An investigation into the Biological Treatment of Platinum Refinery Effluent using the plant Azolla Filiculoides

By

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Dedicated to

My daughters Kerry-Ann and Christie.

ABSTRACT

In order to understand the effects of metals contained in effluent and to define effluent quality suitable for safe discharge to natural water streams, it is essential to understand the effects of the interaction of metal ions with plants. The availability of metal ions and their ability to bind to plants are dependent on the chemical speciation of metals and on the biological factors governing the availability of metals within the plant cells.

This thesis will address both aspects and thereby propose a combination of an appropriate chemical and biological approach to the investigation of bioaccumulation of the plant *Azolla Filiculoides*. Laboratory studies have shown that varying concentrations of free metal ions in solution determine efficiency of metal uptake and that metal toxicity can also be detrimental to plant life and efficiency of metal recovery from solution. Many questions however, remain unanswered with regard to the application of a biological treatment for effluent discharge. This thesis includes the determination of metal speciation combined with the study of bioaccumulation of metals in plants and their effects from test-work utilising effluent generated from a Precious Metals Refinery (PMR).

Plant species are known to differ widely in their tolerance to metals, however despite an abundant knowledge on molecular, biochemical and physiological effects of metals to plants, only a few general principles have been proposed to guide the prediction of tolerance differences. The properties of protective cellular responses as well as of the molecular target sites are important components in determining the intrinsic tolerance of a particular species to a metal. The role of the whole assembly of cellular ligands in buffering metal ions within the cells will be evaluated.

Standard preparation methods combined with use of Inductively Coupled Plasma Emission Spectrophotometer (ICP) used for analytical analysis will be included to reflect analytical data in providing evidence to support a conclusion. The outcome of the test work utilising the aquatic plant *Azolla* has proven that it can be used as a process step to re-mediate effluent generated from Precious Metal Refining operations. This process offers an alternative to the classical chemical methods widely used in the Precious Metals Refining industry proving economically viable and ensuring environmental sustainability in comparison to the current known methods of effluent treatment.

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LIST OF ABBREVIATIONS:

SA	South Africa
PGM / PGM's	Platinum Group Metal/s
PMR	Precious Metals Refinery
ppm	Parts Per Million
ppb	Parts Per Billion
PTE	Potentially Toxic Elements
IAP	Invading Alien Plants
ERWAT	East Rand Water Care Works ?
PVC	Poly-vinyl-chloride
ICP	Inductively Coupled Plasma
R & D	Research and Development
XPS	X-ray Photoelectron Spectroscopy
Pt	Platinum
Pd	Palladium
Au	Gold
Rh	Rhodium
Ru	Ruthenium
Ir	Iridium
Cu	Copper
Ni	Nickel
Fe	Iron
Pb	Lead
Ag	Silver
Se	Selenium
As	Arsenic
Te	Tellurium
Zn	Zinc
Al	Aluminium

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CHAPTER ONE: INTRODUCTION

1.1 Water Quality Management

Water quality management can be described as the understanding of the physical, chemical and biological characteristics of water and of the requirements associated with various uses of water. It can also be described as the method of predicting changes in water quality due to environmental change and of the method of modification of water quality (Tchobanoglous & Schroeder, 1987).

In Terms of the Constitution of the Republic of South Africa, 1996 (Act No. 108 of 1996) the management of water resources is an exclusive National competency. The National Water Act, 1998 (Act No. 36 of 1998) mandates the Minister of Water Affairs and Forestry to ensure that water is protected, used, developed, conserved, managed and controlled in a sustainable and equitable manner for the benefit of all persons.

The Minister of Water Affairs and Forestry, supported by the Department of Water Affairs and Forestry, acts as the public trustee of the Nation's water resources. Within the Department of Water Affairs and Forestry, the Directorate of Water Quality Management and the various Regional Offices are jointly responsible for the governance of water quality in South Africa.

1.2 Heavy Metal Pollution

Pollution of water and soil by effluent deposited by mining and industrial operations is a major 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. (Nedelkoska, *et al.*, 1998).

The effects of polluted water on human health, on the aquatic ecosystem (aquatic biota and in-stream and riparian habitats) and on various sectors of the economy, including agriculture, industry and recreation, can be disastrous. Deteriorating water quality leads to increased treatment costs of potable and industrial process water and decreased agricultural yields due to increased level of salinity in irrigation water. However, not all health, productivity and ecological problems associated with deteriorating water quality are ascribed to man's activities (http://www.dwaf.gov.za/Dir_WQM/wqm.htm).

Many water quality related problems are inherent in the geological characteristics of the source area. The occurrence, transport and fate in the aquatic environment of numerous persistent and toxic metals and organic compounds (e.g. pesticides) have given cause for serious concern. Contamination of groundwater resources, or of sediments deposited in riverbeds, impoundments and estuaries by toxic and persistent compounds can cause irreversible pollution, sometimes long after the original release to the environment has ceased (http://www.dwaf.gov.za/Dir_WQM/wqm.htm).

1.3 Current Situation in SA

The loudest cry in South Africa is still for safe, clean and accessible drinking water and sanitation services. Access to water at its source is, in fact, only a small element of access to water services; for these, infrastructure, technical and management skills and adequate funds are usually the critical elements. The most important contribution to achieving equitable access to water services is the provision of funds and the regulation and direction of the institutions whose task it is to provide the services. Access can be improved by changing the rules about access to water resources, but this will not in itself meet the needs and desires of people for whom piped water is still a dream (Water Policy, White Paper).

The sustainable use of water resources means that, even where the immediate demands for development are very high, society must find different development approaches which, is to make sure that the use of water resources does not destroy their ability to recover. This approach is in keeping with Section 24 of the Constitution, which/states that any development and use of our natural resources (including water resources) must be environmentally sustainable (Water Policy, White Paper).

1.4 Metal Refining

Base metals are removed from a converter matte either by leaching or by a combination of magnetic separation and leaching processes. The majority of the problem elements such as selenium, arsenic and tellurium are also removed at this stage. The resulting concentrate is sent for further processing into refined precious metals. Base metals are a valuable by-product of PGM extraction. Their further refining by the various producers is largely dictated by economies of scale.

Precious metals refining processes have developed considerably in recent years. The older or "classical" process involved first roasting the PGM concentrate. This makes the rhodium, iridium and ruthenium insoluble in aqua regia. The platinum, palladium and gold are then dissolved and separated by a series of sequential precipitations. Pyro-metallurgical and leaching processes then upgraded the remaining residue before being separated into individual metals.

Final purification of all metals is by repeated dissolution and precipitation. Improved separation and refining procedures have become available for all of the precious metals. These commonly involve operations such as solvent extraction or ion exchange. They are being introduced either to replace procedures in the classical process or as part of completely new refining processes. Advantages such as improved precious metals recovery, lower refining costs and shorter processing times are being claimed.

1.5 Effluent

On a global basis, trace metal pollution is among the most pervasive and serious environmental problems facing the biosphere. Trace metals pose particular problems to the environment because of their chemical stability, they must be sequestered from the effluent that they are contained within.

The recovery of precious metals from a wide assortment of precious metal-bearing materials, effluent or waste solutions, is an attractive proposition. Process recycling is essential for the effective utilisation of raw materials and reduction of mounting waste disposal problems. In many cases, acid leaching from disposable material precedes the separation and recovery of individual metals (Edson, 1985).

Carbon adsorption, ion exchange and some chemical processes are among the methods used for the recovery of metals. The property of certain microbial biomass types has the ability to remove and concentrate metals such as Palladium, Copper, Nickel, Zinc, Cadmium, Gold, Mercury and Uranium (Kuyucak, 1988).

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Bio sorption can be exploited as a new and potentially economical alternative to the conventional methods (Kuyucak, 1986). Some bio-sorbent materials show different degrees of affinity for the different species of metals. An advantage can be taken of bio-sorbent selectivity in the development of bio-sorbents for the recovery of specific metal (Kuyucak, 1988).

Effluent generated from a precious metals refinery (Acid and Alkaline) contains a degree of metal contaminants relative to the content of concentrate material processed. Various chemical processes are adapted for the efficient removal of Platinum Group Metals (PGM's); certain base metals remain, as they are not processed for removal. Table 1.1 is a typical representation of the metals contained in the concentrate received for processing. The chemical processing of PGM's is based on the change in state of the metallurgical structure; this is controlled by the rate of oxidation or reduction by the addition of certain chemicals.

Table 1.1: Various percentages of metals that make up the average months concentrate received from the base metals refinery. Nett concentration weight being 3567.972 Kg, dry weights 3311.353 Kg with a moisture level of 7.192%.

Metal	Percentage	Metal	Percentage
Antimony (Sb)	0.385	Manganese (Mn)	0.015
Arsenic (As)	1.818	Nickel (Ni)	3.318
Bismuth (Bi)	0.144	Palladium (Pd)	14.886
Calcium (Ca)	1.032	Platinum (Pt)	32.465
Chromium (Cr)	0.624	Rhodium (Rh)	5.126
Cobalt (Co)	0.144	Ruthenium (Ru)	8.856
Copper (Cu)	6.275	Selenium (Se)	0.496
Gold (Au)	0.792	Silver (Ag)	0.905
Iridium (Ir)	1.952	Tellurium (Te)	0.786
Iron (Fe)	2.335	Titanium (Ti)	0.054
Lead (Pb)	1.321	Zinc (Zn)	0.001
Magnesium (Mg)	0.410		

The current method for handling effluent generated from concentrate, volumes shown in (Table 1.1), adopted at the refinery is one of chemically treating the liquor until a PGM content of 10 parts per million (ppm) per element or less is achieved for all metals. The liquor is then filtered off and sent for disposal at a hazardous waste facility and the solids returned to process. In conjunction with this process and over a period of time, liquors have been stored in what is commonly known as "evaporation ponds" (Appendix A, Figure 1.1) before final disposal. A potential environmental problem exists with this procedure, as the stored liquor contains combinations of toxic, base and precious metals and depending on the condition of the storage facility can come into contact with underground water bodies by seepage or even over-flow in the event of un-natural rain falls.

Table 1.2: Effluent discharged for the year 2001, January through December (Western Platinum Refinery Monthly Report). Note: This is the acid effluent only.

Month	Volume (Kl)	Month	Volume (Kl)
January	1, 110, 000	July	1, 404, 000
February	1, 300, 000	August	1, 850, 000
March	881, 571	September	2, 570, 000
April	1, 320, 000	October	230, 000
May	1, 760, 000	November	1, 150, 000
June	1, 770, 000	December	1, 010, 000

In addition to this method of disposing being potentially harmful to the environment, in the form of co-disposing, this method commonly used by the disposal agency is also not financially viable. Co-disposal is the ratio of a portion of solid waste mixed with a portion of liquid waste (effluent) and disposed of in certain areas where the soil is not permeable to underground water bodies or streams.

The costs for this form of disposal amounts to millions of Rands per year depending on the quantity of effluent generated and secondly this method is not environmentally sustainable, thus the search for a cost effective, environmentally friendly, method for the treatment and/or disposal of effluent liquors is essential.

1.6 Effluent Discharge

As per section 21 of the Water Act and regulations (54 of 1956): purification and disposal of water used for industrial purposes and effluent, states that any person using water for industrial purposes, including sea water brought ashore, shall purify or otherwise treat the water and any effluent produced by or resulting from such use.

After complying with the paragraph above, one may discharge the purified or treated water, including water recovered from any effluent, in a manner and subject to certain requirements prescribed in the Act. It may be discharged into a canal, sewer or other conduit controlled by the Minister or the relevant local authority, body or person, as the case may be.

Water used for industrial, urban or domestic purposes and which is discharged for purposes of the purification, treatment or disposal thereof into a canal, sewer or other conduit controlled by a local authority, body or person having authority to undertake the purification, treatment or disposal of water or effluent, shall be deemed to be water used by that local authority, body or person for industrial purposes. Effluent which is discharged into a canal, sewer or other conduit controlled by a local authority shall be deemed to be effluent produced or which resulted from the use by that local authority, body or person, of water for industrial purposes.

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1.7 Effluent Standard

The following quality standards are adopted for waste water or effluent arising in a catchment area, draining water to any river or a tributary thereof, at any place between the source thereof and the point of such catchment area, situated within the territory of the Republic of South-Africa (Government Notice No 991).

Table 1.3: Quality standards for wastewater or effluent arising in a catchment area.

Colour, odour or taste.	The wastewater or effluent shall not contain any substance in a concentration capable of producing any colour, odour or taste.
pH.	Shall be between 5,5 and 7,5.
Dissolved oxygen.	Shall be at least 75 per cent saturation.
Typical (faecal) coli.	The wastewater or effluent shall contain no typical (faecal) coli per 100 millilitres.
Temperature.	Shall be a maximum of 25 ^{0}C.
Chemical oxygen demand.	Not to exceed 30 milligrams per litre after applying the chloride correction.
Oxygen absorbed:	The oxygen absorbed from acid N/80 potassium permanganate in 4 hours at 27 ^{0}C shall not exceed 5 milligrams per litre.
Conductivity.	Not to be increased by more than 15 per cent above that of the intake water.
Suspended solids.	Not to exceed 10 milligrams per litre.
Sodium content:	Not to be increased by more than 50 milligrams per litre above that of the intake water.
Soap, oil or grease:	None.

The wastewater of effluent shall contain no other constituents in concentrations, which are poisonous or injurious to trout or other fish or other forms of aquatic life. Other constituents are included in Table 1.4.

Table 1.4: Other constituents listed in the Government Notice No 991.

Maximum concentration in milligrams per litre	
Residual chlorine (as Cl)	Nil
Free and saline ammonia (as N)	1.0
Nitrates (as N)	1.5
Arsenic (as As)	0.1
Boron (as B)	0.5
Total chromium (as Cr)	0.05
Copper (as Cu)	0,02
Phenolic compounds (as phenol)	0,01
Lead (as Pb)	0,1
Soluble ortho phosphate (as P)	1,0
Iron (as Fe)	0,3
Manganese (as Mn)	0,1
Cyanides (as Cn)	0,5
Sulphides (as S)	0,05
Fluoride (as F)	1,0
Zinc (as Zn)	0,3
Cadmium (as Cd)	0,05
Mercury (as Hg)	0,02
Selenium (as Se)	0,02

1.8 Bioremediation

Bioremediation is a term indicating the use of biological agents to reclaim soils and water polluted by substances hazardous to human health and/or the environment. Technically, bioremediation targets the xenobiotics, which means of foreign origin and of toxic pollutants or contaminants either synthetic or alien to the environment. The human population's unbalanced activities, along with uncontrolled release of recalcitrant, xenobiotic and toxic compounds have raised the need for the development of additional effective remediation processes (National Institute for Nuclear Technology Research, 2000).

Traditional and modern industrial activities involved in metal mining and other metal-related industries, discharge toxic heavy metals that contaminate the soil and water resources. The processes of detoxification of these potentially toxic elements (PTE's) or heavy metals are limited and often unsatisfactory. Modern agricultural practices, including injudicious use of fertilizers, herbicides and pesticides, if not monitored and left unchecked may lead to environmental deterioration (National Institute for Nuclear Technology Research, 2000).

Innovation requires the application of novel biotechnological techniques to unique or common industrial problems. Conventional treatment technologies, which include chemical precipitation, chemical oxidation or reduction, filtration, electrochemical treatment, application of membrane technology and evaporative recovery, have proven to be either ineffective or extremely expensive to operate.

Therefore the need for the development of new bio-sorbent or sorbent processes, which can become directly competitive, both economically and performance wise with modern treatment methods, does exist (http://www.wrc.org.za/wrcpublications/wrcbulletin/v24_4/sludge.htm).

By far the greatest demand for metal sequestration comes from the need of immobilizing the metals, partially lost through human technological activities. Heavy metals need to be removed at the source in specially designed, low cost, pre-treatment steps, which prevent contamination and discharge to the environment can be done under controlled conditions, if the need arises. The search for efficient and particularly cost-effective remedies is ongoing (Volesky, 1999).

It has been established beyond doubt that dissolved, heavy metals escaping into the environment, can pose a serious health hazard that has the potential to last for many years. Man receives pre-concentrated metal toxicity, which accumulates in living tissues throughout the food chain with humans at the top; therefore a need for controlling the heavy metal emissions into the environment is necessary.

Specific advantages of bio-sorption include competitive performance, heavy metal selective, cost effectiveness, regenerative, no sludge generation and also metal recovery is possible (Volesky, 1999). Bio sorption will be discussed in more detail in the following chapter.

1.9 Bio sorption

Bio sorption promises to fulfil the requirements of avoiding toxic metals threatening the environment and utilises biomass such as raw materials, which are either abundant (seaweeds) or wastes from other industrial operations (fermentation wastes). The metal-sorbing performance of certain types of biomass can be more or less selective for heavy metals that depend on (1) the type of biomass and its preparation, (2) the mixture in the solution and (3) the chemico-physical environment (Volesky, 1999).

Numerous investigations carried out over past years testify to the ability of microbial cells to concentrate substantial amounts of metals from a medium, no matter whether these metals are of physiological significance or whether their involvement in the metabolic processes has been established. Virtually in all cases microbial biomass can retain more or less significant amounts of metal ions by means of "passive" sorption and/or complex formation.

This type of interaction is called bio-sorption and is characteristic of both live and dead cells. However, in a number of cases it is possible to observe "active" concentration of metal ions by live cells, which is markedly dependant upon the metabolic activity of the organism. Such a type of interaction has been given the generalised name bioaccumulation. Generally, the process of concentration includes two phases:

- The short term initial phase of physiochemical binding of metal ions, and

- The subsequent prolonged phase of energy dependant accumulation in the cell.

The first phase takes several minutes and the duration of the second phase is several hours (Karamushka, *et al.*, 1994).

It is important to note that uptake during sorption can be manipulated by the properties of a bio-sorbent, or upon de-sorption during the regeneration cycle of the bio-sorbent. Metal removal in bio-sorption processes is comparable to commercially used competitors, namely the ion-exchange treatment. Effluent qualities in the order of only ppb (µg/L) of residual metal(s) can be achieved. While commercial ion exchange resins are rather costly, the price tag of bio-sorbents can be an order of magnitude cheaper, (1/10 of the ion exchange resin cost).

The attraction of bio-sorption is its cost effectiveness, the levels of uptake in various plants and a waste that could be re-used. While ion exchange can be considered a mature technology, bio-sorption is in its early developmental stages and further improvements in both performance and costs can be expected. Thus, bio-sorption may be useful in providing a solution for environmental problems (Volesky, 1999).

While the bio-sorption process could be used even with a low degree of understanding of its metal-binding mechanisms, better understanding of metal binding will result in more effective and optimised applications. This poses a scientific challenge with ongoing Research and Development (R&D) efforts. In addition, even the same type of industrial activity can produce effluents that differ from each other a great deal. Bio sorption does offer a competitive wastewater treatment alternative; the basis of this treatment alternative needs to be well understood in order to prevent possible application failures (Http://ww2.mcgill.ca/biosorption/publication/BVspain/BVspain.htm).

1.10 Biological Options

Although the plant species *Azolla Filiculoides* is to be used for experimental work, due to the availability of the plant in the Gauteng area, there are other plant species available for biological treatment and have already been tested in terms of heavy metal sorption, with numerous studies performed on gold accumulation using various plants and algae species. Below is some examples of other plant / algae species.

1.10.1 *Eichhornia Crassipes* (Water Hyacinth)

The most common floating aquatic plant used for treatment of wastewater is the hyacinth. They are widely used to provide nutrient removal and upgrade the performance of Waste stabilisation ponds. The use of submerged macrophytes is to provide a final polishing step after primary and secondary treatment. (http://www.scienceinafrica.co.za/2001/september/wsp.htm).

1.10.2 *Spirulina Platensis*

Spirulina Platensis is a filamentous cyanobacterium that is biotechnologically important due its high nutritional value. The nutritional value derives from its high protein content (about 70%) and its type of lipids (g-linolenic acid) (Ciferri and Tiboni, 1985; Henrikson, 1989). This micro organism also finds application in environmental technology (Pulz and Scheibenbogen, 1998).

1.10.3 *Salvinia*

Salvinia is a floating aquatic plant, which grows wildly in sub-tropical and tropical regions. It has not been subject of extensive research as in the case of other aquatic plants until recently. Research has been carried out to define its capacity to remove heavy metals from diluted solutions. It was found (Olguin *et al*, 1997), that bioaccumulation factors were in the range of 2595-2932 for cadmium removal, in the range of 2730-3211 for lead removal and around 783-784 for chromium removal. The removal efficiency was a function of the pH of the solution, in all cases.

1.10.4 Kelpak

Kelpak, seaweed concentrate made from brown alga *Ecklonia Maxima* that is harvested off the southern African west coast. Kelpak is made using a unique "cell burst" process, where high and low pressure causes the cell walls to expand and rupture, releasing the cell contents. This is filtered and the solution used as a concentrate for agriculture and horticultural application. No chemicals or heat are involved in the production process. The waste product consists of the ruptured cell walls (Stirk, *et. al.,* 2001).

1.11 *Azolla Filiculoides* (Red Water Fern)

The heightened awareness of human welfare, environmental protection and the irreversible damage to soil and water, has raised interest in biological solutions for potentially toxic elements (PTE) detoxification using bacterial, fungal, algal and plant-based technologies. Quite well known is the use of *Azolla filiculoides*, an aquatic fern in the bio filtration of heavy metal effluents from metal industries (National Institute for Nuclear Technology Research, 2000).

Red water fern, *Azolla* (Figure 1.2) is a small, rarely longer than 25mm, green to reddish, free-floating annual or perennial plant. The short branched stem bears roots and is densely covered with small leaves, which are between 1 and 1.5 mm long and which, overlap in a scale-like manner. Each leave is bi-lobed. The upper lobe contains chlorophyll, while the lower one is colourless.

A blue green alga~~e~~ is present in the cavities of the upper lobe of each leaf. From October to February spores are produced as rounded fruiting bodies borne in the leaf axis. Red water fern is fertile under South African conditions and also propagates vegetatively. Red water fern is also considered a declared weed, invader plant (Department of Water Affairs and Forestry, 1997).

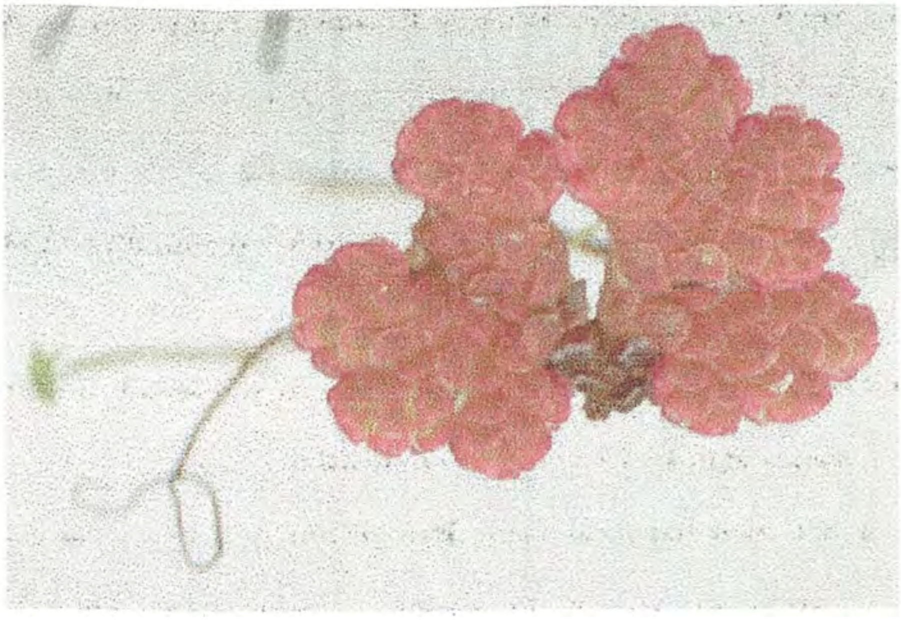


Figure 1.2:Red *Azolla* leaf.
Under nutrient deficient and strong light conditions, *Azolla* becomes red.

Invading alien plants (IAP's) are the single biggest threat to plant and animal biodiversity. IAP's have become established in over 10 million hectares of land in South Africa. The cost of controlling IAP's in South Africa is estimated at R600 million a year over 20 years. If IAP's are left uncontrolled, the problem will double within 15 years. IAP's waste 7% of our water resources; reduce our ability to farm; intensify flooding and fires; cause erosion, destruction of rivers, siltation of dams and estuaries and poor water quality and can cause a mass extinction of indigenous plants and animals (Department of Water Affairs and Forestry, The Working for Water Programme, 1995).

Not only are *Azolla* species prolific vegetative reproducers, they also have a very interesting and uniquely specialized sexual cycle. Like all ferns, *Azolla* produces spores (Figure 1.3); however, unlike most ferns, *Azolla* produces two kinds of spores. If one carefully examines *Azolla filiculoides,* during the summer months, one can easily find numerous spherical structures called sporocarps on the undersides of the branches.

The sporocarp of *Azolla* is homologous to the sorus of other ferns and the sporocarp wall represents modified indusium's. The male sporocarp is a greenish or reddish case about two millimetres in diameter and inside are numerous male sporangia, which look like the egg mass of an insect or spider inside a transparent case. Male spores (microspores) are extremely small and are produced inside each microsporangium. When growing in full sunlight, particularly in late summer and fall, *Azolla* may produce reddish anthocyanin in the leaves, in contrast with the bright green carpets of duckweed and filamentous green algae (Armstrong, 1979).

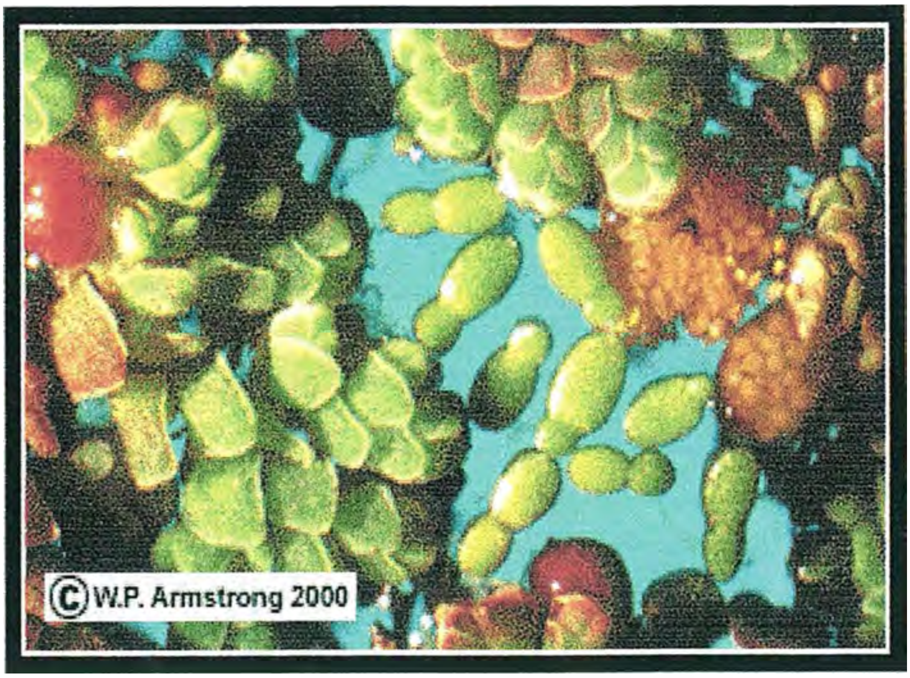


Figure 1.3: Close-up view of *Azolla filiculoides.*
Scale like, overlapping leaves and several globose reproductive structures called sporocarps. The male sporocarp (middle right) contains micro sporangia that resemble eggs inside an egg sac. One sporocarp has broken open releasing many spore clusters or massulae. The minute ovoid plants in center are *Wolffia borealis*, a minute flowering seed plant.

Azolla absorbs nutrient from water, when it is floating on water. However, since phosphorus diffusion from soil to water is slow, field population of floating *Azolla* is generally deficient in phosphorus. The application of phosphorus fertilizer (water soluble, applied on top of an *Azolla* mat) is effective to enhance its growth. Live *Azolla* prefers an acidic media of pH 4. *Azolla* plants have a long life and will absorb various metals, with some metals as much as 500 times their concentration in common effluents. It was found that because heavy metals bind to the cell walls of the plant, *Azolla* could be equally effective when dried and pressed (Ashkenazy, 1999).

This attribute makes it possible to transform the *Azolla* into a bio filter, rather than use it as a living plant that must be nurtured and attended. Bio filters can also be placed anywhere, especially close to a source of a potential pollutant. This reduces the amount of effluent that must be treated and optimises the efficiency of the bio filter, which can be targeted to treat one kind of metal, instead of a complex chemical mixture of various compounds in a larger body of water (Ashkenazy, 1999).

1.12 Common use of *Azolla*

Azolla has been used for centuries to enrich rice paddies with fixed nitrogen. Before planting rice, the farmer allows the surface of the rice paddy to become densely covered with the fern. As the rice plants grow, they eventually crowd out the *Azolla-Anabaena* mixture, leading to the death of the fern and release fixed nitrogen, which is assimilated by the rice plants (Brock, 2000).

Azolla has been used as feed for pig, duck, and fish. It has high content of protein (20-30% on dry weight basis). Because its protein lacks in methionine and cystein, a combination with cereals is needed. Nutritional values of *Azolla* to animals vary greatly with *Azolla* species. A. Microphylla is the best and palatability by fish is better than other species. On a dry weight basis, *Azolla* can be mixed up to 10% of the purchased animal feed (http://www.asahi-net.or.jp/~it6i-wtnb/azolla~E.html#ch1.1).

Many benefits of using *Azolla* are recognized. As a result, the integrated use of *Azolla* with rice and fish farming has been developed at Fujian Academy of Agricultural Sciences, China. The integrated approach can increase a farmer's income, while reducing the use of pesticides and fertilizers, and, consequently, environmental pollution. A Filipino farmer, Mr. Fantilanan, also developed an integrated use. He combined *Azolla* culture with rice and vegetables culture, and rearing pigs and ducks. The excreta from pigs and ducks were introduced to a house scale biogas plant and the effluent from biogas plant was returned to wetland rice fields with *Azolla* (http://www.asahi-net.or.jp/~it6i-wtnb/azolla~E.html#ch1.1).

CHAPTER TWO: METALS

2.1 Metal Speciation

The overall toxicity in an environment is not so much determined by total metal concentration but, instead by the metal species, which determines the bio-availability (Roane *et al.*, 1996). The speciation of a metal in the environment is the result of the combined effects of pH, redox potential and to a lesser extent, ionic strength (Sposito, 1989). The speciation of the metal ion will determine whether precipitation is predominantly a function of pH or of alkalinity. The exact composition of a precipitate depends on the species of the metal ion, the concentration of metal and anion, pH and thermodynamic and kinetic factors (Uusitalo, 1995).

Metal bio-availability is determined by the solubility of the metal species present and the sorption of metal species by solid surfaces, including organic matter and colloidal materials. Bio-availability metals are therefore metals in solution that are not bound to solid phase particles (Roane *et al.*, 1996). Studies have shown that metals in solution are most available to uptake and are easily accumulated.

It's known that heavy metal ions are accumulated by plant species and are valuable as an aid in removing metal ions from solutions. Biological processes for removal can be divided into three general categories:

- Bio sorption (adsorption) of metal ions onto the surfaces of micro organisms.

- Intracellular uptake of metal ions, and
- Chemical transformation of metal ions by micro organisms.

The latter two processes, intracellular uptake of metal ions and chemical transformation of metal ions by micro organisms, have found some use in water purification and mining processes, however, they suffer from a disadvantage that living organisms are required. The process of bio-sorption, however, may occur under conditions that would normally be toxic to living organisms. Adsorption occurs through interaction of the metal ions with functional groups that are found in the cell wall biopolymers of either living or dead organisms. (Environmental Science Technology, Volume 20. 1986).

2.2 Redox

The conversion of energy from chemical reactions in living organisms involves oxidation-reduction (also called redox) reactions. Chemically, an oxidation is defined as the removal of an electron or electrons from a substance. A reduction is defined as the addition of electron or electrons to a substance. In biochemistry oxidations and reductions frequently involve the transfer of not just electrons but whole hydrogen atoms. A hydrogen atom (H) consists of an electron plus a proton. When the electron is removed, the hydrogen atom becomes a proton (or hydrogen ion, H^+). It will be necessary, on occasion, to distinguish between oxidation-reduction reactions involving electrons only or hydrogen atoms only (Brock, 2000).

Table 2.1: Typical species to be found for PGM's. Also listed is the reaction and the redox potentials associated with that reaction (Bard *et al., 1985*).

Element	Species	Reaction	Redox Potential
Platinum	Pt^{2+}	$Pt^{2+} + 2e = Pt$	1,188
	Pt^{4+}	$Pt^{4+} + 4e = Pt$	1.115
	$PtCl_4{}^{2-}$	$PtCl_4^{2-} + 2e = Pt + 4Cl^-$	0,758
	$PtCl_4^{2-}$	$PtCl_4^{2-} + 4e = Pt + 4Cl^-$	0,744
	$PtCL_6^{2-}$	$PtCl_6^{2-} + 2e = PtCl_4^{2-} + 2Cl^-$	0,726
	$Pt(NH_3)_4^{2+}$	$Pt(NH_3)_4^{2+} + 2e = Pt + 4NH_3$	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
	$IrCl_5(H_2O)^-$	$IrCl_5(H_2O)^- + e = IrCl_5(H_2O)^{2-}$	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

2.3 pH

The pH of natural waters is determined largely by geological and atmospheric influences. Most fresh waters are relatively well buffered against a change in pH and are more or less neutral with pH ranges around 7 and 8. pH determines the form and mobility of many chemical compounds and thus, the potential toxicity of water resources (*e.g.* aluminium is mobilised by acidification). Human-induced acidification in fresh waters is normally the result of industrial effluents, mine drainage and acid precipitation. The metals that are most likely to have negative impacts on users as a result of lowered pH are iron, aluminium, cadmium, cobalt, copper, mercury, manganese, nickel, lead and zinc. Studies on the changes in the acidity of rivers have indicated that fluctuations in pH have a severe effect on freshwater biota. Fluctuations in pH also affect domestic users, industry, mining and agriculture (http://www.gov.za/whitepaper/1997/pollution.htm).

In water treatment or mining applications, it might be desirable to recover bound metal ions from micro organisms. Since the bio-sorption of Cu^{2+}, Pb^{2+}, Zn^{2+}, Cd^{2+}, Ni^{2+} and U^{5-} by different micro organisms is dependent on pH. In some cases pH adjustments or addition of ligands reverse binding. It has been determined that with the alga~~e~~ *Chlorella vulgaris*, the bio-sorption and elution of Au^{3+}, Hg^{2+} and Ag^{+} is very much different than that of the aforementioned metal ions. Furthermore, it's shown that some of the adsorbed metal ions may be selectively recovered from

Not even a sentence

the algae by use of an appropriate elution scheme (Environmental Science Technology, Volume 20. 1986).

2.4 Ligands

Defined in the "Handbook of Chemistry and Physics, 58th edition 1977-1978" an atom is known as the central or nuclear atom and all other atoms which are directly attached to the central or nuclear atom are known as coordinating atoms. These other atoms or groups of atoms are known as ligands. A group containing more than one potential coordinating atom is termed a multidentate ligand, with the number of potential coordinating atoms being indicated by the terms unidentate, bidentate etc. A chelate ligand is a ligand attached to one central atom through two or more coordinating atoms, while a bridging group is attached to more than one atom. The whole assembly of one or more central atoms with their attached ligands is referred to as a complex, which may be an uncharged molecule or an ion of either polarity.

2.5 Metal Binding

To fully exploit the potential of plants in concentrating metals, a better understanding of both the binding mechanism and the surface functional groups involved in the metal binding is required. The complex nature of the surface of the micro organisms however renders analytical techniques difficult. X-ray photoelectron spectroscopy (XPS) has been applied to study cellular surface (Armory *et al.*, 1988).

This surface analytical technique can provide elemental analysis of the outermost 2 – 5 nm of the surface. The roles of metal ions must ultimately reflect their fundamental properties, namely charge, size, electronic configuration (and hence redox properties) and rates of ligand exchange. The IA cations do not bind ligands strongly and so in solution behave as mobile hydrated cations, whose distribution is controlled by membranes. This gives rise to charge and concentration gradients across membranes, which, can be exploited in various ways (Hughes and Poole, 1989).

The intrinsic ability of various bacteria, fungi and algae to sequester and concentrate metals from dilute aqueous solutions has long been recognised (Ruchoft, 1949). Various micro organisms reportedly possess high metal uptake capacities and several patents have appeared in the literature (Darnell *et al.*, 1986; Volesky & Kuyucak, 1988; Brierley *et al.*, 1990). Naturally occurring benthic algal mats reportedly accumulated significant quantities of gold and platinum from sea water (Dissanayake & Krisotakis, 1984).

Other metal ions bind to ligands more tightly and this can be done selectively. A metal cation can complex with two ligands and so cross-link groups, with stabilisation of structure or control of orientation of reactants. Coordination of the ligand to the metal may effect the charge distribution in the ligand and hence, its reactivity.

Finally, the nature of the ligand bound to a transition metal species will affect its redox potential. Thus, metal-ligand interaction is associated with selectivity, stabilisation, cross-linking, orientation, reactivity and tuned redox potential (E^0) values – all terms that relate positively to important functions in biology (Hughes and Poole, 1989).

It must be emphasised that the relationship between the metal ion and its binding groups is a reciprocal one, in that these groups help control the behaviour of the metal ion by providing certain ligands in particular irregular geometries. It should also be noted that a metal ion may fulfil alternative roles under different sets of conditions and that, in general, metal ions serve as neutralisers of negative charge of organic biopolymers and help to maintain osmotic balance inside the cell (Hughes and Poole, 1989).

2.6 Polarisation

According to Hughes and Poole, 1989, the complexing power of a metal depends upon its polarising power, that is, on the charge/radius ratio of the ion. A cation of high polarising power is seen by the ligand as a centre of high density of positive charge with resulting strong interaction. Ionic sizes decreases from left to right across the Periodic Table due to the effect of increased nuclear charge.

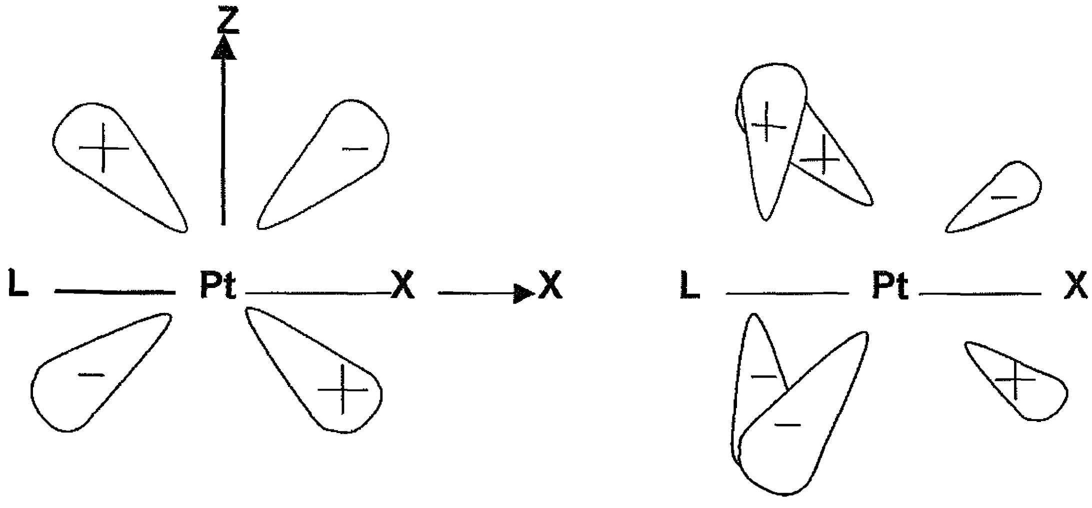
This means that the alkali metal cations interact very weakly with ligands, but the alkali earth cations Mg^{2+} and Ca^{2+} interact more strongly. In general, for small ligands, Mg^{2+} will interact more strongly than does Ca^{2+}, as shown by the data in Table 2.2. Other factors, including geometry, can disturb this sequence.

Table 2.2: Formation constants for ligands of biological importance at 25°C.

Log formation constant (K_1)										
Ligand	**Mg^{2+}**	**Ca^{2+}**	**Mn^{2+}**	**Fe^{3+}**	**Fe^{2+}**	**Co^{2+}**	**Ni^{2+}**	**Cu^{2+}**	**Cu^{+}**	**Zn^{2+}**
Glycine	3.44	1.38	3.44		4.3	5.23	5.77	8.62		5.52
Cysteine			4.1			9.3			19.2	
Aspartic acid	2.43	1.60	3.74			5.94	7.12	8.57		5.84
Histidine			7.74*		9.3*	13.9*	15.9*	18.3*		12.9*
Glutamic acid	1.9	2.05	3.3		4.6	5.06	5.90	7.85		5.45
Citric acid	3.6	3.3	3.7	11.4	4.4	5.0		5.9		5.0
Polyphosphate	3.0	3.2	5.5*	6.5*	3.0	3.0		5.5*		6.0*
EDTA	9.12	11.0	14.0	25.1	13.9	16.2	18.6	18.8		16.3
*** Values of β_2.**										

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 (Hartley, 1973).

A result of this the Pt-X bond (Platinum) becomes weakened and lengthened. It may not always be the case, since the kinetic trans-effect may arise simply because the trans–group (L) owns rather more of the empty $p_\sigma$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 (Hartley, 1973).



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

Figure 2.1: Schematic representation of the bonding mechanism for the trans effect (Hartley, 1973).

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 (Hartley, 1973)

2.7 Dipole moments

An important feature of many molecules is that they have a dipole moment, although the total charge is zero, this is because there are an equal number of protons and electrons. However, the positive and negative charges are separated, resulting in an overall electric field in the region near the molecule that can then interact with other molecules to form bonds. The dipole moment of water is one of its important physical properties.
(http://www.mth.uct.ac.za/~webpages/ellis/cos3.html).

2.8 Ions

Ions are charged atoms or molecules. Atoms and molecules are normally electrically neutral. However, if they gain an electron they become a negatively charged ion and if they lose an electron they become a positively charged ion; for example: water can be ionised to give H+ ions and OH- ions. Acidity is a measure of to what degree water has more H+ ions than OH- ions present. An acid has a H-bond that can be broken to cause an increase in the numbers of H+ ions and a base can remove H+ ions. The pH scale measures the degree of acidity. Strong acids and strong bases are both corrosive. Important acids are hydrochloric acid (HCl), sulphuric acid (H_2SO_4) and nitric acid (HNO_3). Important bases are caustic soda (NaOH) and ammonia (NH_3) (http://www.mth.uct.ac.za/~webpages/ellis/cos3.html).

2.9 Noble Metals

2.9.1 Platinum

(*Platina*) symbol: Pt, atomic weight 195.08, atomic number 78 and melting point 1772^{0}C. Discovered in South America in 1735 and also in 1741. Platinum occurs natively, accompanied by small quantities of iridium, osmium, palladium, ruthenium and rhodium, all belonging to the same group of metals naturally called the PGM's. The metal does not oxidise in air at any temperature however, is corroded by halogens, cyanides, sulfur and caustic alkalis. It is insoluble in hydrochloric and nitric acid, but dissolves when they are mixed as *aqua regia*, forming chloro-platinic acid (H_2PtCl_6), an important compound (Handbook of Chemistry and Physics, 58th edition 1977-1978).

2.9.2 Palladium

(Named after the asteroid, *Pallas*) symbol: Pd, atomic weight 106.42, atomic number 46 and melting point 1552^{0}C. Discovered in 1803. Palladium is along with platinum and other metals of the platinum group, also found associated with nickel-copper deposits. Palladium is attacked by nitric and sulfuric acid. At room temperatures the metal has the unusual property of absorbing up to 900 times its own volume of hydrogen, possibly forming Pd_2H (Handbook of Chemistry and Physics, 58th edition 1977-1978).

2.9.3 Gold

(Sanskrit *Jval*; Anglo-Saxon *gold*) symbol: Au, atomic weight 196.9665, atomic number 79 and melting point 1064.43^{0}C. Known and highly valued from earliest times, gold is found in nature as the free metal and in tellurides, it is very widely distributed and is almost always associated with quartz or pyrite. Gold is unaffected by air and most reagents. A mixture of one part nitric acid with three parts hydrochloric acid is called *aqua regia*, because it dissolves gold, the King of Metals (Handbook of Chemistry and Physics, 58th edition 1977-1978).

2.9.4 Rhodium

(*Rhodon,* rose) symbol: Rh, atomic weight 102.9055, atomic number 45 and melting point 1966 ± 3^{0}C. Discovered in 1803-4 in crude platinum ore. Rhodium occurs native with other platinum metals, also found in nickel-copper sulfide ores. The metal is silvery white and at red heat slowly changes in to the sesquioxide. At higher temperatures it converts back to the element (Handbook of Chemistry and Physics, 58th edition 1977-1978).

2.9.5 Ruthenium

(Ruthenia, Russia) symbol: Ru, atomic weight 101.07, atomic number 44 and melting point 2310^{0}C. Berzelius and Osann, in 1827, examined the residues left after dissolving crude platinum in *aqua regia.* While Berzelius found no unusual metals, Osann thought he found three new metals, one of which he named ruthenium.

Ruthenium does not tarnish at room temperatures however, oxidizes in air at about 800^{0}C. Hot or cold acids or *aqua regia* does not attack the metal, but when potassium chlorate is added to the solution, it oxidizes explosively. It is attacked by halogens and hydroxides (Handbook of Chemistry and Physics, 58th edition 1977-1978).

2.9.6 Iridium

(*Iris*, rainbow) symbol Ir, atomic weight 192.22, atomic number 77 and melting point 2410^{0}C. Discovered in 1803 in the residue left when crude platinum is dissolved in *aqua regia.* It's the most corrosion resistant metal known. Iridium occurs un-combined in nature in the presence of platinum and other metals of this family in alluvial deposits. It is recovered as a by-product from the nickel mining industry (Handbook of Chemistry and Physics, 58th edition 1977-1978).

CHAPTER THREE: PROJECT AIMS AND OBJECTIVES

3.1 Project Aims

The aim of this project is to develop a biological system to recover toxic heavy metals from effluent generated from a precious metals refinery with the intension of polishing effluent to a standard that the effluent can be discharged to a sewer or storm water system without affecting the biological ecosystems contained therein. The concept of recovering precious metals as a financial benefit must not be ignored as they could be returned to process for chemical extraction.

3.2 Objectives

In terms of toxicity of heavy metals entering the environment and the need for research into a bioremediation technique, the following objectives have been formulated with regard to refinery effluent treatment:

Short to?

- To compare *Azolla* efficiency in terms of selectivity and saturation levels.
- To determine an optimal pH of the effluent which the *Azolla* plant prefers.
- To provide a reference for the treatment of refinery effluent with the aim of discharge to sewer / storm water,
- To evaluate the use of the *Azolla* plant as medium for bioremediation of effluent liquors,
- To analyse the status of the treated effluent liquors in comparison to the affect on the *Azolla*.

CHAPTER FOUR: METHODOLOGY

4.1 Methodology

The *Azolla* was gathered from the local sewer works, East Rand Water Care Works (ERWAT) in an abandoned portion of the works. The plant was transferred from the works, in plastic buckets, to the refinery and placed in a Poly-vinyl-chloride (PVC) tank containing municipal water. Although other drying techniques have been tested for example "microwave technology" (Breckenridge, 2001), these will not be discussed as part of this project.

Quantities of *Azolla* where removed and placed in a glass dish for drying in an electric drying oven, a temperature of 50^0C was set, the moisture was allowed to escape through an aperture on top of the oven. Once dried, the plant was inspected and large particles (husks and sticks) where removed. The plant was then stored in plastic sample bottles ready for use. The use of metal equipment in conjunction with the plant was avoided as the plant reacted with "mild steel" trays, used when experimenting with the drying stage.

Two test rigs were built, (dimensions described under the headings 4.2 and 4.3), an up-flow column rig (Appendix B, Figure 4.1) and a raceway system (Appendix C, Figure 4.2). Both systems were used in determining the effectiveness of the *Azolla,* however, the raceway system was used to perform a test using only live *Azolla*.

4.2 Test Rig 1

Test Rig 1, as indicated by Appendix B, Figure 4.1, was constructed of a 100-litre glass liquor storage vessel. The liquor is gravity fed through a valve into a 20-litre glass vessel. From this vessel, the liquor gravity feeds to a pump, which is used for circulation, of which various flow rates can be achieved. The liquor is circulated, via a glass valve, through the bottom of a glass column (1000 mm X 25 mm in size), which contains the packed *Azolla.*

The large glass column allows the option of using various quantities of *Azolla.* From the top of the column, a flexible plastic hose is used to return the liquor to the 20-litre vessel, creating the circulation and/or a sample can be taken. All pipes used between the vessels are constructed from 20 mm PVC piping. Glass fibre wool is used at each end of the glass column to contain the plant within the tube and prevent loss of *Azolla.*

4.3 Test Rig 2

Test Rig 2, as indicated by Appendix C, Figure 4.2, was constructed from 2 PVC, 100 mm pipes, 2000 mm long, creating a raceway effect. The pipes were slotted on the top (50 mm) for placement and removal of the *Azolla* plant and mechanically connected together utilising 40 mm PVC pipe. A 20-litre glass vessel is used to contain the liquor, which gravity feeds through a 20 mm PVC flexible pipe to a variable speed flow pump.

Once processed though the 100 mm raceway pipes, the discharge liquor returned to the 20-litre glass vessel via a 40 mm PVC pipe. A valve is inserted to assist in maintaining a constant flow rate, thus retention time can be adjusted. Because live *Azolla* is to be used, a combination of fluorescent and incandescent lighting was provided in order to simulate natural sunlight to encourage plant growth or at the least maintain the plants stability.

Inserts were designed to limit the *Azolla* plant from entering the pump system and also to prevent the plants from collecting at any of the ends of the raceway however, still maintaining a certain flow rate. The plant naturally floats on top of the liquor with its roots hanging from the leaf region.

CHAPTER FIVE: ANALYTICAL METHODS

5.1 Analytical Method

The purpose of chemical analysis is to establish the composition of naturally or artificially manufactured substances. This is usually done in two distinct steps. First, *qualitative analysis* is used to identify the sample components. This is followed with *quantitative analysis*, by which the relative amounts of these components are determined (Vogel's, 1996).

One of the most accurate instruments utilised in the PGM industry is that of Inductively Coupled Plasma Emission Spectrophotometer (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).

5.2 ICP Standard Preparation

These standards are samples with a matrix similar to that of the samples to be analysed with 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 D).

At the end of the phase of measurement of standards (calibration) linear regression calculations are performed for each element, also indicated in Appendix D. Intensity versus 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 are 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

Indium, an internal standard, was used 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 and gas sample up-take, etc. may produce variations in the intensity of elements measured however, this will be the same degree for the internal standard. Consequently, the ratio of intensities will remain relatively constant.

The results will be obtained by using the Jobin Yvon (JY) 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 the grating size. The grating of the JY spectrometer is thermally controlled thereby having negligible thermal expansion and zero distortion on the surface.

CHAPTER SIX: EXPERIMENTAL METHODS

6.1 Base Line Experiment

A base line experiment was conducted to determine the impact of PGM change contained in the acid effluent at various pH levels. A range of adjustments, (Table 7.1) from pH 4 to pH 7 was completed to determine the most suitable solution to suit the *Azolla* optimising the removal of PGM's. Caustic (Sodium Hydroxide) solution was added to chemically adjust the pH. For each adjustment, 200 ml of acid effluent was used from which a sample of 50 ml was taken for filtering.

The 50 ml sample was filtered through a Millipore filter unit using a Cellulose Nitrate Filter, with a pore size of 0.45 µm. Samples of the solids were not sent for analysis. Once filtered, a 20 ml sample was taken for analysis by ICP spectrophotometer. The results of the liquors are reflected in Chapter seven (7.1 Base Line Experiment).

6.2 Experiment One

For experiment one a 100 litres of acid effluent liquor was siphoned into the glass storage vessel. The pH of the liquor was chemically adjusted using caustic (Sodium Hydroxide) solution (585ml in total) to the required pH 4. The caustic was added in 50 ml amounts to the column, and the pH checked every half an hour. When pH 3.75 was reached, the caustic was added in 5 ml amounts that resulted in attaining pH of 4.02. The liquor continuously circulated at a flow rate of 2 litres per minute during this process. Once adjusted the liquor was filtered through a (polyester needle felt) filter cloth (Appendix E) and a sample of the filtered liquor taken for analysis, referred to as the "head value".

It was calculated that Rig 2 could only contain 33 litres and therefore this amount was used to fill the rig in order to perform the test. The live *Azolla* was added to the raceway and placed in-between the specially designed retaining inserts. Sufficient plant was used to cover the full surface area of the open raceway, without significant overlapping of the plant leaves, thus providing for a total surface area of 200 cm^2 within the raceway rig.

The flow rate for this experiment, within Rig 2, was set at 3 litres per minute to ensure sufficient contact between the plant and the solution. The temperature was ambient with the rig being indoors, no adjustments to the temperature made.

Because live *Azolla* was used, this experiment was conducted over period of twelve days to determine (a) metal binding of the live plant (b) pH fluctuation and (c) deterioration or growth of the *Azolla* over the specified period of time. At each 24-hour sample period, a liquor and plant sample was taken. The outcome of this experiment is discussed from results in chapter seven (7.2 Experiment One).

6.3 Experiment Two

For this experiment 100 litres of the acid effluent liquor was used. The pH of the liquor was chemically adjusted using caustic solution (1050ml) to pH 4. The same principle of caustic addition was used as with experiment one. It must be noted that more caustic was used to adjust this particular liquor, which was a result of an adjustment to the flow operation.

The circulation flow rate was increased from 2 litres per minute to 4 litres per minute obtaining a higher degree of agitation, which resulted in achieving a higher accuracy of pH value (pH 4.0). The liquor was filtered through a (polyester needle felt) filter cloth (Appendix E) and a sample of the filtered liquor taken for analysis.

To continue this experiment 20 litres of the chemically adjusted liquor was used to fill the glass vessel of Rig 1. The dried *Azolla* (30 grams), as apposed to experiment one, where live *Azolla* was used, was added to the glass column with retaining inserts in conjunction with glass wool, this was used to contain the *Azolla* within the column. The flow rate was set at 120 ml per minute.

30 grams of dried *Azolla* was used in experiment two as a variance to previous experiments conducted by Smith, 2001 where 10 and 20 grams had been used, however, the duration of time remained the same as experiments conducted by Smith. This experiment was conducted over a period of eight hours to determine (a) metal binding of the dried plant (b) pH fluctuation and (c) metals remaining in

solution on completion of experiment. The outcome of this experiment is discussed as results in chapter seven (7.3 Experiment Two).

6.4 Experiment Three

For experiment three 20 litres of effluent liquor was used to fill Rig 1. The pH of the liquor was not adjusted; it was used directly from the acid effluent storage dam. The intention was to ascertain how the liquor containing large amounts of iron, which at a pH of 1.10, reacted with the dried *Azolla*. The liquor was not filtered. As with experiment two, 30 grams of dried *Azolla* was added to the glass column and the effluent was allowed to circulate through the column.

A flow rate was set at 120 ml per minute. This experiment was also conducted over an eight-hour period to determine (a) metal binding of the dried plant (b) pH fluctuation and (c) metals remaining in solution on completion of experiment.
The outcome of this experiment is reflected in chapter seven (7.4 Experiment Three).

6.5 Experiment Four

For this experiment, 25 litres of acid effluent was taken as the head liquor. With this experiment however, the use of caustic to chemically adjust the pH to 4 was replaced by using alkaline effluent. The alkaline effluent being the other main waste stream generated from precious metal refining processes.

To adjust the 25 litres of acid effluent to the desired pH of 4, the same principle of addition as with the caustic was utilised however, 8 litres of alkaline effluent was used as opposed to 1050 ml of caustic used with the previous adjustment in experiment one.

The concept with this experiment was to retain as much of the dissolved iron in solution as possible, then determine the affects of the accumulation efficiency of the plant with its presence. Once the desired pH was achieved, the liquor was filtered through a (polyester needle felt) filter cloth (Appendix E) and a sample of the filtered liquor taken for analysis. It must be noted that most of the solids passed through the filter cloth. To continue experiment four, 20 litres of the chemically adjusted liquor was used to fill Rig 1. The same amount of dried *Azolla* (30 grams) was added to the column.

At the start of the experiment the liquor was allowed to fill the column of Rig 1. The *Azolla* was then probed to ensure no channelling or restriction had taken place after the plant had expanded because of contact with the liquor. This did not prove successful, as the flow was restricted to almost zero. This was due to the fact that the liquor contained a large degree of solids, which had not been removed by filtration prior to commencing test work.

The test was stopped and the 20 litres of liquor including the 30 grams of *Azolla* was transferred to the 100-litre glass storage vessel of Rig 1. The liquor was allowed to circulate through this storage vessel for the eight-hour period.

Utilising this method resulted in a more effective circulation of the liquor whilst the *Azolla* floated on the surface without channelling or restriction obtaining improved contact with the liquor. Table 7.11 indicates the results of the various effluents before and after the liquor was chemically adjusted. The flow rate for this experiment was set at 4 litres per minute.

CHAPTER SEVEN: RESULTS AND DISCUSSION

7.1 Results of Base Line Experiment

Table 7.1: Describes the amount of caustic solution used to adjust the effluent to the various pH levels.

Initial pH of the acid dam was 1.37		
pH required	**Amount of caustic in ml**	**pH achieved**
4	1.6	4.0
5	2.4	5.2
6	2.8	6.4
7	5.1	7.3

From the above analysis (Table 7.1) it was decided to use the pH 4 liquor to perform the experimental test runs. The main reason that pH 4 was selected was a result of:

(1) The research information prior discussed about the plant states that live *Azolla* prefers pH4.

(2) The initial removal of the iron from 1183 ppm to 9 ppm as reflected in the results of Table 7.2 was achieved.

(3) There was a reduction in most of the other base metals, which precipitated out after chemical adjustment to pH 4 except for the zinc, which only precipitated out at pH 7.

(4) The low reduction efficiency experienced with regard to the precious metals removal.

The ultimate, besides polishing the effluent, would be to recover the PGM's, as their value is far greater than that of the base metals. The conclusion to be determined is, do the base metals act as catalysts, specifically iron in efficient PGM removal, or do the base metals saturate the binding site and are detrimental to removal efficiency.

Table 7.2 reflects the metal reduction. Gold reflected less than 1 ppm as a result of efficient removal by current applied chemical processes at the refinery. A marginal reduction in the rhodium and ruthenium was achieved with the arsenic noticeably being totally removed from solution at pH 5. A tremendous reduction in the iron was achieved however, the majority of zinc and all the aluminium and tellurium precipitated out at pH 7.

Table 7.2: PGM's remaining in solution after the liquor had been filtered (all readings are expressed in parts per million).

Element	Acid Dam Head Value	pH 4	pH 5	pH 6	pH 7
Pt	6	7	6	6	5
Pd	3	2	2	2	2
Au	<1	<1	<1	<1	<1
Rh	12	10	7	5	3
Ru	15	10	7	5	1
Ir	15	8	5	3	1
Cu	183	165	79	18	10
Ni	174	165	156	133	9
Fe	1183	9	<1	<1	<1
Pb	306	274	164	49	2
Ag	7	8	7	7	6
Se	28	21	20	18	10
As	34	2	<1	<1	<1
Te	17	3	2	2	<1
Zn	6957	6580	6060	4759	125
Al	573	368	21	2	<1

One could conclude that chemical adjustment offers an effective method of removing metals from the effluent however, the cost of the chemicals used to perform this process, would add up to a substantial amount of money over an extended time period.

7.2 Results of Experiment One

The analytical results from the liquor prepared for the use in experiment one are reflected in Table 7.3 and contain the elemental head value of the live *Azolla*, the liquor value before the chemical adjustment and the value of the elements remaining in solution after filtration.

Table 7.3: Results of analysis of effluent before experiment one commenced (all readings are expressed in parts per million unless otherwise stated).

Element	Live *Azolla* Solids Head Value	Liquor Head Value Before Adjustment	Liquor Value After Chemical Adjustment and Filtration
pH		1.88	3.77
Pt	4	9	10
Pd	9	3	3
Au	1	<1	<1
Rh	<1	20	17
Ru	4	26	18
Ir	5	25	15
Cu	8	49	50
Ni	<1	293	318
Fe	95	1751	40
Pb	24	521	541
Ag	35	23	25
Se	14	42	31
As	8	4	4
Te	95	25	5
Zn	335	1.16%	1.16%
Al	44	903	669

Of particular note is the iron precipitation that occurred, this however, was expected and the theory is to determine how the base metals, especially the iron, affect the binding site of the plant, if any affect at all. There was not a great deal of fluctuation on any of the other elements after chemical adjustment. The results of the twelve-day experiment are listed in Table 7.4.

Table 7.4: Results of analysis of effluent liquor after the completion of experiment one (all readings are expressed in parts per million unless otherwise stated).

Element	Head value	24 Hr	48 Hr	72 Hr	96 Hr	120 Hr	144 Hr	168 Hr	192 Hr	216 Hr	240 Hr	264 Hr
pH	3.77	3.62	3.60	3.60	3.59	3.58	3.56	3.56	3.55	3.54	3.53	3.52
Pt	10	11	13	11	12	12	11	12	13	11	9	10
Pd	3	2	2	2	2	2	2	2	2	2	2	2
Au	<1	1	1	1	1	1	1	1	1	1	<1	<1
Rh	17	18	18	19	19	19	18	20	21	20	18	20
Ru	18	20	21	22	22	22	21	23	24	23	20	22
Ir	15	11	12	13	13	13	12	13	14	13	12	13
Cu	50	330	341	352	363	362	348	382	391	365	338	361
Ni	318	326	335	348	356	356	342	370	382	359	336	352
Fe	40	21	15	11	24	13	14	18	19	19	20	22
Pb	541	572	598	621	642	642	616	680	679	651	602	648
Ag	25	23	24	25	26	26	25	27	28	26	25	26
Se	31	35	38	38	39	41	39	42	43	40	38	40
As	4	4	4	4	4	4	4	4	4	4	3	4
Te	5	9	9	9	10	10	9	10	11	10	8	9
Zn	1.16 %	1.12 %	1.15 %	1.15 %	1.23 %	1.15 %	1.20 %	1.10 %	1.09 %	1.18 %	1.01 %	1.00 %
Al	669	622	649	667	693	691	662	731	755	706	643	703

The conclusion that can be drawn is that there is very little, or no movement at all, of any metals from solution absorbed to the live plant. The noticeable change in this experiment is the decrease of the iron from 40 ppm to as low as 11 ppm at the 72 hour sample recorded. A slight decrease in the pH over the twelve-day period was noted. Iron being the predominant factor in determining how it can affect the binding sites of the plant in relation to PGM recovery. One could conclude that the base metals do perform a task in the binding of the PGM's.

As apposed to the effluent liquor results (Table 7.4), where very little or no movement was apparent, a great amount of absorption in fact had taken place by the plant as can be seen in Table 7.5.

Table 7.5: Results of analysis of the *Azolla* plant after the completion of experiment (all readings are expressed in parts per million unless otherwise stated).

Element	Head value	24 Hr	48 Hr	72 Hr	96 Hr	120 Hr	144 Hr	168 Hr	192 Hr	216 Hr	240 Hr	264 Hr
Pt	42	483	607	936	777	851	716	800	865	1023	829	1001
Pd	20	229	165	159	154	261	134	203	157	330	234	246
Au	5	77	93	93	96	95	92	85	102	102	89	100
Rh	90	147	138	158	151	141	151	147	153	146	152	155
Ru	120	215	303	332	325	317	248	270	328	349	273	329
Ir	168	46	47	75	49	70	82	80	62	65	64	84
Cu	537	3874	3863	3676	3742	3707	3703	3557	3838	4035	3655	3996
Ni	211	1737	1698	1674	1649	1662	1630	1683	1701	1644	1671	1592
Fe	2.48 %	3095	2525	3729	3437	3727	3943	4588	2964	3116	2617	3018
Pb	676	4082	3986	3880	3816	3488	3691	3592	3785	3384	3309	3558
Ag	85	349	292	238	215	437	196	437	228	535	361	438
Se	184	421	511	477	468	430	450	372	436	444	372	440
As	659	145	160	172	170	201	139	161	161	201	140	177
Te	494	1428	1241	875	1057	2232	947	1597	1169	2904	1730	1914
Zn	1.08 %	6.40 %	6.27 %	6.18 %	6.50 %	6.36 %	6.20 %	6.42 %	6.73 %	6.22 %	6.14 %	5.88 %
Al	7249	7658	7757	8440	8387	8588	7998	7911	8395	9118	7704	9399

When using the live *Azolla*, it was evident that the plant was effective in achieving absorption (Table 7.5). Although some of the results are contradictory with the fact that, where good or efficient metal removal from the solution was achieved, the plant should theoretically reflect an increase in the amount of the same metals absorbed, this however, was not always the scenario.

The PGM's reflect almost no movement with the liquor content (Table 7.4) however, the results achieved from the plant reflect a steady increase in collection after each 24-hour period, excluding iridium.

The iridium indicates de-sorption when considering the head value at 168 ppm, a large decrease to 27.38 % of the original value within the first 24 hours and gradually increasing to 50 % at the end of the 264 hour run. The platinum reflected the highest degree of absorption (Figure 7.1).

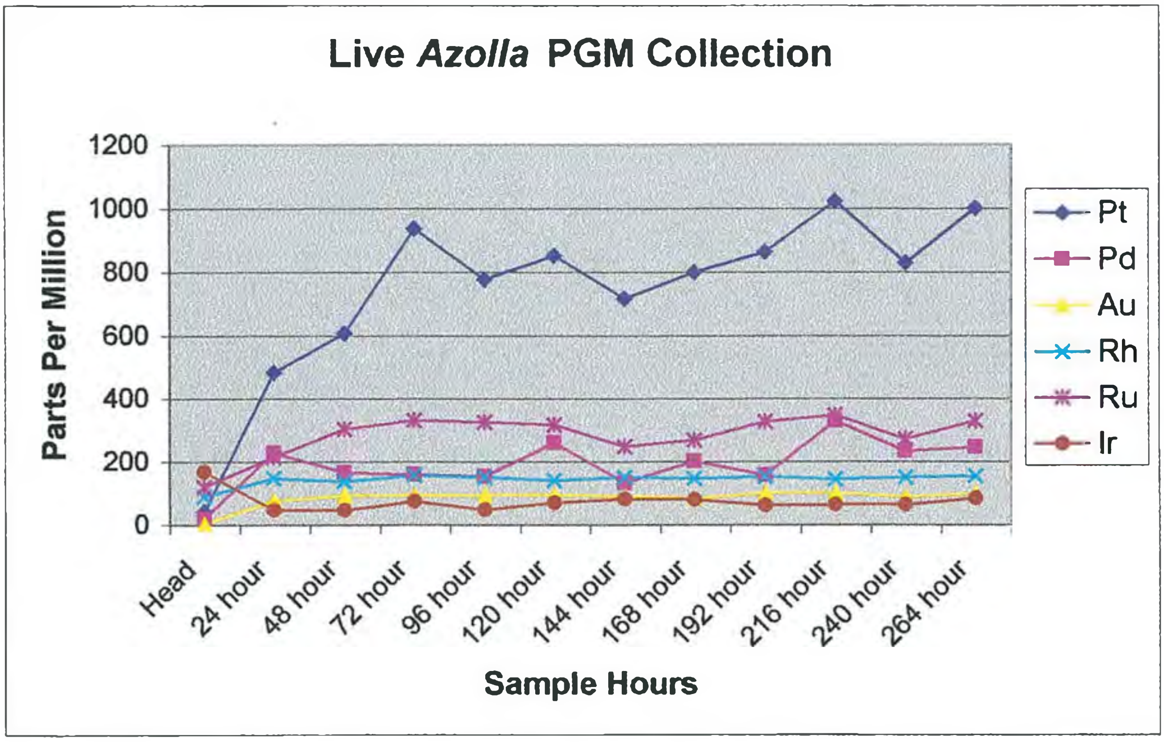


Figure 7.1: This graph reflects the fluctuation and the absorption of the PGM's by the *Azolla* including the initial de-sorption of the iridium with the gradual increase again.

Fluctuation between some of the metals during the different time scales was noted, this fluctuation was expected due to the *Azolla* becoming saturated and then releasing the metals back into solution. It must be noted that there was a distinguishable difference between the head value and the 264-hour result at the end of the experiment. If one considers the difference between the head value and the results of the experiment, it could be said that there was a very high degree of absorption that took place at particular intervals.

The expectation from this experiment was initially to note the activity of the iron in conjunction with the precious metals in relation to the live *Azolla*. The conclusion one can draw is that there can be beneficial long-term benefits from utilising this process, this will however, demand further investigation utilising various growth and cultivation techniques. The average mass of the plant taken for sampling was 0.710 grams.

7.3 Results of Experiment Two

The analysis of liquor to be used for experiment two reflects once again that the iron precipitated out of solution with virtually no loss of other metals as reflected by Table 7.6. Utilising pH 4 again reflects that the iron precipitated from the liquor. A slight loss of the rhodium and ruthenium is evident with very little noticeable movement of other metals in solution. Of particular reference is the arsenic that precipitated out and the zinc that remained.

With the effective pH accuracy adjustment achieved and the effective circulation, most metals remained in solution after the liquor had been filtered as reflected within Table 7.6.

Table 7.6: Results of the effluent, which was used to conduct experiment two, before and after chemical adjustment (all readings are expressed in parts per million unless otherwise stated).

Element	Liquor Head Value Before Adjustment	Liquor Value After Chemical Adjustment and Filtration	Remaining liquor, chemically Adjusted not Filtered
pH	1.9	4.0	4.0
Pt	8	9	9
Pd	1	2	2
Au	<1	<1	<1
Rh	20	17	17
Ru	23	19	20
Ir	23	15	14
Cu	324	315	310
Ni	291	304	311
Fe	1668	3	3
Pb	529	550	554
Ag	20	21	21
Se	39	33	34
As	41	7	6
Te	26	7	7
Zn	1.23%	1.18%	1.16%
Al	945	597	581

Table 7.7: Results of analysis of the effluent liquor after experiment two was completed (all readings are expressed in parts per million unless otherwise stated).

Element	Head	1 Hr	2 Hr	3 Hr	4 Hr	5 Hr	6 Hr	7 Hr
pH	4.0	3.9	4.0	3.9	4.2	3.9	4.0	4.1
Pt	9	8	8	8	8	8	8	8
Pd	2	2	2	2	2	2	2	2
Au	<1	<1	<1	<1	<1	<1	<1	<1
Rh	17	16	16	16	16	15	16	16
Ru	19	19	19	19	19	18	19	19
Ir	15	13	13	13	13	13	13	13
Cu	315	295	293	287	289	290	301	297
Ni	304	291	294	288	290	291	300	298
Fe	3	3	3	4	3	25	9	4
Pb	550	523	524	514	518	517	538	535
Ag	21	20	20	19	19	20	20	20
Se	33	32	32	31	31	31	33	33
As	7	6	6	6	6	5	6	6
Te	7	6	7	7	7	6	7	7
Zn	1.18%	1.23%	1.13%	1.14%	1.01%	1.00%	1.09%	1.15%
Al	597	555	554	540	539	541	551	541

It should be noted that there was a small decrease in some of the base metals for example in the copper, nickel and the aluminium however, the lead and zinc remained constant. The pH remained more or less constant throughout the process as indicated by the various samples.

Due to the long length of the glass column it was decided to take a sample of the *Azolla* at the top of the column and at the bottom to determine if there was a differential between the two areas. Except for the rhodium and iridium, the results were almost identical indicating good contact throughout the column.

It was at this stage that it was noted that the *Azolla* was fairly dry in the top region of the column. The conclusion was drawn that this was a result of the expansion of the plant on commencement of the experiment, which possibly caused some channelling and / or a restriction in flow rate. Table 7.8 reflects the results achieved by the plant on completion of the experiment. There was no significant difference from the results of the top of the column plant and the bottom of the column plant.

The results reflect that the *Azolla* did however, absorb various quantities of the metals over the eight-hour run in relation to the head value. Once again one cannot ignore the fact that when iron is present, absorption of the precious metals is more evident.

Table 7.8: Results of analysis of the *Azolla* after experiment two was completed (all readings are expressed in parts per million unless otherwise stated).

Element	Dried *Azolla* Head Value	Dried *Azolla* bottom of column	Dried *Azolla* top of column
Pt	326	618	600
Pd	367	289	300
Au	144	94	139
Rh	10	109	91
Ru	162	213	248
Ir	2	42	24
Cu	95	6673	7060
Ni	212	1121	937
Fe	1.28 %	1.15 %	1.04 %
Pb	31	4894	5025
Ag	153	200	175
Se	302	331	348
As	127	136	127
Te	1450	1130	1112
Zn	754	4.39 %	3.34 %
Al	4037	1.32 %	1.35 %

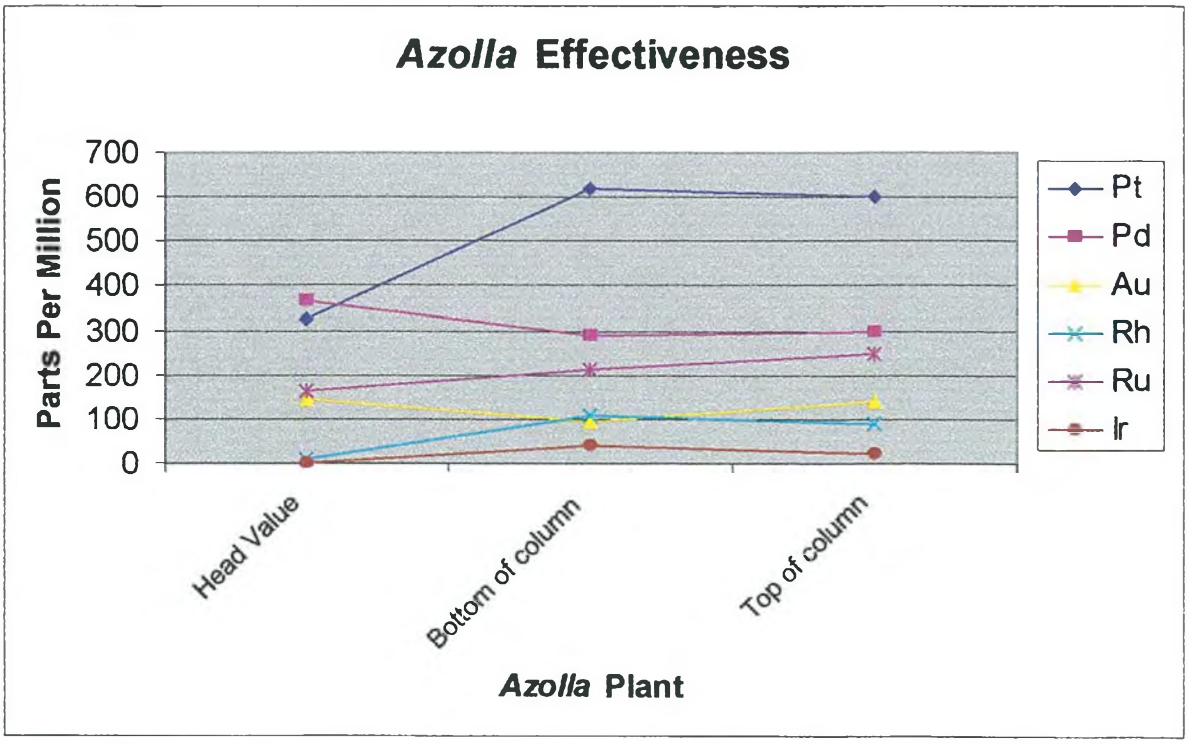


Figure 7.2: Azolla effectiveness compared to the head value, some metals showing absorption whilst others show de-sorption.

7.4 Results of Experiment Three

The Head Value reflected in Table 7.9 is the liquor analysis direct from the acid effluent storage dam. Although the pH remained very constant, there was very little movement between the precious metals. The low pH of the effluent seemed to dissolve the metals already present in the plant, however, the results of the plant analysis reflect differently, as reflected in Table 7.10. The used plant from the column was placed into a container and a representative sample taken, this was done because of the channelling problem experienced with experiment two. With this experiment however, there seemed to be no dry areas or any signs of channelling.

Table 7.9: Results of analysis of effluent after experiment three was completed (all readings are expressed in parts per million unless otherwise stated).

Element	**Head Value**	**1 Hr**	**2 Hr**	**3 Hr**	**4 Hr**	**5 Hr**	**6 Hr**	**7 Hr**
pH	1.10	1.09	1.20	1.01	0.09	1.0	1.0	1.4
Pt	18	17	18	18	19	19	18	21
Pd	1	<1	<1	<1	<1	<1	<1	<1
Au	<1	<1	<1	<1	<1	<1	<1	<1
Rh	38	36	37	36	36	37	36	39
Ru	42	40	41	40	41	42	41	43
Ir	39	38	38	38	39	40	39	42
Cu	888	564	572	567	575	584	570	604
Ni	508	500	497	492	501	510	505	530
Fe	4380	4062	4172	4162	4247	4354	4413	4629
Pb	732	727	724	723	734	754	735	786
Ag	66	66	65	65	66	69	67	72
Se	53	50	53	53	54	56	55	61
As	122	102	117	115	113	110	120	128
Te	38	36	37	36	38	39	38	42
Zn	1.95%	1.90%	1.77%	1.75%	1.98%	1.98%	2.06%	1.99%
Al	1968	1944	1974	1909	1947	1932	2012	2043

The results in Table 7.10 reflect that the plant did absorb the PGM, except for the gold. The base metal and metalloids show great movement in particular copper, arsenic and the iron. The remaining precious metals, except for the gold, showed movement, which proves that the iron again played a significant role in metal binding efficiency. The movement to take cognisance of was with the iron, which showed an increase of 1.28 % to 3.95 %, the copper from 95 ppm to 2177 ppm and the arsenic from 127 ppm to 2239 ppm.

Table 7.10: Results of analysis of the *Azolla* after experiment three was completed (all readings are expressed in parts per million unless otherwise stated).

Element	Dried *Azolla* Head Value	Dried *Azolla* (mixture from top and bottom of column)
Pt	326	542
Pd	367	627
Au	144	90
Rh	10	182
Ru	162	254
Ir	2	100
Cu	95	2177
Ni	212	942
Fe	1.28 %	3.95 %
Pb	31	1367
Ag	153	311
Se	302	880
As	127	2239
Te	1450	1462
Zn	754	3.67 %
Al	4037	6699

7.5 Results of Experiment Four

Table 7.11 reflects the results of the acid effluent and the alkaline effluent used to make up the solution used for experiment four. It also reflects the results of the liquor after the chemical adjustment had been completed, before and after filtering. As experienced with previous experiments, the metals remained in solution after filtration as reflected in Table 7.11 between the "before filtration" analysis results and the "after filtration" analysis results. It must be noted that the same type of filter cloth (polyester needle felt), as reflected in Appendix E, was used for all the experiments.

Table 7.11: Results of analysis of effluent used to prepare liquor for experiment four (all readings are expressed in parts per million unless otherwise stated).

Element	Acid Effluent	Alkaline Effluent	Liquor before Filtration	Liquor after Filtration
pH	1	13.5	4.0	3.9
Pt	18	6	12	11
Pd	1	5	<1	<1
Au	<1	<1	<1	<1
Rh	42	3	25	26
Ru	59	6	24	24
Ir	47	<1	24	24
Cu	713	<1	350	353
Ni	571	<1	429	439
Fe	4871	12	<1	2
Pb	819	<1	573	585
Ag	79	9	41	42
Se	61	71	32	30
As	144	210	3	4
Te	45	33	7	9
Zn	1.99 %	84	1.37 %	1.35 %
Al	2116	103	174	187

Other methods are available to allow for improved filtering for example, another make and type of filter cloth could have been used, filter cloth aids such as "Celite Powder" are also available on the market however, these will not be discussed. An approach for all experiments was to keep the principle methods adopted between the different make-up solutions as similar as possible in order to compare results more effectively.

The results of the analysis of the liquor, reflected in Table 7.12, indicate that there was no movement or loss between any of the metals, however the pH fluctuated slightly. The PGM's remained almost identical at each sample interval with some fluctuation between the base metals. One would have to conclude that this experiment failed as a result of either, contamination when transferring the head liquor from the glass column or as a result of analytical errors experienced at the time of analysing.

Table 7.12: Results of analysis of effluent after experiment four was completed (all readings are expressed in parts per million unless otherwise stated).

Element	**Head Value**	**1 Hr**	**2 Hr**	**3 Hr**	**4 Hr**	**5 Hr**	**6 Hr**	**7 Hr**
pH	3.9	4	3.9	4.1	4.0	4.0	3.9	3.8
Pt	11	11	11	11	11	11	11	12
Pd	<1	<1	<1	<1	<1	<1	<1	<1
Au	<1	<1	<1	<1	<1	<1	<1	<1
Rh	26	24	24	26	26	25	25	26
Ru	24	23	23	25	24	24	24	25
Ir	24	22	22	24	24	23	23	24
Cu	353	325	326	343	343	335	329	350
Ni	439	419	423	440	440	439	430	454
Fe	2	2	<1	<1	<1	<1	2	<1
Pb	585	552	560	591	593	580	569	609
Ag	42	37	37	40	40	40	38	41
Se	30	27	27	28	29	28	29	31
As	4	3	3	3	3	3	3	3
Te	9	7	7	8	8	7	7	8
Zn	1.35 %	1.32 %	1.33 %	1.38 %	1.39 %	1.31 %	1.36 %	1.31 %
Al	187	188	184	200	174	195	177	191

Table 7.13, the results of the plant after the experiment confirms that there was a problem with this test as the *Azolla* dissolved the palladium, gold and ruthenium contained in the head value with some absorption of the platinum, rhodium and iridium. A similar trend within the base metals exists with only the copper reflecting absorption from 95 ppm to 3599 ppm and tellurium de-sorption from 1450 ppm to 425 ppm. To conclude, this experiment should be ignored as all other experiments show absorption of metal to the *Azolla* plant to some degree, which is contradictory to this experiment thus, proving that there was a problem with the way this test was performed or as mentioned, a problem with the analytical results.

Table 7.13: Results of analysis of the *Azolla* after experiment four was completed (all readings are expressed in parts per million unless otherwise stated).

Element	Dried *Azolla* Head Value	Dried *Azolla* from column
Pt	326	595
Pd	367	168
Au	144	4
Rh	10	72
Ru	162	77
Ir	2	42
Cu	95	3599
Ni	212	487
Fe	1.28 %	5660
Pb	31	695
Ag	153	227
Se	302	101
As	127	64
Te	1450	425
Zn	754	1.55 %
Al	4037	3878

CHAPTER EIGHT: CONCLUSION

8.1 Live *Azolla*

Although there was excellent absorption within the base metal group and only a gradual increase in the absorption rate of PGM's, the platinum however, reflected the greatest movement with a huge increase from 42 ppm to 483 ppm in the first 24 hours of "experiment one" and then gradually resulting in 1001 ppm by the end of the 264 hour run. The iron, in this experiment, seemed to fluctuate at every sample interval where as the all other base metals reflected a steady increase, with only a small degree of fluctuation.

An advantage of utilising the live *Azolla* was that no drying of the plant would be required. A disadvantage, in this scenario, was that the *Azolla's* condition deteriorated over the duration of the experiment. What was expected, in conjunction with the artificial lighting provided, was that the plant would extract nutrients from the solution and be in a position to sustain it's growth and at best reproduce under the conditions provided.

However, as mentioned in previous case studies it may be possible to add other nutrient supplements, such as fertilizer, that would prolong the life of the *Azolla* and make it a more efficient metal collector whilst sustaining its own growth and reproduction within the process plant designed for the application.

8.2 Dried *Azolla*

In all experiments, test run 4 excluded, the dried *Azolla* proved successful in metal absorption indicating a potential to develop the plant for biological remediation of industrial effluent. Utilising this method meets the criteria for a cheap alternative process, as it is readily available in large quantities.

Drying of the plant to decrease transport costs and improve storage and subsequent ways to recover the ions and reuse the plant to reduce disposal costs still needs to be addressed. An advantage with using the dried plant is the effect of various pH levels of the solution (effluent) does not seem to affect the plant however, this also requires future study and investigation.

8.3 Comparison

The test work untaken by Smith 2001, and the current work, with no doubt indicates the potential of *Azolla* in having the capability to treat refinery effluent. However, the main draw back at this stage would seem to be with the volume to be treated in conjunction with the quantity of *Azolla* to be used and the contact period needed to obtain acceptable discharge levels. Therefore, it must be stated that more research is required in sustaining plant life and growth, pond treatment and recovery with the possibility of utilising *Azolla* in the form of a system of bio-filters.

8.4 Conclusion

Removal of heavy metal contaminants from industrial wastewater (effluent) is necessary for all industries to maintain not only a competitive economic edge, but also to ensure a sustainable environmental. The use of plants for the removal and recycling of heavy metals are often more economical than currently used chemical processes. It is obvious from other experiments studied, that many different and challenging contributions can be made on the path to developing bio-sorption applications.

The answer, if the presence of iron in solution for effective PGM recovery was necessary, it was then successfully proved that it is an essential part of PGM recovery. This also needs to be explored further in optimising the amount needed to be present, together with the possibility of effluent blending. The possibility of blending of effluent streams will also provide the opportunity of making any biological option more industrial friendly, with endless opportunities to reduce cost of effluent treatment.

It can be stated without doubt, that there definitely is potential for metal recovery by the use of the plant *"Azolla Filiculoides"* (Red Water Fern) in this field, with both the live and the dried plant.

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Appendix A

Figure 1.1: Arial view of the evaporation ponds.
The pond at the bottom of the picture is the one containing the acidic effluent with an average pH between 1 and 2. The quantity in question is roughly three hundred cubic litres.

Appendix B

Figure 4.1: Up-flow construction of Rig 1.

Arrow 1: The 100 litre glass storage vessel.

Arrow 2: The 1 m X 25 mm glass column.

Arrow 3: The 20 litre glass vessel.

Appendix C

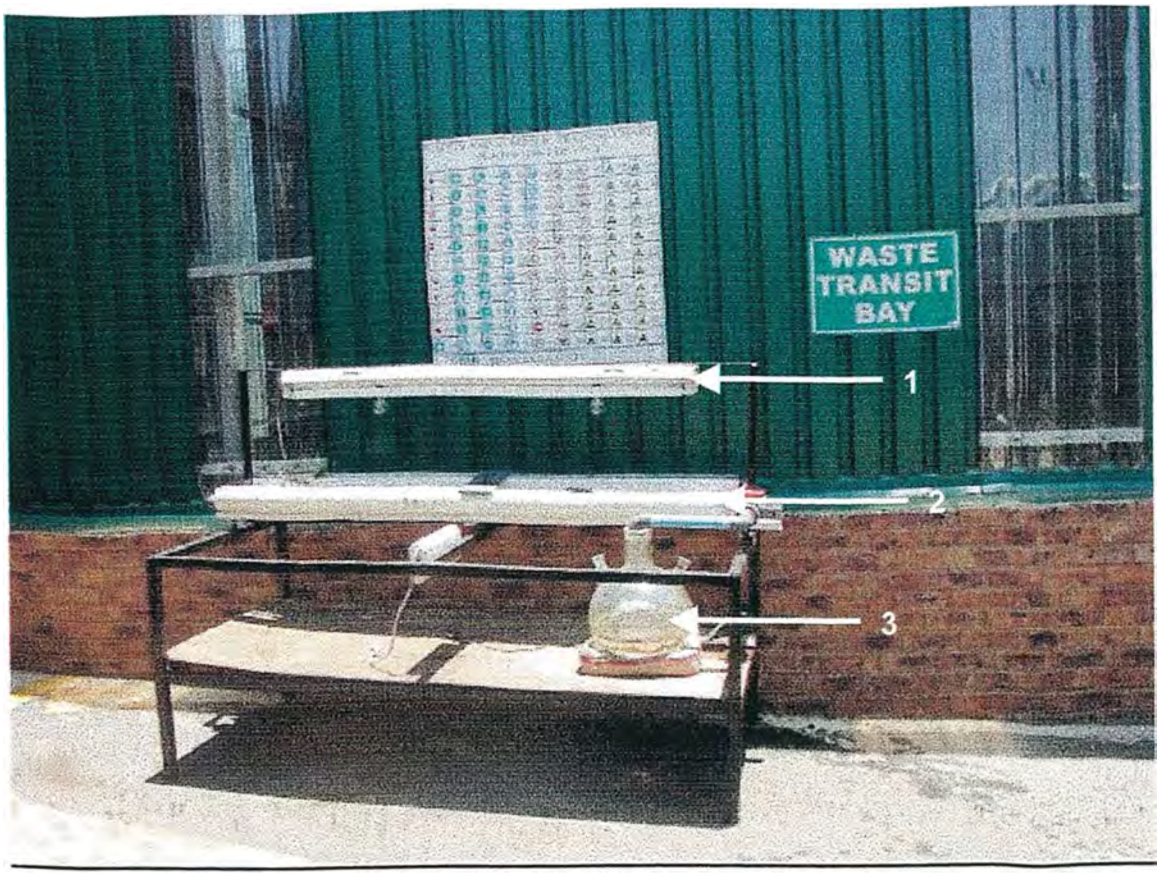


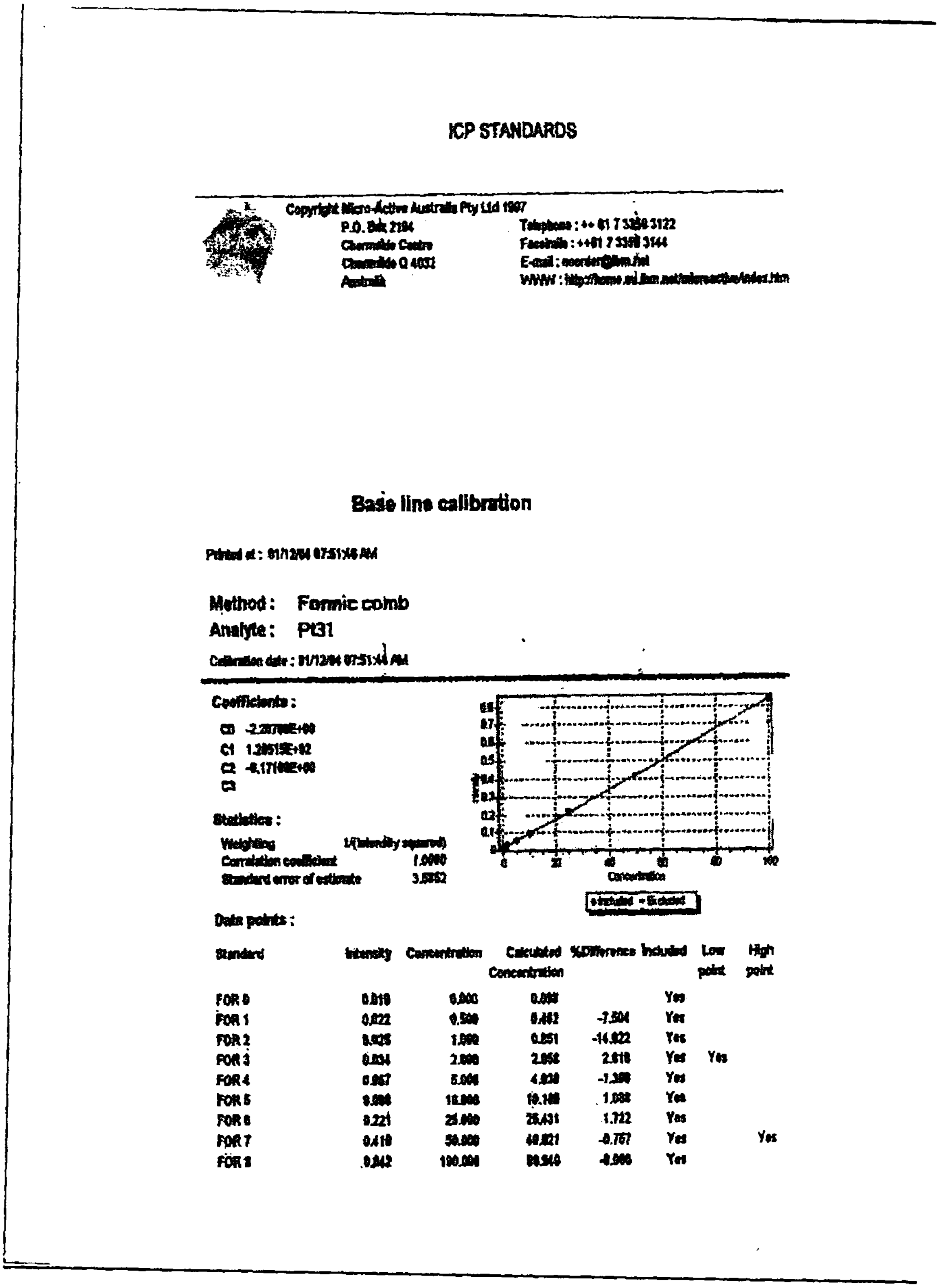
Figure 4.2: Raceway system Rig 2

Arrow 1: Combination of fluorescent and incandescent lighting.

Arrow 2: The 2 PVC, 100 mm pipes, 2000 mm long raceway.

Arrow 3: The 20 litre glass vessel.

Appendix D



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Base line calibration

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

Method : Fonnic comb

Analyte : Pt31

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

Coefficients :

C0 -2.28700E+00
C1 1.28515E+02
C2 -6.17100E+00
C3

Statistics :

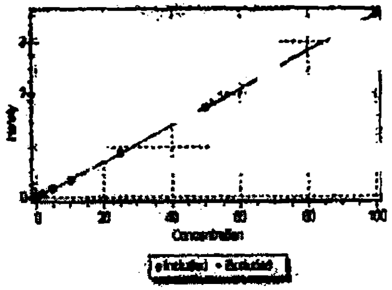
Weighting	1/(Intensity squared)
Correlation coefficient	1.0000
Standard error of estimate	3.5352

Data points :

Standard	Intensity	Concentration	Calculated Concentration	%Difference	Included	Low point	High point
FOR 0	0.018	0.000	0.000		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	2.918	Yes	Yes	
FOR 4	0.057	5.000	4.930	-1.398	Yes		
FOR 5	0.094	10.000	10.108	1.098	Yes		
FOR 6	0.221	25.000	25.431	1.722	Yes		
FOR 7	0.418	50.000	49.621	-0.757	Yes		Yes
FOR 8	0.842	100.000	99.140	-0.860	Yes		

Coefficients :

C0 -8.87700E-03
C1 2.88820E+01
C2 -2.42032E-01
C3



Statistics :

Weighting	1/(Intensity squared)
Correlation coefficient	1.0000
Standard error of estimate	0.4587

Data points :

Standard	Intensity	Concentration	Calculated Concentration	%Difference	Included	Low point	High point
FOR 0	0.000	0.000	0.008		Yes		
FOR 1	0.017	0.500	0.487	-2.500	Yes		
FOR 2	0.035	1.000	1.008	0.838	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	10.038	0.380	Yes		
FOR 6	0.870	25.000	25.278	1.112	Yes		
FOR 7	1.723	50.000	49.625	-0.750	Yes		Yes
FOR 8	3.550	100.000	100.078	0.078	Yes		

APPENDIX D

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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

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Base line calibration

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

Method : Formic comb

Analyte : Au7

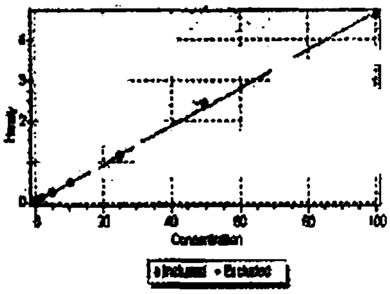
Calibration date : 01/12/04 07:50:40 AM

Coefficients :

C0 -8.86671E-01
C1 2.18577E+01
C2 -1.18708E-01
C3

Statistics :

Weighting	1/(intensity squared)
Correlation coefficient	0.9987
Standard error of estimate	0.0005



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	0.464	-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	0.282	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	51.870	3.752	Yes		Yes
FOR 8	4.841	100.000	98.435	-1.565	Yes		

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Base line calibration

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Method : Formic comb
Analyte : Au26

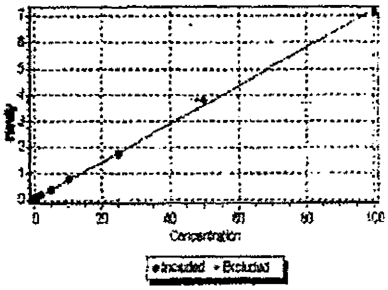
Calibration date : 01/12/04 07:50:31 AM

Coefficients :

C0 -4.15672E-01
C1 1.40588E+01
C2 -3.33400E-02
C3

Statistics :

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

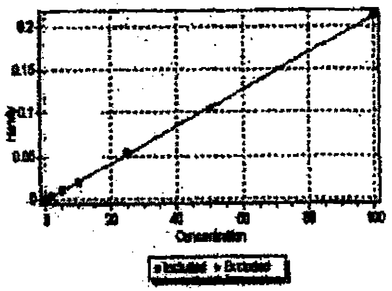


Data points :

Standard	Intensity	Concentration	Calculated Concentration	%Difference	Included	Low point	High point
FOR 0	0.030	0.000	0.006		Yes		
FOR 1	0.062	0.500	0.455	-9.048	Yes		
FOR 2	0.100	1.000	0.992	-0.838	Yes		
FOR 3	0.182	2.000	2.142	7.093	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	7.196	100.000	98.582	-1.418	Yes		

Coefficients :

C0 -8.47811E-02
C1 4.84749E+02
C2 -6.59888E+01
C3



Statistics :

Weighting	1/(Intensity squared)
Correlation coefficient	0.9999
Standard error of estimate	11.893

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	0.476	-4.873	Yes		
FOR 2	0.002	1.000	1.033	3.293	Yes		
FOR 3	0.004	2.000	1.998	-0.061	Yes	Yes	
FOR 4	0.010	5.000	4.979	-0.427	Yes		
FOR 5	0.021	10.000	9.950	-0.490	Yes		
FOR 6	0.054	25.000	25.813	3.253	Yes		
FOR 7	0.105	50.000	48.927	-2.145	Yes		Yes
FOR 8	0.213	100.000	100.318	0.318	Yes		

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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

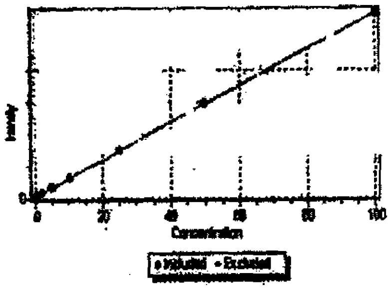
Method : Formic comb

Analyte : Ru5

Calibration date : 01/12/96 07:52:41 AM

Coefficients :

C0 -1.41301E+00
C1 6.87422E+01
C2 8.83071E-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	0.168	10.000	10.214	2.138	Yes		
FOR 6	0.340	25.000	24.854	-0.584	Yes		
FOR 7	0.742	50.000	50.062	0.125	Yes		Yes
FOR 8	1.448	100.000	99.004	-0.996	Yes		

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Base line calibration

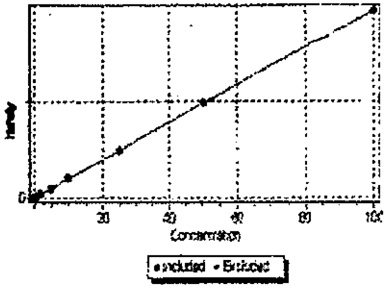
Printed at : 01/12/04 07:52:03 AM

Coefficients :

C0 4.98800E-02
C1 4.98580E+01
C2 1.04507E+00
C3

Statistics :

Weighting	1/(intensity squared)
Correlation coefficient	0.9998
Standard error of estimate	1.0817



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	2.261	Yes	Yes	
FOR 4	0.096	5.000	4.858	-2.838	Yes		
FOR 5	0.198	10.000	9.970	-0.297	Yes		
FOR 6	0.483	25.000	24.358	-2.568	Yes		
FOR 7	0.981	50.000	50.017	0.035	Yes		Yes
FOR 8	1.938	100.000	100.737	0.737	Yes		

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Analyte : Ir15

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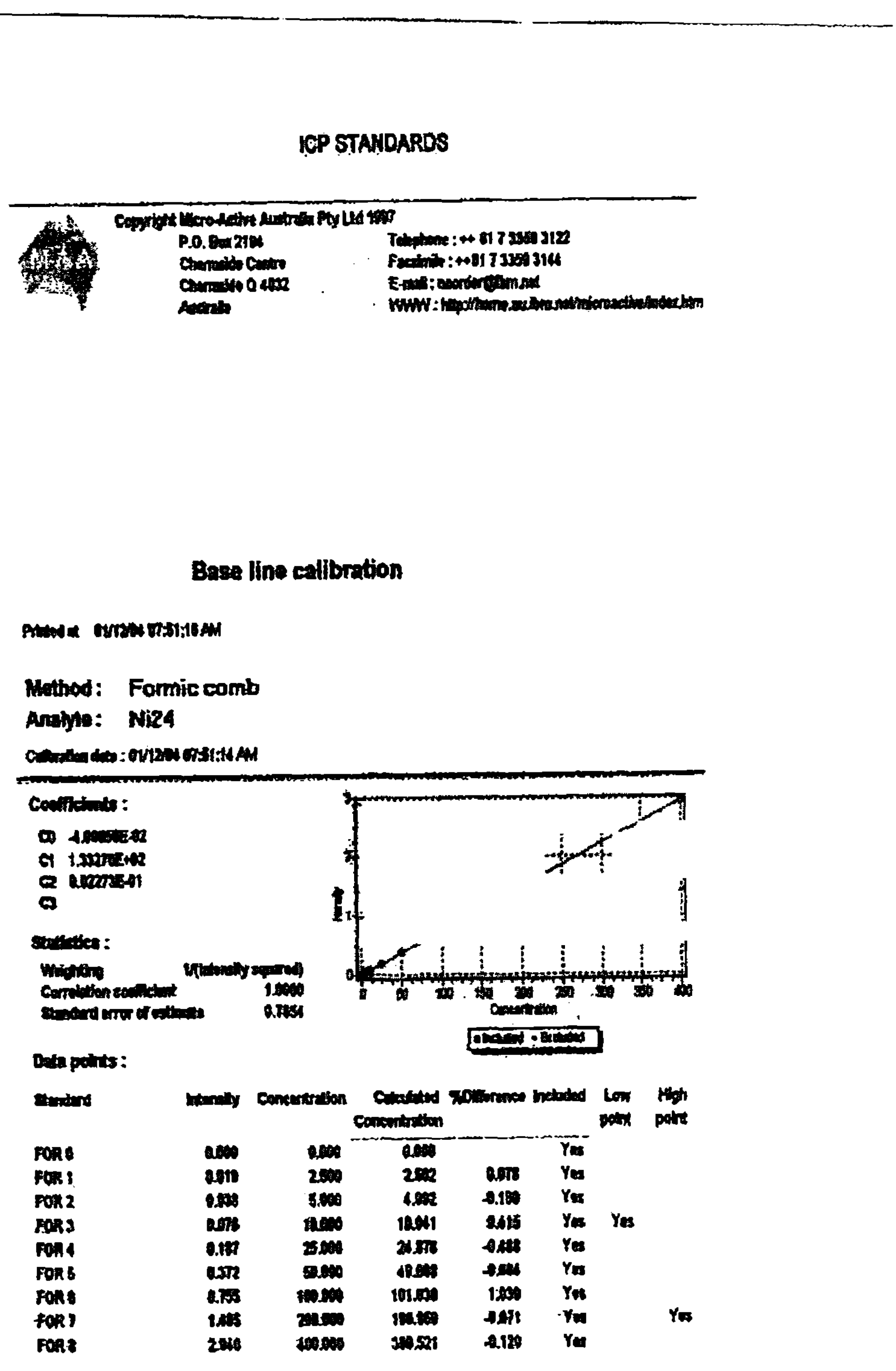

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Base line calibration

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Method : Formic comb
Analyte : Ni24

Calibration date : 01/12/04 07:31:14 AM

Coefficients :

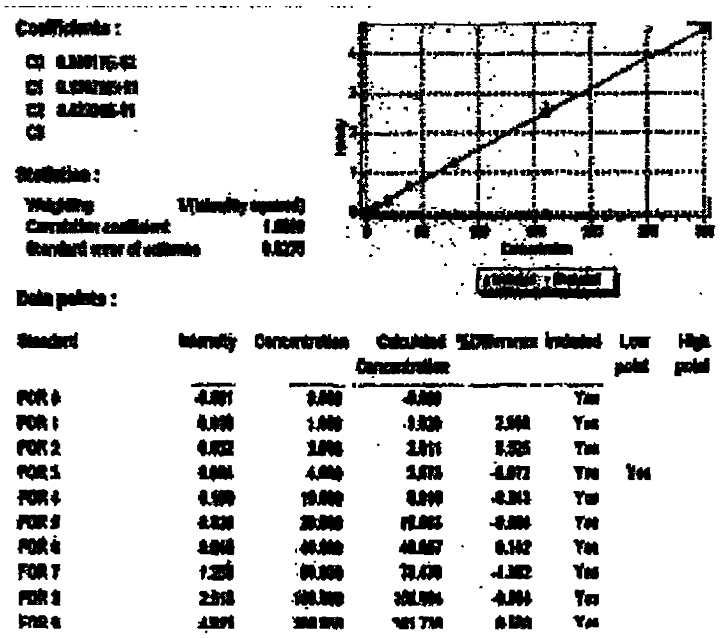
C0 -4.90050E-02
C1 1.33270E+02
C2 0.02273E-01
C3

Statistics :

Weighting	1/(intensity squared)
Correlation coefficient	1.0000
Standard error of estimate	0.7854

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	-0.159	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	49.908	-0.184	Yes		
FOR 6	0.755	100.000	101.030	1.030	Yes		
FOR 7	1.485	200.000	199.059	-0.471	Yes		Yes
FOR 8	2.946	400.000	399.521	-0.120	Yes		



Coefficients :

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C1 [illegible]
C2 [illegible]
C3

Statistics :

Weighting 1/(Intensity squared)
Correlation coefficient [illegible]
Standard error of estimate [illegible]

Data points :

Standard	Intensity	Concentration	Calculated Concentration	%Difference	Included	Low point	High point
FOR 0	[illegible]	[illegible]	[illegible]		Yes		
FOR 1	[illegible]	1.000	[illegible]	[illegible]	Yes		
FOR 2	[illegible]	[illegible]	[illegible]	[illegible]	Yes		
FOR 3	[illegible]	4.000	[illegible]	[illegible]	Yes	Yes	
FOR 4	[illegible]	10.000	[illegible]	[illegible]	Yes		
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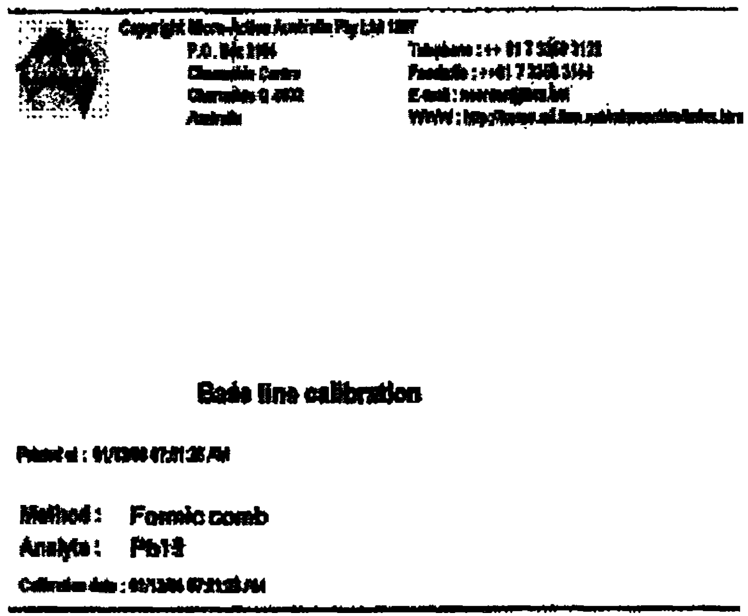

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Base line calibration

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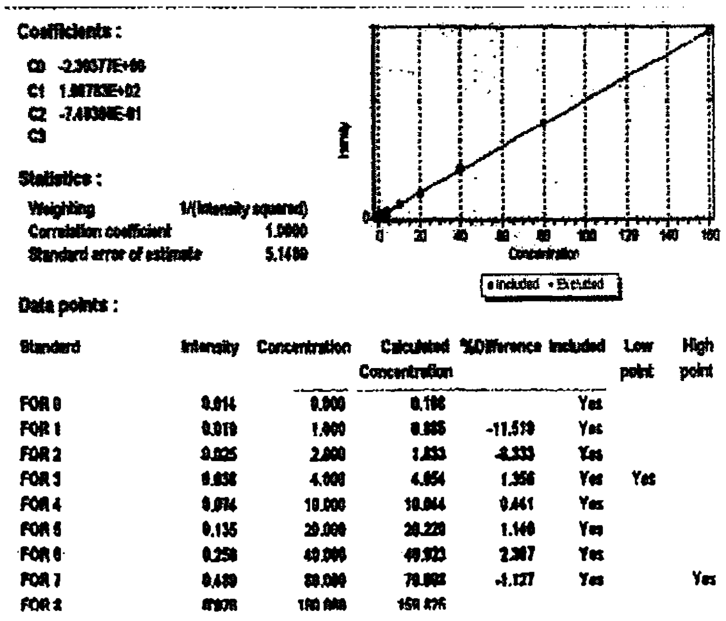
Analyte : Pb13

Calibration date : 08/13/96 07:21:23 AM



Coefficients :

C0 -2.30377E+00
C1 1.80783E+02
C2 -7.40390E-01
C3

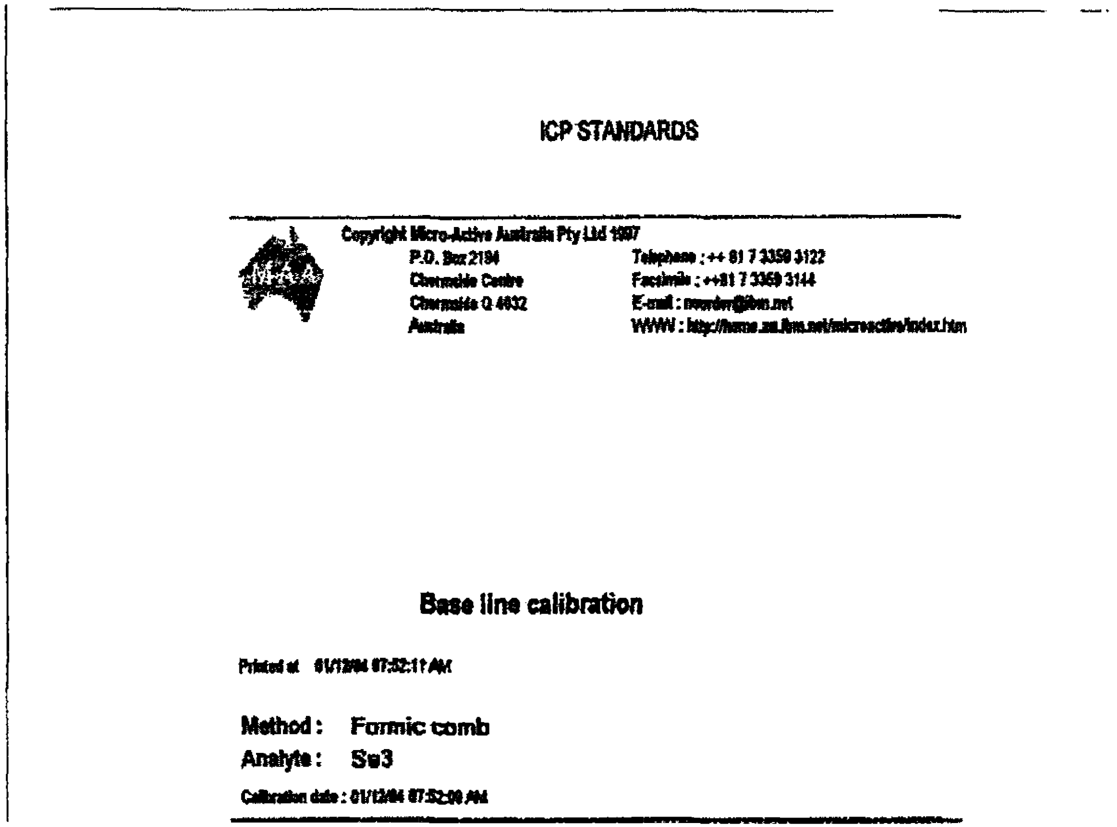
Statistics :

Weighting	1/(Intensity squared)
Correlation coefficient	1.0000
Standard error of estimate	5.1409

Data points :

Standard	Intensity	Concentration	Calculated Concentration	%Difference	Included	Low point	High point
FOR 0	0.014	0.000	0.196		Yes		
FOR 1	0.019	1.000	0.885	-11.519	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	1.148	Yes		
FOR 6	0.258	40.000	40.923	2.307	Yes		
FOR 7	0.439	80.000	79.098	-1.127	Yes		Yes
FOR 8	0.878	160.000	159.876				

APPENDIX D



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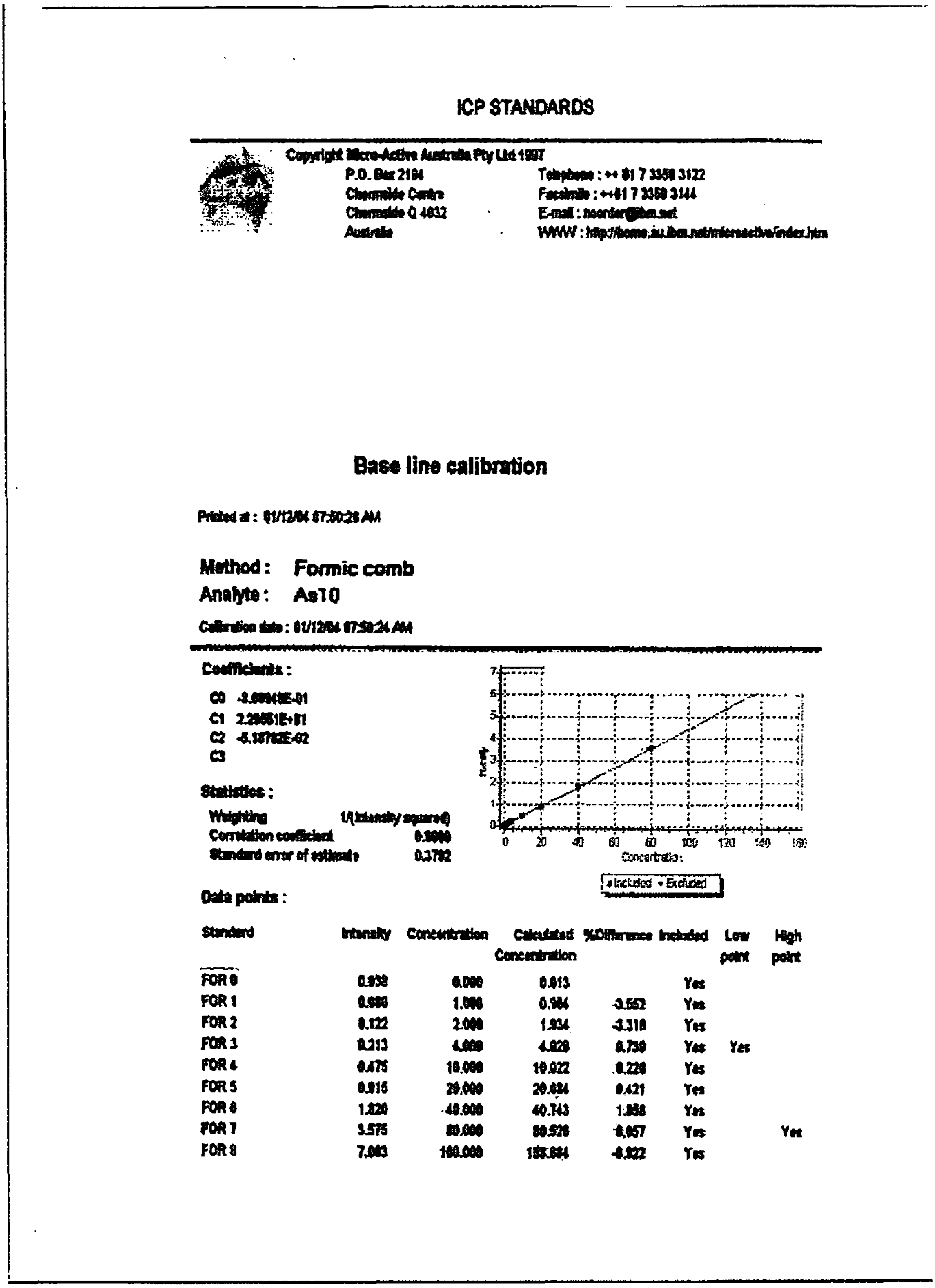
Analyte : Se3

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



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Analyte : As10

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

Coefficients :

C0 -3.00940E-01
C1 2.28651E+01
C2 -5.18702E-02
C3

Statistics :

Weighting	1/(Intensity squared)
Correlation coefficient	0.9990
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	0.083	1.000	0.964	-3.562	Yes		
FOR 2	0.122	2.000	1.934	-3.310	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	0.916	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	159.484	-0.322	Yes		

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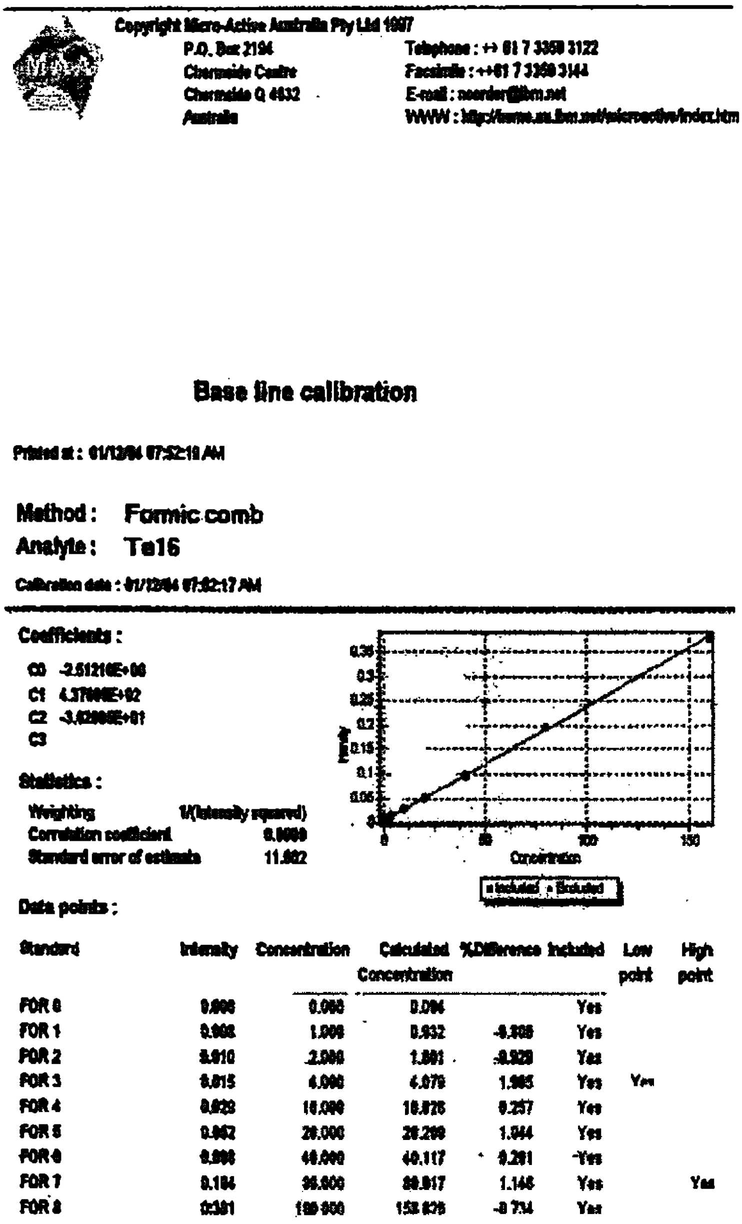

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Base line calibration

Printed at : 01/12/04 07:52:10 AM

Method : Formic comb
Analyte : Te16

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

Coefficients :

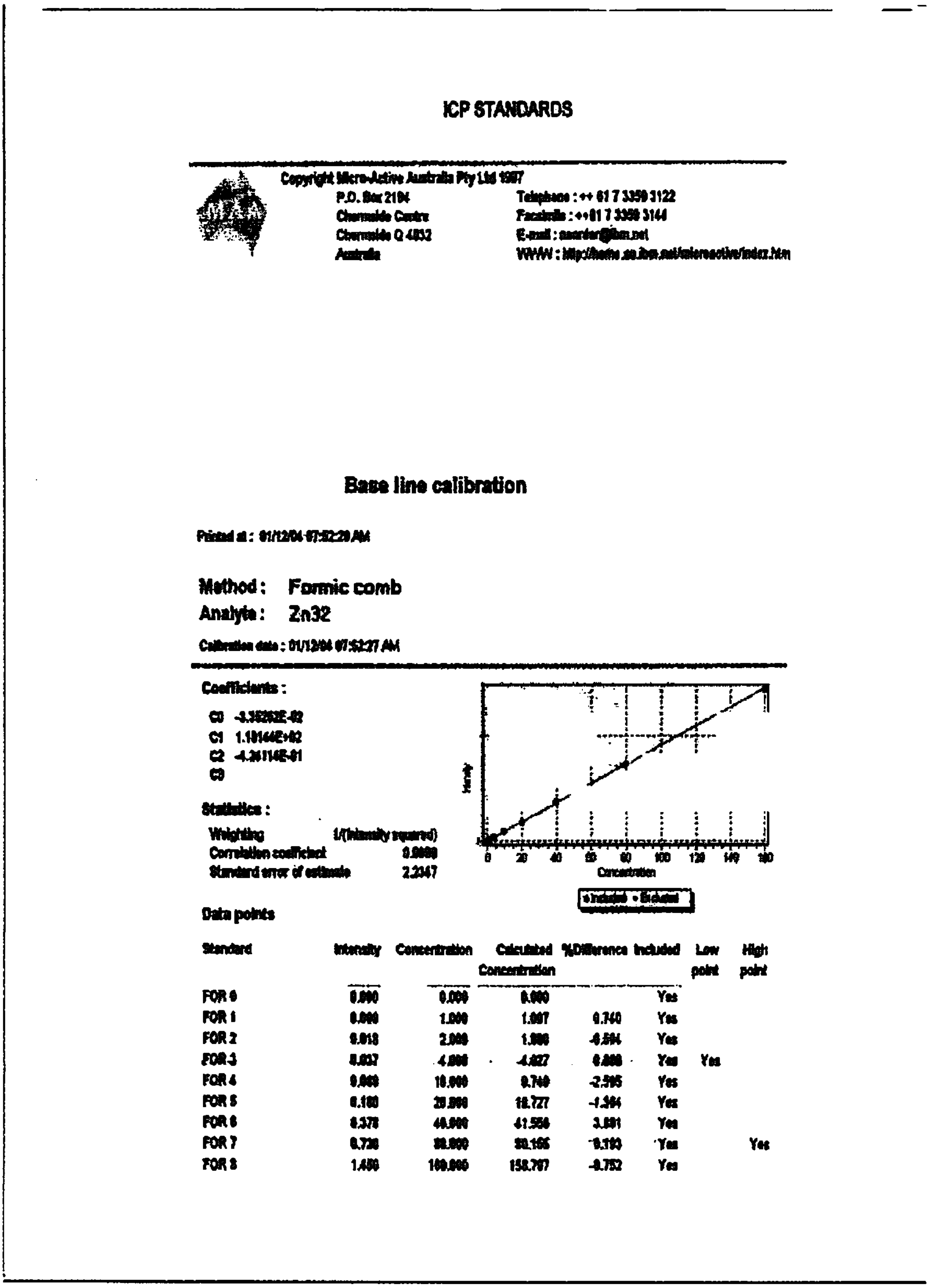
C0 -2.51210E+00
C1 4.37900E+02
C2 -3.02000E+01
C3

Statistics :

Weighting	1/(Intensity squared)
Correlation coefficient	0.9999
Standard error of estimate	11.982

Data points :

Standard	Intensity	Concentration	Calculated Concentration	%Difference	Included	Low point	High point
FOR 0	0.006	0.000	0.004		Yes		
FOR 1	0.008	1.000	0.992	-0.808	Yes		
FOR 2	0.010	2.000	1.981	-0.929	Yes		
FOR 3	0.015	4.000	4.079	1.975	Yes	Yes	
FOR 4	0.028	10.000	10.026	0.257	Yes		
FOR 5	0.047	20.000	20.209	1.044	Yes		
FOR 6	0.096	40.000	40.117	0.291	Yes		
FOR 7	0.184	80.000	80.917	1.146	Yes		Yes
FOR 8	0.361	155.000	153.826	-0.734	Yes		



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Base line calibration

Printed at : 01/12/04 07:52:28 AM

Method : Formic comb
Analyte : Zn32

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

Coefficients :

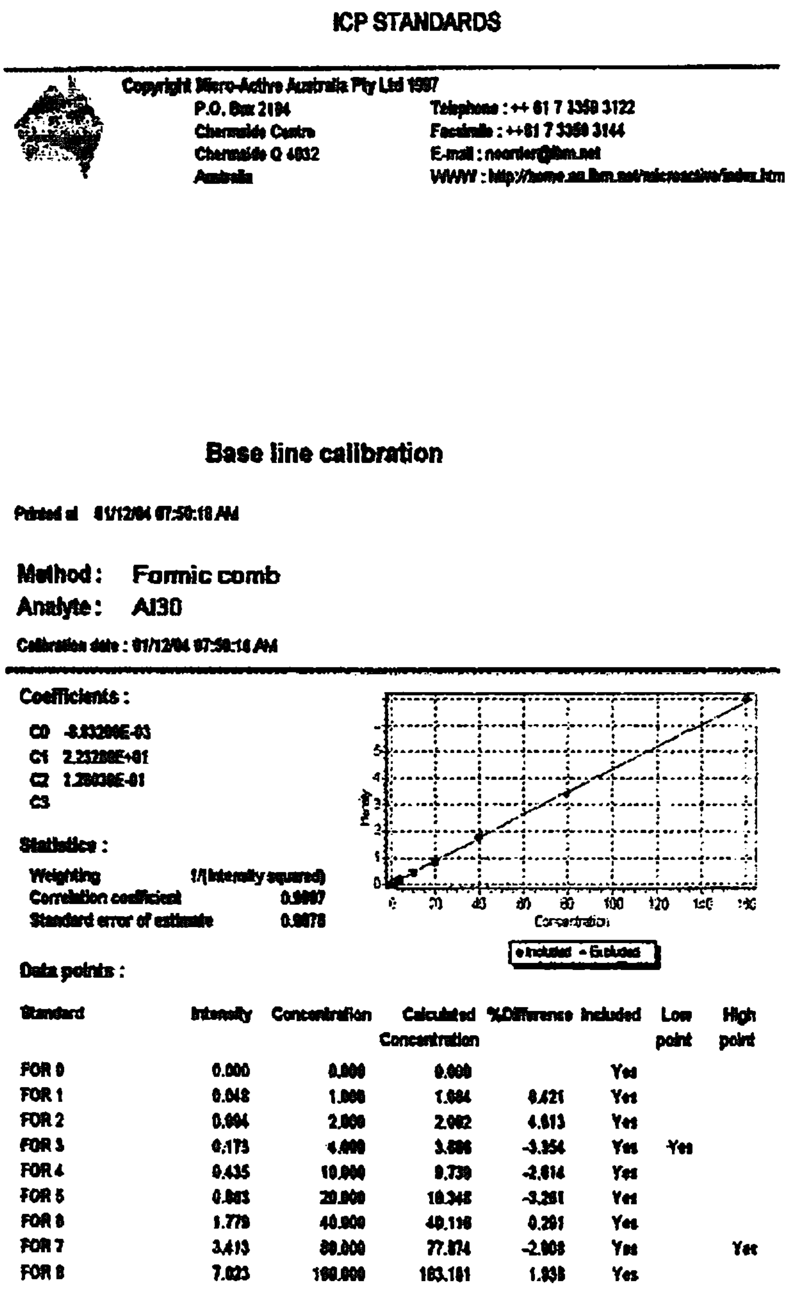
C0 -3.35262E-02
C1 1.10144E+02
C2 -4.26114E-01
C3

Statistics :

Weighting	1/(Intensity squared)
Correlation coefficient	0.9999
Standard error of estimate	2.2047

Data points

Standard	Intensity	Concentration	Calculated Concentration	%Difference	Included	Low point	High point
FOR 0	0.000	0.000	0.000		Yes		
FOR 1	0.008	1.000	1.007	0.740	Yes		
FOR 2	0.018	2.000	1.868	-6.594	Yes		
FOR 3	0.037	4.000	4.027	0.688	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	0.738	80.000	80.155	0.193	Yes		Yes
FOR 8	1.450	160.000	158.797	-0.752	Yes		



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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 -8.83200E-03
C1 2.23200E+01
C2 1.28030E-01
C3

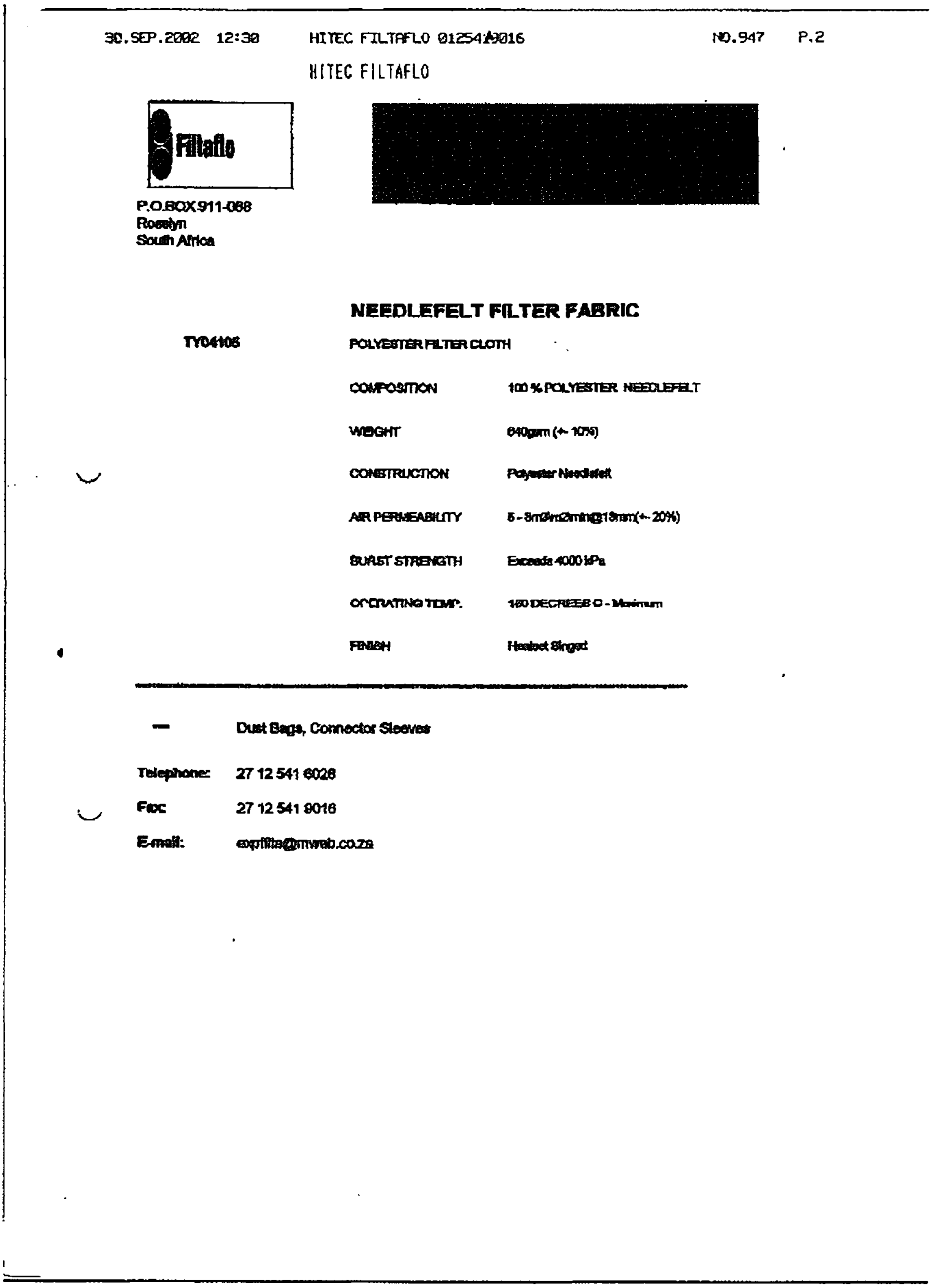
Statistics :

Weighting	1/(Intensity squared)
Correlation coefficient	0.9997
Standard error of estimate	0.0078

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	1.004	0.421	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	163.181	1.988	Yes		

Appendix E



30.SEP.2002 12:30 HITEC FILTAFLO 0125419016 NO.947 P.2

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Filtaflo

[redacted]

P.O.BOX 911-068
Rosslyn
South Africa

NEEDLEFELT FILTER FABRIC

TY04106 POLYESTER FILTER CLOTH

COMPOSITION	100 % POLYESTER NEEDLEFELT
WEIGHT	640gsm (+- 10%)
CONSTRUCTION	Polyester Needlefelt
AIR PERMEABILITY	5 - 8m3/m2/min@13mm(+- 20%)
BURST STRENGTH	Exceeds 4000 kPa
OPERATING TEMP.	150 DEGREES C - Maximum
FINISH	Heatset Singed

— Dust Bags, Connector Sleeves

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