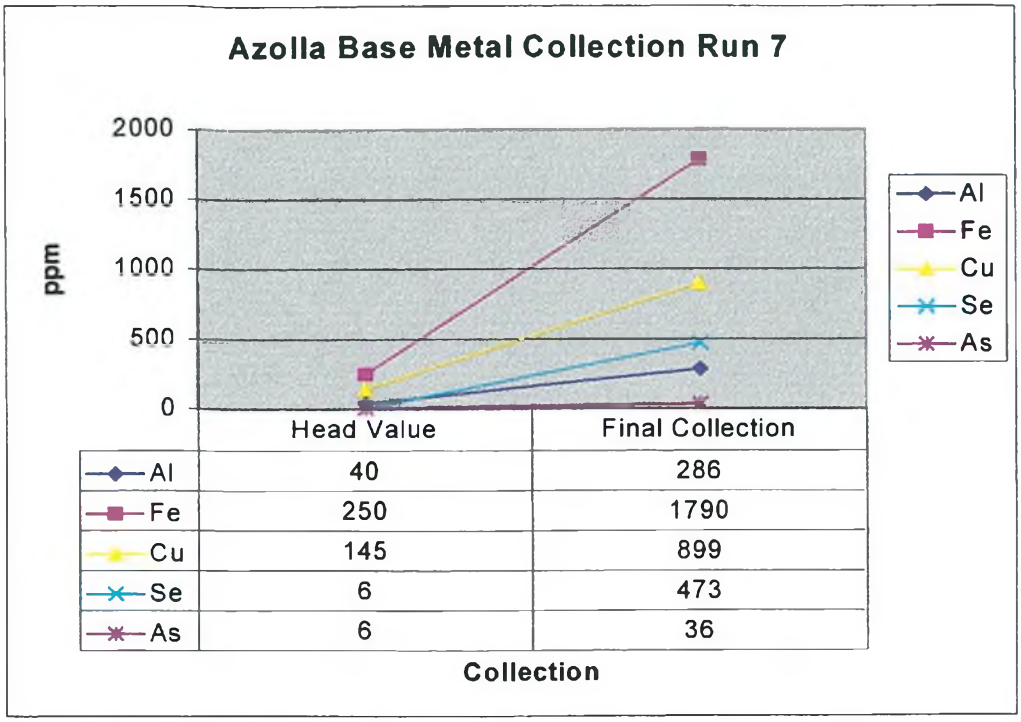


Figure 12.4 Showing Azolla head value and again excellent base metal collection can be observed.



	Head Value	Final Collection
Al	40	286
Fe	250	1790
Cu	145	899
Se	6	473
As	6	36

Figure 12.5 Showing Azolla head value and again excellent base metal collection can be observed.

CHAPTER THIRTEEN: - CONCLUSION

13.1 Discussion and Observations of Test Runs

In all test runs excluding run 4 (used liquor from run 3) there seems to be significant activity taking place between the head value (H) and the first sample (A) and thereafter a more stable up-take often resulting in a re-release of metal. This release is possibly due to saturation of Azolla.

During test runs it can also be observed the largest variations in redox potential also occur between these two points.

This is even more evident in the runs 1, 2 and 7, which utilised 50^{0}C Azolla when making comparisons with results from runs, which had used the sun dried Azolla.

Could this be the point at which there is the most ligand activity, which in-turn stimulates the transition of metals and thereafter metal collection is performed by absorption.

The major fluctuations in the range of pH occurred in liquors with the lower pH (runs 1 and 2), which saw an increase in pH.

This was not however so evident in run 3 and 4, which had low pH, however had utilised sun dried Azolla.

In all other tests there is almost no effect on the range of pH and it appears fairly constant throughout the tests.

At this stage it is not clear if the difference in high or low pH hinders collection but it could impact on metal speciation, thus inadvertently effect the efficiency of metal collection by Azolla.

Throughout the tests base metal collection was always prominent especially Fe collection. It must be noted that there appears to be conflict with the results obtained during run 5/6, Fe was not detected in liquor head value by ICP analysis, was re-checked with same results however was detected in final analysis. This could be due to contamination when taking Azolla from the columns prior to analysis therefore those results for Fe cannot be deemed reliable and should be ignored.

What cannot be ignored is the success of base metal collection and the question is there metal competition for binding sites, which may be detrimental to pgm collection.

Varied flow rates were utilised during test runs, the real effect cannot be determined of those varying rates because considerable metal collection is evident in all runs.

Though metal collection is evident, no run obtained a level of full pgm depletion (<10ppm), which is required for safe disposal, this maybe due to Azolla saturation. However what was proven that the Azolla was not metal selective and was capable of collection of all pgm elements.

Contact time may however play a significant part in removal when observing that metal continued to bind from the same liquor that was circulated twice (test run 4).

From comparison of test results it is apparent that dried Azolla is preferable and achieves the best results.

One failure of the test work was the omission to make a comparison of the results obtained from Azolla in the double columns.

Extracted Azolla from each column should have been analysed separately to compare the effect of initial contact.

13.2 The Ability of Azolla to treat Refinery Effluent

The test work included many varied scenarios to test the ability of Azolla to treat effluent.

- Low / high pgm content
- pH ranges 2.3 to 4.5
- Single / double packed columns
- Flow rates changes
- Sun dried / 50^0C Azolla

All scenarios proved from results taken that Azolla does have the ability to treat refinery effluent containing pgm, however they do not indicate the most appropriate method of metal collection.

13.3 Recommendation

From test work results it is evident that there is the potential to treat refinery effluent with Azolla however there must be more test work undertaken to provide that best method.

During this test it was not possible to determine the effect of pH in relation to metal collection, it was noted that pH will effect the type of metal species formed and that species is important in metal transition or binding.

Therefore new test work should determine speciation before each test run, this would provide evidence, which metal species is most susceptible to collection by Azolla.

When future tests provide information, which is the most appropriate metal state, effluent could then be chemically pre-treated to alter their state before biological treatment to optimise metal removal.

The refinery does not have Chromatography equipment that could provide this information and a third party analyst should be identified prior to new tests. Then their services should be utilised to provide this resource.

From all test results, ignoring 5/6 it is clearly evident that Fe removal is a dominant feature in all tests and could play a very important part in pgm removal.

Does Fe compete with the other metals and occupy the majority of binding sites first, from results this may well be the correct assumption.

Note should be taken that Fe and pgm exist in section VIII of the periodic table and should naturally compete during stages of transition.

To verify this, consideration should be given to Fe removal prior to future tests.

This can be done with simple chemical adjustment (sodium hydroxide) to change the pH and precipitate base metal before commencing test.

A test should then be undertaken using depleted Fe liquor and Fe rich liquor to compare pgm collection.

If contact time or surface area is a factor and the majority of collection takes place on initial contact then various other options should be explored this could include multiple columns, larger columns, series of circulation tanks or even shallow raceways after column treatment, the possibilities are endless?

The demonstrated effect of the change in redox potential as witnessed by the result of metal removal from head value to sample (A), should also be explored further. Consideration should be taken of a chemical addition (hydrogen peroxide or HCL) prior to commencement of test to alter the state of oxidisation or reduction again to identify optimal conditions for metal removal.

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APPENDIX A
Groundwater Sampling & Hydro-Chemical Evaluation

TABLE 8: HEAVY METAL SPECIATION IN HEAVILY CONTAMINATED SAMPLES.

Metal	BH-1	SRK-1	SRK-10d	SRK-12s	Acid dam
Cu	3.5%$CuCO_{3\,(aq)}$ 94.3%$Cu(CO_3)^{2-}$ 2.2%$Cu(OH)_2$	52.2%Cu^{2+} 40%$CuC1^{+}$ 7.5%$CuC1_{2(aq)}$	86.0%Cu^{2+} 13.1%$CuC1^{+}$	88.1%Cu^{2+} 9.5%$CuC1^{+}$ 1.5%$CuHCO_3$	15.4%Cu^{2+} 54.1%$CuC1^{+}$ 27.6%$CuC1_{2(aq)}^{+}$ 2.6%$CuS0_{4(aq)}$
Fe	72.7%Fe^{2+} 8.2%$FeOH^{+}$ 18.9%$FeSO_{4(aq)}$	99.5%Fe^{2+}	99.8%Fe^{2+}	99.9%Fe^{2+}	87.2%Fe^{2+} 12.8%$FeS0_{4(aq)}$
Mn	28.8%Mn^{2+} 33.0%$mnC1^{-}$ 3.8%$MnC1_{2(aq)}$ 7.7%$MnSO_{4(aq)}$ 24.5%$MnHCO_3$	43.2%Mn^{2+} 49.7%$MnC1^{+}$ 4.7%$MnC1_{2(aq)}$	80.8%Mn^{2+} 18.5%$MnC1^{+}$	85.8%Mn^{2+} 13.8%$MnC1^{+}$	10.3%Mn^{2+} 54.6%$CuC1^{+}$ 14.1%$MnC1_{2(aq)}$ 19.5%$MnC1_3$
Ni	2.6%$NiCO_{3(aq)}$ 97.4%$Ni(CO_3)^{2-}$	38%Ni^{2+} 27.2%$NiC1^{+}$ 34.5%$NiC1_{2(aq)}$	83.5%Ni^{2+} 11.9%$NiC1^{+}$ 4.4%$NiC1_{2(aq)}$	88.1%Ni^{2+} 8.8%$NiC1^{+}$ 2.2%$NiC1_{2(aq)}$	6.4%Ni^{2+} 20.8%$NiC1^{+}$ 71.8%$NiC1_{2(aq)}$
Pb	98.2%$Pb(CO_3)$ 1.8%$PbCO_{3(aq)}$	3.7%Pb^{2+} 41.4$PbC1^{+}$ 22.8%$PbC1_{2(aq)}$ 19.3%$PbC1_3$ 12.1%$PbC1_4^{2-}$	26.8%Pb^{2+} 60.4%$PbC1^{+}$ 9.8%$PbC1_{2(aq)}$	35.3%Pb^{2+} 55.9%$PbC1^{+}$ 6.2%$PbC1_{2(aq)}$	9.9%$PbC1^{+}$ 14.9%$PbC1_{2(aq)}$ 36.2%$PbC1_3$ 38.8%$PbC1_4^{2-}$
Zn	99.8%$Zn(CO_3)^{2-}$	38.4%Zn^{2+} 29.4%$ZnC1^{+}$ 10.7%$ZnC1_{2(aq)}$ 12.9%$ZnC1_3$ 8.4%$ZnC1_4$	85%Zn^{2+} 12.9%$ZnC1^{+}$	89.1%Zn^{2+} 9.6%$ZnC1^{+}$	3.0%Zn^{2+} 10.4%$ZnC1^{+}$ 10.4%$ZnC1_{2(aq)}$ 35.7%$ZnC1_3$ 39.9%$ZnC1_4^{2-}$

8.0 CONCLUSIONS

Based on the available information, the following conclusions have been reached:

- The water levels in BH-1 and SRK-1 have fallen significantly, while the others have remained relatively constant. This represents lower recharge due the low winter rainfall.
- The results strongly indicate that the storage dams have been the source of the heavy metals, acidity and chloride.

- BH-1 has changed from acidic to alkaline between 1995 and 2001, with the corresponding reduction in the presence of the heavy metals. The most likely explanation for this is leakage from the alkaline ponds.
- SRK-1 has shown a slight improvement in water quality, although the zinc and manganese concentrations remain 10000 and 1000 times in excess of the EEC values.

- SRK-7, SRK-10d, SRK-12s and SRK-12d, which previously showed little or no contamination, show signs of increased metal contamination as well as reduced pH and increased conductivity.
- The results indicate that the contamination plume has spread and affected a large area.
- Chloride and sulphate are the major anionic species.
- The nitrate levels are high in BH-1, SRK-1, SRK-10d, SRK-12s and the acid dam. These high nitrate levels represent a serious health risk.
- The presence of high concentrations of chloride in SRK-1 and the acid dam, as well as high alkalinity in BH-1 leads to the formation of chloride and carbonate complexes with the heavy metals, reducing their bio-availability. This is not observed in the samples further from the source, so it is unlikely to be a major factor.
- The presence in the groundwater samples of concentrations of calcium, magnesium and manganese that are higher than the source ponds indicate leaching or ion exchange interactions with the underlying sub-strata.
- Zinc is the most mobile metal. The concentration of zinc detected in the contaminated samples are at a higher percentage, relative to the source, than the other heavy meals. This indicates that the less mobile metals are being immobilised in the soil.
- The charge balance results indicate that an accurate and complete picture has been obtained.
- In terms of section 19 of the National Water Act 36 of 1998 (i.e. prevention and remedying the effects of pollution), Western Platinum Refinery is in non-compliance.

9.0 RECOMMENDATIONS

1) The acid pond must be decommissioned as soon as possible and the extent of any contaminated soils evaluated and classified as per DWA&F (1998).
2) The same sample sites must be re-sampled early on a quarterly basis, beginning early in November, to assess the impact of seasonal variations in rainfall and to determine if the contaminant plume has moved further.
3) Soil samples be collected from the vicinity of the storage ponds to assess the degree of heavy metal accumulation and the risk of future mobilisation of the accumulated metal. In addition the buffering capacity of the contaminated soil relatively to an uncontaminated site needs to be assessed.

4) Considering the fact that SRK-7 and SRK-12 have shown signs of contamination, a single set of shallow (10m) and deep (30M) borehole needs to be drilled approximately 30m south east of SRK-12 to determine the extent of the plume migration in both aquifers. This should be done prior to the proposed November sampling run.
5) A further borehole set should be installed hydraulically "up-gradient" of the acid/alkaline dams to quality the background water quality.
6) In light of the issue of legal non-compliance in term of the National Water Act, the regulatory authorities must be informed of the current situation so that a cost-effective and realistic remediation strategy can be agreed upon.
7) The process required for remediation, in line with current DWA&F policy, is attached in Appendix III.

Robert van Hille M.Sc

Jan Rasmussen Pr.Sci.Nat.

APPENDIX B: Section 28 Nema

COMPLIANCE, ENFORCEMENT AND PROTECTION

Part 1: Environmental Hazardous

Duty of care and remediation of environmental damage

28. (1) Every person who causes, has caused or may cause significant pollution or degradation of the environment must take reasonable measures to prevent such pollution or degradation from occurring, continuing or recurring, or, in so far as such harm to the environment is authorised by law or cannot reasonably be avoided or stopped, to minimise and rectify such pollution or degradation of the environment.

(2) Without limiting the generality of the duty in subsection (1), the persons on whom subsection (1) imposes an obligation to take reasonable measures, include an owner of land or premises, a person in control of land or premises or a person who has a right to use the land or premises on which or in which-

(a) any activity or process is or was performed or undertaken; or

(b) any other situation exists, which causes, has caused or is likely to cause significant pollution or degradation of the environment.

(3) The measures required in terms of subsection (1) may include measures to-

(a) investigate, assess and evaluate the impact on the environment;

(b) inform and educate employees about the environmental risks of their work and the manner in which their tasks must be performed in order to avoid causing significant pollution or degradation of the environment;

(c) cease, modify or control any act, activity or process causing the pollution or degradation.

(d) contain or prevent the movement of pollutants or the causant of degradation;

(e) eliminate any source of the pollution or degradation; or

(f) remedy the effects of the pollution.

(3) The Director-General or a provincial head of department may, after consultation with any other organ of state concerned and after having given adequate opportunity to affected persons to inform him or her of their relevant interests, direct any person who fails to take the measures required under subsection (1) to-

(a) investigate, evaluate and assess the impact of specific activities and report thereon;

(b) commence taking specific reasonable measures before a given date;

(c) diligently continue with those measures; and

(d) complete them before a specified reasonable date:

Provided that the Director-General or a provisional head of department, when considering any measure or time period envisaged in subsection (4), must have regard to the following:

(a) the principles set out in section 2;

(b) the provisions of any adopted environmental management plan or environmental implementation plan;

(c) the severity of any impact on the environment and the costs of the measures being considered;

(d) any measures proposed by the person on whom measures are to be imposed;

(e) the desirability of the State fulfilling its role as custodian holding the environment in public trust for the people;

(f) any other relevant factors.

(6) if a person required under this Act to undertake rehabilitation or other remedial work on the land of another, reasonably requires access to, use of or a limitation on use of that land in order to effect rehabilitation or remedial work, but is unable to acquire it on reasonable terms, the Minister may-

(a) expropriate the necessary rights in respect of that land for the benefit of the person undertaking the rehabilitation or remedial work, who will then be vested with the expropriated rights; and

(b) recover from the person for whose benefit the expropriation was effected all costs incurred.

(7) Should a person fail to comply, or inadequately comply, with a directive under subsection (4), the Director-General or provisional head of department may take reasonable measures to remedy the situation.

(8) Subject to subsection (9), the Director-General or provincial head of department may recover all costs incurred as a result of it acting under subsection (7) form any or all of the following persons-

(a) any person who is or was responsible for, or who directly or indirectly contributed to, the pollution or degradation or the potential pollution or degradation;

(b) the owner of the land at the time when the pollution or degradation or the potential for pollution or degradation occurred, or that owner's successor in title;

(c) the person in control of the land or any person who has or had a right to use the land at the time when-

(i) the activity or the process is or was performed or undertaken ; or
(ii) the situation came about; or

(d) any person who negligently failed to prevent-

(i) the activity or the process being performed or undertaken; or
(ii) the situation from coming about;

Provided that such person failed to take the measures required of him or her under subsection (1).

(9) The Director-General or provincial head of department may in respect of the recovery of costs under subsection (8), claim proportionally from any other person who benefited from the measures undertaken under subsection (7).

(10) The costs claimed under subsections (6), (8) and (9) must be reasonable and may include, without being limited to, labour, administrative and overhead costs.

(11) If more than one person is liable under subsection (8), the liability must be apportioned among the persons concerned according to the degree to which each was responsible for the harm to the environment resulting from their respective failures to take the measures required under subsection (1) and (4).

(12) Any person may, after giving the Director-General or provincial head of department 30 day's notice, apply to a competent court for an order directing the Director-General or any provincial head of department to take any of the steps listed in subsection (4) if the Director-General or provincial head of department fails to inform such person in writing that he or she has directed a person contemplated in subsection (8) to take on of those steps, and the provisions of section 32(2) and (3) shall apply to such proceedings with the necessary changes.

(13) When considering any application in terms of subsection (12), the court must take into account the factors set out in subsection (5).

APPENDIX C

CONCENTRATE COMPOSITE ANALYSIS

WESTERN PLATINUM REFINERY
(INCORPORATED IN THE REPUBLIC OF SOUTH AFRICA)
REG NO. 71/11391/06

P O BOX 1021
BRAKPAN
1540
(011) 813 – 2100/1/2/3/

07/11/2001

MONTHLY COMPOSITE ANALYSIS
October-01

W.P. CONCENTRATE LOT: 2002/001 – 2002/018

NETT WEIGHT: 3567.9720
NETT DRY WEIGHT: 3311.3531
MOISTURE: 7.1923

	AVERAGE
% Pt	32.465
% Pd	14.886
% Au	0.792
% Rh	5.126
% Ru	8.856
% Ir	1.952
% Cu	6.275
% Ni	3.318
% Fe	2.335
% Pb	1.321

CHIEF CHEMIST _____

c.c. Metal Accounting

APPENDIX C

CONCENTRATE COMPOSITE ANALYSIS

WESTERN PLATINUM REFINERY
(INCORPORATED IN THE REPUBLIC OF SOUTH AFRICA)
REG NO. 71/11391/06

P O BOX 1021
BRAKPAN
1540
(011) 813 – 2100/1/2/3/

07/11/2001

MONTHLY COMPOSITE ANALYSIS
October-01

W.P. CONCENTRATE LOT: 2002/001 – 2002/018

NETT WEIGHT: 3567.9720
NETT DRY WEIGHT: 3311.3531
MOISTURE: 7.1923

		%
Silver	Ag	0.905
Selenium	Se	0.496
Arsenic	As	1.818
Tellurium	Te	0.786
Zinc	Zn	0.001
Calcium	Ca	1.032
Chromium	Cr	0.624
Titanium	*Ti*	0.054
Cobalt	Co	0.144
Manganese	Mn	0.015
Bismuth	Bi	0.144
Magnesium	Mg	0.410
Antimony	Sb	0.385

CHIEF CHEMIST _____

c.c. Metal Accounting

APPENDIX D

Azolla Preparation

Initial Work Conducted

Moisture content after allowing excess wash solution to drain off;

Relative density of Azolla dried at 50 degrees centigrade;

Preparation of dried Azolla;

Material was screened to obtain a product suitable for the test work

(1) A + 2800 mu screening was used to remove husks and foreign particles

(2) A + 1700 mu / -2800 mu product separated out (husks and large Azolla particles)

(3) A + 600 mu / -1700 mu product separated out for test work

(4) A – 600 mu product removed which could cause restriction to flow and channeling

	gm	Vol ccs	Tap Density (hand tap for 3 min. in vol flask)
+ 2800 mu	12.75		
+ 1700 / -2800 mu	8.84		
+ 600 mu / -1700 mu	207.02	1634	0.1267
- 600 mu	56.58	300	0.1886

Moisture Content dried at 50 degrees C

Wet	Dry	Wet	Dry	Wet	Dry
67.6911	48.80451	74.49928	55.53229	65.62449	47.1738
-47.89587	-47.8959	-54.5193	-54.5193	-46.2275	-46.2275
19.27324	0.90864	19.97996	1.01297	19.39699	0.9463
% Moisture	95.28548	94.93007		95.12141	
		Mean Moisture Value %		95.11232	

Expansion Test Of Dried Azolla

20 cc of dried Azolla transferred to 100ml measuring cylinder water added and Azolla allowed to absorb the water.

Start	20 cc
Final Vol	35 cc increase in vol 15 ml = 75%

APPENDIX E

ICP STANDARDS

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Base line calibration

Printed at : 01/12/04 07:50:18 AM

Method : Formic comb

Analyte : Al30

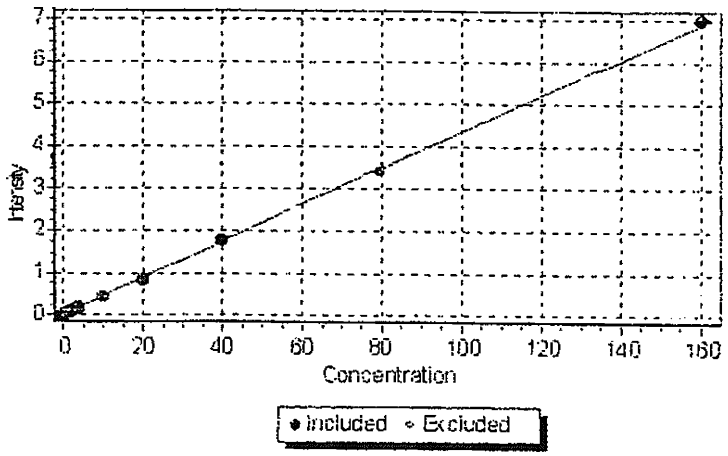
Calibration date : 01/12/04 07:50:16 AM

Coefficients :

C0 -8.83209E-03
C1 2.23269E+01
C2 1.28039E-01
C3

Statistics :

Weighting	1/(Intensity squared)
Correlation coefficient	0.9997
Standard error of estimate	0.9076



Data points :

Standard	Intensity	Concentration	Calculated Concentration	%Difference	Included	Low point	High point
FOR 0	0.000	0.000	0.000		Yes		
FOR 1	0.048	1.000	1.064	6.421	Yes		
FOR 2	0.094	2.000	2.092	4.613	Yes		
FOR 3	0.173	4.000	3.866	-3.354	Yes	Yes	
FOR 4	0.435	10.000	9.739	-2.614	Yes		
FOR 5	0.863	20.000	19.348	-3.261	Yes		
FOR 6	1.779	40.000	40.116	0.291	Yes		
FOR 7	3.413	80.000	77.674	-2.908	Yes		Yes
FOR 8	7.023	160.000	163.101	1.938	Yes		

APPENDIX E

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Base line calibration

Printed at : 01/12/04 07:50:26 AM

Method : Formic comb

Analyte : As10

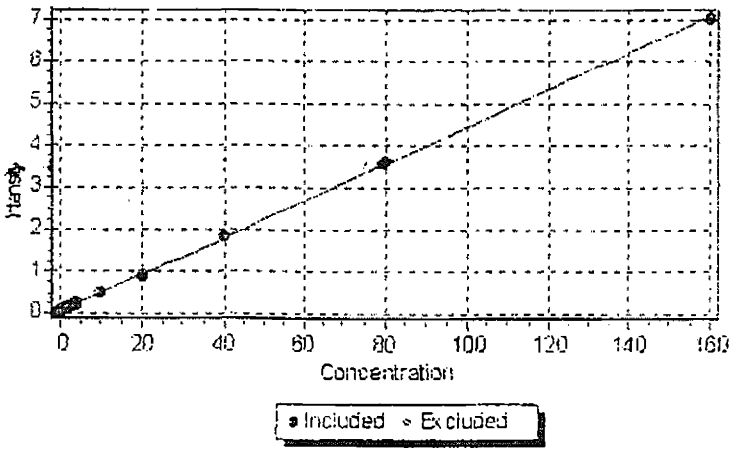
Calibration date : 01/12/04 07:50:24 AM

Coefficients :

C0 -8.68948E-01
C1 2.29551E+01
C2 -5.18782E-02
C3

Statistics :

Weighting	1/(Intensity squared)
Correlation coefficient	0.9999
Standard error of estimate	0.3792

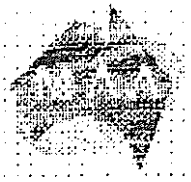


Data points :

Standard	Intensity	Concentration	Calculated Concentration	%Difference	Included	Low point	High point
FOR 0	0.038	0.000	0.013		Yes		
FOR 1	0.080	1.000	0.964	-3.552	Yes		
FOR 2	0.122	2.000	1.934	-3.316	Yes		
FOR 3	0.213	4.000	4.029	0.730	Yes	Yes	
FOR 4	0.475	10.000	10.022	0.220	Yes		
FOR 5	0.915	20.000	20.084	0.421	Yes		
FOR 6	1.820	40.000	40.743	1.858	Yes		
FOR 7	3.575	80.000	80.526	0.657	Yes		Yes
FOR 8	7.063	160.000	158.684	-0.822	Yes		

APPENDIX E

ICP STANDARDS



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Base line calibration

Printed at : 01/12/04 07:50:33 AM

Method : Formic comb

Analyte : Au26

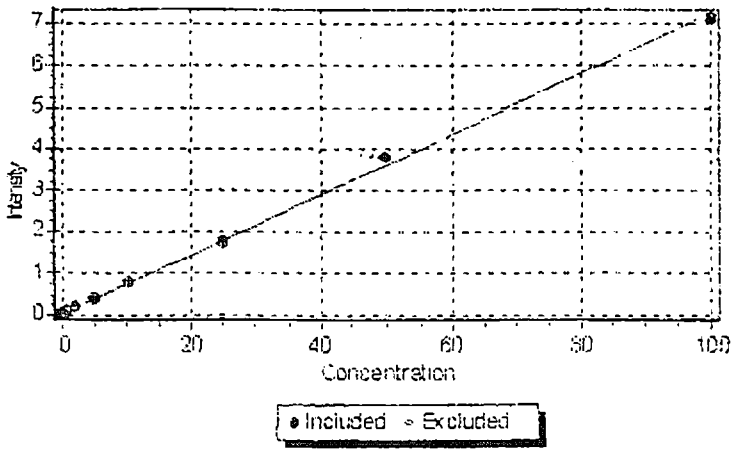
Calibration date : 01/12/04 07:50:31 AM

Coefficients :

C0 -4.15672E-01
C1 1.40508E+01
C2 -3.33468E-02
C3

Statistics :

Weighting	1/(Intensity squared)
Correlation coefficient	0.9997
Standard error of estimate	0.5275



Data points :

Standard	Intensity	Concentration	Calculated Concentration	%Difference	Included	Low point	High point
FOR 0	0.030	0.000	0.008		Yes		
FOR 1	0.062	0.500	0.455	-9.048	Yes		
FOR 2	0.100	1.000	0.992	-0.830	Yes		
FOR 3	0.182	2.000	2.140	7.003	Yes	Yes	
FOR 4	0.377	5.000	4.873	-2.533	Yes		
FOR 5	0.745	10.000	10.033	0.329	Yes		
FOR 6	1.786	25.000	24.571	-1.714	Yes		
FOR 7	3.754	50.000	51.865	3.730	Yes		Yes
FOR 8	7.166	100.000	98.562	-1.438	Yes		

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Base line calibration

Printed at : 01/12/04 07:50:42 AM

Method : Formic comb

Analyte : Au7

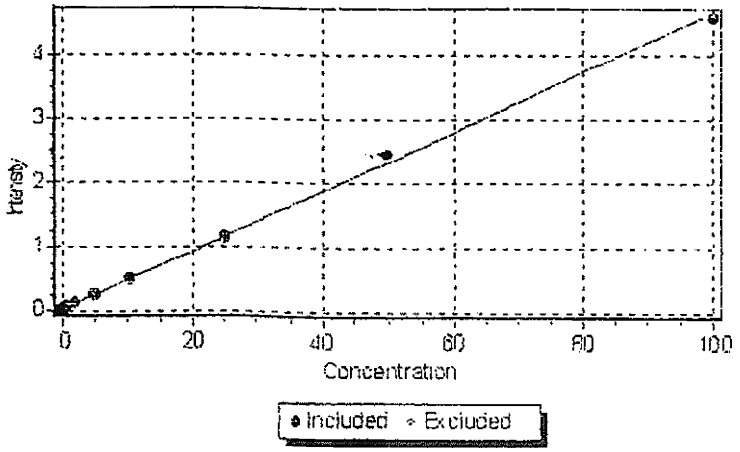
Calibration date : 01/12/04 07:50:40 AM

Coefficients :

C0 -8.86671E-01
C1 2.19577E+01
C2 -1.19709E-01
C3

Statistics :

Weighting	1/(Intensity squared)
Correlation coefficient	0.9997
Standard error of estimate	0.6995



Data points :

Standard	Intensity	Concentration	Calculated Concentration	%Difference	Included	Low point	High point
FOR 0	0.041	0.000	0.021		Yes		
FOR 1	0.061	0.500	0.454	-9.229	Yes		
FOR 2	0.084	1.000	0.966	-3.424	Yes		
FOR 3	0.137	2.000	2.123	6.136	Yes	Yes	
FOR 4	0.262	5.000	4.848	-3.036	Yes		
FOR 5	0.501	10.000	10.074	0.738	Yes		
FOR 6	1.173	25.000	24.703	-1.186	Yes		
FOR 7	2.435	50.000	51.876	3.752	Yes		Yes
FOR 8	4.641	100.000	98.435	-1.565	Yes		

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Base line calibration

Printed at : 01/12/04 07:51:16 AM

Method : Formic comb
Analyte : Ni24

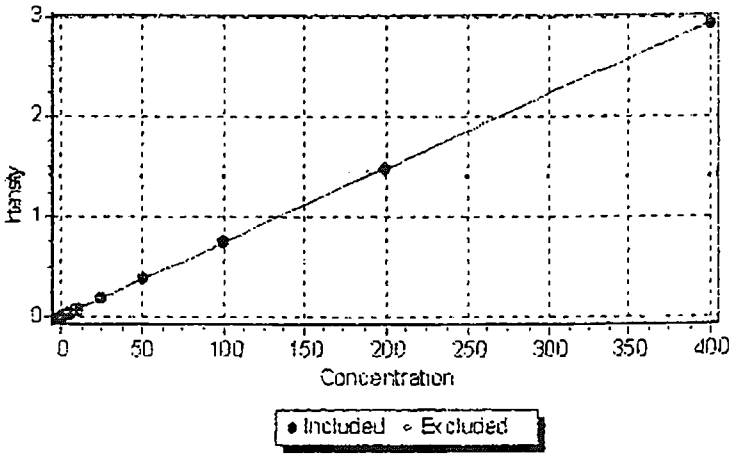
Calibration date : 01/12/04 07:51:14 AM

Coefficients :

C0 -4.00956E-02
C1 1.33270E+02
C2 9.02273E-01
C3

Statistics :

Weighting	1/(Intensity squared)
Correlation coefficient	1.0000
Standard error of estimate	0.7654

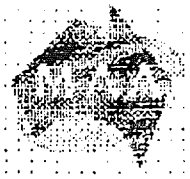


Data points :

Standard	Intensity	Concentration	Calculated Concentration	%Difference	Included	Low point	High point
FOR 0	0.000	0.000	0.000		Yes		
FOR 1	0.019	2.500	2.502	0.078	Yes		
FOR 2	0.038	5.000	4.992	-0.169	Yes		
FOR 3	0.076	10.000	10.041	0.415	Yes	Yes	
FOR 4	0.187	25.000	24.878	-0.488	Yes		
FOR 5	0.372	50.000	49.668	-0.664	Yes		
FOR 6	0.755	100.000	101.039	1.039	Yes		
FOR 7	1.485	200.000	199.859	-0.071	Yes		Yes
FOR 8	2.940	400.000	399.521	-0.120	Yes		

APPENDIX E

ICP STANDARDS



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Base line calibration

Printed at : 01/12/04 07:51:09 AM

Method : Formic comb

Analyte : Ir15

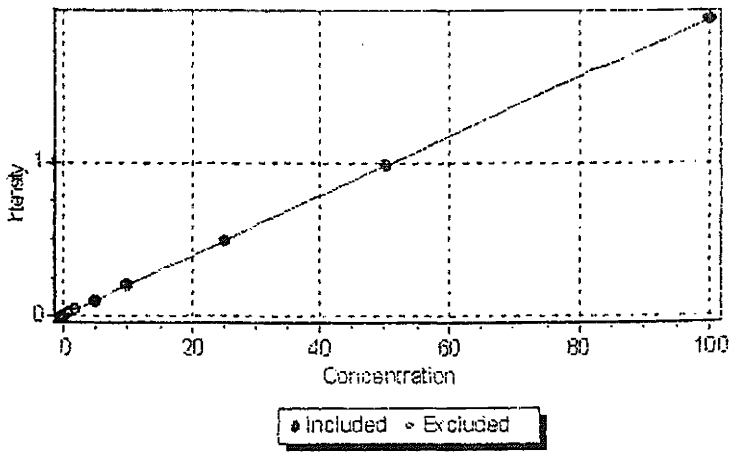
Calibration date : 01/12/04 07:51:05 AM

Coefficients :

C0 4.66600E-02
C1 4.98580E+01
C2 1.08597E+00
C3

Statistics :

Weighting	1/(Intensity squared)
Correlation coefficient	0.9999
Standard error of estimate	1.0617

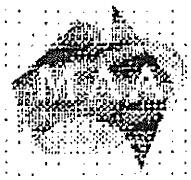


Data points :

Standard	Intensity	Concentration	Calculated Concentration	%Difference	Included	Low point	High point
FOR 0	-0.001	0.000	-0.000		Yes		
FOR 1	0.009	0.500	0.511	2.182	Yes		
FOR 2	0.019	1.000	1.002	0.232	Yes		
FOR 3	0.040	2.000	2.045	2.251	Yes	Yes	
FOR 4	0.096	5.000	4.859	-2.830	Yes		
FOR 5	0.198	10.000	9.970	-0.297	Yes		
FOR 6	0.483	25.000	24.358	-2.566	Yes		
FOR 7	0.981	50.000	50.017	0.035	Yes		Yes
FOR 8	1.938	100.000	100.737	0.737	Yes		

APPENDIX E

ICP STANDARDS



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E-mail : noorder@ibm.net
WWW : http://home.au.ibm.net/microactive/index.htm

Base line calibration

Printed at : 01/12/04 07:51:25 AM

Method : Formic comb

Analyte : Pb19

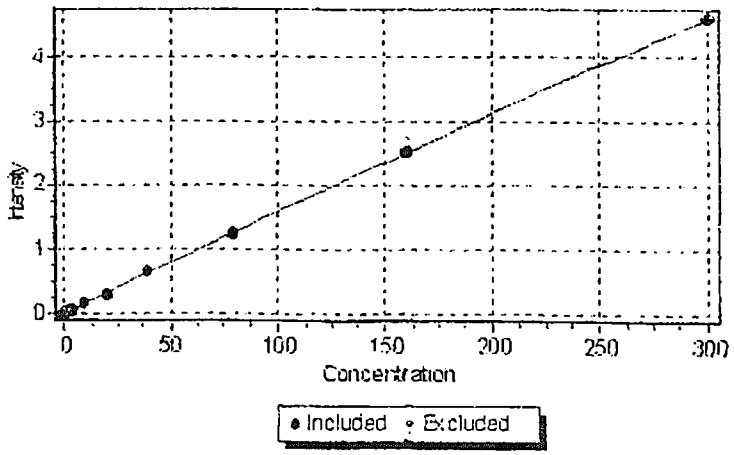
Calibration date : 01/12/04 07:51:23 AM

Coefficients :

C0 6.64617E-02
C1 6.13978E+01
C2 8.42234E-01
C3

Statistics :

Weighting	1/(Intensity squared)
Correlation coefficient	1.0000
Standard error of estimate	0.9275



Data points :

Standard	Intensity	Concentration	Calculated Concentration	%Difference	Included	Low point	High point
FOR 0	-0.001	0.000	-0.000		Yes		
FOR 1	0.016	1.000	1.029	2.909	Yes		
FOR 2	0.032	2.000	2.011	0.525	Yes		
FOR 3	0.064	4.000	3.973	-0.672	Yes	Yes	
FOR 4	0.160	10.000	9.916	-0.843	Yes		
FOR 5	0.320	20.000	19.803	-0.984	Yes		
FOR 6	0.646	40.000	40.057	0.142	Yes		
FOR 7	1.255	80.000	78.479	-1.902	Yes		
FOR 8	2.518	160.000	159.994	-0.004	Yes		
FOR 9	4.621	300.000	301.739	0.580	Yes		Yes

APPENDIX E

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Base line calibration

Printed at : 01/12/04 07:51:37 AM

Method : Formic comb

Analyte : Pd25

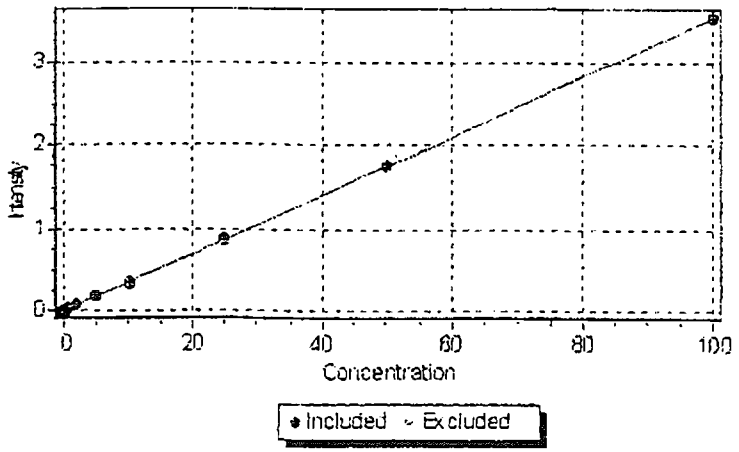
Calibration date : 01/12/04 07:51:35 AM

Coefficients :

C0 -9.97709E-03
C1 2.89829E+01
C2 -2.42832E-01
C3

Statistics :

Weighting	1/(Intensity squared)
Correlation coefficient	1.0000
Standard error of estimate	0.4507



Data points :

Standard	Intensity	Concentration	Calculated Concentration	%Difference	Included	Low point	High point
FOR 0	0.000	0.000	0.000		Yes		
FOR 1	0.017	0.500	0.487	-2.590	Yes		
FOR 2	0.035	1.000	1.009	0.938	Yes		
FOR 3	0.071	2.000	2.040	1.990	Yes	Yes	
FOR 4	0.171	5.000	4.945	-1.091	Yes		
FOR 5	0.348	10.000	10.039	0.390	Yes		
FOR 6	0.879	25.000	25.278	1.112	Yes		
FOR 7	1.738	50.000	49.625	-0.750	Yes		Yes
FOR 8	3.559	100.000	100.076	0.076	Yes		

APPENDIX E

ICP STANDARDS

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Base line calibration

Printed at : 01/12/04 07:51:46 AM

Method : Formic comb
Analyte : Pt31

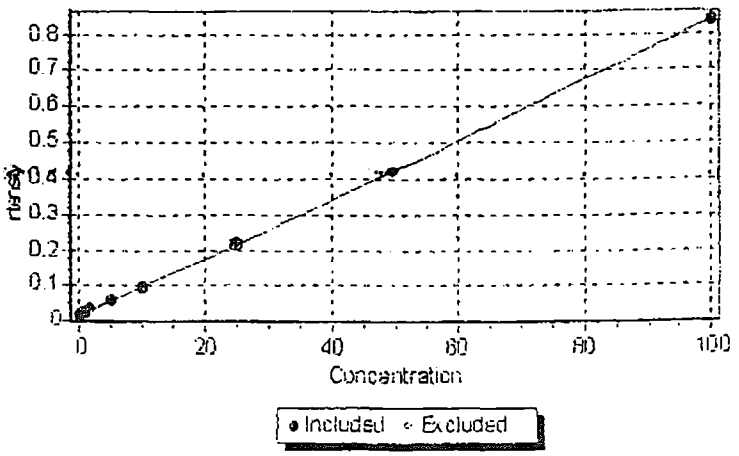
Calibration date : 01/12/04 07:51:44 AM

Coefficients :

C0 -2.26799E+00
C1 1.26515E+02
C2 -6.17169E+00
C3

Statistics :

Weighting	1/(Intensity squared)
Correlation coefficient	1.0000
Standard error of estimate	3.5852



Data points :

Standard	Intensity	Concentration	Calculated Concentration	%Difference	Included	Low point	High point
FOR 0	0.019	0.000	0.098		Yes		
FOR 1	0.022	0.500	0.462	-7.504	Yes		
FOR 2	0.025	1.000	0.851	-14.922	Yes		
FOR 3	0.034	2.000	2.058	2.916	Yes	Yes	
FOR 4	0.057	5.000	4.930	-1.396	Yes		
FOR 5	0.098	10.000	10.109	1.088	Yes		
FOR 6	0.221	25.000	25.431	1.722	Yes		
FOR 7	0.419	50.000	49.621	-0.757	Yes		Yes
FOR 8	0.842	100.000	99.940	-0.060	Yes		

APPENDIX E

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Base line calibration

Printed at : 01/12/04 07:51:54 AM

Method : Formic comb

Analyte : Rh27

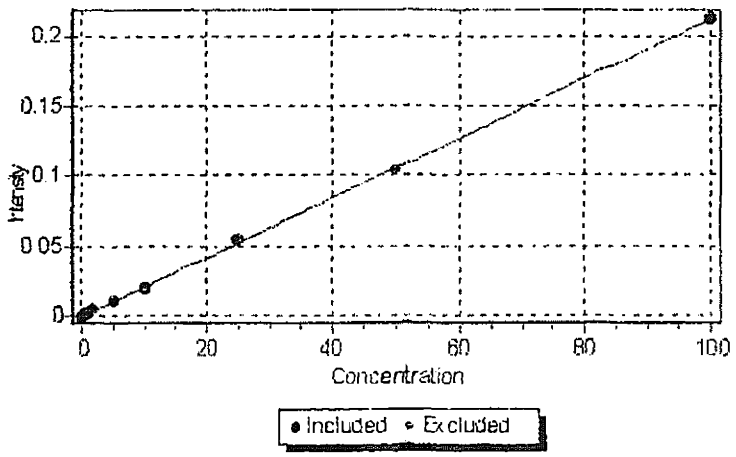
Calibration date : 01/12/04 07:51:52 AM

Coefficients :

C0 -9.47911E-02
C1 4.84749E+02
C2 -6.50805E+01
C3

Statistics :

Weighting	1/(Intensity squared)
Correlation coefficient	0.9999
Standard error of estimate	11.865



Data points :

Standard	Intensity	Concentration	Calculated Concentration	%Difference	Included	Low point	High point
FOR 0	0.000	0.000	0.000		Yes		
FOR 1	0.001	0.500	0.480	-4.073	Yes		
FOR 2	0.002	1.000	1.033	3.263	Yes		
FOR 3	0.004	2.000	1.999	-0.051	Yes	Yes	
FOR 4	0.010	5.000	4.979	-0.427	Yes		
FOR 5	0.021	10.000	9.950	-0.499	Yes		
FOR 6	0.054	25.000	25.813	3.253	Yes		
FOR 7	0.103	50.000	48.927	-2.145	Yes		Yes
FOR 8	0.213	100.000	100.319	0.319	Yes		

APPENDIX E

ICP STANDARDS

P.O. Box 2184
Chermside Centre
Chermside Q 4032
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Telephone : ++ 61 7 3359 3122
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E-mail : noorder@ibm.net
WWW : http://home.au.ibm.net/microactive/index.htm

Base line calibration

Printed at : 01/12/04 07:51:46 AM

Method : Formic comb

Analyte : Pt31

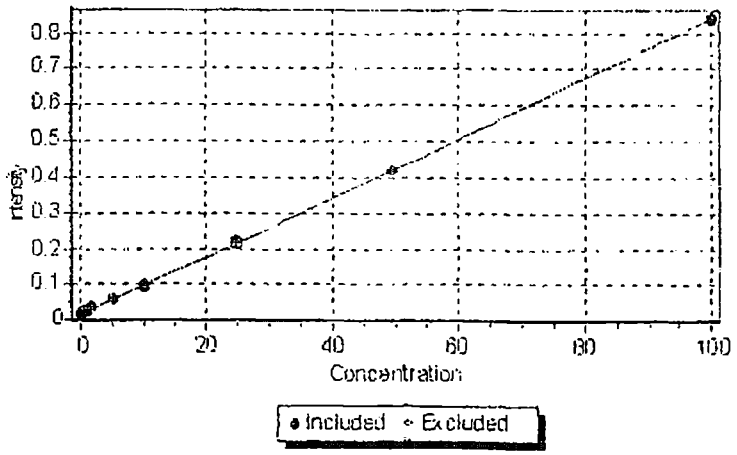
Calibration date : 01/12/04 07:51:44 AM

Coefficients :

C0 -2.26799E+00
C1 1.26515E+02
C2 -6.17169E+00
C3

Statistics :

Weighting	1/(Intensity squared)
Correlation coefficient	1.0000
Standard error of estimate	3.5852



Data points :

Standard	Intensity	Concentration	Calculated Concentration	%Difference	Included	Low point	High point
FOR 0	0.019	0.000	0.098		Yes		
FOR 1	0.022	0.500	0.462	-7.504	Yes		
FOR 2	0.025	1.000	0.851	-14.922	Yes		
FOR 3	0.034	2.000	2.058	2.916	Yes	Yes	
FOR 4	0.057	5.000	4.930	-1.396	Yes		
FOR 5	0.098	10.000	10.109	1.088	Yes		
FOR 6	0.221	25.000	25.431	1.722	Yes		
FOR 7	0.419	50.000	49.621	-0.757	Yes		Yes
FOR 8	0.842	100.000	99.940	-0.060	Yes		

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WWW : http://home.au.ibm.net/microactive/index.htm

Base line calibration

Printed at : 01/12/04 07:52:29 AM

Method : Formic comb
Analyte : Zn32

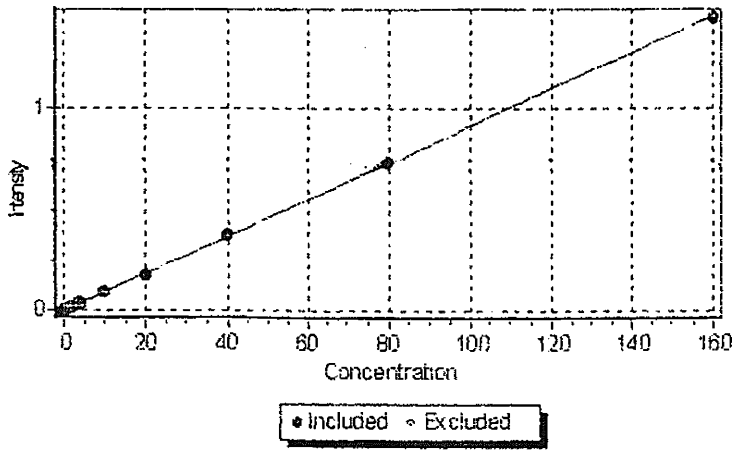
Calibration date : 01/12/04 07:52:27 AM

Coefficients :

C0 -3.35252E-02
C1 1.10144E+02
C2 -4.24114E-01
C3

Statistics :

Weighting	1/(Intensity squared)
Correlation coefficient	0.9999
Standard error of estimate	2.2347



Data points :

Standard	Intensity	Concentration	Calculated Concentration	%Difference	Included	Low point	High point
FOR 0	0.000	0.000	0.000		Yes		
FOR 1	0.009	1.000	1.007	0.740	Yes		
FOR 2	0.013	2.000	1.990	-0.504	Yes		
FOR 3	0.037	4.000	4.027	0.666	Yes	Yes	
FOR 4	0.089	10.000	9.740	-2.595	Yes		
FOR 5	0.180	20.000	19.727	-1.364	Yes		
FOR 6	0.378	40.000	41.556	3.891	Yes		
FOR 7	0.730	80.000	80.155	0.193	Yes		Yes
FOR 8	1.450	160.000	158.797	-0.752	Yes		

APPENDIX E

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Base line calibration

Printed at : 01/12/04 07:52:29 AM

Method : Formic comb

Analyte : Zn32

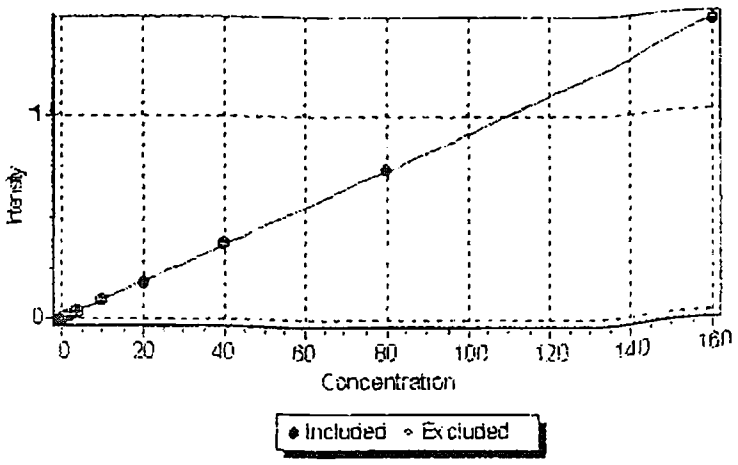
Calibration date : 01/12/04 07:52:27 AM

Coefficients :

C0 -3.35252E-02
C1 1.10144E+02
C2 -4.24114E-01
C3

Statistics :

Weighting	1/(Intensity squared)
Correlation coefficient	0.9999
Standard error of estimate	2.2347



Data points :

Standard	Intensity	Concentration	Calculated Concentration	%Difference	Included	Low point	High point
FOR 0	0.000	0.000	0.000		Yes		
FOR 1	0.009	1.000	1.007	0.740	Yes		
FOR 2	0.018	2.000	1.990	-0.504	Yes		
FOR 3	0.037	4.000	4.027	0.666	Yes	Yes	
FOR 4	0.089	10.000	9.740	-2.595	Yes		
FOR 5	0.180	20.000	19.727	-1.364	Yes		
FOR 6	0.378	40.000	41.556	3.891	Yes		
FOR 7	0.730	80.000	80.155	0.193	Yes		Yes

APPENDIX E

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WWW : http://home.au.ibm.net/microactive/index.htm

Base line calibration

Printed at : 01/12/04 07:52:19 AM

Method : Formic comb
Analyte : Te16

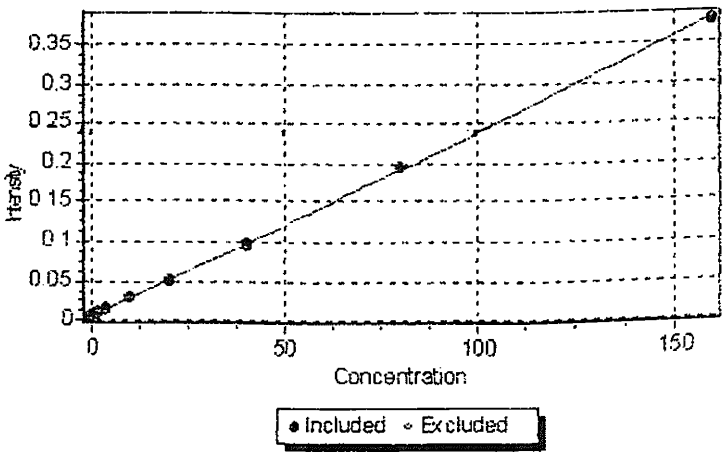
Calibration date : 01/12/04 07:52:17 AM

Coefficients :

C0 -2.51216E+00
C1 4.37699E+02
C2 -3.62065E+01
C3

Statistics :

Weighting	1/(Intensity squared)
Correlation coefficient	0.9999
Standard error of estimate	11.602



Data points :

Standard	Intensity	Concentration	Calculated Concentration	%Difference	Included	Low point	High point
FOR 0	0.006	0.000	0.094		Yes		
FOR 1	0.008	1.000	0.932	-6.800	Yes		
FOR 2	0.010	2.000	1.801	-9.929	Yes		
FOR 3	0.015	4.000	4.079	1.965	Yes	Yes	
FOR 4	0.029	10.000	10.026	0.257	Yes		
FOR 5	0.052	20.000	20.209	1.044	Yes		
FOR 6	0.098	40.000	40.117	0.291	Yes		
FOR 7	0.194	80.000	80.917	1.146	Yes		Yes
FOR 8	0.381	160.000	158.826	-0.734	Yes		

APPENDIX E

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Base line calibration

Printed at : 01/12/04 07:52:11 AM

Method : Formic comb
Analyte : Se3

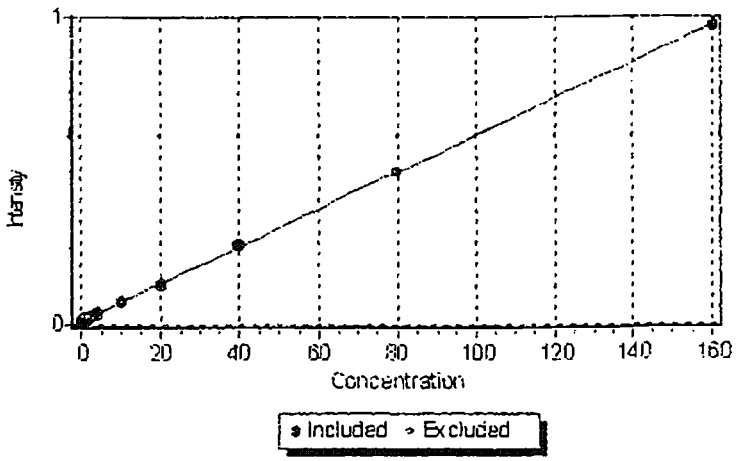
Calibration date : 01/12/04 07:52:09 AM

Coefficients :

C0 -2.30377E+00
C1 1.66783E+02
C2 -7.49300E-01
C3

Statistics :

Weighting	1/(Intensity squared)
Correlation coefficient	1.0000
Standard error of estimate	5.1469

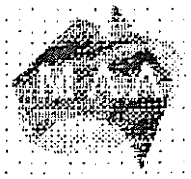


Data points :

Standard	Intensity	Concentration	Calculated Concentration	%Difference	Included	Low point	High point
FOR 0	0.014	0.000	0.108		Yes		
FOR 1	0.019	1.000	0.885	-11.518	Yes		
FOR 2	0.025	2.000	1.833	-8.333	Yes		
FOR 3	0.038	4.000	4.054	1.356	Yes	Yes	
FOR 4	0.074	10.000	10.044	0.441	Yes		
FOR 5	0.135	20.000	20.229	1.146	Yes		
FOR 6	0.259	40.000	40.923	2.307	Yes		
FOR 7	0.489	80.000	79.098	-1.127	Yes		Yes
FOR 8	0.976	160.000	159.825	-0.109	Yes		

APPENDIX E

ICP STANDARDS



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WWW : http://home.au.ibm.net/microactive/index.htm

Base line calibration

Printed at : 01/12/04 07:52:03 AM

Method : Formic comb

Analyte : Ru5

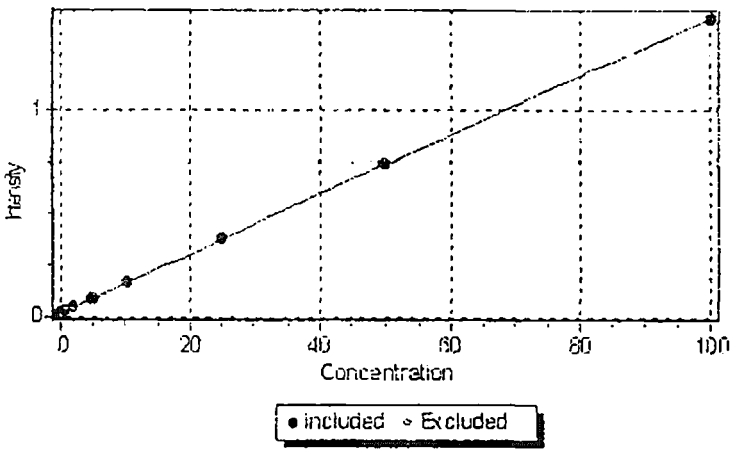
Calibration date : 01/12/04 07:52:01 AM

Coefficients :

C0 -1.41301E+00
C1 6.87422E+01
C2 9.03971E-01
C3

Statistics :

Weighting	1/(Intensity squared)
Correlation coefficient	1.0000
Standard error of estimate	1.5116



Data points :

Standard	Intensity	Concentration	Calculated Concentration	%Difference	Included	Low point	High point
FOR 0	0.021	0.000	0.047		Yes		
FOR 1	0.027	0.500	0.449	-10.124	Yes		
FOR 2	0.034	1.000	0.938	-6.162	Yes		
FOR 3	0.050	2.000	2.023	1.133	Yes	Yes	
FOR 4	0.093	5.000	5.008	0.153	Yes		
FOR 5	0.169	10.000	10.214	2.138	Yes		
FOR 6	0.380	25.000	24.854	-0.584	Yes		
FOR 7	0.742	50.000	50.062	0.125	Yes		Yes
FOR 8	1.446	100.000	99.904	-0.096	Yes		

APPENDIX E

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WWW : http://home.au.ibm.net/microactive/index.htm

Base line calibration

Printed at : 01/12/04 07:51:54 AM

Method : Formic comb

Analyte : Rh27

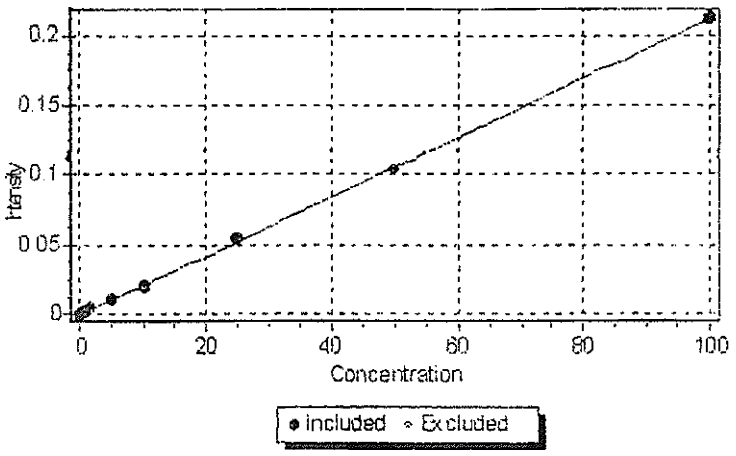
Calibration date : 01/12/04 07:51:52 AM

Coefficients :

C0 -9.47811E-02
C1 4.84749E+02
C2 -6.50805E+01
C3

Statistics :

Weighting	1/(Intensity squared)
Correlation coefficient	0.9999
Standard error of estimate	11.865



Data points :

Standard	Intensity	Concentration	Calculated Concentration	%Difference	Included	Low point	High point
FOR 0	0.000	0.000	0.000		Yes		
FOR 1	0.001	0.500	0.480	-4.073	Yes		
FOR 2	0.002	1.000	1.033	3.263	Yes		
FOR 3	0.004	2.000	1.999	-0.051	Yes	Yes	
FOR 4	0.010	5.000	4.979	-0.427	Yes		
FOR 5	0.021	10.000	9.950	-0.499	Yes		
FOR 6	0.054	25.000	25.813	3.253	Yes		
FOR 7	0.103	50.000	48.927	-2.145	Yes		Yes
FOR 8	0.213	100.000	100.319	0.319	Yes		

ICP WORKSHEET

AZOLLA SAMPLES (ppm Value Results)

Sample	pH	mv	Pt	Pd	Au	Rh	Ru	Ir	Cu	Ni	Fe	Pb	Ag	Se	As	Te	Zn	Al
Head Value - Run 1	2.3	507	96	46	<1	13	11	3	90	114	797	9	16	62	5	5	10	412
500ml Sample	2.63	432	79	11	<1	13	10	4	78	113	721	8	16	37	<1	4	16	407
1Litre Sample	2.76	444	85	19	<1	14	11	4	89	118	805	10	17	49	<1	5	11	444
1,5Litre Sample	2.41	453	92	29	<1	14	11	4	95	118	800	10	17	50	2	5	10	437
2Litre Sample	2.35	457	100	35	<1	15	12	4	102	128	883	11	18	53	2	5	12	466
2,5Litre Sample	2.33	454	93	34	<1	14	11	4	95	119	815	10	17	51	3	5	11	432
Head Value - Run 2	2.34	507	106	51	<1	15	13	4	89	128	913	11	18	69	7	6	11	441
15min. Sample	2.65	435	86	26	<1	13	9	3	85	119	787	10	16	44	4	3	10	394
30min. Sample	2.75	436	85	24	<1	13	9	3	85	119	790	10	17	44	4	3	10	399
45min. Sample	2.64	465	84	26	<1	12	9	3	84	116	768	10	16	42	4	3	10	389
60min. Sample	2.63	454	84	24	<1	12	9	3	84	117	779	10	16	43	4	3	10	392
75min. Sample	2.33	460	84	24	<1	13	9	3	83	117	783	10	16	43	4	3	10	390
90min. Sample	2.39	452	86	25	<1	13	10	3	86	121	806	10	17	44	4	3	10	397
105min. Sample	2.66	442	82	23	<1	12	9	3	82	116	774	10	16	41	3	2	10	380
Head Run 3	2.75	278	17	<1	<1	15	3	7	<1	70	2091	4	<1	87	1	<1	12	63
A	2.83	280	17	<1	<1	13	2	7	<1	71	2292	4	<1	87	1	<1	15	55
B	2.8	282	16	<1	<1	12	1	7	<1	68	2178	4	<1	82	1	<1	14	53
C	2.76	284	15	<1	<1	11	1	7	<1	66	2135	4	<1	80	1	<1	14	52
D	2.77	284	15	<1	<1	11	2	7	<1	68	2176	4	<1	83	1	<1	14	54
E	2.76	285	15	<1	<1	11	1	7	<1	67	2179	4	<1	82	1	<1	13	53
Head Run 4	2.83	281	15	<1	<1	11	1	7	<1	66	2144	4	<1	79	1	<1	14	51
A	2.81	282	15	<1	<1	11	1	7	<1	68	2188	4	<1	80	1	<1	15	50
B	2.83	282	15	<1	<1	11	1	7	<1	66	2170	4	<1	79	1	<1	15	49
C	2.84	282	15	<1	<1	11	1	7	<1	66	2152	4	<1	78	1	<1	15	49
D	2.83	282	15	<1	<1	11	1	7	<1	65	2138	4	<1	78	1	<1	14	49
E	2.83	283	14	<1	<1	11	1	7	<1	65	2138	4	<1	76	1	<1	14	48

Laboratory Manager Signature: _____

ICP WORKSHEET

EFFLUENT LIQUOR SAMPLES FROM AZOLLA TEST

Sample	pH	mv	Pt	Pd	Au	Rh	Ru	Ir	Cu	Ni	Fe	Pb	Ag	Se	As	Te	Zn	Al
Head Run 5	4.58	420	190	140	<1	6	<1	1	48	599	<1	21	114	116	6	<1	<1	<1
A	4.59	357	181	118	<1	6	<1	1	38	562	<1	19	109	110	5	<1	2	<1
B	4.6	379	180	122	<1	5	<1	1	40	522	<1	19	110	106	5	<1	1	<1
C	4.56	374	175	119	<1	5	<1	1	40	564	<1	19	107	106	5	<1	<1	<1
D	4.55	393	182	128	<1	6	<1	1	43	586	4	19	110	106	5	<1	<1	<1
E	4.57	401	184	129	<1	6	<1	1	44	592	<1	20	112	110	5	<1	<1	<1
Head Run 6	4.57	373	181	119	<1	6	<1	1	40	571	<1	19	110	108	5	<1	1	<1
A	4.56	372	176	113	<1	5	<1	1	40	566	<1	19	108	106	5	<1	2	<1
B	4.57	370	181	112	<1	5	<1	1	42	570	<1	19	110	110	5	<1	2	<1
C	4.57	367	178	112	<1	5	<1	1	40	562	<1	19	108	107	5	<1	2	<1
D	4.62	360	173	107	<1	5	<1	1	39	571	7	18	107	97	4	<1	2	<1
E	4.56	371	182	121	<1	6	<1	1	41	576	<1	20	112	110	5	<1	1	<1
Head Run 7	4.51	405	218	190	<1	42	<1	<1	79	235	<1	6	190	819	6	<1	3	<1
A	4.49	370	202	159	<1	39	<1	<1	57	208	<1	6	180	788	5	<1	5	<1
B	4.52	383	192	157	<1	34	<1	<1	57	210	<1	5	174	763	5	<1	5	<1
C	4.5	390	200	172	<1	35	<1	<1	63	220	<1	5	179	779	6	<1	4	<1
D	4.48	394	192	169	<1	33	<1	<1	59	210	<1	5	175	763	6	<1	4	<1
E	4.49	397	202	180	<1	34	<1	<1	65	216	<1	6	184	798	7	<1	5	<1

Laboratory Manager Signature: _____

ICP WORKSHEET AZOLLA SAMPLES (ppm Value Results) Ex Microwave

Sample	pH	mv	Pt	Pd	Au	Rh	Ru	Ir	Cu	Ni	Fe	Pb	Ag	Se	As	Te	Zn	Al
Blank 50^0			<8	23	<2	<5	<5	<5	145	60	250	16	<5	<6	<6	<10	<0.8	40
Azola - Run 1			2639	173	<2	74	<3	<5	751	91	8883	76	<5	2850	731	62	<0.8	1712
Azola - Run 2			2022	1844	<2	71	<3	<5	979	246	9664	105	<5	2710	974	80	<0.8	1093
Blank - Sun Dried			<4	<6.9	<0.8	<5.6	<2.8	<2.8	21	53	1018	67	5	27	<4	<5.6	218	401
Azola - Run 3			133	109	5	129	183	68	108	388	8230	108	5	317	32	<1	64	842
Azola - Run 4			586	2480	13	43	27	55	930	3840	2290	251	5	534	<1	15	72	1
Azola - Run 5 & 6			319	406	7	244	170	132	133	361	7980	142	5	256	34	260	<1	885
Blank 50^0			<8	23	<2	<5	<5	<5	145	60	250	16	<5	<6	<6	<10	<0.8	40
Azola - Run 7			1000	1010	17	207	17	237	899	3850	1790	286	5	473	36	97	<1	286

APPENDIX F

Laboratory Manager Signature: _____