A STUDY OF THE ACCUMULATION TOXICITY AND DETERMINATION OF PLATINUM GROUP METALS IN PLANTS

A thesis presented for the degree of
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of Science of the University of London

by

Patrick Jeremy Parsons

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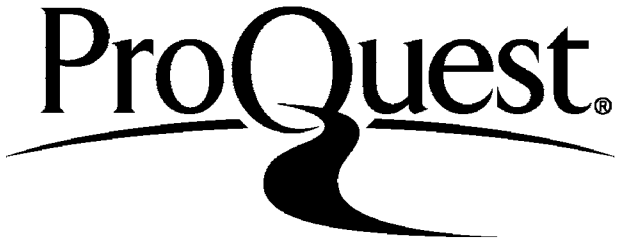

TO MY PARENTS

I shoot the Hippopotamus with bullets made of platinum, because if I use leaden ones his hide is sure to flatten 'em.

(from 'The Bad Child's Book of Beasts')

Hillaire Belloc's

CAUTIONARY VERSES

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ABSTRACT

A range of analytical techniques were investigated and compared for the determination of platinum group metals in plant tissues. These included ICP, DC arc AES and PIXE. Particular attention was paid to problems associated with determination by ETA AAS and INAA. PIXE was suitable as a routine method and gave results in good agreement with those from INAA. The ICP method was satisfactory only at relatively high concentrations.

The biological effects of platinum group metals on *Eichhornia crassipes* were studied. At an applied metal concentration of 10ppm, the relative toxicity was found to be: $Pt^{2+} \approx Pd^{2+} > Os^{4+} \approx Ru^{3+} > Ir^{3+} > Pt^{4+} >> Rh^{3+}$. The metals were deposited mostly in plant roots though varying amounts were transported to the tops. Toxic symptoms of Pt^{2+} complexes included the appearance of reddish-brown streaks in the leaves, whilst Rh^{3+} appeared to exhibit a tonic effect. In contrast, the grass *Setaria verticillata* was growth stimulated by Pt^{2+} at low levels.

Two complexed forms of platinum were investigated further because of the difference in relative toxicity. Platinum applied as the anti-tumour complex cis $[Pt(NH_3)_2Cl_2]$ was found to be toxic at high levels. When applied at low levels some 47.9% of the platinum in the leaves was associated with α-cellulose and lignin; 16.1% was removed by the proteolytic enzyme pronase, and 20.8% found with water soluble pectates. A similar distribution was found in the floats of *Eichhornia crassipes* and in the plant roots the values were 35%, 9.5% and 14.2% respectively. In the roots however, a further 23.1% was removed with low molecular weight alcohol soluble materials and 12.0% with polar water soluble materials. The amino acid constituents of control and platinum treated plants are also presented.

When applied as Pt^{4+}, platinum appeared relatively non-toxic. Analytical electron micrographs revealed Pt deposits concentrated in the epidermis of the root with lesser amounts extending up to the endodermis. XPS confirmed the deposits on the root surface as Pt and the binding energy suggests it is Pt^{4+}. Ruthenium also has been detected in the epidermis of Ru treated root samples.

CONTENTS

A STUDY OF THE ACCUMULATION TOXICITY AND DETERMINATION OF PLATINUM GROUP METALS IN PLANTS

Foreword

To sustain normal healthy growth, higher plants require some eight metals of which five are classified as transition elements. Many plant species can accumulate quite high levels of both essential and non-essential transition elements; such species are called tolerant. The mechanism(s) by which these plants can tolerate such high metal levels, normally considered toxic, is far from fully understood. Little is known about the accumulation and toxicity of the six heaviest members of Group VIII: the platinum group metals (or platinum metals).

This study is an attempt to investigate and compare the biological effects of the platinum group metals on plants. The aquatic plant *Eichhornia crassipes* (water hyacinth) was selected for detailed study because of its remarkable ability to assimilate high levels of transition metals and hence its potential as an agent in the control of heavy metal pollution in rivers, ponds and streams. In the introduction, sections on the anatomy and chemical composition of plants set the scene for later investigations. A review of the inorganic biochemistry of the platinum group metals with particular reference to plant studies is included.

This work has brought to light many of the problems involved in using instrumental techniques for the determination of the platinum group metals in biological matrices. It is for this reason, that analytical methods have featured so prominently in this thesis. The principles and applications of the major techniques are presented as a prelude to uptake experiments.

CHAPTER I

INTRODUCTION

1.1 The plant cell

The structure of a typical plant cell can be divided into two main regions: the cell wall and the protoplast. The latter contains the nucleus, the cytoplasm, consisting of various membrane bound organelles and various organic molecules responsible for the metabolic activity of the cell. Although these features are common to the majority of plant cells there is a large degree of variation in size, shape and structure depending on function, age and other factors.

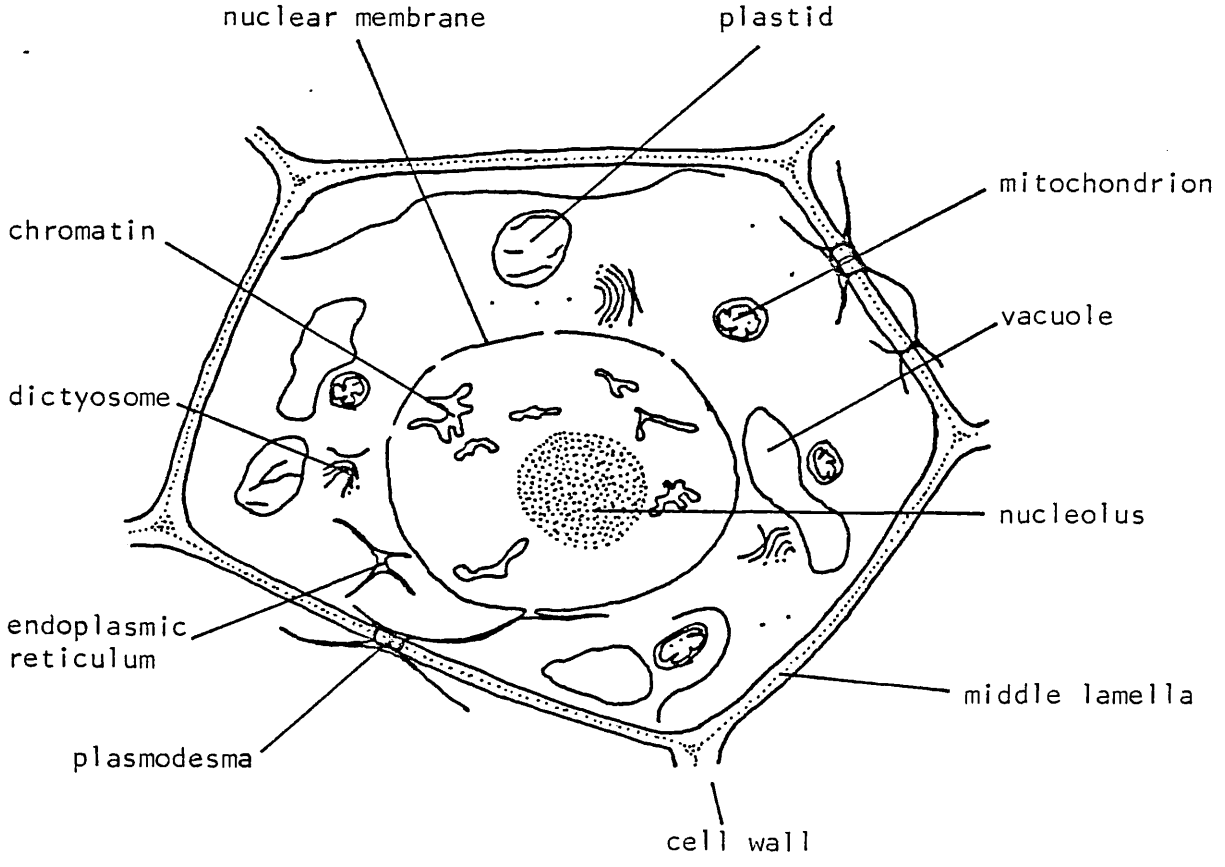


Figure 1. Ultrastructural details of a young plant cell (after Esau 1965).

The majority of higher plant cells fall within the range 20-30 μm in length or diameter. Each cell is protected by an outer membrane, the plasmalemma. This acts as a semipermeable barrier allowing water to pass but restricting the passage of solutes. The plasmalemma is itself bounded by a cell wall which imparts rigidity and protects the cell against attack by pathogens. Another feature of plant cells is that they are not isolated. Plasmodesmata are cytoplasmic interconnections lined by plasmalemma. Their exact function is not understood but they may provide a channel through which material can be transported between cells.

The cytoplasm is a translucent viscous mass of which 80-90% is water. In a young plant cell the cytoplasm may contain one or two sap filled cavities called vacuoles. As the cell matures these vacuoles become enlarged and combine until they dominate the cell. They are separated from the cytoplasm by a membrane known as the tonoplast. Plant vacuoles may contain salts, sugars, organic acids, proteins and even fatty substances either in solution or in a colloidal state; pigments and tannins have also been found.

The most dominant of the differentiated protoplasmic structures is the nucleus, a spherical or ellipsoidal body bounded by a nuclear membrane. Suspended in the nuclear sap are one or more nucleoli and the chromosomes, which arise during nuclear division from the chromatin, a diffuse tangle of separate fine threads. It has been shown that chromatin consists of a complex of proteins, ribonucleic acid (RNA) and deoxy-ribonucleic acid (DNA), which is characteristic of the nucleus and is the carrier of the hereditary genetic material.

Various other protoplasmic bodies include plastids, mitochondria, microsomes and the Golgi apparatus. Plastids are viscous bodies which

vary in structure and function. Colourless plastids are called leucoplasts, pigmented ones chromoplasts, of which chloroplasts, the green plastids containing chlorophylls, are the most dominant, being involved in photosynthesis.

Mitochondria appear as filamentous rod-shaped bodies between 0.2 and 2 μm in length and are found in the cytoplasm of virtually all living cells. In the electron microscope each is seen to be bound by a double membrane. Mitochondria are involved in the breakdown of complex food molecules and oxidation of organic acids to provide energy which is stored in adenosine triphosphate (ATP). Microsomes are bodies of the order of 0.1 μm which are thought to originate in the nucleus. Rich in RNA, they are extruded in the cytoplasm and are associated with the endoplasmic reticulum, a complex system of membranes identified by electron microscopy. Also identified by electron microscopy are the Golgi bodies or dictyosomes bounded by a single membrane; very little is known about their function.

1.2 Physiological anatomy of the root

Of the elements required by higher plants to sustain healthy growth, only three are absorbed via aerial parts; carbon, hydrogen and oxygen. The other elements are absorbed and transported throughout the plant by a highly complex mechanism which begins at the root. Some roots also serve as a means of anchorage and these are found to extend well below the soil surface whilst those roots with an absorbing function tend to be nearer the surface. The plant's root system may cover a surface area which is much larger than that of its aerial parts. The area of contact between soil and root is enhanced considerably by the growth of root hairs which are replaced rapidly by new hairs as the older ones die. Figure 2 is a diagram of a root cross section about 1 cm from the tip. The root is bound by a single

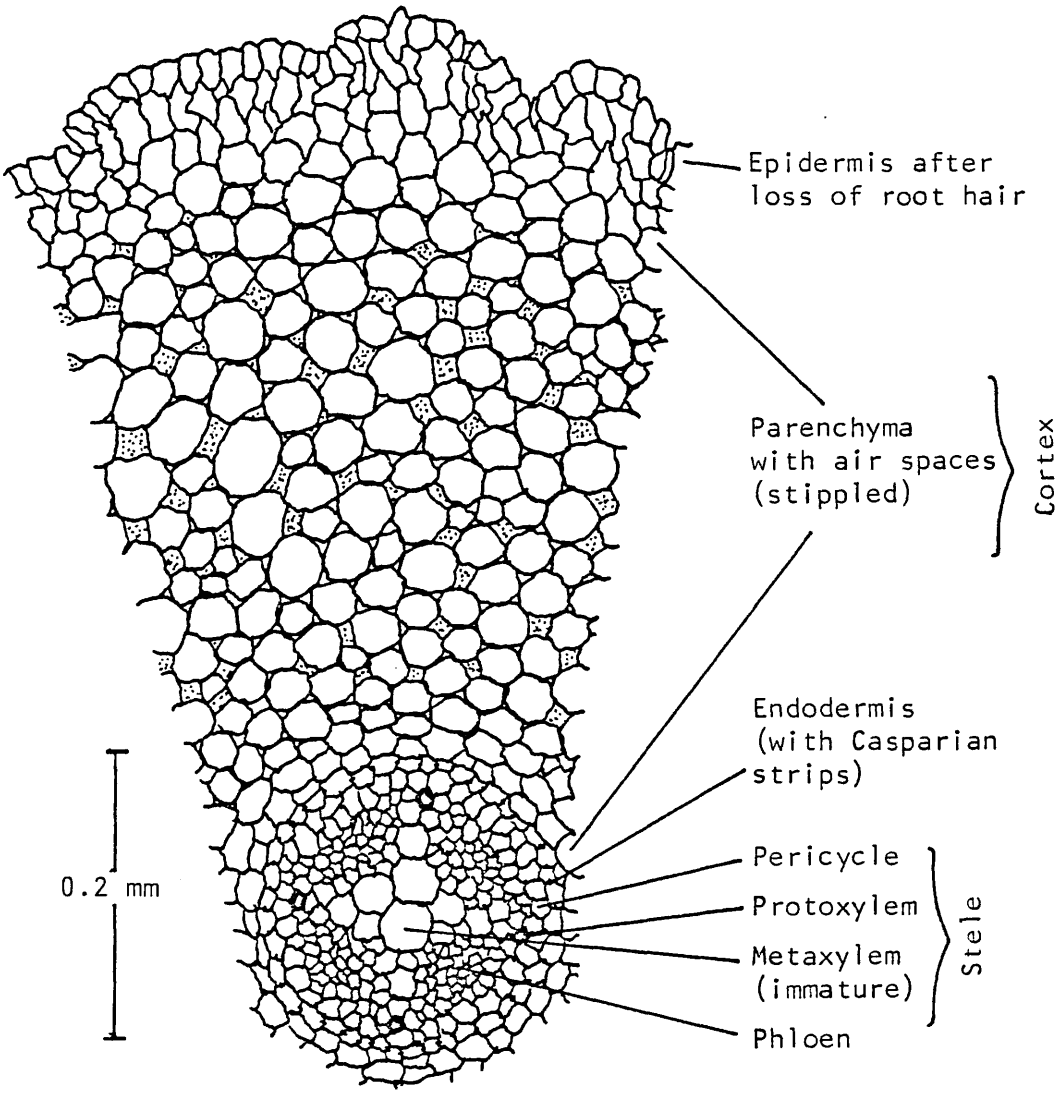


Figure 2. Transverse section of a root of *Ranunculus repens* about 1 cm from the apex (after Bowling 1976).

external layer of cells called the epidermis. The root hairs, which grow outwards, are merely extentions of the outer epidermal cell wall. The majority of the root structure consists of relatively large and loosely arranged parenchyma cells, with many air spaces, known collectively as the cortex. The innermost part of the root, called the stele, is separated from the cortex by a single layer of elongated cells called the endodermis.

The chief feature of the endodermis is the appearance of a ribbon like strip surrounding the radial walls of each cell (Figure 3). This is the Casparian strip or band, the chemical composition of which appears to be a combination of cellulose and suberin, a wax like substance, though this combination is not well understood (Van Fleet 1961).

The major features of the stele are the vascular tissues which are responsible for transporting minerals, food and water throughout the plant. The xylem is a structurally and functionally complex tissue which is responsible for the upward movement of water and nutrients to the plant

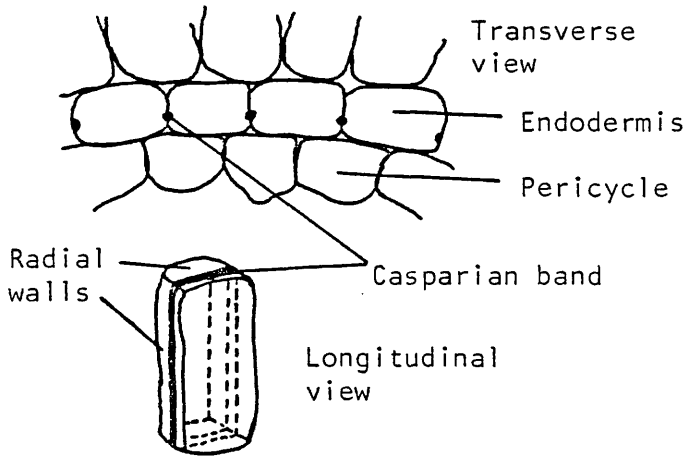


Figure 3. The location of the Casparian strip in the endodermis (after Bowling 1976).

aerial parts. The phloem is a complex tissue also, which is closely associated and alternates with the xylem. It functions as a conductor of carbohydrates and organic molecules from the leaves to the roots. The major cells involved in phloem transport are the sieve-tube members. Both the xylem and phloem are surrounded by their smaller thin walled parenchymatic cells. The stele is encased by a single layer of thin walled cells adjacent to the endodermins, called the pericycle, from whence lateral roots originate.

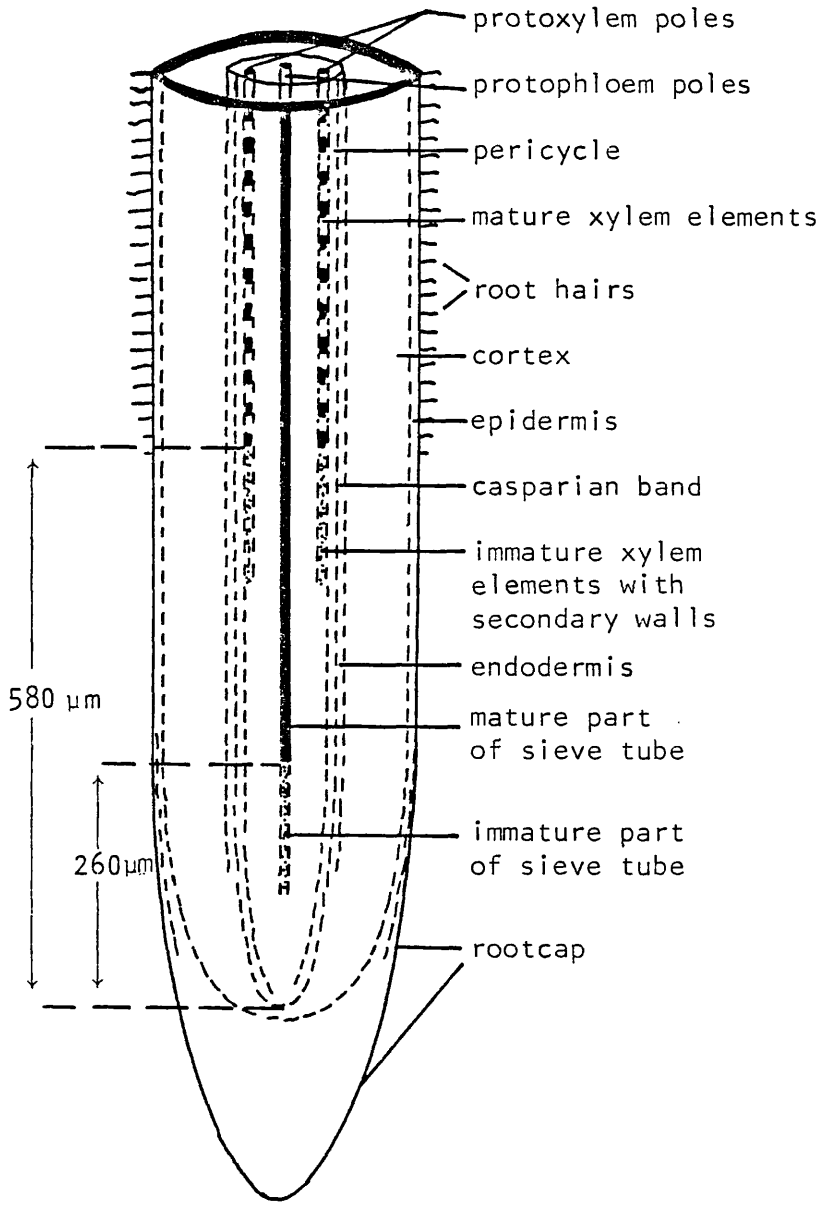


Figure 4. Longitudinal section of a root tip of *Nicotiana* (tobacco) (after Esau 1953).

Most of the root growth takes place behind the root cap in the meristematic cells. It is behind the apex that cell elongation takes place, but before this is complete the phloem will have matured, unlike the protoxylem which matures after elongation (Figure 4). The arrangements of the xylem and phloem in vascular bundles varies between species and even between the same plants. As the cells age they become impregnated, mainly with lignin, which causes the cell walls to thicken. This is particularly important in monocotyledons where the thickening of vascular cell walls imparts rigidity to the root structure.

1.3 Chemical composition and structure of the cell wall

The cell walls of plants may be classified as either primary or secondary. In young cells only the primary wall exists; it is usually thin (1-3 μm) and associated with the living protoplast, as in the meristematic cells. Later, as the cell develops, it may become coated with a thicker layer from within the protoplast; this forms the secondary cell wall, which is usually multilayered and between 5-10 μm thick. This imparts rigidity and shape to the cell. The primary wall of each cell is separated from other cells by an intercellular substance known as the middle lamella. The composition of the cell wall varies from species to species and between the various plant tissues. It has been suggested that the structure of the cell wall resembled that of reinforced plastic with a framework of cellulose impregnated with pectins and hemicelluloses (Northcote 1972). Secondary walls are thickened further with deposits of lignin, various pigments, waxes, fats and proteins. The primary cell walls contain more pectic substances than do secondary walls which undergo lignification as the cells mature.

Table 1. Composition of primary and secondary cell walls

Component	% of dry material			
	Oat		Wheat	
	Pr.	Sec.	Pr.	Sec.
Cellulose	42	44	36	39
Pectic substances	8	1	13	1
Hemicellulose	38	32	30	32
Lignin	-	19	-	17

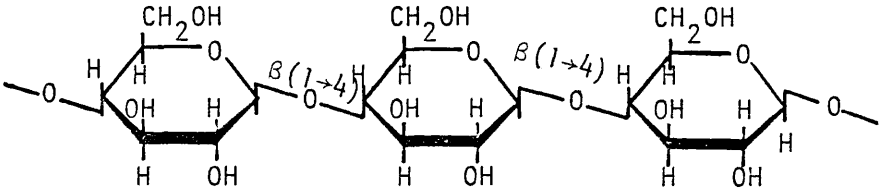
Key: Pr. = The primary cell walls of growing seedling tissues

Sec. = Secondary wall material from mature grass tissues

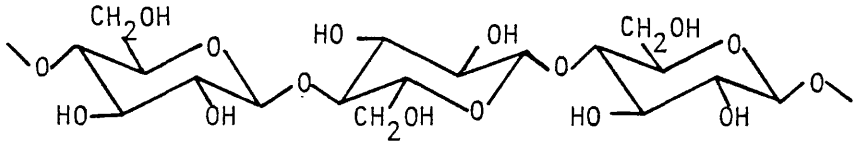
(adapted from Bonner 1950)

The backbone of the cell wall, cellulose, is a linear polymer of β (1 → 4) linked glucopyranose units. The resultant glucan chains have a length ranging from 1 - 7 μm and number 2 000 - 14 000 glucose units. The molecular weight is approximately 1000 000. Hydrogen bonding aids the arrangement of the chains into orderly 3-D lattices called micelles which are packed together in bundles known as microfibrils. The microfibrils are part of a larger complex macrofibril, which contains interspersed non-cellulosic material.

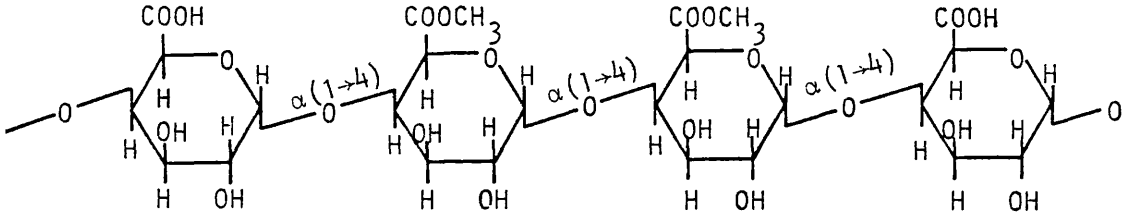
The capillaries between the microfibrils are able to absorb large amounts of water, thus allowing the passage of water-soluble substances. Pectic substances are regarded as the most soluble of polysaccharides and are highly hydrophilic. They occur in three forms, protopectin, pectin and pectic acid and are found largely in the middle lamella as calcium and magnesium salts. They usually contain polymers of D-galacturonic acid, L-arabinose, D-galactose and L-rhamnose.



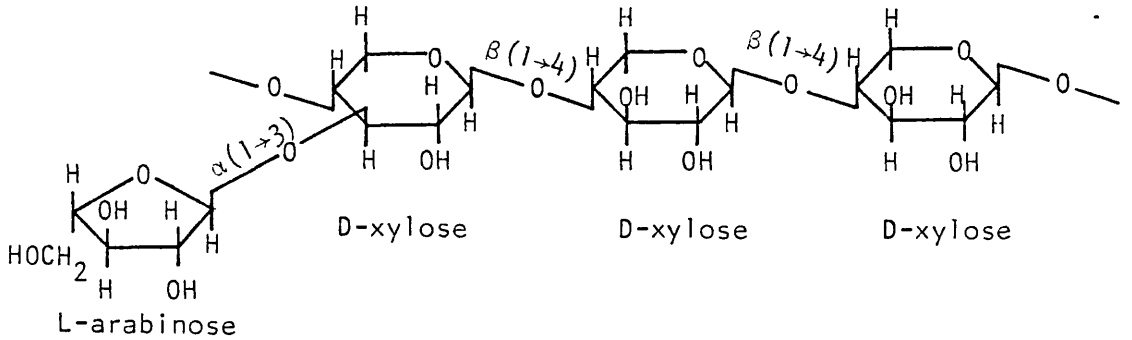
(a) Cellulose; β(1→4) linked glucose residues (Haworth formula)



(b) Cellulose; β(1→4) linked glucose residues (Chair configuration)



(c) α-D-(1→4) linked galacturonic acid residues found in Pectin.

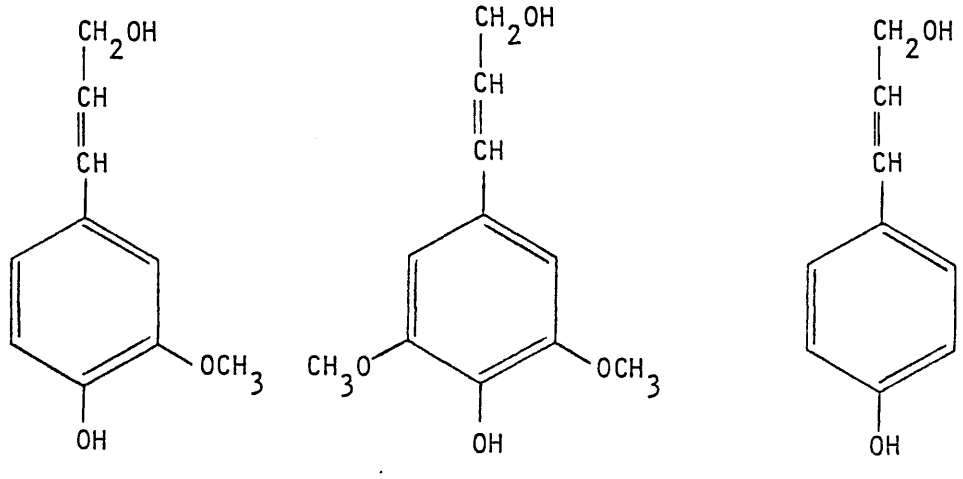


(d) Arabinoxylan

Figure 5 Structures of cell wall polysaccharides

Other sugars may be present also (Aspinall 1970). It is the carboxyl groups of the D-galacturonic acid residues which undergo dissociation, resulting in the build up of a negative charge. This charge is responsible for the strong cation binding characteristic of the cell wall.

Hemicelluloses are a complex class of polysaccharides which vary in structural units, extent of branching and in solubility. They can be extracted with alkali from cell wall material after removal of pectin. They can be isolated further into hemicelluloses A and B by neutralisation with acetic acid (Northcote 1963). Some examples from this class include the homopolymers, xylans, galactans, mannans and glucans, as well as the copolymers, glucomannans, galactomannans and arabinoxylans. Of these, the more important are the xylans and glucomannans. The xylans are based on a backbone of β (1 → 4) linked xylopyranose units with various other sugars attached either as units or branches. The glucomannans have backbones which comprise varying amounts of both D-glucose and D-mannose linked by β (1 → 4) glycosidic bonds (Figure 5).



(a) Coniferyl alcohol (Guaiacyl)

(b) Sinapyl alcohol (Syringyl)

(c) *p*-Hydroxycinnamyl alcohol

Figure 6. Structural components of lignin (after Hall, Flowers and Roberts 1974)

Finally, lignin, as an end product of cell metabolism, is one of the more important features of the secondary cell wall. The structure of lignin is complex and far from clear, but appears to be that of an aromatic polymer of high molecular weight containing various *p*-hydroxyphenylpropane units, some of which may be methoxylated on the aromatic ring (Figure 6). Cross-linkage of the units provides the cement with which the cellulose fibrils are strengthened and bound.

1.4 Inorganic composition of plants

Fresh plant material comprises 80-90% water and of the remainder over 90% consists of the elements carbon, oxygen and hydrogen. In the chemical analysis of plant material, the water is removed by drying and the organic matter by ashing. The remaining 1.5% of the plant's fresh weight represents its mineral composition. Table 2 gives the typical elemental composition of the corn plant, *Zea mays*.

Table 2. Elemental composition of the corn plant *Zea mays* (After Miller, 1938).

Element	% of dry weight	Element	% of dry weight
O	44.43	P	0.20
C	43.57	Mg	0.18
H	6.24	S	0.17
N	1.46	Cl	0.14
Si	1.17	Al	0.11
K	0.92	Fe	0.08
Ca	0.23	Mn	0.04

Essentiality

The fact that an element is detected in the plant tissue does not necessarily mean it is essential. Since any given soil sample is likely

to contain almost all the elements in the Periodic Table, some at ultra-trace levels, it is hardly surprising that with the sophisticated methods of analysis available today, most of them are detectable in plant tissues. Plant roots do not discriminate between essential and non-essential elements.

Various criteria of essentiality have been proposed by Arnon and Stout (1939).

1. Omission of the element in question must result in abnormal growth, failure to complete the life cycle or premature death of the plant.

2. The element must be specific and not replaceable by another.

3. The element must exert its effect directly on growth or metabolism and not by some indirect effect such as by antagonising another element present at a toxic level.

The difficulties involved in such experimental investigations in eliminating trace elements from the water used, has resulted in some criticism of the criteria (Hewitt, 1966). Chlorine is a good example of this criticism. It is an extremely difficult element to eliminate from water supplies and enough is present in the atmosphere, especially near the coast, to satisfy the plant's needs. Essentiality was not fully accorded chlorine until demonstration of its deficient effects (Broyer *et al.*, 1954) and its apparent involvement in the photo-oxidation of water in isolated chloroplasts (Arnon, 1961). The question of replacement has led to arguments about the criteria also (Nicholas, 1957). Chlorine is clearly an essential element, but can be replaced by bromine or iodine.

A more recent qualification of these criteria has been proposed by Epstein (1965). An element is essential (a) if, without it, the plant cannot complete its life cycle, and (b) if it is part of the molecule of an essential plant constituent or metabolite.

The experimental evidence for essentiality came from solution culture methods. (Arnon and Hoagland, 1948; Stout, 1956). By the beginning of the 20th century some ten elements were recognised as being essential for healthy plant growth. As well as carbon, hydrogen and oxygen, the elements nitrogen, phosphorus, sulphur, potassium, calcium and magnesium were collectively referred to as macronutrients on account of the relatively large concentrations required by plants. At that time the only exception was iron, known to be essential but required at a minimal concentration.

Micronutrient elements

During the first half of the 20th century experimental techniques and the quality of water used in hydroponic experiments improved considerably. Nutrient solutions based on the supply of essential elements at appropriate concentrations were developed, (Arnon and Hoagland, 1940) and these enabled investigators to distinguish another group of essential elements which, like iron, were required in very small amounts and were therefore termed the micronutrients (Stout, 1961).

With the exception of chlorine and boron, the five other essential micronutrients, iron, manganese, zinc, copper and molybdenum are all transition or 'd block' elements. It is because these elements have vacant or partially filled d orbitals that they exhibit some common physical and chemical characteristics. These include variable oxidation states, catalytic properties and most important, the ability to form complexes readily. This thesis is concerned with the six group VIII elements, platinum, palladium, rhodium, iridium, ruthenium and osmium, known collectively as the platinum group metals (P.G.M.), or just 'platinum metals'.

There are several plant species which are known to require elements other than the sixteen already discussed; some may require additional nutrients, others may find a replacement. Certain species (halophytes) adapted to

growing on saline soils can tolerate quite high levels of sodium, some eg: *Atriplex vesicaria* may even require sodium as an essential micronutrient (Brownell, 1965).

Those plants which rely on nitrogen fixation by bacteria in root nodules, require cobalt as an essential element since vitamin B_{12}, required by the bacteria, is an elaborate complex of cobalt. (Reisenauer, 1960; Hallsworth, Wilson and Greenwood, 1960). One species, *Trifolium subterraneum*, has been shown to require cobalt irrespective of the nitrogen fixing bacteria. (Wilson and Nicholas, 1967).

Silica is found in appreciable amounts in most plants, however, uncertainty remains as to its essentiality, though it does function in the growth of some plants (Mitsui and Takatoh, 1960, 1963; Lanning, Ponnaiya and Crumpton, 1958).

1.5 Mechanism of uptake

The mechanism by which plants take up metal ions and transport them to various organs has been the subject of much controversy among plant physiologists. Bowling (1976) and Epstein (1972) have discussed the current theories of ion uptake and movement within plant roots but the process is far from fully understood.

Early experimenters in this field soon discovered that metal ions are able to diffuse passively in and out of the root, occupying the porous cell wall spaces of the epidermis and cortex. However, metal ions are halted at the endodermis by the Casparian strip, an impermeable barrier to water and ionic solutes. The volume occupied by the diffusion of metal ions into the plant root can be calculated (Epstein, 1955) and is known as the "outer" space of the root; it is given as:

$$\text{"Outer" space in } cm^3\ g^{-1} = \frac{\text{[Diffusible ions] in } \mu \text{ mol } g^{-1} \text{ fresh wt}}{\text{[External concentration] in } \mu \text{ mol } cm^{-3}}$$

However, some of the metal ions, which diffuse into the root cannot diffuse out because they are electrostatically held by negatively charged sites on the cell wall. This region of the wall has dissociated carboxyl groups, giving rise to the negatively charged sites, and is known as the Donnan phase; the volume occupied by metal ions in the Donnan phase is called the Donnan Free Space (D.F.S.) (Briggs, Hope and Pitman, 1958). The two components of free space, the freely diffusing "outer" space and the Donnan Free Space are known collectively as the Apparent Free Space (A.F.S.). Once within the A.F.S., metal ions must find their way to the stele, which contains the vascular system, a dual plumbing network which extends throughout the plant body. Water and nutrients are distributed to aerial parts via the xylem whilst the products of photosynthesis are transported in the phloem. Unable to cross the endodermis, metal ions are prevented from diffusing into the protoplasm of living cells by the plasma membrane, the plasmalemma.

It is generally agreed at this point that the metal ion must cross the plasmalemma in the cortex by a process requiring energy; this process is termed active transport, and involves the action of a specific carrier which forms a complex with the metal ion on the outer surface of the plasmalemma. The carrier-ion complex then moves to the inner surface adjacent to the cytoplasm. By some molecular or mechanistic rearrangement the metal ion is released into the cytoplasm; the metal ion has crossed from the apoplasm to the symplasm (Figure 7). Since the metal ion cannot be transported back to the apoplasm, the carrier must undergo a change in conformation at the inner surface preventing complexation; the process

is irreversible. The chemical nature of the carrier has not yet been elucidated, but it is believed to be related to similar carriers which operate in animal cells, and since the process requires metabolic energy, it is likely that the carriers involved are complex enzymes of the adenosine triphosphatase (A.T.Pase) group (Epstein, 1973) though no correlation between metal uptake and A.T.Pase activity has yet been found. The next stage in the ion transport system is not so well agreed. Once in the cytoplasm of cortical cells, the ion is free to diffuse to an adjoining cell via cytoplasmic connections called plasmodesmata; thus the ion can cross the endodermis into the stele (Figure 7).

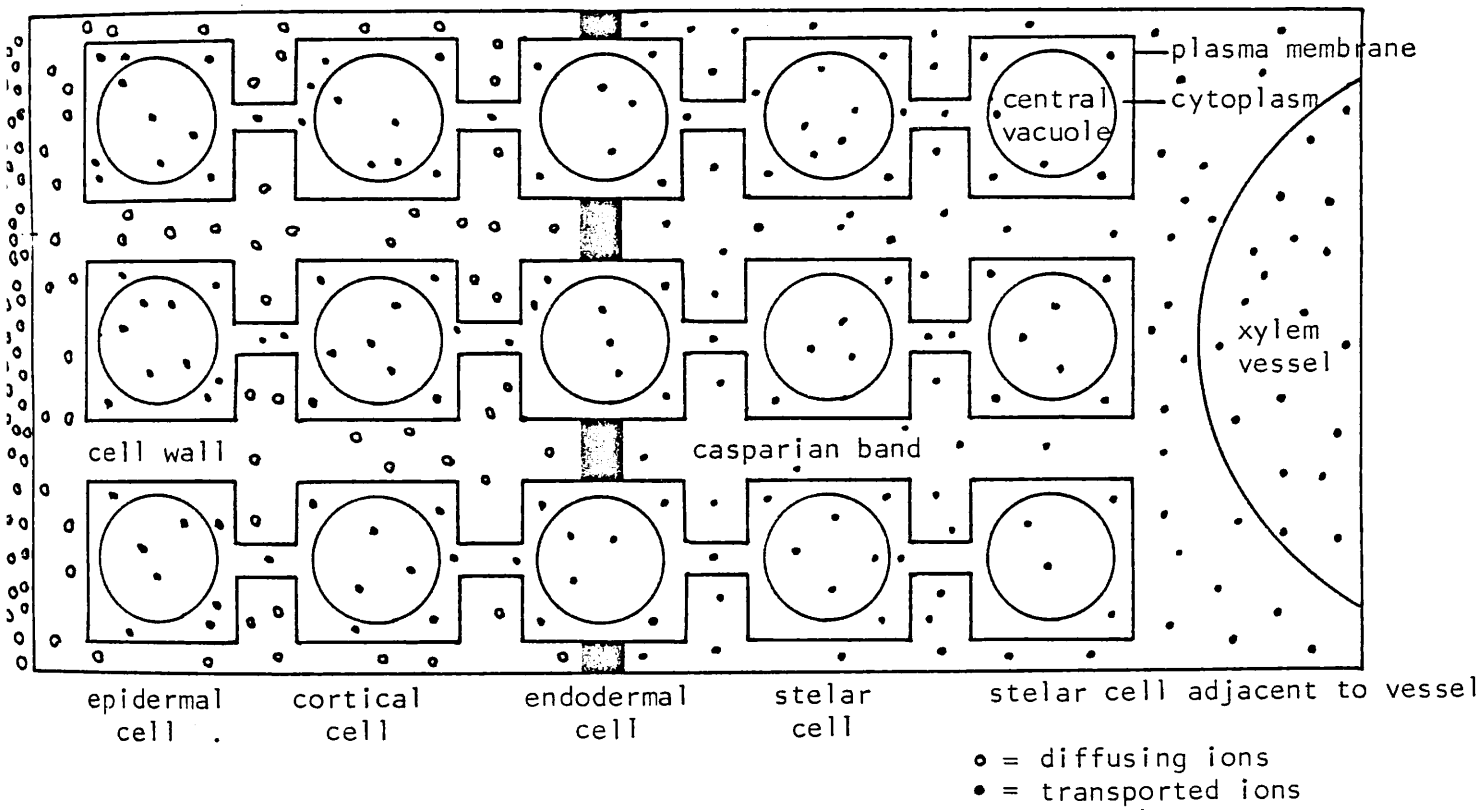


Figure 7. Ion transport across a plant root (after Epstein, 1973)

At one time, the mechanism of ion transport across the plasmalemma of stelar cells was thought to be passive diffusion. This has been criticised by Epstein, (1973) who proposed that a "reverse" carrier mechanism takes place in which metal ions are pumped out of the stelar symplasm into the porous cell wall spaces of the stele, where they diffuse into the xylem vessels and are transported throughout the plant.

Recent research, based on measuring a potential difference across the root with microelectrodes, has led to an application of the Nernst equation. It has been shown that the polar ion flux is powered by active transport of anions; the cations follow to maintain the ionic balance (Bowling, 1976).

Uptake kinetics

The main characteristics of ion uptake are that it is highly specific and selective for the various ions, as well as irreversible. In addition, the rate of ion absorption (ν) can be monitored and when plotted as a function of the external concentration, it is found to follow Michaelis-Menten kinetics (Figure 8) given by:

$$\nu = \frac{V_{max} \cdot [S]}{K_m + [S]} \qquad 1.1$$

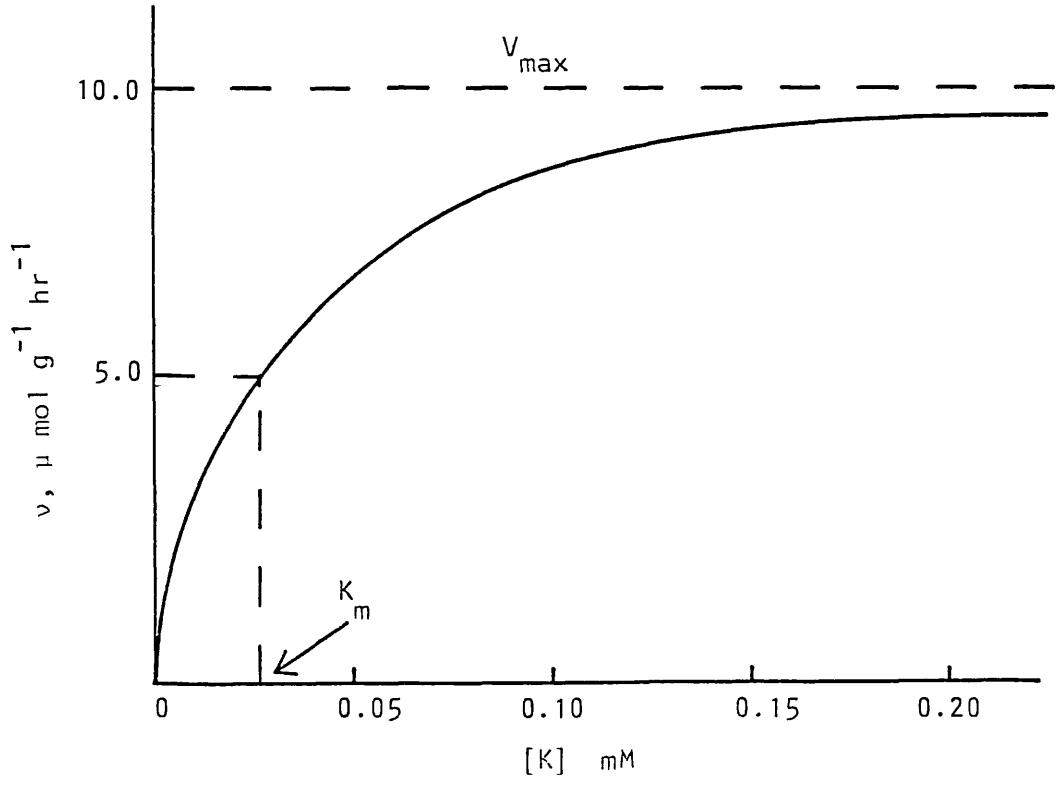


Figure 8. Rate, ν, of absorption of potassium as a function of potassium concentration. (after Epstein, 1972).

where, V_{max} = maximum velocity of uptake at which the carrier is saturated.

K_m = Michaelis constant, ie. the concentration of the ion giving half the maximum rate of absorption.

[S] = ionic concentration.

There have been many reports of ion uptake following Michaelis-Menten kinetics involving various plant species and metal ions, including transition metal ions eg: Mn^{2+}, Zn^{2+}, Cu^{2+}.

The Michaelis-Menten equation can undergo linear transformation to yield straight line plots, (Figure 9) which are useful for checking the fit of experimental parameters and calculating V_{max} and K_m; eg. by taking reciprocals on both sides of the equation:

$$\frac{1}{v} = \frac{K_m}{V_{max}[S]} + \frac{1}{V_{max}} \qquad 1.2$$

At low ionic concentrations (< 1 m mol dm^{-3}) where simple Michaelis-Menten kinetics are followed, the mechanism is highly specific for a particular cation; even elements in the same group of the Periodic Table have no effect on uptake; the mechanism is indifferent to anion uptake also.

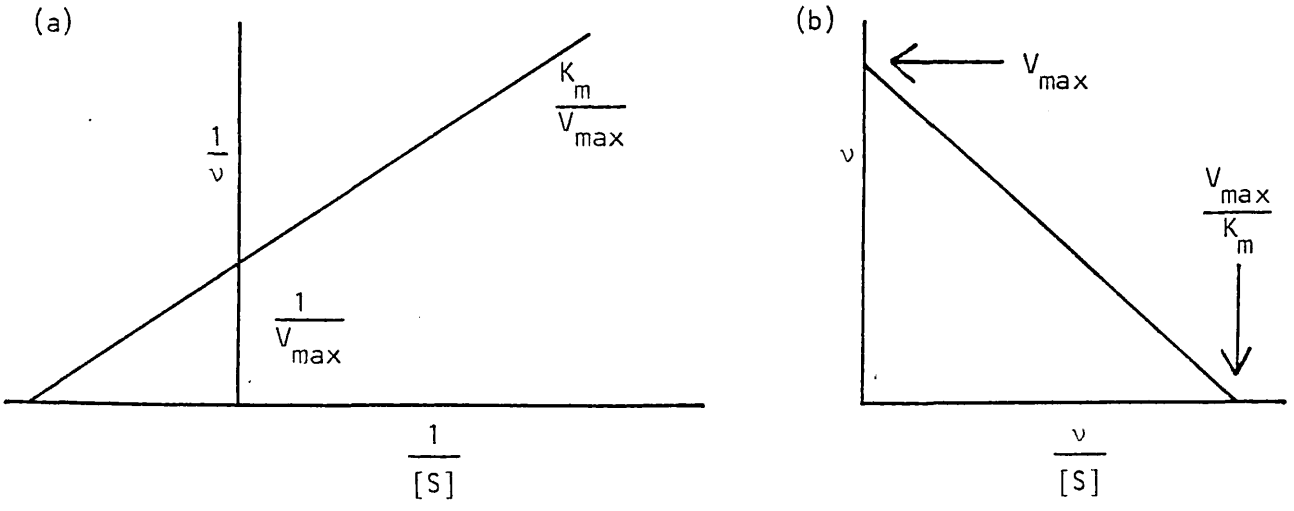


Figure 9. (a) Lineweaver and Burk plot; (b) Hofstee plot. (after Epstein.1972).

When the ionic concentration exceeds that of V_{max} (> 1 m mol dm^{-3}) all carrier sites for that cation are assumed to be occupied; the carrier system is saturated. A plot of rate of potassium uptake against increased potassium concentration, for example, suggests that, like the first mechanism, Michaelis-Menten kinetics are operating but for a second quite distinct mechanism (Figure 10). This second uptake mechanism at the higher concentration is different from the first because other cations interfere

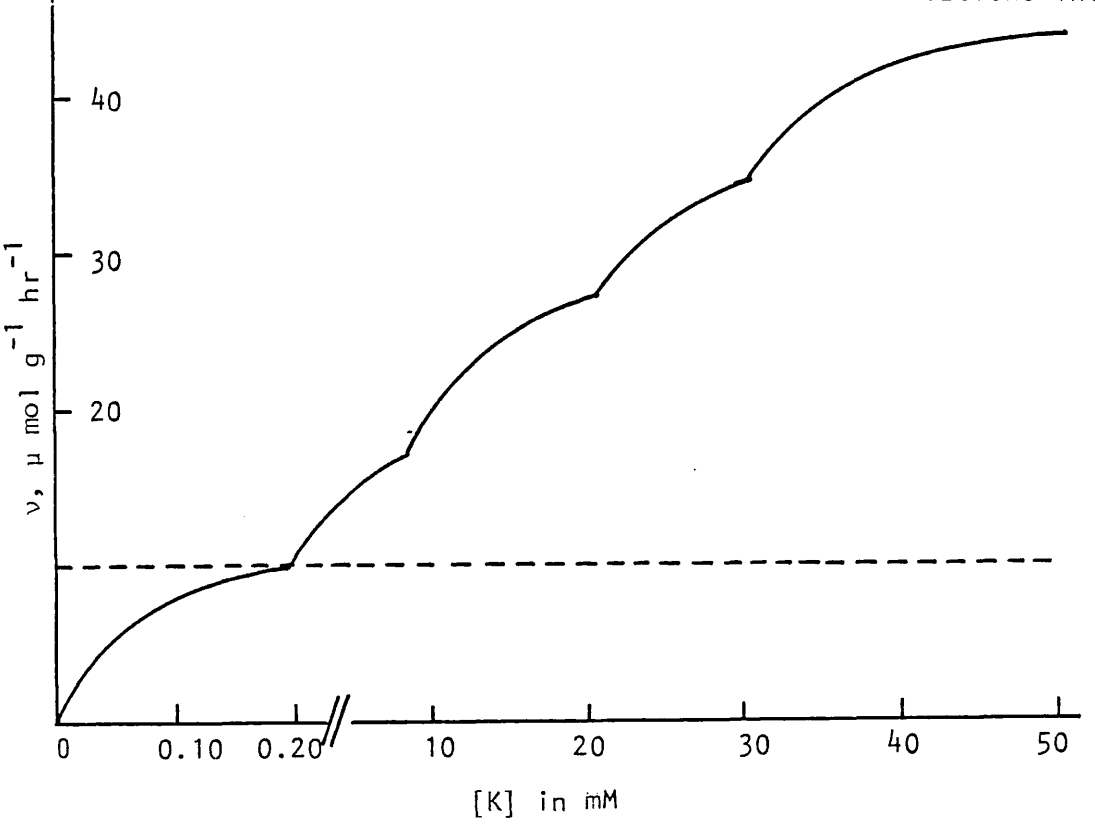


Figure 10. Rate of potassium absorption by barley roots. (Modified after Epstein and Rains, 1965).

with it and it is sensitive to the anion used. Further studies have suggested that over a range of concentrations, a multiphase mechanism may operate, where mechanism one is an active process following Michaelis-Menten kinetics at low concentrations (Figure 10).

It is generally agreed that the location of mechanism one for most ions is in the plasmalemma. The second mechanism which operates at higher concentrations is not well understood and is suggested by some to operate

in parallel with mechanism one, i.e. in the plasmalemma also (Epstein, 1965, 1966). Other workers, recognising the two distinct mechanisms have suggested two locations; as well as the plasmalemma for the first mechanism, the tonoplast is suggested as the site of the second mechanism, which operates in series with the first, (Laties, 1969). This has been criticised on the grounds of an unlikely assumption that the plasmalemma becomes more permeable to ions at concentrations greater than 1 m mol dm^{-3} and the mechanism on the tonoplast becomes the rate determining step (Bowling, 1976).

Although, experimentally, the rate of uptake can be determined quite easily, the rate ν, the Michaelis constant K_m and V_{max} are entirely dependent on a great many factors, such as temperature, root radius and length, quantity and quality of light, soil particle size, soil pH, oxygen availability, competing ions, etc., which all contribute to the rate of uptake.

1.6 Toxicity and tolerance

Indicator plants

It is well known that excesses or deficiencies of metal ions can have profound effects on the growth of plants. Many plant communities can serve as 'indicators' of areas where mineralisation is likely to occur. Some examples of unique floras which are characteristic of soils containing high levels of heavy metals include the serpentine floras, which can be used to locate chromite deposits (Lyon et. al., 1968) and the galmei floras which indicate zinc deposits (Brooks, 1972).

Indicator plants can occur on mineralised sites which are natural, such as ore bodies, or which are manmade; the result of mining spoils, industrial waste tips and the deposition of tailings. The factors which govern the colonisation of such sites include the type of mineralisation

present, the status of nutrients, drainage and soil pH. Many plant species find high concentrations of heavy metals toxic and are unable to survive; some species which can survive assimilate the heavy metals present to a degree where they become accumulators. An accumulator is defined as having a metal concentration in the tissues which is greater than that in the soil (Peterson, 1981). Indicators may not necessarily be accumulators.

Some classic examples of specific indicator species include *Viola lutea* (Schwickerath, 1931) for zinc, *Becium homblei* (Horizon, 1959) and *Armeria maritima* (Henwood, 1857) for copper. The *Astragalus* species accumulate selenium, but indicate uranium since the two elements are often found together (Cannon, 1952).

The science of indicator geobotany is now well established and lists of indicator species have been reported (Cannon, 1960) (Brooks, 1972). The multidisciplinary nature of this field has brought important contributions from many specialised areas. The commercial application of the technique in various parts of the world has led to contributions from the U.S.S.R. (Malyuga, 1964) (Chikishev, 1965) (Viktorov et al., 1964), Canada, Australia, South Africa and the U.S.A. (Cannon, 1960) (Cole, 1965) (Nicolls et al., 1965).

Toxicity

Elements may be classified according to their toxicities. Bowen (1966) has suggested three groups:

1. Very toxic: those elements such as beryllium, copper, mercury, silver and tin, which are toxic at concentrations of less than 1 ppm in the soil.

2. Moderately toxic: toxic symptoms become apparent at concentrations ranging from 1-100 ppm. Examples are the 'd' block and

most of groups III, IV, V and VI of the periodic table.

3. Scarcely toxic: this group includes the macronutrients nitrogen, phosphorus and sulphur, the 's' block and the halogens. Heavy metal toxicity in plants often results in changes in morphology such as chlorosis, dwarfism, abnormally shaped fruits, necrosis of leaves and stunting of root growth. The physiology of metal toxicity in plants has been reviewed recently (Foy et. al., 1978). Among transition metals, chromium toxicity results in chlorosis of leaves (Hewitt, 1953); cobalt increases the chlorophyll in some species and causes chlorosis in others, whilst copper results in chlorosis and dwarfism (Duvigneaud, 1959) (Hewitt, 1953). Iron toxicity gives rise to a darkening of leaves (Burghart, 1956) and manganese, chlorosis with white blotches (Löhnis, 1950, 1951). The toxic effects of molybdenum were reported as the formation of abnormally coloured shoots (Warington, 1937) and nickel causes chlorosis and necrosis of leaves (Hewitt, 1953).

The most common toxic effect of heavy metal ions is chlorosis, which is a characteristic symptom of iron defficiency. In a study carried out by Hewitt (1948), the effects of some heavy metal cations were monitored and the severity of chlorosis was observed to be in the order of:

$$Co^{2+} > Cu^{2+} > Zn^{2+} > Ni^{2+} > Cr^{3+} > Mn^{2+} > Pb^{2+}$$

However, other toxic symptoms were also evident, principally dwarfing and necrosis, which were quite distinct from the induced chlorosis. On this basis a second toxicity order was established:

$$Ni^{2+} > Co^{2+} >> Zn^{2+} > Cu^{2+} >> Cr^{3+} = Mn^{2+} = Pb^{2+}$$

That the induced chlorosis was due to a failure in iron metabolism was shown by painting the leaf surface with iron(II)sulphate solution which

restored chlorophyll production. The mechanism by which heavy metal chlorosis is induced is not fully understood but is believed to be related to the tendency for metal toxicity to increase with the stability constant of various organometallic complexes (De Kock, 1956) (Mellor and Maley, 1948). In a review of toxic effects in animal cells, it was reported that metals such as copper, gold, lead and mercury were found to reduce the permeability of the cell membrane, preventing the transport of potassium,sodium and organic molecules (Passow et. al., 1961).

Tolerance

Plants which are able to grow on soils with metal concentrations which are normally toxic are called metal tolerant. Various mechanisms of tolerance have been proposed to explain how some plants have been able to cope with toxic conditions and further, how some species have developed their own tolerant ecotypes (Antonovics et al., 1971) (Wainwright and Woolhouse, 1975); individual mechanisms of metal tolerance have been reviewed recently (Farago, 1981).

Mechanisms are generally divided into external and internal types. The former covers very few situations in which the metal is rendered unavailable to the root. Within the root the latter operates preventing toxicity. Internal mechanisms are numerous but can be grouped loosely under four headings (Farago, 1981):

1. Metal is available to plant root but is not taken up.

   e.g. excretion of chelating agent rendering toxic metal unavailable.

2. Metal is taken up but rendered harmless to metabolism within the plant.

   e.g. deposition in the cell wall or vacuole.

3. Metal is taken up but excreted

e.g. by guttation, leaching or leaf fall

4. Metal is taken up but metabolism is altered drastically to accommodate increased concentration.

e.g. increase in specific enzymes affected.

The binding of the metal as a highly stable complex, deposition in the cell wall or vacuole and metabolic adaption are some of the more important of these internal mechanisms and have been discussed in detail elsewhere (Mullen, 1980) (Pitt, 1977).

Several methods are available for measuring heavy metal tolerance using rooting tests (Wilkins, 1957, 1978) (Jowett, 1958, 1964). The 'parallel method' described by Jowett (1964) was modified later by McNeilly and Bradshaw (1968) and used to measure the index of tolerance for copper in *Agrostis stolonifera* (Lin Wu, Bradshaw and Thurman, 1975). Tillers of comparable size and age were grown in a solution of calcium nitrate ($0.5g\ dm^{-3}$) and in calcium nitrate plus a known concentration of copper. The index of tolerance is given as

$$\text{Index} = \frac{\text{Mean length of longest root in solution with copper}}{\text{Mean length of longest root in solution without copper}}$$

The 'series method' devised by Wilkins (1957) differs slightly; plants were grown in calcium nitrate solution for six days and the longest roots measured for a given period. The plants were then transferred to calcium nitrate solution containing copper and the root growth measured again over a given period. The measurements were used to calculate the tolerance index as before. Further studies have concluded that plants which are tolerant to one particular metal are not necessarily tolerant to high concentrations of other heavy metals. For *Agrostis* it was found that the tolerance mechanism is highly specific for copper, zinc and lead individually (Gregory and Bradshaw, 1965).

1.7 Inorganic biochemistry of the platinum group metals

1.7.1 Background

The six heaviest elements in Group VIII are known collectively as the platinum group metals: platinum, palladium iridium, ruthenium, rhodium and osmium. They are rare metals, platinum being the most abundant, and their increasing industrial importance is due to various technological uses, such as in the manufacture of high octane petrol, fertilisers, pharmaceuticals, vitamins, antibiotics and laser crystals. The metals are used also in coinage, dentistry, jewellery, ceramics, electronics and in scientific apparatus. Their useful properties, such as chemical inertness and high melting points earned them the name 'Noble Metals'.

Because of the increased demand for the platinum group metals (P.G.M.) doubts have arisen as to the future availability of adequate supplies. Almost all industrial supplies are met by the three primary sources of P.G.M.'s: South Africa, Canada and the U.S.S.R. In 1924 Hans Merensky discovered extensive deposits of platinum bearing ores, now called 'the Merensky Reef' in South Africa, and today, both Canada and South Africa provide for the greater part of the western world's needs (Hunt, 1971). The platinum ores of Canada and the U.S.S.R. are mined primarily for copper and nickel with the P.G.M.'s as by products. The Merensky Reef is worked primarily for the P.G.M.'s; copper and nickel are by products, therefore an increased world demand for platinum causes the South African mines to respond by increasing production without being swamped with stocks of copper and nickel (Hung and Lever, 1971).

Two recent scientific advances have extended the use of platinum but have, at the same time given rise to an interest in the interactions

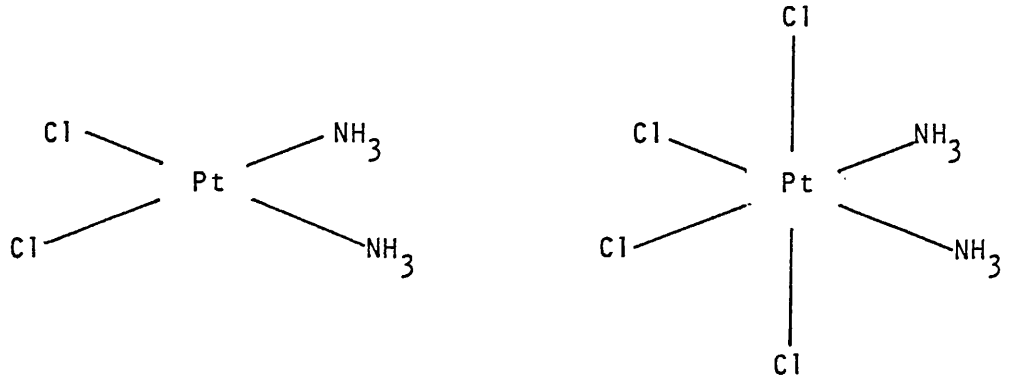
of platinum metals and their complexes in biological systems. (Le Roy, 1975) (Moore et al., 1975). The amendments to the Clean Air Act (1970) in the U.S.A. have meant that all cars from 1975 must have a catalytic converter containing platinum and palladium, which can reduce the concentration of carbon monoxide and hydrocarbons in the exhaust gases by catalytic oxidation to carbon dioxide and water. Some are concerned, particularly about the possibilities of further heavy metal pollution of the environment (Johnson et al., 1975) (Duffield et al., 1976). It is not clear how much platinum (if any) is emitted from such converters, or in what form. Brubaker et al., (1975) claim an estimated loss per car of 1.49g per unit and there is one report of an emission of 20µg Pt per vehicle-mile (Newkirk et al., 1973).

The second recent advance has been in the field of cancer chemotherapy where platinum is administered as a drug; this has led to toxicological interest in the P.G.M.'s (Wiester, 1975) (Holbrook et al., 1975).

1.7.2 Cancer chemotherapy

The initial discovery of platinum antitumour activity happened when Professor B. Rosenberg of Michigan State University was investigating the effects of an electric field on the bacteria *Escherichia coli* using platinum electrodes. The cells stopped dividing but growth continued producing long filamentous bacteria (Rosenberg, 1965). Some very extensive detective work led to the conclusion that the entity blocking cell division came from the platinum electrodes (Rosenberg et al., 1967). It was discovered that the active species was a coordination complex of platinum formed by photochemical reactions in the culture medium. Two active complexes were found: cis diamminedichloroplatinum (II) and cis diamminetetrachloroplatinum (IV) (Figure 11).

A series of investigations revealed that only certain specific configurations of platinum complexes were biologically active; the cis isomers generally achieve inhibition of growth or cell division whilst the trans isomers are inactive. The neutral complex of platinum (II) (fig.11(a)) was given a standard antitumour test to determine its suitability as a candidate for more detailed testing (Rosenberg et al., 1969). These results were the first evidence that platinum complexes



(a) (b)

Figure 11. Platinum antitumour complexes:
(a) cis diamminedichloroplatinum (II)
(b) cis diamminetetrachloroplatinum (IV)

were active antitumour agents.

The complexes were submitted to the U.S. National Cancer Institute for screening against a standard leukaemia tumour in mice. The results confirmed antitumour properties. Further research (Rosenberg and Van Camp, 1970) has shown that cis diamminedichloroplatinum (II) can effect a complete cure i.e. where treated animals not only produced no further tumour growth but had also developed an immunity when rechallenged with the same tumour. Various developments of the drug from these early trials to the official approval for cancer treatment, have been reviewed recently (Rosenberg, 1971, 1978, 1980) (Wiltshaw, 1979).

The first clinical trials with humans were conducted in 1972 (De Conti, et al., 1973) (Connors and Roberts, 1974); further trials followed (Yagonda et al., 1976). Toxic side effects were reported, particularly damage to the kidney and to a lesser extent bone marrow; distribution studies had indicated that platinum accumulated in the liver and kidney (Lange et al., 1972, 1973). Minor effects included irritation of the intestinal lining causing intense nausea and vomiting along with some hearing defects. Because of its toxic effects on those cells which divide most rapidly, the drug is classed as cytotoxic (Leonard et al., 1971).

Further studies have established that combination therapy, involving use of the platinum complex and another chemotherapeutical agent e.g. adriamycin, offers the best treatment for a wide variety of tumours (Bruckner et al., 1976). From March 1979, the Department of Health and Social Security in the U.K. have given official approval for the complex cis diamminedichloroplatinum (II) to be classified as a drug for the treatment of testicular and ovarian tumours. In the U.K. it is formulated under the trade name 'Neoplatin'; in the U.S.A., it is known as 'Cisplatin'.

The antitumour mechanism by which cisplatin operates is not well understood. Inorganic chemists have contributed in the area of structure-activity relationships (Williams, 1972) (Cleare and Hoeschele, 1972) (Braddock et al., 1975) (Tobe and Khokhar, 1977) (Cleare et al., 1978; 1980). These studies have examined the relationships between the variation in stereochemistry, physical and chemical properties of a range of platinum complexes, with observed antitumour activity. Although it is still difficult to predict antitumour activity, several common features have emerged from their work. For those complexes exhibiting

antitumour activity:

1. Only some of the ligands exchange rapidly.
2. Complexes should be electrically neutral, though the active form may be charged within the cell.
3. Geometry: should be square planar or octahedral.
4. Ligands: leaving group; one bidentate or two cis monodentate; trans form inactive.
5. Ligands: rates of exchange must be limited.
6. Leaving groups should be 0.3-0.4nm apart.
7. The non-exchanging ligands should be strongly bonded inert amine systems.

Within the body the cisplatin molecule is transported throughout the plasma, where a high chloride concentration prevents exchange of the chloro ligands which are kinetically more labile than ammine ligands. The half life for the drug within the body is about one hour; it is mostly excreted. About 5% of the drug is associated with proteins. Some of the drug crosses the cell membrane as the neutral complex, with no active transport. The chloride concentration inside the cell is much lower and exchange of the chloro ligands takes place:

$$[Pt(NH_3)_2Cl_2] + H_2O \rightleftharpoons [Pt(NH_3)_2(H_2O)Cl]^+ + Cl^-$$

$$[Pt(NH_3)_2(H_2O)Cl]^+ + H_2O \rightleftharpoons [Pt(NH_3)_2(H_2O)_2]^{2+} + Cl^-$$

The exchange could involve hydroxide ions (OH^-) but this would depend on the pH inside the cell.

Research has shown that in mammalian cells, cisplatin can form crosslinks between the two strands of the double helix of D.N.A. (Harder and Rosenberg, 1970). It does not bind with the sugar-phosphate backbone, but with the nitrogen bases. Other investigations have shown

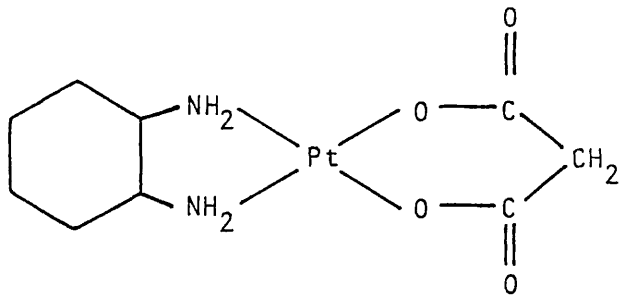
how the various platinum (II) complexes can stimulate renaturation of D.N.A., confirming formation of interstrand cross-links (Horacek et al., 1972) (Drobnik and Horacek, 1973). Recent studies have led to some controversy over the exact mode of binding between D.N.A. and cisplatin (Kelman and Peresie, 1979). It would appear that a variety of reactions with D.N.A. is possible, including interstrand and intrastrand cross-linking as well as reactions with individual bases, the most likely of which is guanine at the O^6 position (Rosenberg, 1980, 1981). This is supported by X-ray photoelectron spectroscopy, which indicates that chelation of N_7(Gua).O^6(Gua) occurs with cisplatin (Millard et al., 1975).

Since the development of cisplatin as a viable antitumour drug, there has been further interest in complexes of the other members of the platinum group and second generation platinum complexes are now being screened for similar antitumour properties. Several platinum containing analogues, selected on the basis of preliminary structure-activity investigations (Cleare et al., 1978), have been examined further and recommended for clinical trials, (Wolpert-De Filippes, 1980) pending toxicity testing (Figure 12). Some thirty different complexes of palladium, rhodium and ruthenium have been screened for antitumour activity already, and more than 5% were found to be active (Wolpert-De Filippes, 1980). At present platinum complexes appear to be the most active.

1.7.3 Interaction with microorganisms

The first report of interaction with bacteria was in 1903 (Zinno and Cutolo, 1903) when a solution of iridium chloride exhibited anti-bacterial activity. It wasn't until Rosenberg discovered that platinum complexes inhibited the growth of *E. coli.* that further

(a)



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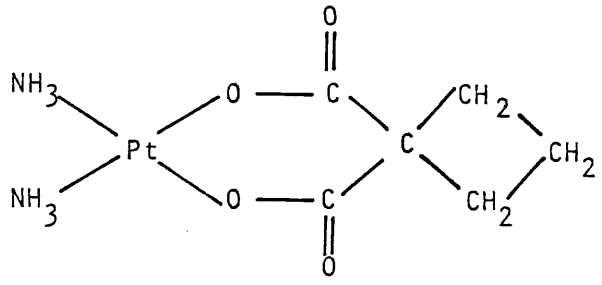

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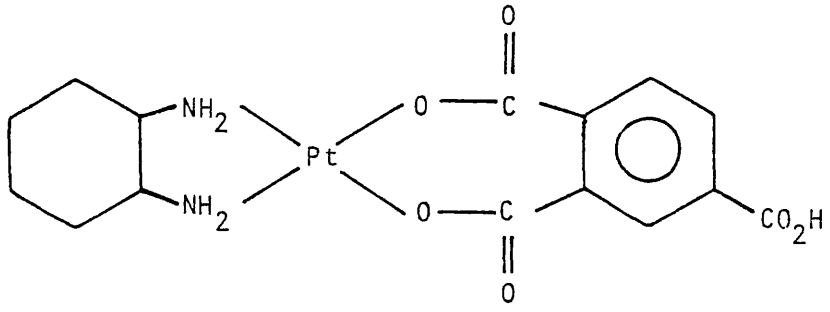


Figure 12 Cisplatin analogues: (a) 1,2-diaminocyclohexane malonatoplatinum (II); (b) Diammine (1,1-cyclobutane dicarboxylato) platinum (II); (c) 1,2-diaminocyclohexane (4-carboxyphthalato) platinum (II).

investigations were undertaken to examine the interaction with micro-organisms. Much of the work was aimed at elucidating the mechanism by which cisplatin operates; on repression and induction of enzymes (Drobnik et al., 1972); and on bacterial, viral and tissue culture studies (Roberts, 1974).

Escherichia coli have been investigated extensively as to the effects cisplatin has: on various strains (Beck, 1974) (Drobnik, 1973)

(Matlova, 1975); on inhibition of cell division (Gringrich and Beck, 1978); and on mutagenic properties (Beck and Brubaker, 1975). Ruthenium, as the complex dichlorotetrakis dimethyl sulphoxide ruthenium (II) induced filamentation in *E. coli* (Monti-Bragadin, et al., 1975). Similar effects were observed with the neutral complex cis trisammine trichloro ruthenium (III) and the charged complex potassium aquopentachlororuthenate (III), but the palladium complexes investigated were toxic at high concentrations and exhibited no filamentation at low concentrations (Durig et al., 1976). Further studies with a range of ruthenium complexes have confirmed such effects and it has been shown that for dimeric ruthenium complexes, filament inducing activity is lost when the metal is oxidised from the (II, III) state to the (III, III) state. (Gibson, Hughes and Poole, 1980) (Gibson, 1981) Some effects of organorhodium complexes e.g. trans dichloro tetrapyridyl rhodium (III) chloride, are reported with a variety of strains of *E. coli* (Bromfield et al., 1969) (Gillard, 1981).

Toxicity

With the introduction of platinum car exhaust catalysts, in 1975, interest in the toxicity of the platinum group metals increased. Hysell et al., (1975) found that rats exposed to the exhaust gases emitted with the new catalyst, contained no detectable concentrations of platinum or palladium in their tissues. Detailed studies of the nature of P.G.M. toxicity have been carried out on rats largely (Fisher et al., 1975), and have included; cardiovascular actions of palladium compounds in rats (Wiester, 1975); distribution of various platinum compounds in rats using radioactive platinum - 191 (Moore et al., 1975); tissue culture methods have been used also (Waters et al., 1975). Holbrook, et al. (1975) used rats to evaluate the toxicity of various salts of lead,

manganese, platinum and palladium. It was found that the toxicity was in some cases dependent on the method of administration. The toxicity order for oral administration was:

$$PtCl_4;\ Pt(SO_4)_2.4H_2O > PdCl_2.2H_2O;\ RuCl_3 > MnCl_2.4H_2O;$$

$$PdSO_4;\ PbCl_2;\ PtCl_2 > PtO_2 > MnO_2;\ PdO.$$

The most toxic compounds were the two soluble Pt^{IV} salts. When administered by intraperitoneal injection, the order of toxicity becomes

$$PdCl_2.2H_2O > Pt(SO_4)_2.4H_2O;\ MnCl_2.4H_2O;\ PdSO_4;\ PtCl_4 >$$

$$PbCl_2;\ PtCl_2$$

In another study by Schroeder and Mitchener (1971) the toxic effects of rhodium and palladium on mice were investigated along with several other metals. Rhodium was found to have least effect on body weight, and although palladium supressed growth in male mice, those fed with palladium in their diets lived significantly longer than controls. Similar results were recorded for rhodium fed mice. Both palladium and rhodium appeared tumourigenic and carcinogenic. Oral toxicity, based on growth rate supression, was assigned the following order:

$$Ga > Yt > Sc > In = CrO_4^{2-} > Pd > Rh.$$

As part of the catalyst research program of the U.S.A. Environment Protection Agency, Duffield et al., (1976) carried out human autopsy analysis for platinum. The aim was to establish baseline data for platinum content of various human tissues prior to the widespread introduction of car exhaust catalysts. The range of platinum

concentrations detected was 0.003 - 1.46μg g^{-1} (wet tissue), the highest levels being found in subcutaneous fat, followed by the kidney, pancreas and liver. In a previous programme, Johnson et al., (1975) had analysed samples of blood, hair, urine and faeces from people living in Southern California. Detectable levels of platinum were only found in composite blood samples (0.049 - 0.180μg $100cm^{-3}$). Platinum and palladium were not detected in any individual samples.

It is a well known fact that platinum can cause an asthmatic condition in certain sensitised people who come into contact with soluble platinum salts in the course of their employment. This condition has been observed frequently in staff at platinum refineries (Hunter, et al., 1945) (Roberts, 1951). Research into the cause of this allergy, called Platinosis, is scarce, but it is believed that platinum complexes, particularly the hexachloroplatinate IV anionic complex, are responsible for the development of platinosis in humans (Saindelle, et al., 1968). The evidence so far indicates that the platinum salt does not act directly but may release toxic substances, such as histamine, in the body. (Parrot, et al., 1969) (Saindelle and Ruff, 1969).

Osmium tetroxide is well known as a highly toxic and volatile substance which can cause burns and irritate the respiratory tract. It has become well established as a fixative in histology, though the mechanism is not well understood. More recently it has become useful as a fixative in electron microscopy and some of its reactions with biological tissues are known. It can react in lipid forming cross links between the double bonds (Bahr, 1954) (Korn, 1967).

Uptake by plants

There are very few references to the uptake and effects of the platinum group metals in plants. The earliest studies carried out were

limited to platinum and palladium. Brenchley (1934, 1946) reported that palladium interfered with plant growth and Somers (1959) observed that palladium was toxic to fungi. In 1942, Hamner demonstrated that platinum applied as hexa chloroplatinic IV acid could adversely affect tomato and bean plants grown by sand culture methods.

More recently, the uptake of palladium by Kentucky bluegrass (*Poa pratensis L)* and its effect on plant growth have been investigated (Sarwar et al., 1970). Small quantities of palladium (II) chloride added to the nutrient solution were found to stimulate growth but high concentrations were toxic. Palladium has been detected in limber pine (*Pinus flexilis*) (Fuchs and Rose, 1974) and the uptake of palladium by vegetation has been investigated by Kothny (1976, 1979) where palladium content for several species was reported, including *Quercus chrysolepsis* with a value of 400ppb in plant ash. Seasonal variations were noted in the concentration of palladium in ash of *Juglans hindsii* leaves and stalks. In one report (Rudolph and Moore, 1972) several species were analysed for platinum, and one, *Eritrichium chamissonis D.C.* known as the platinum flower appears to indicate indirectly platinum deposits.

Platinum group metals were among those tested for their effects on the nicotine level of tobacco plants (*Nicotiana tabacum L.)* grown hydroponically; platinum and palladium definitely increased nicotine yield (>25%); rhodium and ruthemium possibly (<25%) increased the yield whilst iridium and osmium possibly decreased the nicotine yield (Tso, et al., 1973). The environmental aspects of ruthenium are a special case, since various radioactive isotopes, in particular ^{103}Ru and ^{106}Ru, are produced during the fission of nuclear fuels and form a significant amount of nuclear waste (Brown, 1976).

In two independent studies conducted at about the same time,

platinum uptake by horticultural crops was investigated (Pallas and Jones, 1978) (Farago, Mullen and Payne, 1979). Tomato, bean and corn plants were grown hydroponically in the presence of platinum. Both studies concluded that platinum is taken up by the roots and some is transported to the tops; dry weights are affected and chlorosis is evident at the highest levels. Both confirmed Hamner's findings that the water content of plants treated with platinum is affected. Pallas and Jones added that a decrease in manganese uptake accompanied the increase in platinum uptake.

Farago et al. extended their study to include palladium and rhodium. They found a similar uptake took place, though rhodium appeared to be less toxic at high levels.

Several papers report detailed investigations of P.G.M. complexes and plant cells. (Bournique, et al., 1976) (Scheuchenko, 1977) (Benedict, 1970). Ivanov et al. (1976) have shown that there is a correlation between the ability of these complexes to inhibit cell division in corn roots and their known antitumour activities.

A recent patent claimed that the fruit of banana and citrus plants could be used to extract a range of rare and precious metals, including platinum and rhodium (Bumbalek, 1977, 1978). The dubious claims were substantiated with results of analyses by neutron activation; some 200-8.00g Rh were reported present in 1kg of banana ash. It would seem from studies of NAA. of platinum at least, in plant materials, that the technique suffers from many interferences which can lead to erroneous results (Zeister and Greenberg, 1981) (Farago and Parsons, 1980).

REFERENCES

Antonovics, J., Bradshaw, A.D. and Turner, R.G. (1971), Adv. Ecol. Res., 7 1.

Arnon, D.I. (1961), Bull. Torrey Bot. Club, 88 215.

Arnon, D.I. and Hoagland, D.R. (1940), Soil Sci., 50 463.

Arnon, D.I. and Hoagland, D.R. (1944), Biol. Rev., 19 55.

Arnon, D.I. and Stout, P.R. (1939), Plant Physiol., 14 371.

Aspinall, G.O. (1970), Polysaccharides, Pergamon Press, Oxford.

Bahr, G.F. (1954), Expt. Cell Res., 9 277.

Beck, D.J. and Brubaker, P.E. (1975), Muta. Res., 17 (2) 181.

Beck, D.J., Vazquez, D. and Cook, R. (1977), J. Clinical Haematol. Oncol., 7 (2) 726.

Benedict, W.G. (1970), Can. J. Bot., 48 (1) 91.

Bonner, J. (1950), Plant Biochemistry, Academic Press New York (p134), cited in Hall *et al.*, (1974).

Bournique, C.P., Deysson, G., Chottard, J.C. and Comptes, R. (1976), Seance's Soc de Biologie Ses Fil., 170 (1) 20.

Bowen, H.J.M. (1966), Trace elements in biochemistry, Macmillan, London.

Bowling, D.J.F. (1976), Uptake of ions by plant roots, Chapman and Hall, London.

Braddock, P.D., Connors, T.A., Jones, M., Khokhar, A.R., Melzack, D.H. and Tobe, M.L. (1975), Chem-Biol. Interactions, 11 145.

Brenchley, W.E. (1934), Annals of Appl. Biol., 21 398.

Brenchley, W.E. (1946), Chem. Prod., 9 61.

Briggs, G.E., Hope, A.B. and Pitman, M.G. (1958), J. Expt. Bot., 9 128.

Bromfield, R.J., Dainty, R.H., Gillard, R.D. and Heaton, B.T. (1969), Nature, 222 735.

Brooks, R.R. (1972), Geobotany and Biogeochemistry in Mineral Exploration, Harper and Row, London.

Brown, K.W. (1976), Ruthenium: Its behaviour in plant and soil systems, Report EPA-600/3-76-019 U.S. Environ. Prot. Agency.

Brownell, P.F. (1965), Plant Physiol., 40 460.

Broyer, T.C., Carlton, A.B., Johnson, C.M. and Stout, P.R. (1954), Plant Physiol., 29 526.

Brubaker, P.E., Moran, J.P. and Hueter, F.G. (1975), Environ. Health Persp., 10 39.

Bruckner, H.W., Cohen, C.J., Gusberg, S.B., Wallach, R.C., Kabakow, B., Greenspan, E.M. and Holland, J.F. (1976), Proc. Am. Assoc. Cancer Res., in Am. Soc. Clin. Oncol., 17 287.

Bumbalek, A. (1977), British Patent No.1489 557.

Bumbalek, A. (1978), Ger. Offen. No. 2 702 638.

Burghart, H. (1956), Flora (Jena), 143 1. Cited in Brooks (1972).

Cannon, H.L. (1952), Am. J. Sci., 250 735.

Cannon, H.L. (1960), Science, 132 591.

Chikishew, A.G. (1965), Plant indicators of soils, rocks and subsurface water, Consultants Bureau, New York.

Cleare, M.J. and Hoeschele, J.D. (1972), Adv. Antimicrob. Antineoplastic Chemother. Proc. Int. Congr. Chemother. 7th, 1971, 2 193.

Cleare, M.J., Hydes, P.C., Malerbi, B.W. and Watkins, D.H. (1978), Biochimie, 60 835.

Cleare, M.J., Hydes, P.C., Hepburn, D.R. and Malerbi, B.W. (1980), in Cisplatin, edited by Prestayko *et al.*, (p149).

Cole, M.M. (1965), Proc. Commonw. Min. Metall. Congr. 8th, 6 429.

Connors, T.A. and Roberts, J.J. (1974), editors; Platinum Coordination Complexes in Cancer Chemotherapy. Proceedings of the 2nd International Symposium on Platinum Coordination Complexes in Cancer Chemotherapy, Oxford, April 1973; Springer Verlag, Berlin; Heidelberg, New York.

De Conti, R.C., Toftness, B.R., Lange, R.C. and Creasey, W.A. (1973), Cancer Research, 33 1310.

De Kock, P.C. (1956), Annals Bot., 20 133.

Drobnik, J. and Horacek, P. (1973), Chem-Biol. Int., 7 (4) 223.

Drobnik, J. and Ruzickova, J. (1972), Adv. Antimicrob. Antineoplastic Chemother. Proc. Int. Congr. Chemother. 7th, 1971, 2 223.

Drobnik, J., Urbanokova, M. and Krekulova, A. (1973), Mutat. Res., 17 (1) 13.

Duffield, F.V.P., Yoakum, A., Bumgarner, J. and Moran, J. (1976), Environ. Health Persp., 15 131.

Durig, J.R., Danneman, J., Behnke, W.D. and Merces, E.E. (1976), Chem-Biol. Interact., 13 (3-4) 287.

Duvigneaud, P. (1959), Soc. R. Bot. Belgique Bull., 91 111, cited in Brooks (1972).

Epstein, E. (1955), Plant Physiol., 30 329.

Epstein, E. (1965), Mineral metabolism , in : Plant Biochemistry, edited by Bonner, J. and Varner, J.E., Academic Press, New York and London (p438).

Epstein, E. (1966), Nature, 212 1324.

Epstein, E. (1972), Mineral nutrition of plants principles and perspectives, Wiley, London.

Epstein, E. (1973), Sci. Am., 222 48.

Esau, K. (1965), Plant Anatomy, 2nd Ed., Wiley, New York.

Farago, M.E. (1981), Coord. Chem. Rev., 36 155.

Farago, M.E., Mullen, W.A. and Payne, J.B. (1979), Inorg. Chim. Acta., 34, 151.

Farago, M.E. and Parsons, P.J. (1980), Meeting of the Inorganic Biochemistry Discussion Group of the Royal Society of Chemistry, London.

Fisher, R.F., Holbrook, D.J., Leake, H.B. and Brubaker, P.E. (1975), Environ. Health Persp., 12 57.

Foy, C.D., Chaney, R.L. and White, M.C. (1978), Ann. Rev. Plant Physiol., 29 511.

Gibson, J.F., Hughes, M.N. and Poole, R.K. (1980), Soc. for Gen. Microbiol. Quart., 8 35.

Gibson, J.F. (1981), Ph.D. Thesis, Univ. of London.

Gillard, R.D. (1981), Int. Conf. Chem. PGMs, Bristol, England (A8).

Gregory, R.P.G. and Bradshaw, A.D. (1965), New Phytol., 64 131.

Gringrich, D.A. and Beck, D.J. (1978), Micron., 9 (1) 45.

Hall, J.L., Flowers, T.J. and Roberts, R.M. (1974), Plant Cell Structure and Metabolism, Longmans, London.

Hallsworth, E.G., Wilson, S.B. and Greenwood, E.A.N. (1960), Nature, 187 79.

Harder, H.C. and Rosenberg, B. (1970), Int. J. Cancer, 6 207.

Henwood, W.J. (1857), Edinburgh New Phil. J., 5 61.

Hewitt, E.J. (1948), Nature, 161 489.

Hewitt, E.J. (1953), J. Expt. Bot., 4 59.

Hewitt, E.J. (1966), Sand and Water Culture Methods Used in the Study of Plant Nutrition, Tech. Comm. No.22, Commonwealth Agricultural Bureau, England.

Holbrook, D.J., Washington, M.E., Leake, H.B. and Brubaker, P.E. (1975), Environ. Health Persp., 10 95.

Horacek, P. and Drobnik, J. (1972), Adv. Antimicrob. Antieoplastic. Chemother., Proc. Int. Congr. Chemother, 7th, 2 223.

Horizon, (1959), R.S.T. Group, 1 35, (anonymous), cited in Cannon (1960).

Hunt, L.B. (1971), Platinum Metals Review, 15 207.

Hunt, L.B. and Lever, F.M. (1971), Platinum Metals Review, 15 126.

Hunter, D., Milton, R. and Perry, K.M.A. (1945), Brit. J. Ind. Med., 2 92, cited in Roberts (1951).

Hysell, D.K., Moore, W., Hinners, R., Malanchuk, M., Miller, R. and Stara, J.F. (1975), Environ. Health Persp., 10 57.

Ivanov, V.B., Chel'tsov, P.A. and Shchelokov, R.N. (1976), Dokl. Akad. Nauk. SSSR., 229 (2) 484.

Johnson, E.W., Tillery, J.B. and Prevost, R.J. (1975), Environ. Health Persp., 12 27.

Jowett, D. (1958), Nature, 182 816.

Jowett, D. (1964), Evolution, Lancaster, Pa., 18 70.

Kelman, A.D. and Peresie, H.J. (1979), Cancer Treat. Rep., 63 (9-10) 1445.

Korn, E.D. (1967), J. Cell Biol., 34, 627.

Kothny, E.L. (1976), Palladium uptake by Vegetation, presented, 31st N.W. Regional Meeting of the ACS, Reno, Nevada.

Kothny, E.L. (1979), Plant and Soil, 53 547.

Lange, R.C., Spencer, R.P. and Harder, H.C. (1972), J. Nucl. Med., 13 (5) 328.

Lange, R.C., Spencer, R.P. and Harder, H.C. (1973), J. Nucl. Med., 14 (4) 191.

Lanning, F.C., Pannaiya, B.W. and Crumpton, C.F. (1958), Plant Physiol., 33 339.

Laties, G.G. (1969), Ann. Rev. Plant Physiol., 20 89.

Leonard, B.J., Eccleston, E., Jones, D., Todd, P. and Walpole, A. (1971), Nature, 234 43.

LeRoy, A.F. (1975), Environ. Health Persp., 10 73.

Löhnis, M.P. (1950), in Trace elements in plant physiology: Lotsya, 3 63.

Löhnis, M.P. (1951), Plant and Soil, 3 193.

Lyon, G.L., Brooks, R.R., Peterson, P.J. and Butler, G.W. (1968), Plant and Soil, 29 225.

Malyuga, D.P. (1964), Biochemical methods of prospecting, Consultants Bureau, New York.

Matlova, S. (1975), Folia Microbiol (Prague), 20 (4) 332.

McNeilly, T. and Bradshaw, A.D. (1968), Evolution, Lancaster, Pa, 22 108.

Mellor, D.P. and Maley, L. (1948), Nature, 161 436.

Millard, M.M., Macquet, J.P. and Theophanides, T. (1975), Biochimica et Biophysica Acta 402 166.

Miller, E.C. (1938), Plant Physiology, 2nd ed., McGraw-Hill Book Co. Inc., New York and London, cited in Epstein (1972).

Mitsui, S. and Takatoh, H. (1960), J. Sci. Soil Manure, 30 335.

Mitsui, S. (1963), Soil Sci. and Plant Nutr. (Tokyo), 9, 49.

Monti-Bragadin, C., Ramani, L., Samer, L., Mestroni, G. and Zassinovich, G. (1975), Antimicrob. Agents Chemother., 7 (6) 825.

Moore, W., Hysell, D., Hall, L., Campbell, K. and Stara, J. (1975), Environ. Health Perspectives, 10 63.

Moore, W., Malanchuk, M., Crocker, W., Hysell, D. and Cohen, A. (1975), Environ. Health Perspectives, 12 35.

Mullen, W.A. (1980), Ph.D. Thesis, University of London.

Newkirk, H.W. and Goluba, R.W. (1973), Livermore Calif. U.C.I.D. - 16321, cited in Brubaker (1975).

Nicholas, D.J.D. (1957), J. Sci. Food Agric., 8 515.

Nicolls, O.W., Provan, D.M.J., Cole, M.M. and Tooms, J.S. (1965), Bull. Inst. Min. Metall., 74 695.

Nissen, P. (1971), Physiol. Plant., 24 315.

Nissen, P. (1974), Ann. Rev. Plant Physiol., 25 38.

Northcote, D.H. (1963), Int. Rev. Cyt., 14 223.

Northcote, D.H. (1972), Ann. Rev. Pl. Physiol., 23 113.

Pallas, J.E. and Jones, J.B. (1978), Plant and Soil, 50 (1) 207.

Parrot, J.L., Hébert, R., Saindelle, A. and Ruff, F. (1969), Arch. Environ. Health, 19 (5) 685.

Passow, H., Rothstein, A. and Clarkson, T.W. (1961), Pharmac. Rev., 13 185.

Peterson, P.J. (1981), Int. Sym. Uptake Utilis. Metals by Plants; Phytochemical Soc. of Europe, Hull.

Pitt, M.J. (1977), Ph.D. Thesis, University of London.

Prestayko, A.W., Crooke, S.T. and Carter, S.K. (1980), eds. Cisplatin: current status and new developments, Academic Press, London.

Reisenauer, H.M. (1960), Nature, 186 375.

Roberts, A.F. (1951), Arch. Indus. Hyg. and Occup. Med., 4 549.

Roberts, J.J. (1974), Recent Results Cancer Res., 48 79.

Rosenberg, B., Van Camp, L. and Krigas, T. (1965), Nature, 205 698.

Rosenberg, B., Van Camp, L., Grimley, E.B. and Thompson, A.J. (1967), J. Biol. Chem., 242 (6) 1347.

Rosenberg, B., Van Camp, L., Trosko, J.E. and Mansour, V.H. (1969), Nature, 222 385.

Rosenberg, B. and Van Camp, L. (1970), Cancer Res., 30 197.

Rosenberg, B. (1971), Platinum Metals Rev., 15 42.

Rosenberg, B. (1978), Interdiscip. Sci. Rev., 3 (2) 134.

Rosenberg, B. (1980), in Cisplatin, eds. Prestayko *et al.*, (1980), (pg9).

Rosenberg, B. (1981), Int. Conf. Chem. PGMs., Bristol, UK (A1).

Rudolph, W.W. and Moore, J.R. (1972), Alaska Construction and Oil Rep., (p40).

Saindelle, A., Ruff, F., Hébert, R. and Parrot, J.L. (1968), Rev. Fr. Allerg., 8 (4) 205.

Saindelle, A. and Ruff, F. (1969), Brit. J. Pharm., 35 313.

Sarwar, M., Thibert, R.J. and Benedict, W.G. (1970), Can. J. Plant Sci., 50 91.

Schroeder, H.A. and Mitchner, M. (1971), J. Nutrit., 101 (10) 1431.

Schwickerath, M. (1931), Beitr. Nat. Denk. Pflege, 14 463, cited in Brooks (1972).

Shevchenko, V.V., Grinikh, L.I. and Ivanov, V.B. (1977), Genetika (Moscow), 13 (9) 1543.

Somers, E. (1959), Nature, 184 475.

Stout, P.R. (1956), Agric. Food Chem., 4 1000.

Stout, P.R. (1961), Proc. 9th Ann. Calif. Fertil. Conf., (p21).

Tobe, M.L. and Khokhar, A.R. (1977), J. Clin. Haematol. and Oncol., 7 114.

Tso, T.C., Sorokin, T.P. and Engelhaupt, M.E. (1973), Plant Physiol., 51 (4) 805.

Van Fleet, D.S. (1961), Bot. Rev., 27 165, cited in Bowling (1976).

Viktorov, S.V., Vostokova, Y.A. and Vyshirkin, D.D. (1964), Short guide to geobotanical surveying, Pergamon Press, Oxford.

Wainwright, S.J. and Woolhouse, H.W. (1975), in; The ecology of resource degradation and renewal; 15th Symp. Brit. Ecol. Soc., eds. Chadwick, M.J. and Goodman, G.T., Blackwell Scientific, Oxford (p231).

Warington, K. (1937), Annls. Appl. Biol., 24 475.

Waters, M.D., Vaughan, T.O., Abernethy, D.J., Garland, H.R., Cox, C.C. and Coffin, D.L. (1975), Environ. Health Perspec., 12 45.

Wiester, M. (1975), Environ. Health Perspec., 12 41.

Wilkins, D.A. (1957), Nature, 180 37.

Wilkins, D.A. (1978), New Phytol., 80 623.

Williams, R.J.P. (1972), Adv. Antimicrob. Antieoplastic Chemother., Proc. Int. Congr. Chemother, 7th, 2 197.

Wilson, S.B. and Nicholas, D.J.D. (1967), Phytochem., 6 1057.

Wiltshaw, E. (1979), Platinum Metals Rev., 23 (3) 90.

Wolpert-DeFilippes, M.K. (1980), in Cisplatin eds. Prestayko *et al.*, (1980).

Yagonda, A., Watson, R.C. Gonzalez-Vitale, J.C., Grubstald, H. and Whitmore, W.F. (1976), Cancer Treat. Rep., 60 (7) 917.

Zeisler, R. and Greenberg, R.R. (1981), 6th Int. M.T.A.A. Conf. Toronto, Canada.

Zinno, A. and Cutolo, A. (1903), Napoli Gior. Assoc. Med. Nat., 14 82, cited in Le Roy (1975).

CHAPTER II

ANALYTICAL METHODS

2.1 Plant tissue analysis

Background

Most trace analysis of plant materials involves three basic steps. First, homogeneity must be ensured by careful sample preparation. The plant material is either oven dried at around 110°C, to constant weight, or alternatively, freeze dried, since the plant's fresh weight can vary with atmospheric relative humidity. Though drying can be imprecise, standardised conditions can achieve a reproducibility of between 0.5 and 1% (Bowen, 1974). The dried plant material is ground in a ball mill to provide a representative sample for analysis, the results of which are normally quoted on a dry weight basis.

The next step, in preparing plant material for trace metal determination, is to remove the organic matter present by oxidation. This is necessary for most modern analytical techniques except those described as 'non-destructive' methods, such as instrumental neutron activation analysis (INAA) and several X-ray methods (e.g. X.R.F., P.I.X.E.).

Dry ashing

This method of oxidising organic matter is satisfactory up to 500°C for most elements, (Roche Lynch, 1954) though some volatile elements such as Hg, As and Se may be lost. The sample is usually placed in a silica container and heated in a muffle furnace so as to control the temperature and maintain a steady flow of air over the sample. The ash may be dissolved in acid for metal determination.

This technique is not without its problems; as well as the loss

of volatile elements, errors may occur which are due to spray and dust, and reactions with the crucible and some apparent losses are due to incomplete working up of the ash (Bock, 1979). It has been reported that good results can be obtained even with some volatile elements if various precautions are taken (Gorsuch, 1970).

Wet ashing

The main alternative to dry ashing is to digest the plant material in concentrated acid. This method, known also as 'wet ashing', involves much lower temperatures (around 350°C) than those used for dry ashing, thus preventing losses of some volatile elements. The advantages and disadvantages of both techniques have been well documented (Gorsuch, 1959, 1970) (Adrian and Stevens, 1977).

Various acid combinations have been proposed as optimum for biological materials, including nitric and sulphuric acids; nitric, sulphuric and perchloric acids; nitric and perchloric acids. The potential hazard involved in the use of perchloric acid is well known and its use in this study was avoided on safety grounds. Throughout this study, wherever wet ashing was employed, the method used was based on that of Middleton and Stuckey (1954) where nitric acid is the only reagent used in quantity. Acid washed silica antibumping granules were used to prevent the digest boiling violently.

Standard biological materials

In recent years, attempts to standardise laboratory analyses of biological materials have led to the distribution of various standard reference materials. The major source of these standard biological materials has been the National Bureau of Standards (N.B.S.) Washington, D.C. in the U.S.A. They currently distribute the following biological reference materials (Cali, 1976): Orchard leaves [1571],

Bovine liver [1577], Brewer's Yeast [1569], Spinach [1570], Tomato leaves [1573], Pine needles [1575]. More recently, a new standard has become available; Citrus leaves [1572] has replaced Orchard leaves which are in short supply (Benton Jones, 1981) (Zeisler and Greenberg, 1981). The composition of these biological standards is certified; in the case of Orchard leaves, for some twenty-five elements.

Another useful biological standard has been distributed by Bowen (1967). This is carefully prepared kale, now certified for forty-nine elements, though some problems associated with its analysis, particularly by N.A.A., have been found (Bowen, 1974).

Determinations of the platinum group metals in these biological standards have been very few. This is because the levels (if any) of the P.G.M.'s are well below the detection limits of most analytical techniques with the possible exception of NAA. Zeisler and Greenberg (1981) developed a novel technique using NAA to obtain a positive value of 200×10^{-12} g/g Pt in NBS Orchard leaves. Previously reported values were orders of magnitude higher (Nadkarni and Morrison, 1977) (Gladney, 1980). Values for the P.G.M.'s in Bowen's kale (given in ngX/g) come from NAA work.

Ir	< 21, < 13, < 0.5
Pd	26
Pt	198
Ru	4.5

(Bowen, 1980)

2.2 Atomic Spectroscopy

Background

Atomic spectroscopy underlies both classical and modern instrumental methods of analysis; its origins date back to 1859, when

Kirchoff and Bunsen established the origin of the Fraunhofer lines in the solar spectrum, though these had been observed earlier by Sir Isaac Newton, in 1666. Classical emission spectrography was largely qualitative and though it later became semi-quantitative, it was superseded by atomic absorption spectroscopy, a closely related technique.

Theory

When atoms are excited, they absorb energy (E) from either photons or by thermal collisions with other atoms and their electrons are raised to higher energy levels (E_j). The atoms lose this energy (E_j-E_0) when their electrons return to the ground state (E_0), and it is dissipated as photon emission of a particular wavelength (λ) given by

$$E = \frac{hc}{\lambda} \qquad 2.1.$$

where h = Planck's constant.

c = velocity of light.

λ = wavelength of the spectral line.

$E = E_j-E_0$; the radiant energy.

The electrons may be excited to a number of different energy levels within the atom and since each element has its own characteristic electron configuration, it follows that each element will have its own characteristic line spectrum. Figure 2.1 shows three types of electronic transitions which may occur between the ground state and first excited state.

Case A represents the principle on which atomic absorption measurements are taken. Atoms in their ground states can absorb photons of energy $E = E_1-E_0$ and become excited. In an atomic absorpiton spectrophotometer a spectral source is used which can radiate the

emission spectrum of a particular element. The source is usually a Hollow Cathode lamp; the cathode is constructed from the particular element and filled with low pressure inert gas. An electric current causes a discharge which ionises the gas; cations strike the cathode ejecting excited atoms of the element into the discharge; an action known as sputtering. The emission spectrum of the element is produced and this passes through a cell (flame or carbon tube), a dispersion system (monochromator) and falls on the detector system (photomultiplier tube).

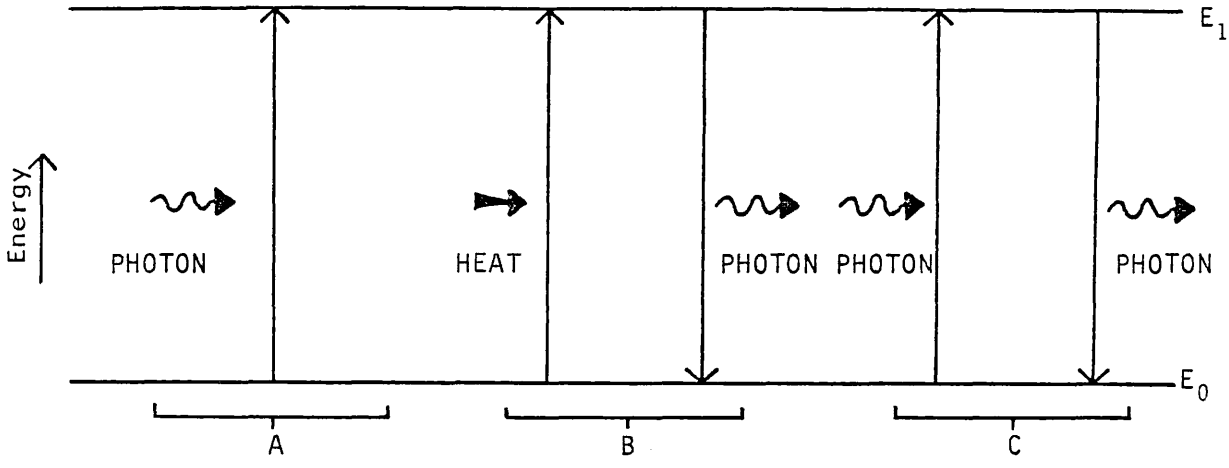


Figure 2.1 Transition diagrams for

A. Atomic Absorption.

B. Atomic Emission.

C. Atomic Fluorescence (after Whiteside, 1979).

The radiation emitted by the hollow cathode lamp may be absorbed by unexcited atoms in their ground states, present in the cell. This atomic absorption will only take place at certain resonance wavelengths, where there is good overlap between absorption lines and emission lines (figure 2.2).

The relative intensities of spectral lines depends on the

population of the ground and excited states. The ratio of the number of atoms in the excited state to the number of atoms in the ground state

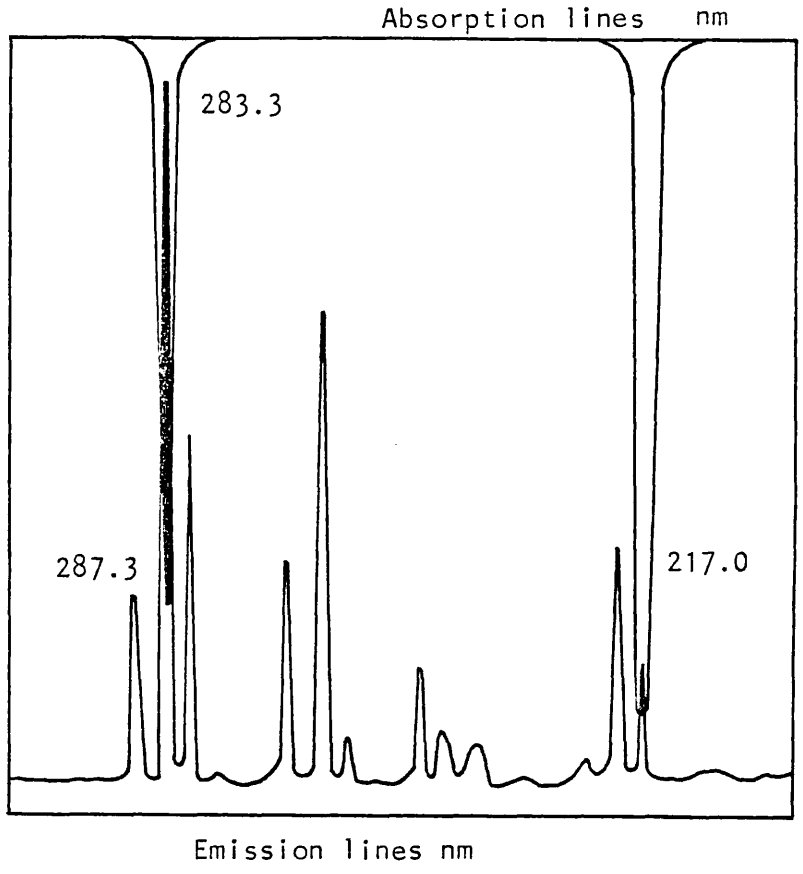


Figure 2.2 Absorbtion and emission spectral lines for lead (after Brooks, 1972)

is given by the Maxwell-Boltzmann law:

$$\frac{N_j}{N_0} = \frac{g_j}{g_0} \exp \frac{E_0 - E_j}{kT} \qquad 2.2.$$

where N is the number of atoms in states 0 or j.

g is the statistical weight for states 0 or j.

T is the absolute temperature.

k is the Boltzmann constant.

Thus for flame temperatures of up to 3000K the majority of atoms will be in their ground states and available for atomic absorption, hence AAS is

inherently a more sensitive technique than AES.

The absorption of radiation by atoms in their ground states follows an exponential law similar to the Beer-Lambert law governing molecular absorption:

$$I_\lambda = I_0 e^{-k\lambda l} \qquad 2.3.$$

where I_0 = Intensity of spectral source at wavelength.

I_λ = Intensity of transmitted radiation.

k_λ = absorption coefficient.

l = cell path length.

Taking logarithms of both sides of equation 2.3 gives a relationship between absorbance (A) and the atomic concentration, the product $k\lambda l$:-

$$A = \log \frac{I_0}{I_\lambda} = k\lambda l \qquad 2.4.$$

In practice most atomic absorption calibration curves are non-linear. This is due to a number of factors, principally stray light effects, thus curve corrections are applied to compensate for this.

2.2.1 Flame Atomic Absorption Spectroscopy (FAAS)

Although the phenomenon of atomic absorption has been known for many years, it was not until 1955 that Walsh first proposed its use for the determination of a wide range of metals in solution. Since then, a great deal of research has been conducted into the nature and various applications of atomic absorption spectroscopy; several reviews of the field have been published (Elwell and Gidley, 1966) (Price, 1972) (Massmann, 1974).

In flame atomic absorption (FAAS), the sample in solution is drawn into a nebulisation system by the pneumatic action of the fuel and oxidant gases as they are mixed. A spray is formed which enables the

sample to flow into an elongated flame. In the flame the sample is decomposed into free atoms (atomisation). These free atoms absorb incident radiation from a hollow cathode lamp (atomic absorption). The gases used for the flame are usually acetylene and air (2500°K) or acetylene with nitrous oxide as the oxidant (3200°K), though other combinations can be used to achieve flames with various temperatures.

Atomic absorption is considered generally to be free of the spectral interferences which plague atomic emission spectroscopy; the few cases of spectral overlap affecting atomic absorption are well documented (Lovett et al., 1975) (Price, 1979). There are other forms of interference which can shift the equilibrium of free atoms in the flame; these are called chemical interferences:

(i) Stable compound formation: anions such as phosphates and sulphates can depress absorption. This can be remedied by adding a releasing agent or using a hotter flame.

(ii) Ionisation: this is only a problem for elements with low ionisation potentials in very hot flames.

(iii) Matrix effects: large excesses of any other elements can give rise to interelement effects.

(iv) Viscosity and Surface tension effects: These affect the rate at which the sample is drawn into the nebuliser; this is important with organic solvents particularly

(v) Scattering and Molecular Absorption effects: The formation of some products in the flame can cause attenuation of the beam. This effect is more serious with flameless work (see section 2.2.2).

Application of FAAS to analysis of plants

The suitability of FAAS for the determination of low levels of metals in plant material was soon realised, especially for zinc (David,

1958). Today, most metals in plant digests can be determined by FAAS to within limits of a few ppm ($\mu g\ cm^{-3}$) (Christian and Feldman, 1970) (Pinta, 1978). A review of this field was published recently by David (1978).

Performance

Analytical performance is defined in terms of a sensitivity and detection limit for each individual element usually. In FAAS, sensitivity refers to that concentration of an element required to produce a 1% absorption; the concentration is quoted in ppm ($\mu g\ cm^{-3}$ or $mg\ dm^{-3}$). The detection limit refers to that concentration of an element (in p.p.m.) required to give a signal which is twice the standard deviation of the background. The detection limit will depend very much on the type of matrix present. The values will depend also on how well the particular instrument is optimised and this includes factors such as the hollow cathode lamp current; monochromator bandpass; fuel flow rate; impact bead ajustment.

2.2.2. Electrothermal Atomisation Atomic Absorption Spectroscopy (ETA AAS)

In conventional FAAS, the source of atomisation is a flame. Other atomisation devices have been investigated, ranging from rods (West and Williams, 1969) and cuvettes (L'Vov, 1970), constructed from graphite, to metal strips or ribbons made from tantalum. These devices belong to a branch of AAS known as electrothermal atomisation atomic absorption spectroscopy (ETA AAS), or occasionally as flameless atomic absorption. The furnace is heated to temperatures up to $3000^{o}C$ within a few seconds by an electric current. The first carbon furnace was constructed by King (1908), but not used for atomic absorption measurements until adapted by L'Vov (1961).

Most modern flameless atomisers are based on the cuvette designed

by Massmann (1968), into which a small aliquot of the sample is injected via a central port hole. The furnace head, containing the electrodes, is water-cooled and the carbon tube enclosed in an inert atmosphere to prevent oxidation of the graphite at high temperatures. The tube is constructed from high purity graphite and may be pyrolitically coated with graphite. This solves the problem of porosity and improves sensitivity for some elements. The relative merits of various flameless atomisers have been described by Fuller (1974); the state of development of ETA AAS has been reviewed recently (Massman, 1976).

With a carbon furnace, the processes: drying, thermal decomposition and atomisation can be carried out as separate stages. In the flame, these are millisecond events which occur continuously. In ETA AAS, the sample matrix may be removed prior to atomisation in a stage labelled ashing or charring. During atomisation, the analyte is vapourised and subsequently flushed from the light path by the inert gas (though the gas flow may be suspended during this stage on some modern instruments). The result is a transient absorption signal which is recorded as a peak usually (figure 2.3). The complex nature of the peak profile depends on factors such as the furnace geometry, slit width etc. The peak precision is not as good as for flame technqiues (RSD 2-5%); this can be due to pipetting errors, though this is reduced with modern instruments incorporating autosampling devices (Dymott, 1981).

Interferences

Interferences encountered with furnace work include stray emission from the walls of the carbon tube which behave like a 'black body' source at high temperatures. This can be reduced by using narrow slit widths and avoiding low hollow cathode lamp currents. Another serious

interference is the non-specific atomic absorption due to the volatilisation of matrix salts (Pritchard and Reeves, 1976). Carbon particles may be sputtered from the tube walls also, especially at high temperatures causing beam attenuation. Most modern furnace systems incorporate a background correction facility to cope with non-atomic absorption. It is usually a deuterium lamp which radiates a continuum from 190-325nm. Both beams (hollow cathode and deuterium) are focused at the centre of

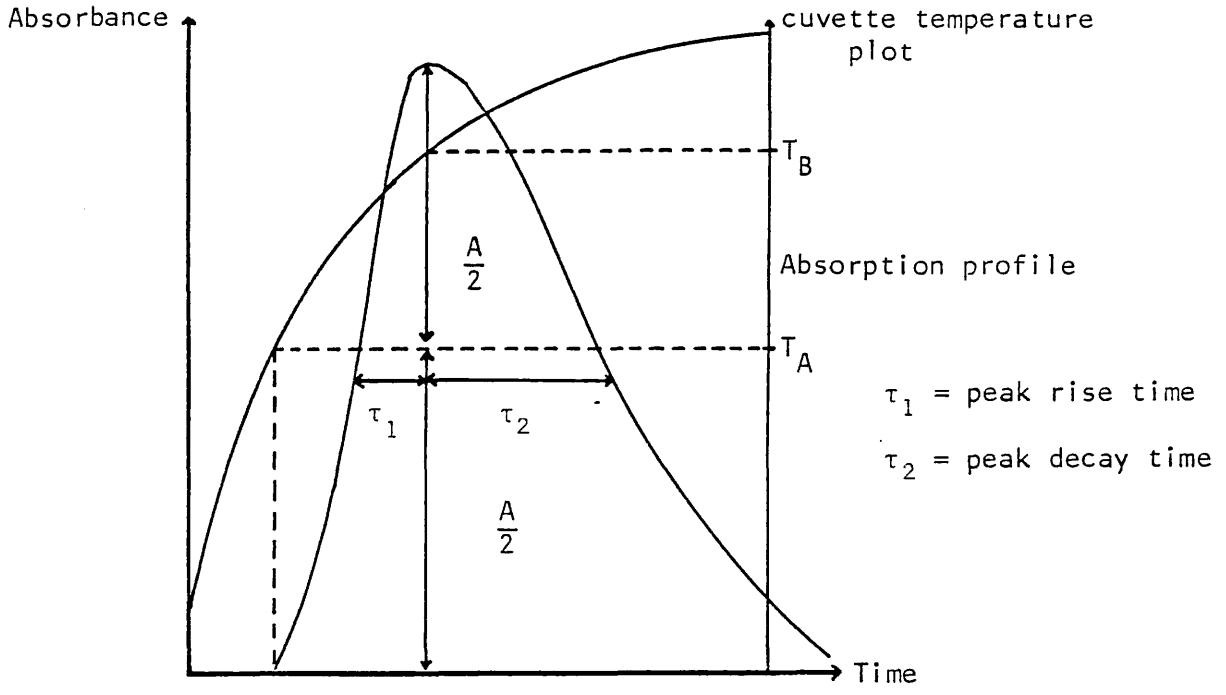


Figure 2.3 Typical furnace absorption profile (after Dymott, 1981)

the tube. Suitable electronics can distinguish between the two types of absorption.

There has been a great deal of interest in this area of ETA AAS since deuterium background correction is limited and the system can be overloaded as well as misaligned leading to erroneous results (Pritchard and Reeves, 1976) (Hendrikx- Jongerius and De Galan, 1976) (Manning, 1972). It has been found for some elements that the presence of nitrate and sulphate anions causes signal depression (Eklund and Holcombe, 1979).

The main advantage of ETA AAS over flame methods is the improved sensitivities and detection limits for many elements; the small sample volume required is also advantageous, but the poorer precision is not. The application to biological samples has brought trace metal analysis within the scope of many laboratories; e.g.clinical laboratories where analysis of biological fluids can be performed by their direct injection into the furnace without the need for pretreatment (Hudnik et al., 1972) (Aggett, 1973).

2.2.3 Atomic Emission Spectroscopy (AES)

The advantage of atomic absorption methods over atomic emission include high sensitivity for many elements and the relative simplicity of identifying atomic spectra compared to complex emission spectra. The basic instrumentation for atomic emission spectroscopy consists of:

1. An excitation source: flame, arc (a.c. or d.c.), spark, plasma or laser.
2. A dispersing unit: quartz prism spectrograph or diffraction grating spectrophotometer.
3. A detection system: photographic plates or a series of photomultiplier tubes and data processing equipment.

In classical d.c. arc spectrography, the samples are loaded into graphite electrodes. The discharge produced between the two electrodes is sufficient to excite the atoms of most elements, thus producing their characteristic line spectra. The theory and practice of this method, along with variations in the excitation source, electrode geometries and measuring systems have been reviewed (Ahrens and Taylor, 1961) (Pinta, 1978).

Flames were some of the first excitation sources used in atomic emission spectroscopy; the colours of flames containing the metal ions of groups I and II are well known. Flames such as air-acetylene and

air-propane are still used today in a branch of AES known as Flame Photometry. The low excitation energy of the flame is thermal and particularly suited to the determination of lithium, sodium and potassium. The technique is in routine use by clinical chemists who need to determine potassium and sodium levels in biological fluids with precision and speed.

High Energy Plasma excitation

There has been a recent revival in AES with the introduction of plasma excitation sources, which can achieve temperatures as high as 10000 K. When a sample is introduced into the plasma, a highly stable emission source is formed. One of the first sources used was the 'plasma jet', in which a d.c. arc discharge in argon provided the plasma: a gas with a high concentration of cations and electrons.

The most popular excitation source in this field has been the inductively coupled plasma (Greenfield, 1971). The structure, called the torch, consists of a hollow quartz tube through which argon gas flows, and which is surrounded by an induction coil. Passing an a.c. current through the coil sets up a magnetic field parallel to the tube axis. This causes an eddy current of ions and electrons whose motion generates intense heat in a continuously ionised flow of gas. The plasma is initiated by a spark which generates enough ions to be circulated by the magnetic field, thus sustaining ionisation.

Applications of AES

An advantage of using emission spectroscopy for the trace metal analysis of biological materials is its multielement capability, coupled with the relatively good sensitivity with ICP sources. Methods for multielement analysis by direct emission spectrography of dried

plant and animal tissues have been suggested (Bedrosian, et al., 1968); careful matrix matching of standards is required. Recently improved spectrographic methods still rely on the dry ashing of plant materials prior to analysis (Bhale, 1979). Gordon et al., (1973) have reviewed methods of trace analysis of biological materials, where a d.c. arc in an inert atmosphere was the excitation source.

The advent of ICP has renewed interest in the multielement analysis of biological samples (Greenfield, 1972) (Knisely et al., 1973). The d.c.-argon plasma was used recently to analyse NBS standard reference biological materials, and good agreement with certified values was found (Reednick, 1979). Little is known about spectral interferences with biological material in plasma excitation. In one study on the determination of trace elements in plant materials by ICP, interferences were reported in the case of zinc (Scott and Strasheim, 1975). In another study, analyses of animal tissues and food material by ICP were carried out and compared favourably with AAS methods (Munter et al., 1979).

2.3 Neutron Activation Analysis (NAA)

2.3.1 Principles

Neutron Activation Analysis (NAA) is a highly sensitive nuclear method of analysis which unlike many, can be a 'non-destructive' technique. When used as such, it is referred to as Instrumental Neutron Activation Analysis (INAA) whilst radiochemical NAA incorporates a separation step either prior to, or after irradiation. During irradiation, the sample is bombarded with neutrons and this can result in the production of radioactive nuclides which then decay to other products. As they decay, various ionising radiations may be emitted;

these include gamma photons of characteristic energies (γ, keV). A common nuclear reaction involving absorption of a neutron and the prompt emission of a gamma ray is:

$$ {}^{A}_{Z}X + {}^{1}_{0}n \rightarrow {}^{A+1}_{Z}X' + \gamma $$

(n,γ) reaction.

The activity (a) induced in a target (X) of mass (m) is given by:

$$ a = \frac{mNf\phi\sigma}{A} e^{-\lambda td}[1-e^{-\lambda t}] \qquad 2.5. $$

where a = activity in d.p.s.

m = mass of X.

N = Avogadro's number.

f = the fractional abundance of the parent nuclide activated (X)

ϕ = neutron flux in n m^{-2} sec^{-1}.

σ = the cross section for the activation in m^2 per nucleus.

A = the relative atomic mass of X.

λ = radioactive decay constant S^{-1}

$\lambda = \frac{\ln 2}{t_{\frac{1}{2}}}$

$t_{\frac{1}{2}}$ = half life of radioisotope produced (X') in sec.

t = irradiation time in sec.

t_d = delay between activation and counting in sec.

The count rate is proportional to the activity, provided the counting efficiency of the detector remains constant. In practice absolute counting, according to equation 2.5, is rarely used because of the experimental uncertainty of parameters ϕ, σ and the counter efficiency. Instead, a standard (C_2) of known mass (m_2) is activated and counted under identical conditions to the unknown sample (C_1) and the mass (m_1) calculated from:

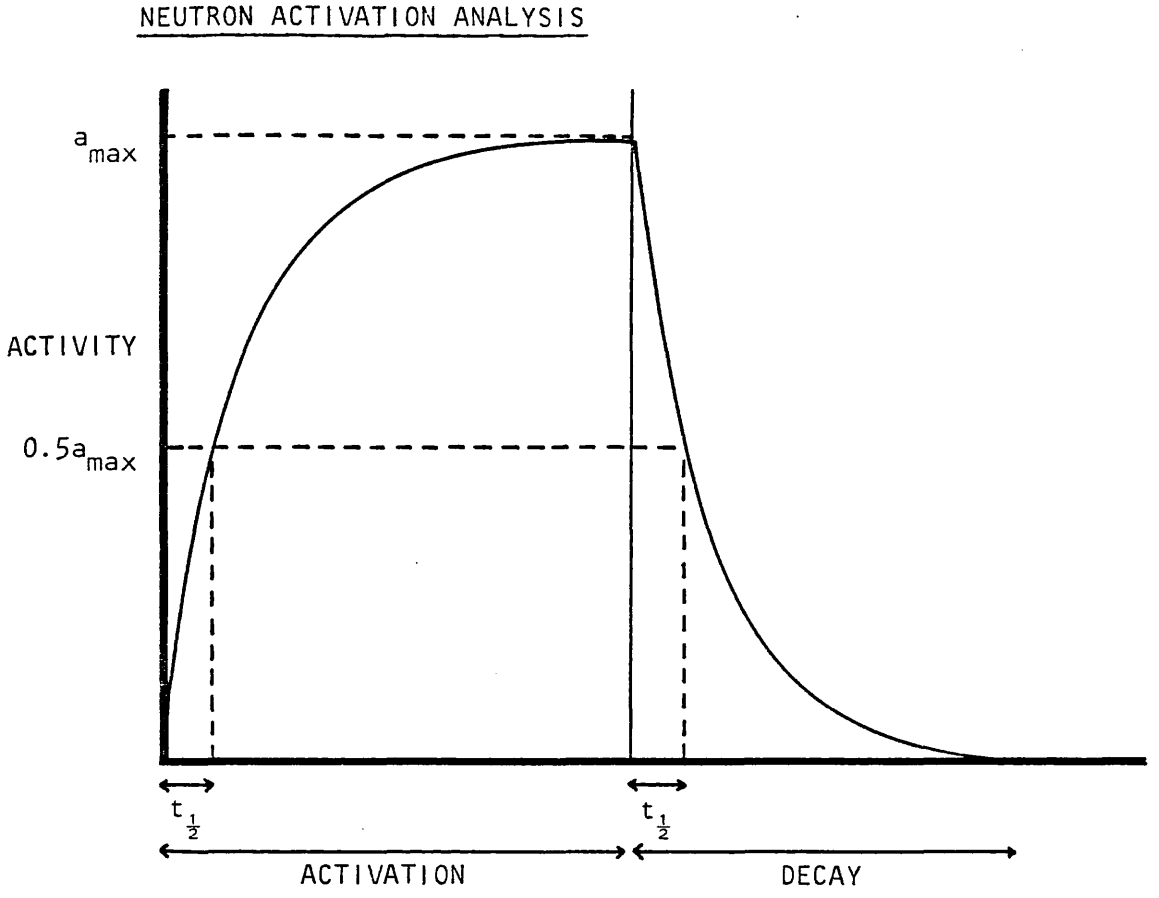


Figure 2.4 Radioactivity plotted against time for activation and decay (after Bowen, 1980)

$$m_1 = \frac{m_1 \times C_1}{C_2} \qquad 2.5.$$

where C = count rate of standard (2) and sample (1).

The relationship between induced activity and time is shown in figure 2.4. In order to obtain reasonable sensitivity, t is set to around $t_{\frac{1}{2}}$, which will produce 50% of the maximum possible activity. The decay time, t_d, is selected to allow short-lived isotopes to decay and improve the background. The selection of a particular isotope for measurement will depend on the half life and the product of the cross section with isotopic fraction, which should be as large as possible.

2.3.2 Neutron Sources

For neutron activation techniques, one of the most abundant sources of neutrons is a nuclear reactor. Nuclear fission of ^{235}U results in the release of γ rays, neutrons, electrons and α particles. Samples for irradiation are introduced into the reactor core, where they undergo various nuclear reactions. At various positions, the neutron flux (ϕ), changes, though the overall reproducibility of the flux is very good; in modern research reactors, neutron fluxes of the order of 10^{11}-10^{13} n cm^{-2} sec^{-1} are achieved. In some cases isotopic neutron sources such as ^{252}Cf may be used for activation purposes, but their fluxes tend to be small and unsymmetrical.

Figure 2.5 shows the various neutron energy distributions in a nuclear reactor. Most of the neutrons fall into an energy region where they are in equilibrium with the moderator. The function of the moderator in a nuclear reactor is to slow down the neutrons produced by the fission of ^{235}U. Light water, heavy water or graphite may be used as a moderator. The slow or thermal neutrons are available for nuclear reactions of the (n,γ) type and are typically about 0.025eV in energy. Neutrons of energies between 1eV and 0.5MeV are referred to as epithermal or resonance neutrons, and finally, those neutrons with an energy distribution around 1MeV constitute the fast neutron flux.

Irradiation with Epithermal Neutrons

Thermal neutron activation is the most common method of activation analysis, where neutrons from the whole reactor spectrum contribute to the formation of various nuclides. Some nuclides may be excited individually by bombarding with neutrons corresponding to the resonance energy of that nuclide. The nuclear cross section changes dramatically with

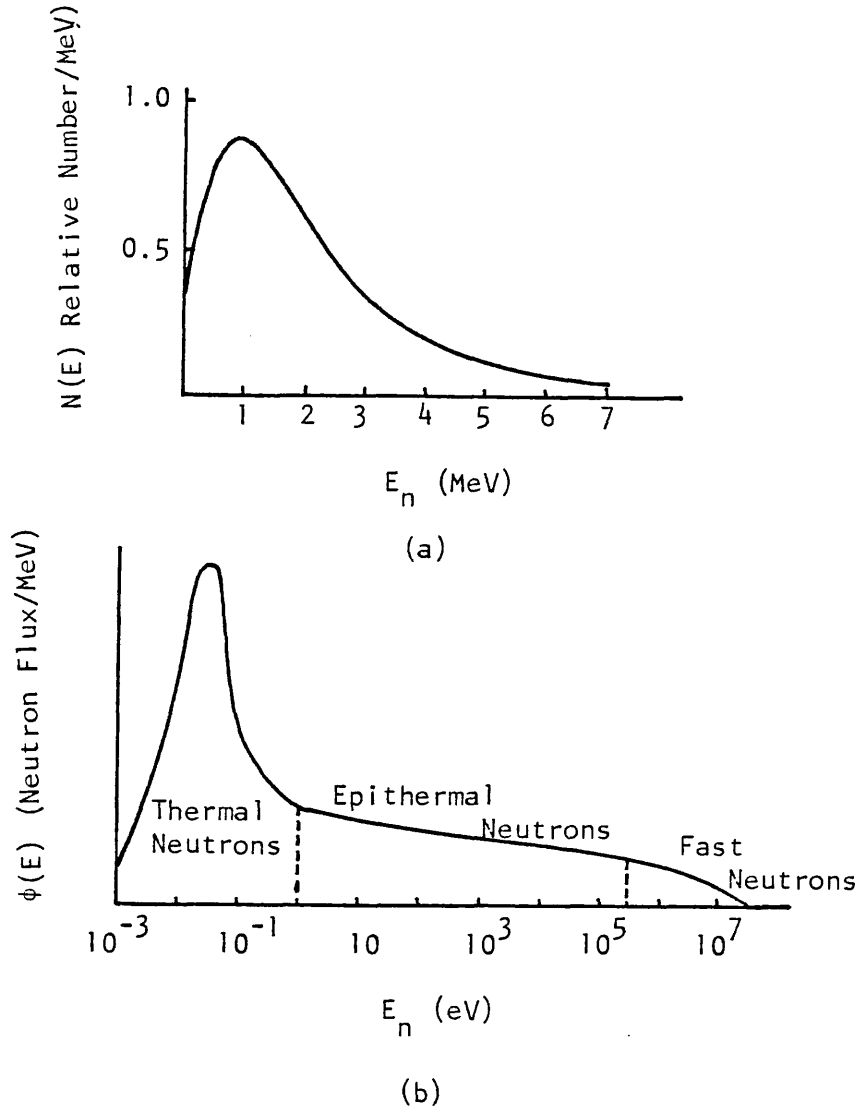


Figure 2.5 (a) Energy distribution of neutrons from ^{235}U fission; (b) Typical distribution of neutron energies in a thermal reactor (after Kruger, 1971).

the variation in energy of the incident neutrons (figure 2.6). Nuclear reactions of this type are called resonance reactions and epithermal neutrons will be responsible for activating the nuclide.

Samples for epithermal irradiation are wrapped in cadmium foil, which can effectively screen out thermal neutrons. The cut-off of thermal neutrons depends on the thickness of the foil; the cadmium ratio R_{Cd} is given as:

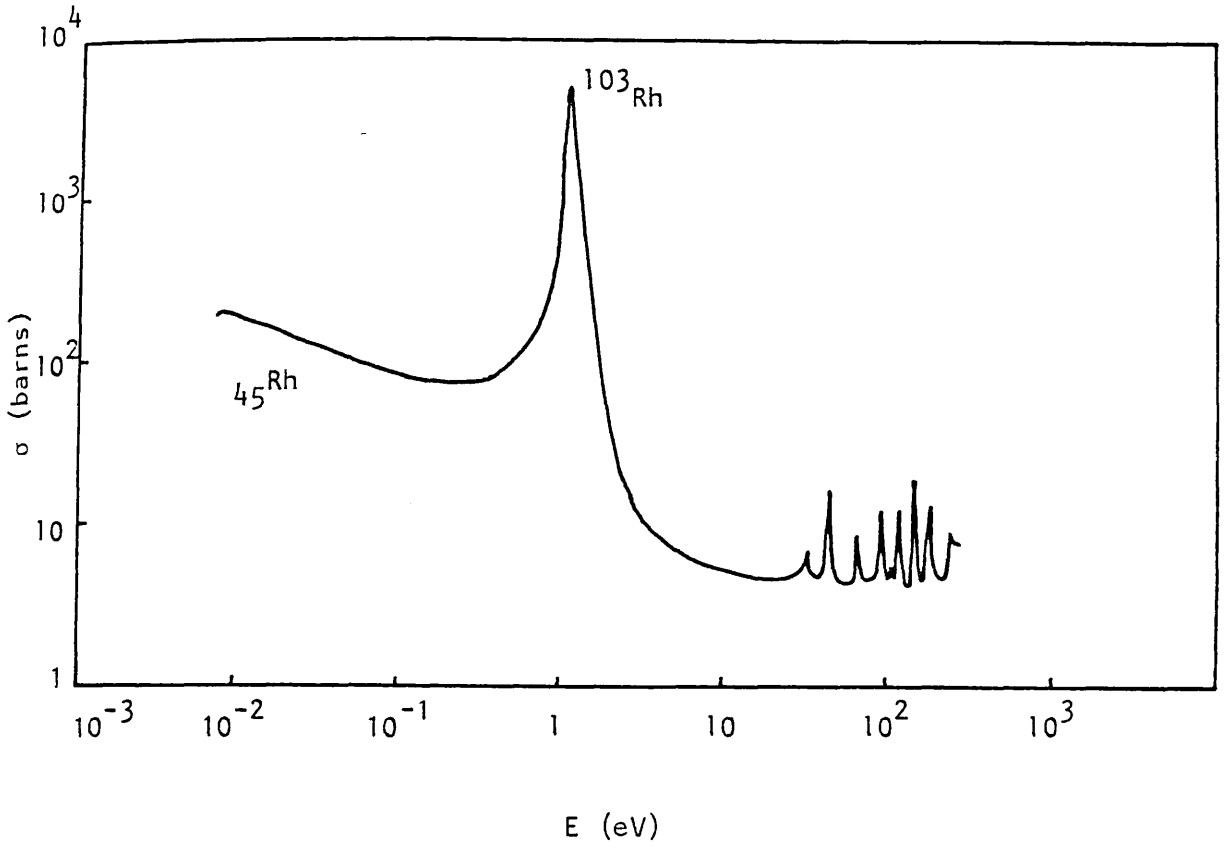


Figure 2.6 Variation in nuclear cross section with incident neutrons for ^{103}Rh (after De Soete et al., 1972)

$$R_{Cd} = \frac{\text{activity without Cd foil}}{\text{activity with Cd foil}} .$$

Epithermal irradiations have some advantages over thermal irradiations for some elements (Steinnes, 1971), though it depends on the variation in cross section with neutron spectrum for each nuclide involved. Epithermal irradiation can reduce the activation of ^{23}Na and ^{41}K, making it particularly suitable for the determination of short lived nuclides in biological materials (Cesana et al., 1978) (Hanna, et al., 1977). The area of activation analysis of biological materials has been reviewed by Bowen (1980).

2.3.3 Cyclic activation analysis

Theory

Cyclic activation analysis is a recent improvement in NAA which enables optimum use to be made of the experimental time available for the counting of short lived isotopes. The signal to noise ratio for the radionuclide of interest is enhanced if the sample is irradiated, allowed to decay and counted for a short period of time; the sample is reirradiated and the process repeated for 'n' cycles. Spyrou (1981) has reviewed the principles and applications of cyclic activation analysis. Figure 2.7 gives the variation in isotope activity with time and cycle number; the detector response for the first counting period (D_1) is given by

$$D_1 = \frac{\epsilon I m N f \sigma \phi}{A\lambda} (1-e^{-\lambda t_i})(e^{-\lambda t_w})(1-e^{-\lambda t_c}) \qquad 2.8$$

where

ϵ = efficiency of the detector.

I = intensity of the γ ray of interest.

ϕ = neutron flux.

m = mass of the element of interest.

N = Avogadro's number.

f = fractional abundance of target isotope.

σ = activation cross section.

λ = decay constant of induced radionuclide of interest.

A = relative atomic mass of element.

For 'n' cycles, C_n, the cumulative response is:

$$C_n = \sum_{j=i}^{n} D_j = D_i \left(\frac{1}{1-e^{-\lambda T}}\right)\left(n-e^{-\lambda T}\left(\frac{1-e^{-n\lambda T}}{1-e^{-\lambda T}}\right)\right) \qquad 2.9.$$

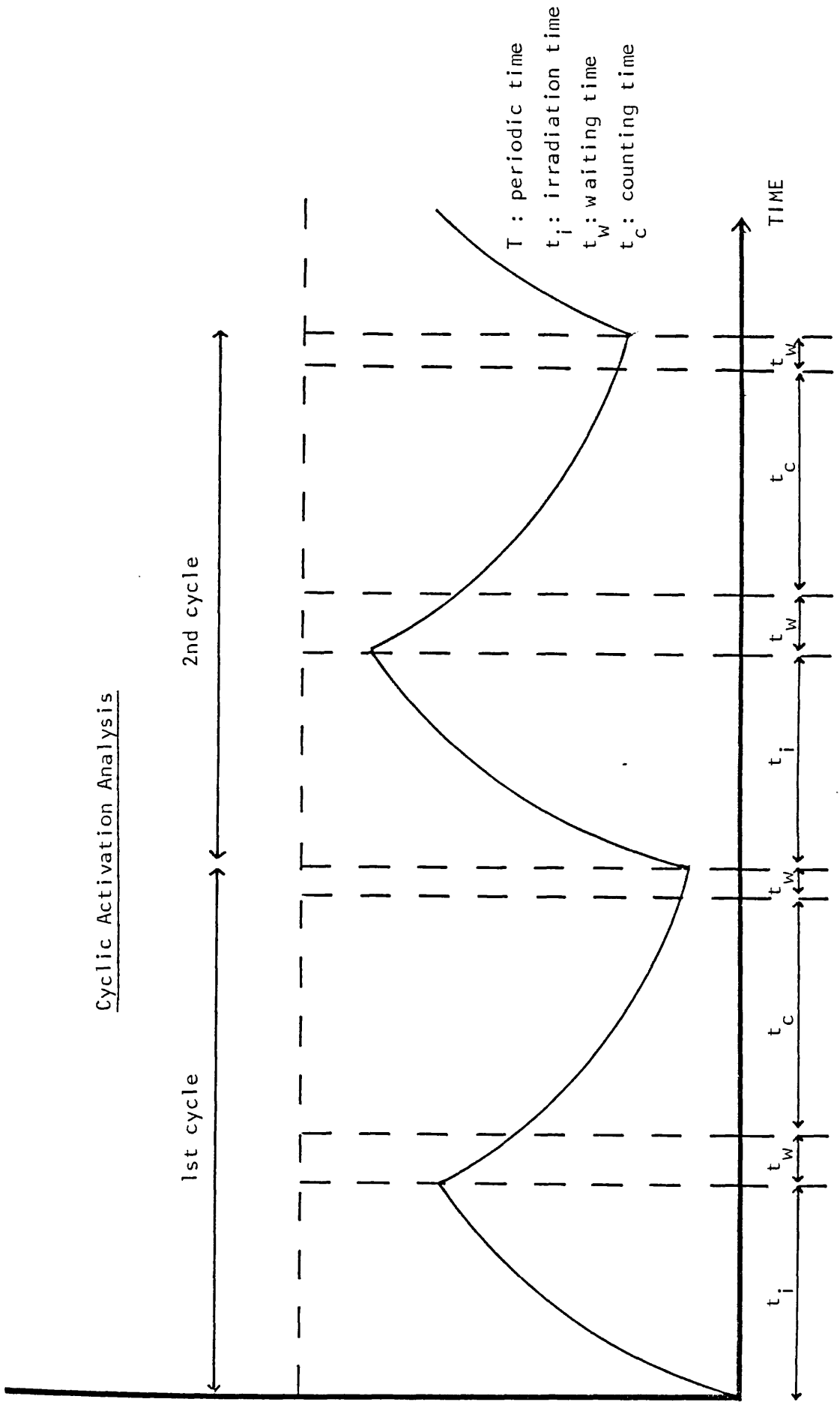


Figure 2.7 Graph of induced activity against time (adapted after Spyrou, 1981)

The problem of rapidly changing Multi Channel Analyser (M.C.A.) dead times has been investigated (Miller and Guinn, 1976); Egan et al., found that values for the ratio (clocktime/live time) from M.C.A. clock data were in good agreement with an exponentially based correction factor (F_D) given by

$$F_D = \frac{\int_0^{t_c} Aoe^{-\lambda t} dt}{\int_0^{t_c} Aoe^{-\lambda t}(1-B-Ce^{-Kt})dt} \qquad 2.10.$$

where

C = actual acquired net counts in the photopeak of interest.

Ao = true initial photopeak count rate.

t_c = length of counting period (clock time).

λ = decay constant of the isotope of interest.

DT(t) = fractional analyser dead time at time t.

$= B+Ce^{-Kt}$.

Advantages in the use of cyclic activation are important in the analysis of biological materials, where activation of ^{23}Na, ^{37}Cl and ^{41}K result in short lived nuclides producing an unfavourable background for the determination of other short lived nuclides. The improvement of detection limits in these cases has been demonstrated (Egan et al., 1977) (Egan and Spyrou, 1977) (Spyrou, 1981). The use of an epithermal neutron flux in conjunction with cyclic activation has been investigated also (Spyrou, 1981).

2.3.4 Gamma Ray Spectrometry

After irradiation by neutrons, the sample contains some radioactive nuclides which decay by emission of γ photons. The measurement of gamma photon energy may be used for both quantitative and qualitative

analysis. The sample is placed next to a detector which records the sample activity; the information is passed on to a multichannel analyser for processing.

Various detecting devices exist for the measurement of radioactivity. For the highly specialised measurements involved in INAA, semiconductor detectors are employed because of their high resolution of γ photons ($\sim$ 2 keV at 1332.4 keV). Scintillation detectors, whilst more efficient, have poorer resolution. Both kinds of detector can be linked to a multichannel analyser which can display the γ activity in the form of a spectrum. The pulse height of each photopeak represents its count rate, which is a measure of the mass of that isotope in the sample.

Germanium-Lithium drifted detectors; Ge(Li)

Detectors used in this study were of the germanium-lithium drifted type. The principle on which a semiconductor detector operates is analogous to a gas ionisation counter. When incident radiation strikes the crystal, which is under an applied potential, electrons drift to the positive electrode. The 'positive' holes which remain move towards the negative electrode. The production of charge carriers result in an output pulse proportional to the incident radiation. Drifting the germanium crystal with lithium changes the electrical properties of the semiconductor such that the detector effieiency is improved over the pure germanium type. The detector has to be cooled with liquid nitrogen to keep the lithium in place and reduce electronic noise.

The measurement of activity is performed by a multichannel analyser linked to the detector; the spectrum may be displayed on a video screen. Modern sophisticated techniques which are computer based,

can process incoming data at a much faster rate, correct for dead time, decay time and calculate concentration automatically.

2.4 X-Ray Spectrometry

2.4.1 Principles of X-ray spectrometry

Unlike the methods of atomic spectroscopy, X-ray spectrometry is a non-destructive method of analysis, both qualitative and quantitative. It has a simultaneous multielement capability which may be used within a wide analytical range. The principles of the method are based on the measurement of X-ray spectra emitted by various elements present in the sample. The excitation of any atom may result in the removal of one or more inner core electrons. The remaining gap(s) are filled by electrons from the upper energy levels; the excess energy is emitted as an X-ray photon. The movement of electrons within the various electron shells (K, L, M, N etc.) gives rise to a line spectrum characteristic of each element (figure 2.8).

The atoms may be excited in several different ways, resulting in the various fields of X-ray spectrometry:

1. Direct X-ray emission spectrometry: the atoms are excited by a beam of electrons or high energy cathode rays (cf. section 2.4.2)
2. X-ray fluorescence spectrometry (X.R.F.): where the atoms are subjected to a specific X-ray beam of sufficient energy to excite the X-ray spectrum (X-ray induced emission).
3. Proton induced X-ray emission (P.I.X.E.): here the sample is bombarded with high energy protons ($\sim$3-4 MeV) which excite the X-ray spectrum (cf. section 2.4.3).

One of the earliest reports on the relative merits of electron, proton and X-ray induced emission for elemental analysis was that of

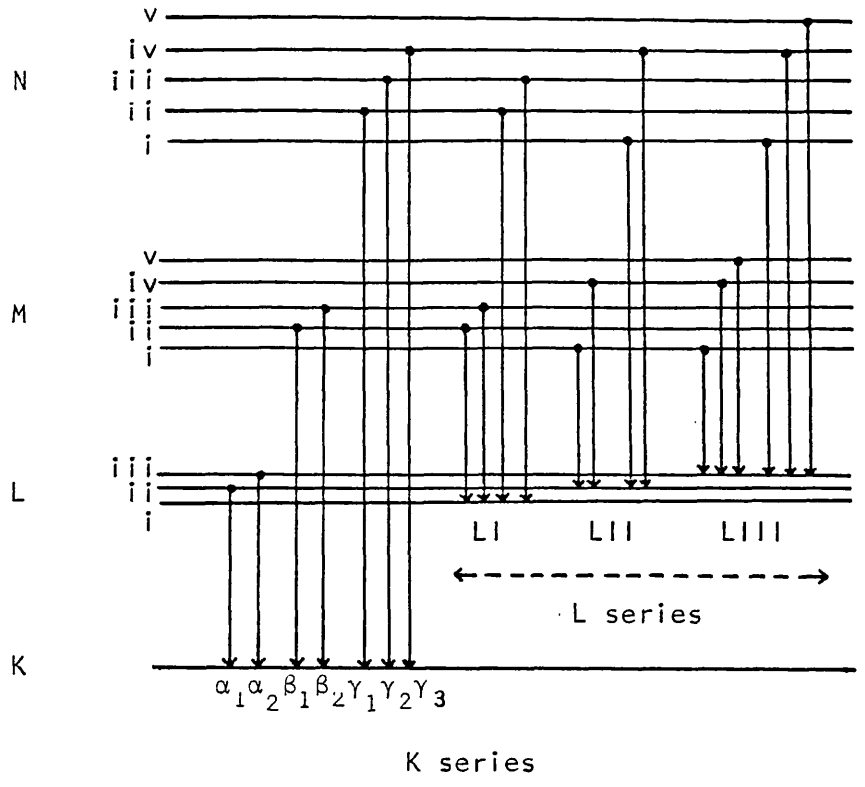


Figure 2.8 Energy level diagram and electronic transitions

(after Pinta, 1978)

Birks (1964). Recent reviews concentrate more on the direct comparison of X.R.F. to P.I.X.E. (Dzubay, 1977). Direct emission techniques are older than fluorescence techniques, though for the general analysis of large samples, X.R.F. is more widely used.

In X.R.F. the sample is placed outside the X-ray tube rather than on the anticathode of the assembly type tube (Pinta, 1978). The analysis of X.R.F. spectra is performed with an X-ray spectrometer, where a rotating crystal diffracts X-rays in accordance with Bragg's Law:

$$n\lambda = 2d \sin\theta \qquad 2.11.$$

where λ is the wavelength diffracted by a crystal of interplanar spacing d at an angle of incidence θ; n is the order of diffraction. The diffraction may be performed by either a plane crystal or curved crystal.

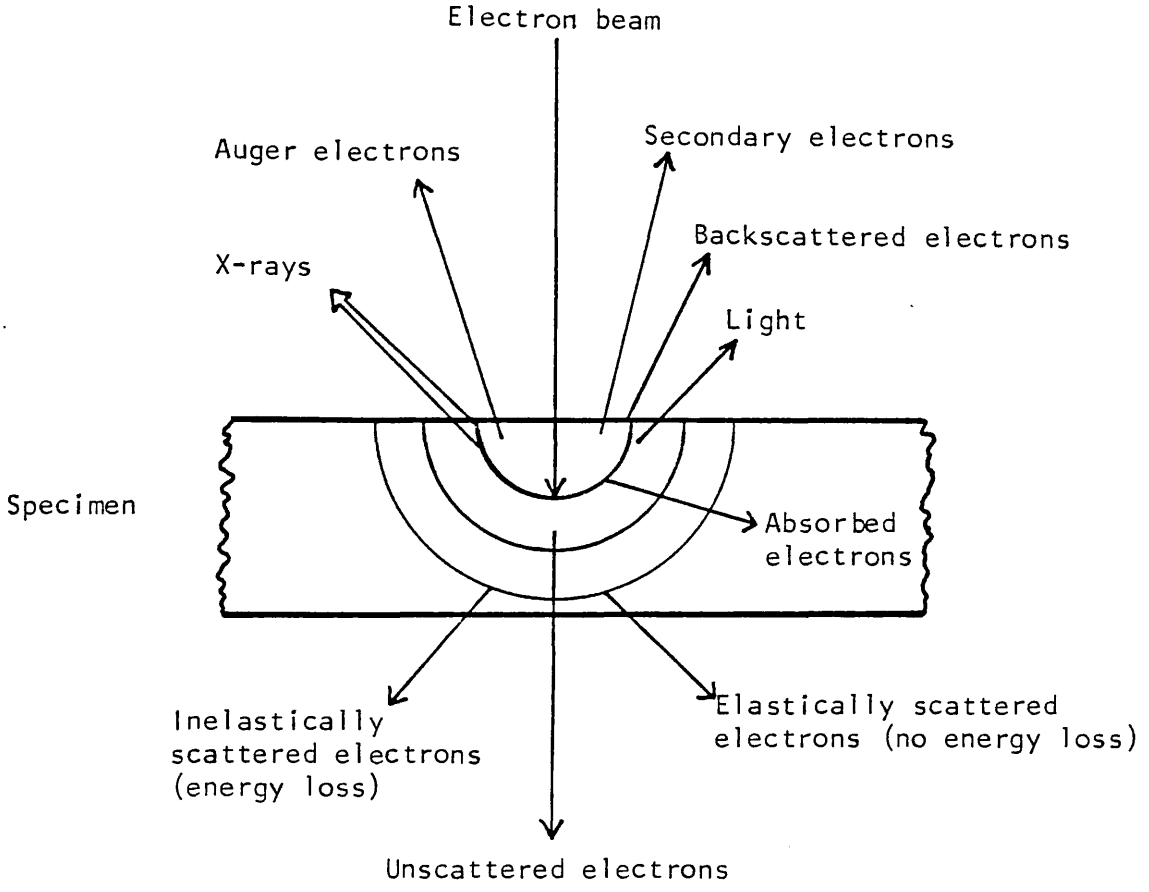


Figure 2.9 Schematic of the interactions between the electrons and the specimen (after Hess, 1977)

The diffracted X-rays are detected by scintillation or gas-flow proportional counters.

2.4.2 X-ray Microanalysis

When a specimen is examined in an electron microscope, it is bombarded with a beam of high velocity electrons. The interactions between the electrons and the specimen are illustrated schematically in figure 2.9.

Energy dispersive analysis (EDXA)

The recent development of solid state detectors has enabled highly efficient X-ray analysis to be carried out by energy dispersive methods. The detector unit consists of a silicon-lithium doped crystal, Si(Li), protected by a beryllium window usually. The principles of detection resemble those of semiconductor devices used in gamma spectrometry (cf. section 2.3.4); the whole detector assembly is cooled with liquid nitrogen to reduce electronic noise and prevent the lithium diffusing out of the crystal.

X-rays of all energies are processed by a microcomputer simultaneously and the energy spectrum is displayed on a C.R.T. screen. The resolution of such detectors is about 150eV at 5.9keV (Jaklevic and Goulding, 1978).

Wavelength dispersive analysis (W.D.)

The classical method of X-ray analysis is the use of a rotating crystal which will diffract X-rays according to equation 2.11. Dedicated electron microprobes are available now, with both energy dispersive and wavelength dispersive facilities. The theoretical principles and instrumentation of W.D. analysis have been discussed by Birks (1978).

Several crystals, with different 'd' spacings, are employed to cover many of the elements in the periodic table. The crystal is preset to a particular angle corresponding to the diffraction of specific X-ray wavelengths. All other wavelengths are eliminated from the detector; the result is a much better spectral resolution and lower detection limits when compared to energy dispersive systems. The disadvantage is that the higher energy electron beams required can damage and distort biological specimens (Erasmus, 1978).

2.4.3 Proton Induced X-Ray Emission (P.I.X.E.)

As well as X-rays and electrons, charged particles such as protons ($^{1}H^{+}$) or alpha particles ($^{4}He^{2+}$) can be used to excite atoms to produce characteristic X-ray emission. The increasing interest in the use of proton beams ($\sim$3MeV) is largely due to the recently improved Si(Li) detectors, which are used in P.I.X.E. analysis (Walter and Willis, 1978).

The P.I.X.E. technique is quite different from Proton Activation Analysis, which is a nuclear excitation method, closely related to neutron activation analysis (section 2.3). P.I.X.E. involves atomic excitation by irradiating the sample with a proton beam and measuring the 'prompt' X-rays emitted during that period. In comparison to X.R.F., P.I.X.E. has better sensitivity and detection limits for some sample types. The applicability of X.R.F. and P.I.X.E. to environmental samples has been compared recently (Dzubay, 1977).

An up to date system can provide the simultaneous determination of forty or more elements from silicon to uranium at detection limits in the 0.005-1 $\mu g\ g^{-1}$ range. With incident electron beams as the excitation source, the X-ray background due to the bremsstrahlung produced, especially in a biological matrix, is quite high, below 12keV; the corresponding background with proton beams of the same energy is about 10^{6} times less intense (Walter and Willis, 1978). Once the X-ray spectrum has been acquired, the data is analysed usually by a complex computer program. This may involve "stripping the spectrum down" using information stored previously, or by representing the background and the peaks as mathematical functions and applying a least squares fit to the spectrum.

REFERENCES

Adrian, W.J. and Stevens, M.L. (1977), Analyst, 102 446.

Aggett, J. (1973), Anal. Chim. Acta., 63 473.

Ahrens, L.M. and Taylor, S.R. (1961), Spectrochemical Analysis, Addison-Wesley, Reading.

Bedrosian, A.J., Skogeboe, R.K. and Morrison, G.H. (1968), Anal. Chem., 40 (6) 854.

Bhale, G.L., Bellary, V.P., Thomas, A. and Dixit, M.N. (1979), Ind. J. Pure Appl. Phy., 17 830

Birks, L.S. (1978), in X-ray Spectrometry, edited by Herglotz, H.K. and Birks, L.S. (1978) ch.1.

Bowen, H.J.M. (1967), Analyst, 92 124.

Bowen, H.J.M. (1974), J. Radioanal. Chem., 19 215.

Bowen, H.J.M. (1980), CRC Critical Reviews in Analytical Chemistry, 10 (2) 127.

Bowen, H.J.M. (1980), private communication.

Cali, J.P. (1976), Anal. Chem., 48 802A.

Cesana, A., Rossitto, F. and Terrani, M. (1978), J. Radioanal. Chem., 45 199.

Christian, G.D. and Feldman, F.J. (1970), Atomic Absorption Spectroscopy: Applications in Agriculture, Biology and Medicine, Wiley, New York.

David, D.J. (1958), Analyst, 83 655.

David, D.J. (1978), Prog. analyt. atom. Spectros., 1 225.

Dymott, T.C. (1981), editor, Atomic Absorption with Electrothermal Atomisation, 2nd ed., Pye Unicam Ltd., Cambridge.

Dzubay, T.G. (1977), editor, X-Ray Fluorescence Analysis of Environmental Samples, Ann Arbor Sci., Michigan.

Egan, A., Kerr, S.A. and Minski, M.J. (1977), Radiochem. Radioanal. Letters, 28 (5-6) 369.

Egan, A. and Spyrou, N.M. (1977), J. Radioanal. Chem., 37 775.

Eklund, R.H. and Holcombe, J.A. (1979), Anal. Chim. Acta, 109 97.

Elwell, W.T. and Gidley, J.A.F. (1966), Atomic Absorption Spectrophotometry, Pergamon, Oxford.

Erasmus, D.A. (1978) editor, Electron probe microanalysis in biology, Chapman and Hall, London.

Fuller, C.W. (1974), Proc. Chem. Conf., 27 67.

Gladney, E.S. (1980), Anal. Chim. Acta, 118 385. Cited in Zeisler and Greenberg (1981).

Gordon, W.A., Mambridge, K.M. and Franklin, M.L. (1973), Prog. Anal. Chem., 6 23. Cited in Pinta (1978).

Gorsuch, T.T. (1959), Analyst, 84 135.

Gorsuch, T.T. (1970), Destruction of Organic Matter: International Series of Monographs in Analytical Chemistry, 39, Pergamon Press Inc., New York.

Greenfield, S.I. (1971), Metron., 3 8. Cited in Pinta (1978).

Greenfield, S.I. and Smith, P.B. (1972), Anal. Chim. Acta, 59 341. Cited in Pinta (1978).

Hanna, A.G. and Al-Shahristani, H. (1977), J. Radioanal. Chem., 31 581.

Hendrikx-Jongerius, C. and De Galan, L. (1976), Anal. Chim. Acta, 87 259.

Hess, W.M. (1977), Conf. on Coffee Rust Control, Paipa, Columbia (Conf. Prog. published by G.T.Z., Eschborn, Germany, 1979), pg215.

Herglotz, H.K. and Birks, L.S. (1978), editors, X-Ray Spectrometry (Practical Spectroscopy Series Vol.2) Marcel-Decker, Inc., New York.

Hudnik, V. and Gomiscek, S. (1972), 2nd Int. Symp. Anal. Chem., Paper NS-40, Ljubliana, Yugoslavia. Cited in Pinta (1978).

King, R.B. (1908), Astrophys. J., 27 353. Cited in Pinta (1978).

Kniseley, R.N., Fassel, U.A. and Butler, C. (1973), Clin. Chem., 19 807.

Lovett, R.J., Welch, D.L. and Parsons, M.L. (1975), Appl. Spectros., 29 (6) 470.

L'vov, B.V. (1961), Spectrochim. Acta, 17 761. Cited in Pinta (1978).

L'vov, B.V. (1970), Atomic Absorption Spectrochemical Analysis, A. Hilger, London.

Manning, D.C. (1972), Atomic Absorption Newsletter, 11 (5) 112.

Massmann, H. (1968), Spectrochim. Acta, 23B 215.

Massmann, H. (1974), Angew. Chem. internat. Edit., 13 (8) 504.

Massmann, H. (1976), Proc. Analyt. Div. Chem. Soc. pg258.

Middleton, G. and Stuckey, R.E. (1954), Analyst, 79 137.

Miller, D.A. and Guinn, V.P. (1976), J. Radioanal. Chem., 32 179.

Munter, R.C., Grande, R.A. and Alin, P.C. (1979), ICP Infor. Newsl. 5 (7) 368.

Nadkarni, R.A. and Morrison, G.M. (1977), J. Radioanal Chem., 38 435.

Pinta, M. (1978), Modern Methods for Trace Metal Analysis, Ann Arbor Sci., Michigan.

Price, W.J. (1972), Analytical Atomic Absorption Spectrophotometry, Heyden and Son Ltd., London.

Pritchard, M.W. and Reeves, R.D. (1976), Anal. Chim. Acta, 82 103.

Reednick, J. (1979), Internat. Lab., (May-June, pg127).

Roche-Lynch, G. (1954), Analyst, 79 137.

Scott, R.H. and Strasheim, A. (1975), Anal. Chim. Acta, 76 77.

Spyrou, N.M. (1981), J. Radioanal. Chem., 61 (1-2) 211.

Steinnes, E. (1971). Activation Analysis in Geochemistry and Cosmochemistry, edited by Brunfelt, A.O. and Steinner, E., Universitetesforlaget, Oslo, (pg113).

Walsh, A. (1955), Spectrochimica Acta, 7, 108.

Walter, R.L. and Willis, R.D. (1978) in X-Ray Spectrometry, edited by Herglotz, H.K. and Birks, L.S. (1978).

West, T.S. and Williams, X.K. (1969), Anal. Chim. Acta, 45 27. Cited in Pinta (1978).

Whiteside, P.J. (1979), An introduction to Atomic Absorption Spectrophotometry, Pye Unicam, Cambridge.

Zeisler, R. and Greenberg, R.R. (1981), 6th International MTAA Conference, Toronto.

CHAPTER III

METHODS FOR THE STUDY OF METAL DISTRIBUTION AND BINDING IN PLANT TISSUES

3.1 Methods for the study of plant growth

Sand and water culture methods

The investigation of metal uptake by plants requires carefully controlled experimental methods. Soils are unsuitable for use in laboratory studies because of their complex and heterogeneous nature. Sand and water culture methods have been developed to overcome such problems; their use dates back to the mid-nineteenth century (Knop, 1865). Since then, many refinements of these methods have taken place, especially in the area of water purity where contaminants have been eliminated, thus permitting the study of trace metal essentiality (Hoagland and Arnon, 1950).

Plants may be grown on an acid washed sand substrate to which is added a solution containing the essential nutrients. Alternatively, plants may be grown hydroponically in a nutrient solution. The plant is supported so that its roots are immersed in the nutrient solution but at the same time, protected from light by darkening the container and lid. The nutrient solution is aerated by bubbling compressed air through the solution, otherwise abnormal root growth results. The technical details of sand and water culture methods have been described extensively by Hewitt (1966).

Nutrient solutions

The composition of most modern nutrient solutions is based on the classical formulations of Hoagland and Arnon (1950). Some slight

Full Strength Nutrient Solution (after Epstein, 1972)

Macronutrients

Compound	Mol. wt.	Concentration of stock solution M	Concentration of stock solution g dm^{-3}	Volume of stock soln. ml dm^{-3} final soln.	Element	Final concentration of element µM	Final concentration of element ppm (mg dm^{-3})
KNO_3	101.10	1.00	101.10	6.0	N	16000	224
$Ca(NO_3)_2.4H_2O$	236.16	1.00	236.16	4.0	K	6000	235
$NH_4H_2PO_4$	115.08	1.00	115.08	2.0	Ca	4000	160
$MgSO_4.7H_2O$	246.49	1.00	246.49	1.0	P	2000	62
					S	1000	32
					Mg	1000	24

Micronutrients*

Compound	Mol. wt.	Concentration of stock solution mM	Concentration of stock solution g dm^{-3}	Volume of stock soln. ml dm^{-3} final soln.	Element	Final concentration of element µM	Final concentration of element ppm (mg dm^{-3})
KCl	74.55	50	3.728	1.0	Cl	50	1.77
H_3BO_3	61.84	25	1.546		B	25	0.27
$MnSO_4.H_2O$	169.01	2.0	0.338		Mn	2.0	0.11
$ZnSO_4.7H_2O$	287.55	2.0	0.575		Zn	2.0	0.131
$CuSO_4.5H_2O$	249.71	0.5	0.125		Cu	0.5	0.032
$Na_2MoO_4.2H_2O$	241.96	0.5	0.121		Mo	0.5	0.05
Fe-EDTAH$_2$	346.08	20	6.922	1.0	Fe	20	1.12

* A combined stock solution is made up of the bracketed micronutrients.

† Ethylenediaminetetraacetic acid dihydrogen iron II

Table 3.1

modifications of 'Hoagland's solution' have taken into account micronutrients also, such as chlorine (Johnson et al., 1957). Each nutrient formulation must include essential macronutrients, the cations: potassium, calcium and magnesium; and the anions: nitrate, phosphate and sulphate; as well as adequate concentrations of the micronutrients. The problem of nutrient solution pH is important because it influences the solubility and oxidation state of various metal ions and can affect the role of H^+ and OH^- ions in active transport. The majority of nutrient solutions have a pH value of between 5 and 7.

The composition of the full strength nutrient solution used in this study is given in Table 3.1 (Epstein, 1972). Four stock solutions of macronutrients were made up (1 mol dm^{-3}) using Analar grade salts. The micronutrients were made up as a single stock solution, apart from iron which was made up separately as an iron II complex with ethylendiamine tetraacetic acid (0.02 mol dm^{-3}). Full or half strength nutrient solutions were made up just before the start of the uptake experiment from stock solutions which had been stored in a refrigerator to prevent bacterial growth.

Contamination

The problem of contamination is significant not only in micronutrient investigations but also in the analytical procedures which may follow. Contamination may arise from either airborne or water sources, containers, glassware or even from the chemicals and reagents employed. Various precautions can be taken to reduce contamination. These include the use of high density polythene containers wherever possible, particularly for the storage of solutions. In this study, all plastic and glassware was acid washed twice (3 M HCl) and rinsed finally in deionised and distilled water. Investigations of the selection and

cleaning of various plastic materials for use in trace analysis have been reported (Karin et al., 1975)(Moody and Lindstrom, 1977).

The major analytical concern of this thesis has been with the determination of the platinum metals. Normally, these 'precious' metals do not feature as trace contaminants either in the water supply or in ambient air sources. However, stringent precautions were still undertaken as a matter of analytical procedure and to ensure analytical accuracy later when some multielement analysis was carried out.

Controlled environment growth rooms

Studies of plant growth also require careful control of the external environment. This is important because various factors such as the temperature, lighting quality and quantity can influence the uptake of nutrients by plants and their growth patterns. For these reasons, hydroponic and sand culture experiments are carried out in controlled environment rooms or cabinets. A typical growth room should provide some means of controlling the quality and quantity of artificial light, thus simulating natural light. This is usually a combination of fluorescent lighting, covering the U.V. and blue end of the spectrum, and tungsten lighting for the red and IR end of the spectrum. The periods simulating day and night should be controlled by preset clocks along with the day and night temperatures.

The plant growth experiments reported in this thesis were conducted in a controlled environment growth room manufactured by Prestcold (Central) Ltd. (Figure 3.1). The internal area of the room measured 12.6m^2 and the floor was finished with 4cm of granolithic concrete. The internal walls were of burnabrite aluminium insulated with 10cm of polystyrene and finished externally with galvanised mild steel sheating. This was surrounded by a wooden hut which contained the control panel for

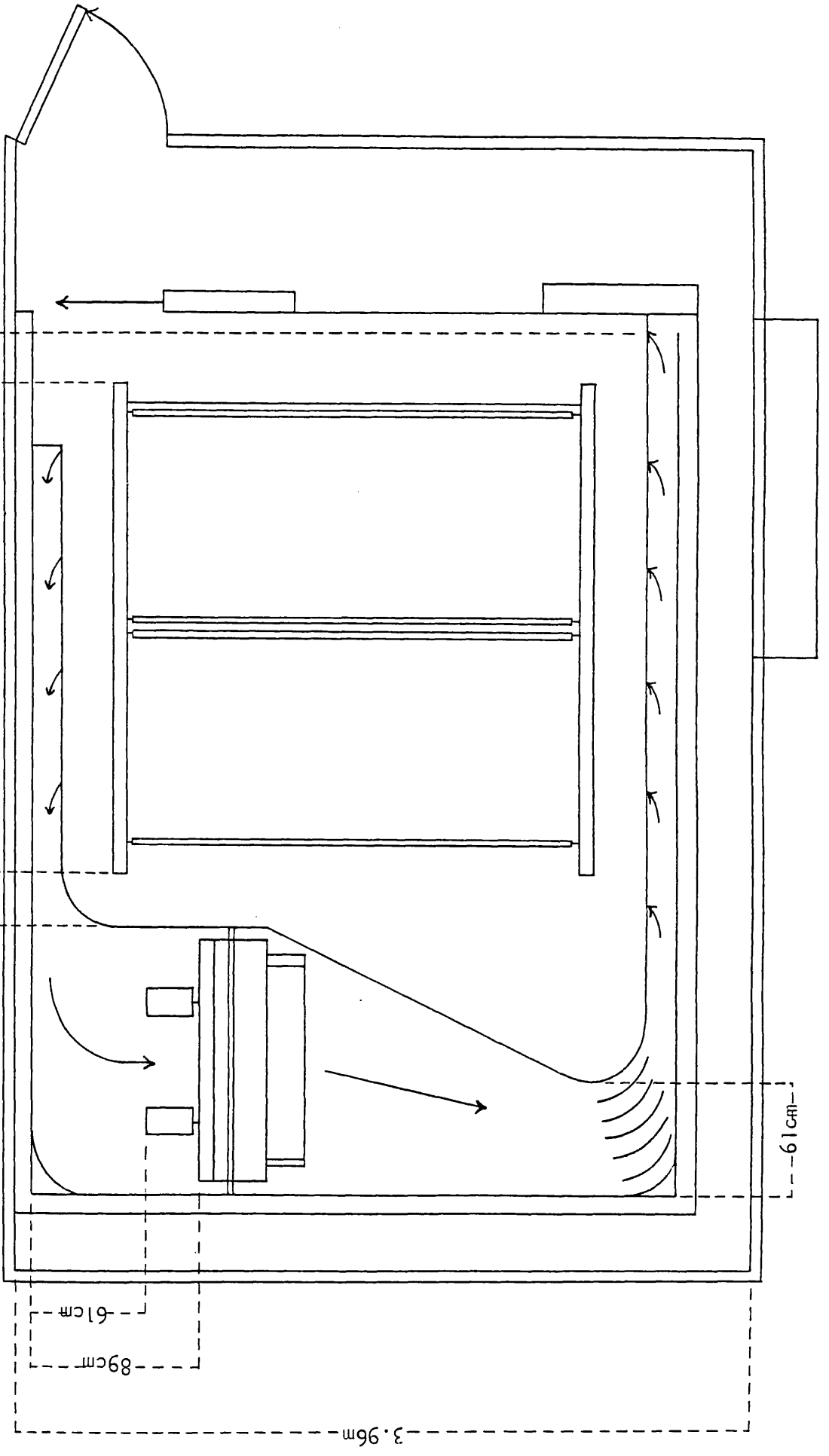


Figure 3.1 The controlled environment growth room (Prestcold)

the lighting system. A perspex panel mounted on a false ceiling approximately 2m high housed the lighting system. This consisted of 75 fluorescent lamps (Philips Daylight, 2.44m 125w/33) situated in 2 banks, one 4cm above the other, and operated under a three phase system with five rows of five lamps to each phase. Tungsten lighting was provided by 4 rows of 5 filament strip lamps (Thorn Opal glass, 60w, 50cm). Together this gave a maximum illumination of 10^3ft candles or 10760 lux at bench height (1m).

Throughout the growth experiments, relative humidity was maintained between 70-75% (UCE 180 floor standing evaporator) along with a 14 hr day and a temperature of 25 ± 1°c (day) and 20 ± 1°c (night). Deionised water was supplied in the growth room (Elgastat C180) for irrigation, the periods of which were also controlled by preset clocks. Regular checks were made on the quality of this water; the Cu, Pb and other trace metal levels never reached more than approximately 0.0006 ppm. Since the growth room featured aluminium as a large part of the internal construction, it is hardly surprising that Al contamination was a serious problem in the subsequent analysis of plant materials. This explains the anomalously high levels of Al found in some plant tissues.

3.2 Methods for the determination of metal binding in plant tissues

Sequential extraction techniques

A variety of solvent extraction techniques for the determination of metal binding in plant tissues have been published (Boroughs and Bonner, 1953) (Bowen et al., 1962) (Diez-Altares and Bornemisza, 1967). However, such schemes serve only as indicators of how the metal is distributed chemically within the plant. The results of extraction studies must be interpreted with caution and cross-checked with other techniques before

any definite conclusions may be drawn.

The extraction of plant material with different solvents can selectively remove various classes of compounds from plant tissues e.g. proteins, α-cellulose, pigments etc. In chapter 8.3 the sequential extraction of plants treated with platinum is reported. The scheme adopted followed that used for the examination of copper distribution in tolerant and non-tolerant species of *Armeria maritima* reported by Mullen (1980), who has reviewed the use of extraction techniques in this area.

The scheme is based on that proposed by Peterson (1969) but with some modifications. The water extracts arising from both the ethanol and the water extraction itself, were investigated further. Soluble proteins were removed as a gel by shaking with trichloromethane and pentan-1-ol (Sevag et al., 1938). In addition to this, the remaining water extracts were treated with acetone to precipitate soluble pectates (Hinton, 1939). The complete scheme is presented in chapter 8, fig. 8.2.

Chromatographic and Electrophoretic techniques

Several workers have examined plant extracts further to determine the metal species present. Soluble metal complexes have been purified, isolated and identified using a range of techniques including ascending, descending and column chromatography; TLC; paper electrophoresis.

Ultracentrifugation

Another technique which has been used to investigate the association of metals with various cell organelles is that of differential ultra-centrifugation (Stocking and Ongun, 1962) (Diez-Altares and Bornemisza, 1967) (Turner, 1970) (Mullen, 1980). The method involves homogenising the fresh plant material which effectively breaks the cell wall, releasing cellular components. A high speed centrifuge can be used to remove selectively various components of different densities: nuclei;

mitochondria; ribosomes.

This technique may be used in combination with autoradiography where the plant is grown in a nutrient solution containing the heavy metal as a radioactive isotope (Rathore et al., 1972) which facilitates its identification in the various cell fractions.

Limitations

The drawbacks and limitations of the experimental methods used in the location and characterisation of the mode of chemical binding of metals in plants have been mentioned briefly already, but must be emphasised. The plant cell is a highly complex and ordered structure which when disturbed may change the binding of heavy metals present.

Since plant material requires a certain amount of preparation prior to analysis, this may result in changes in the character of the metal species present. Once the cell is ruptured, its contents are free to associate with metals they might not meet normally. The contents of the ruptured cell contain a variety of ligands which are available for binding, hence some analyses may lead to erroneous results.

Some metal complexes are only weakly bound and may dissociate during preparation and isolation, therefore, these methods are only useful in isolating and identifying strongly bound metal compounds. The preparation problems are particularly acute when investigating metal species which are kinetically labile. However, the platinum group metals are renowned for being kinetically inert, though the problems outlined still apply, albeit to a lesser extent.

REFERENCES

Boroughs, H. and Bonner, J. (1953), Arch. Biochem. Biophy., 46 279.

Bowen, H.J.M., Cawse, P.A. and Thick, J. (1962), J. Exp. Bot., 13 257.

Diez-Altares, C. and Bornemisza, E. (1967), Plant Soil, 26 175.

Epstein, E. (1972), Mineral Nutrition of Plants: Principles and Perspectives, Wiley, New York.

Hewitt, E.J. (1966), Sand and Water Culture Methods used in the Study of Plant Nutrition, 2nd ed. revised; Commonwealth Bureau of Horticulture and Plantation Crops, East Malling, Tech. Comm. No.22.

Hoagland, D.R. and Arnon, D.I. (1950), The water-culture method for growing plants without soil, Calif. Agric. Expt. Sta. Circ., 347.

Hinton, C.L. (1939), Food Investg. Special Report No.48.

Johnson, C.M., Stout, P.R., Broyer, T.C. and Carlton, A.D. (1957), Plant and Soil, 8 337.

Karin, R.W., Buono, J.A. and Fasching, J.L. (1975), Anal. Chem., 47 (13), 2296.

Knop, W. (1865), Landw. Vers. Sta., 7 93. Cited in Hewitt (1966).

Mahmood, I.M. (1982), Ph.D. Thesis, University of London.

Moody, J.R. and Lindstrom, R.M. (1977), Anal. Chem., 49 (14) 2264.

Mullen, W.A. (1980), Ph.D. Thesis, University of London.

Peterson, P.J. (1969), J. Exp. Bot., 20 863.

Pitt, M.J. (1977), Ph.D. Thesis, University of London.

Sevag, M., Lackman, D.B. and Smolens, J. (1938), J. Biol. Chem., 124 425.

Stocking, C.R. and Ongen, A. (1962), Am. J. Bot., 49 284.

Turner, R.G. (1970), New Phytol., 69 725.

CHAPTER IV

AN INVESTIGATION OF ELECTROTHERMAL ATOMISATION ATOMIC ABSORPTION METHODS FOR THE DETERMINATION OF THE PLATINUM METALS

4.1 Introduction

The application of atomic absorption spectroscopy to determination of the platinum metals was investigated soon after its development as an analytical technique (Lockyer and Hames, 1959) (Strasheim and Wessels, 1963). Today, it has become one of the most widely used methods of determination for the platinum metals. A large number of publications have appeared, dealing with the various applications and interferences (Sen Gupta, 1973). Recently, the analytical chemistry of the platinum metals has been the subject of reviews covering several important techniques including atomic absorption (Van Loon, 1977) (Beamish and Van Loon, 1977).

Recent interest in the medical and industrial significance of the platinum metals (chapter 1.7) has been accompanied by an increasing interest in their determination at low levels. Most studies have been concerned with animal tissues (Pera and Harder, 1977) (Miller and Doerger, 1975), and body fluids (Tillery and Johnson, 1975) (Jones, 1976) (Duffield, 1976). Kothny (1978) has developed a novel spectrophotometric method for the determination of palladium in plants and soils. Because of the poor detection limits of the platinum metals in flame atomic absorption spectroscopy (FAAS), electrothermal atomisation atomic absorption spectroscopy (ETA AAS) is often used for their determination at low levels (Le Roy et al., 1977) (Hendrikx-Jongerius and De Galan, 1976) (Eklund and Holcombe, 1979) (Potter and Waldo, 1979).

One problem with ETA AAS has been the non-atomic absorption from matrix salts volatilised in the graphite furnace. Simultaneous deuterium background correction is now the most widely adopted method for the removal of such errors (Hendrikx-Jongerius and De Galan, 1976). Signal depression when nitrate and sulphate ions are present is another source of error (Eklund and Holcombe, 1979). This last point has some bearing on the preparation of biological samples by the wet ashing method. Dokiya et al., (1975) compared the effects of mineral acids used in the wet digestion of plant material for atomic absorption spectroscopy. They used a Varian Techtron AA1000 instrument equipped with a CRA 63 unit for electrothermal atomisation work, and came to the conclusion that nitric acid was the most suitable medium. Pera and Harder (1977) carried out both wet ashing and analysis of animal tissues in nitric acid solutions. Measurements were made using a Perkin-Elmer 360 spectrometer fitted with the HGA 2100 furnace, and these authors reported that they found no interference from nitrate on signal height when concentrations of nitric acid between 0.0312 and 7.8 mol dm^{-3} were used.

Miller and Doerger (1975) investigated the analysis of tissue samples for platinum also, but converted their nitric acid digestions to chloride for analysis by treatment with sodium chloride and hydrochloric acid. The instrument used for the determinations was a Perkin-Elmer 503 with the HGA 2000 furnace, and with this system Miller and Doerger state: "Several additions of hydrochloric acid are necessary to drive off the nitrate fumes which seem to reduce the sensitivity of the metals." Le Roy et al. (1977) completed wet ashing of animal tissue in a mixture of nitric and perchloric acids, and converted digests to chloride for analysis, although they did not report nitrate interferences in the determination of platinum.

Optimum conditions for ETA AAS of the platinum metals have been reported previously (Adriaenssens and Knoop, 1973) (Everett, 1976). Acid influences on signal heights were investigated using a Perkin-Elmer 403/HGA 70 system (Adriaenssens and Knoop, 1973), and those of nitric acid were found to be more pronounced than those of hydrochloric, with regard to both sensitivity and reproducibility. Everett (1976) reported the furnace parameters for the platinum metals when using a carbon rod atomiser with a Varian Techron AA-5 spectrometer.

In this chapter, methods for the determination of platinum, palladium and rhodium by ETA AAS, as the final step in the analysis of plant material for these metals, are reported. Programme settings have been developed for the Pye Unican SP2900 double beam instrument fitted with the SP9-01 Flameless Atomiser Accessory, and for the Pye Unican SP9 Video Furnace. The SP2900/SP9-01 instrument utilises classical voltage control for atomisation. The SP9 Video Furnace head incorporates an optical temperature feedback facility. The sensitivity of the two methods was investigated using the latter instrument. Acid interferences were investigated using these two systems, and briefly using a Perkin-Elmer 306 together with the HGA 74 furnace.

4.2 Experimental and Results

4.2.1 Instrumentation

The instruments used in this investigation were:

(i) Pye Unican SP2900 double beam spectrophotometer fitted with deuterium background correction and SP9-01 Flameless Atomiser (Plate 4.1). The system was interfaced with a Hewlett-Packard Laboratory Data System (HP 97), which was used to record peak heights, correct for blanks and calculate relative standard

deviations and absolute sensitivities.

(ii) Pye Unican SP9 Video furnace*, equipped with autosampler (FAS1).

(iii) Perkin-Elmer 306 spectrometer† fitted with the HGA 74 furance and equipped also with a Furnace Autosampler Injection facility.

Manual injections (SP2900/SP9-01 system) were made with a 25µl micropipette (Clinipette, Labora Mannheim). All acids were of 'Aristar' grade; solutions were made up with deionised distilled water.

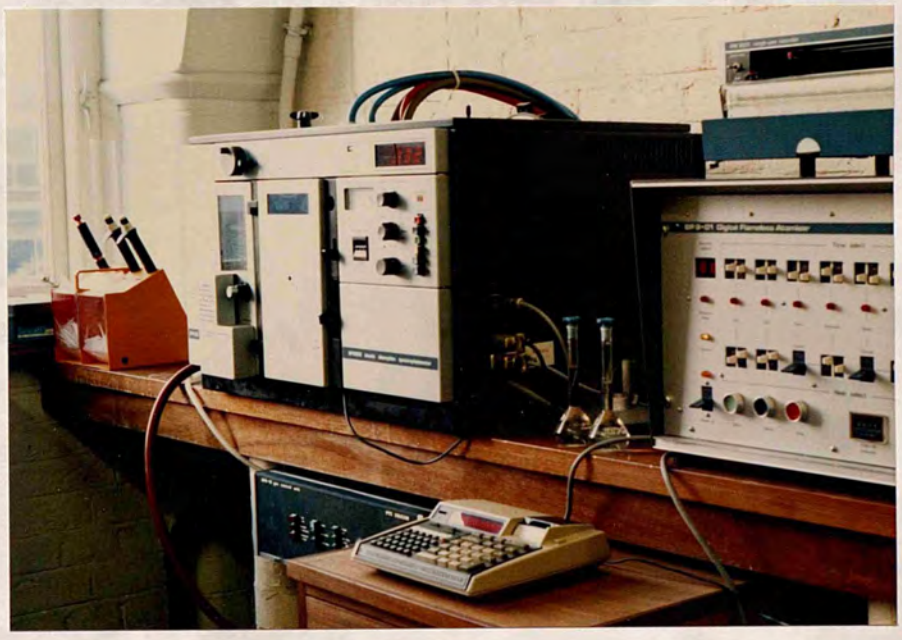


Plate 4.1 Pye Unicam SP2900 Atomic Absorption Spectrophotometer and SP9-01 Flameless Atomiser.

4.2.2 Programme Settings for Platinum and Acid Interferences

Platinum stock solution:- 0.5000g platinum sponge (Johnson Matthey, Specpure) was dissolved in 50ml of aqua regia and evaporated to

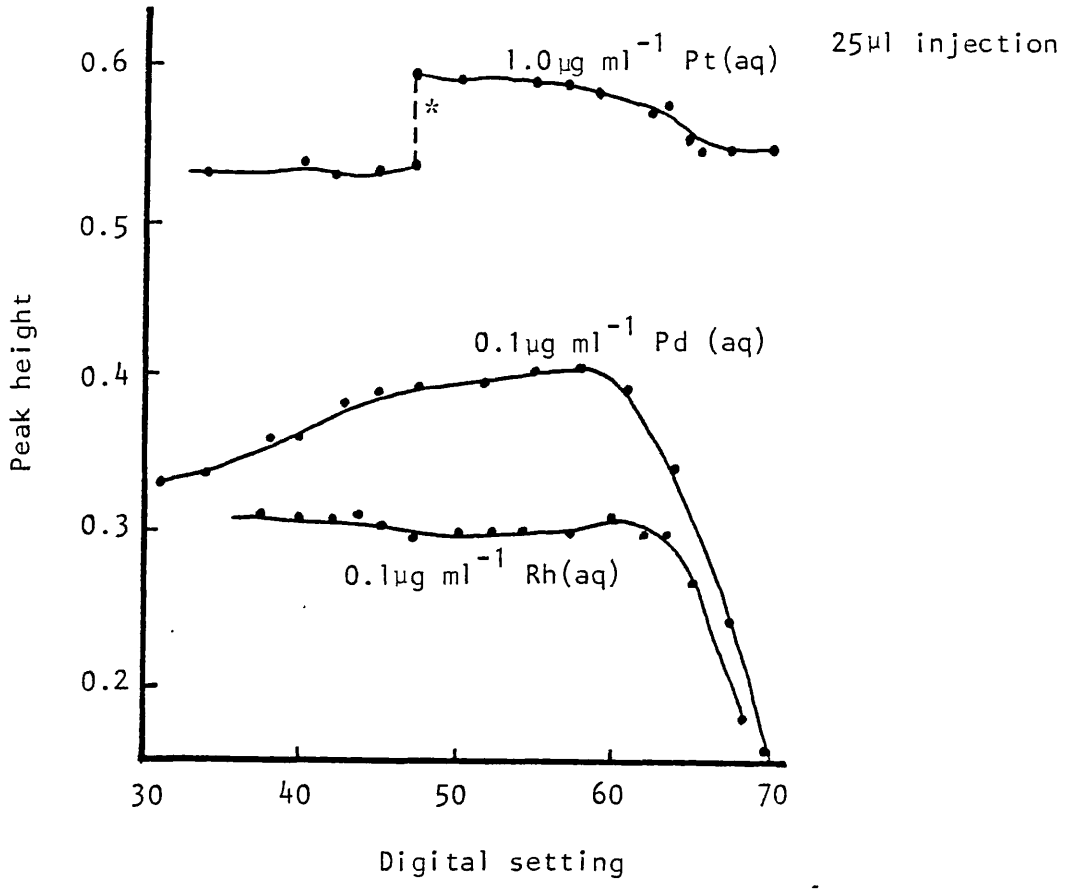
*Facilities provided by the Analytical Dept., Johnson Matthey Research Centre.
†Facilities provided by the Dept. of Clinical Chemistry, Clinical Research Centre, Northwick Park Hospital, Middlesex.

dryness. 25ml of concentrated HCl and 0.5g of NaCl were added to the residue, and the mixture was again evaporated to dryness. The residue was dissolved in 100ml of 50% HCl (v:v) and then diluted to 500ml with water. This stock solution was stored in a polythene bottle.

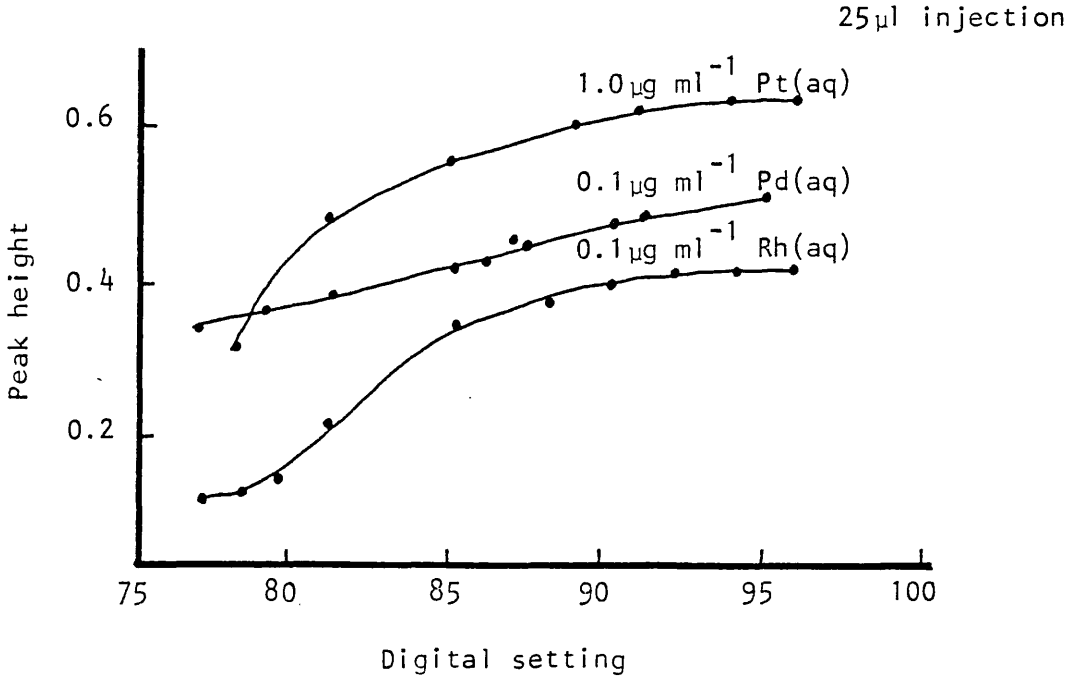
Construction of Atomisation and Ashing Curves, SP2900/SP9-01:- Using an injection of 25µl of a platinum solution (1.0µg ml^{-1}) i.e. 25ng of Pt, a suitable drying time of about 60s at a temperature setting of about 25 ($\sim 240^{o}C$) was established. This was achieved by watching the evaporation of the sample with a dental mirror (care, UV radiation!). The atomisation curve shown in Figure 4.1 was then constructed; similarly, the ashing curve, shown in Figure 4.1, was obtained (see footnotes e and g, Table 4.1). The final settings are shown in Table 4.1.

The Effects of Acids on Peak Heights, SP2900/SP9-01:- The effects of varying concentrations of nitric, sulphuric and hydrochloric acids on peak heights were investigated. The results are shown in Figure 4.2. It can be seen that there is a severe depression of peak height and very poor precision with nitric acid. Sulphuric acid produced a considerable amount of matrix smoke and condensed on the instrument baffles causing damage; its use was therefore abandoned.

Platinum Programme and Acid Effects, PE 306/HGA 74:- The programme settings are shown in Table 4.2. The effects of acids on peak heights were investigated briefly for platinum only. Figure 4.3 shows the effects of varying concentrations of hydrochloric and nitric acids on peak heights. The depression produced by nitric acid is not as severe as in the case of the Pye Unicam SP2900/SP9-01 system, in agreement with previous observations (Le Roy et al., 1977) (Eklund and Holcombe, 1979). In fact, hydrochloric acid gives an enhanced signal with this system (Figure 4.4).



(a) Ashing curves: Pye Unicam SP2900 + SP9-01. (*tube replacement)



(b) Atomisation curves: Pye Unicam SP2900 + SP9-01

Figure 4.1 Ashing and Atomisation curves for the platinum metals:
Pt: λ = 265.95nm; bandpass = 0.2nm
Pd: λ = 247.64nm; bandpass = 0.2nm
Rh: λ = 343.49nm; bandpass = 0.2nm.

Table 4.1 Instrumental guidelines for Pt, Pd and Rh determination using the Pye Unicam SP2900/SP9-01 (ETA AAS).

		Pt	Pd 340.46	Rh
Wavelength, nm		265.95	247.64[a]	343.49
Bandpass, nm		0.2	0.2	0.2
Lamp current, mA		10	5.5	10
Recorder range, mV[b]		10	10	10
Recorder speed, mm min^{-1}		20	20	20
Furnace parameters:[c,d]				
Dry:	time, s	65	60	65
	indicated temperature, °C	300	300	300
	digital setting	28	28	28
Ash:	time, s[e]	45	30	30
	indicated temperature, °C	1375	1375	1800
	digital setting	50	50	58
Delay, s[f]		10	10	10
Atomisation:	time, s[g]	5	5	5
	indicated temperature, °C	3025	2950	3000
	digital setting	93	90	92
Delay, s		10	10	10
Tube Clean:	time, s	5	5	5
	indicated temperature, °C	3175	3175	3175
	digital setting	99	99	99
Delay, s		10	10	10
Tube blank:	time,s	5	5	5
	indicated temperature, °C	3025	2950	3000
	digital setting	93	90	92

[a]The line at 340.46nm is more suitable for samples with high background, and was used subsequently for the analysis of plant samples; [b]Philips PM 8251 pen recorder; [c]Argon flow rate, 3 l min^{-1}; [d]Isothermal heating; [e]Optimum ash times and temperatures are matrix dependent; values given were determined empirically for control plant samples by ensuring the removal of matrix smoke prior to atomisation. This gave an acceptable blank signal during atomisation; [f]peak height read initiated (time, 15s); [g]Atomisation times were determined by observing the peak profile with a fast response recorder and calculating the time taken for the peak to form and decay.

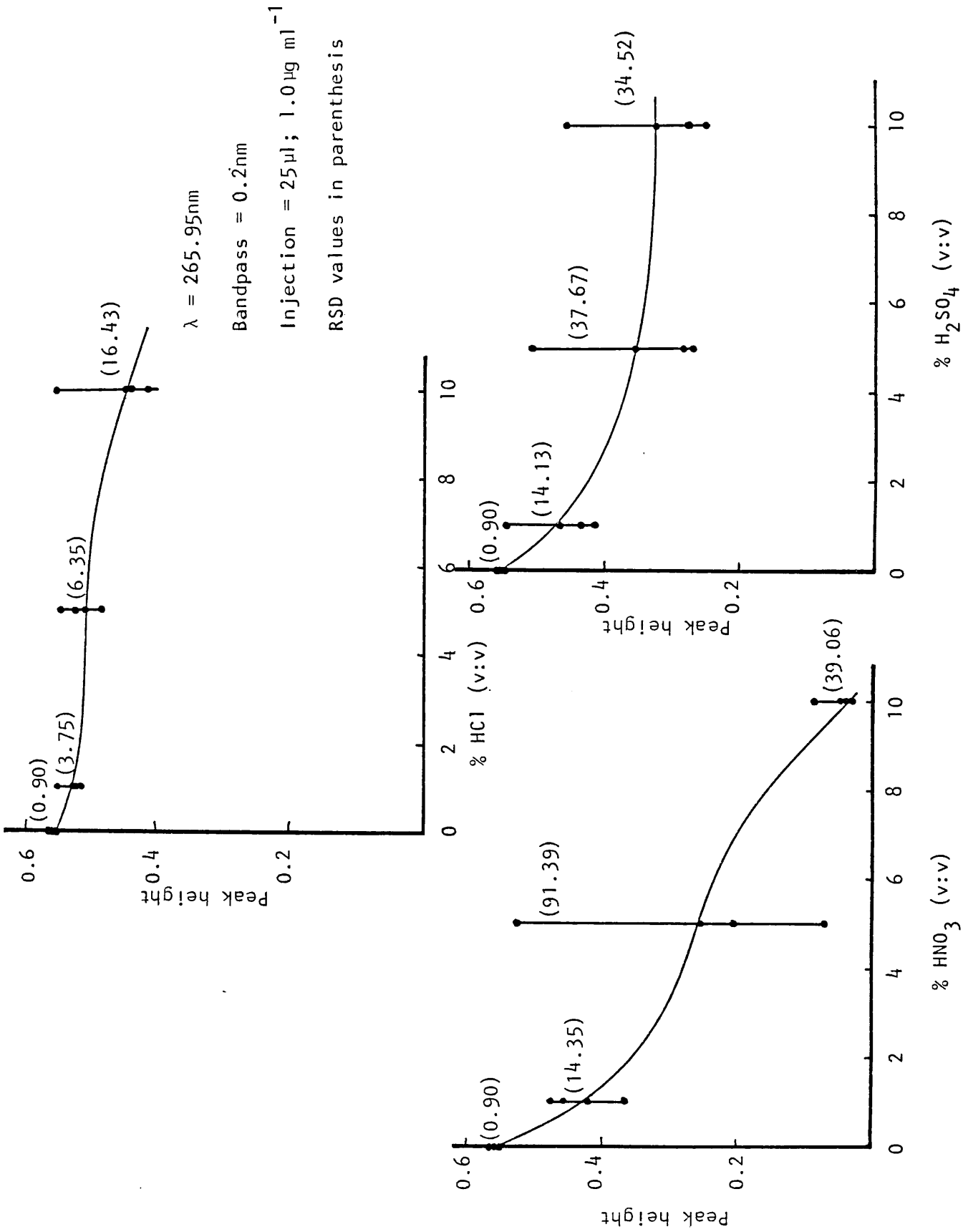


Figure 4.2 Effects of acids on peak heights of platinum in ETA AAS; Pye Unicam SP2900 + SP9-01

Table 4.2 Parameters for the investigation of acid effects on the determination of Platinum by ETA AAS using the Perkin Elmer 306/HGA 74, with Autosampler AS-1[a].

Wavelength, nm			265.95
Bandpass, nm			0.7
Lamp Current, mA			10
Recorder range, mV			10
Recorder speed, cm s^{-1}			10
Furnace parameters:[b]			
Dry:	time, s		60
	indicated temperature, ^{o}C		90
Ash:	time, s		26
	indicated temperature, ^{o}C		1630
Atomisation:[c]	time, s		8
	indicated temperature, ^{o}C		2700
Tube Clean:	time, s		10
	indicated temperature, ^{o}C		2700

[a]Injection, 20µl; [b]adapted from Pera and Harder (1977), and Miller and Doerger (1975); [c]with gas stop facility.

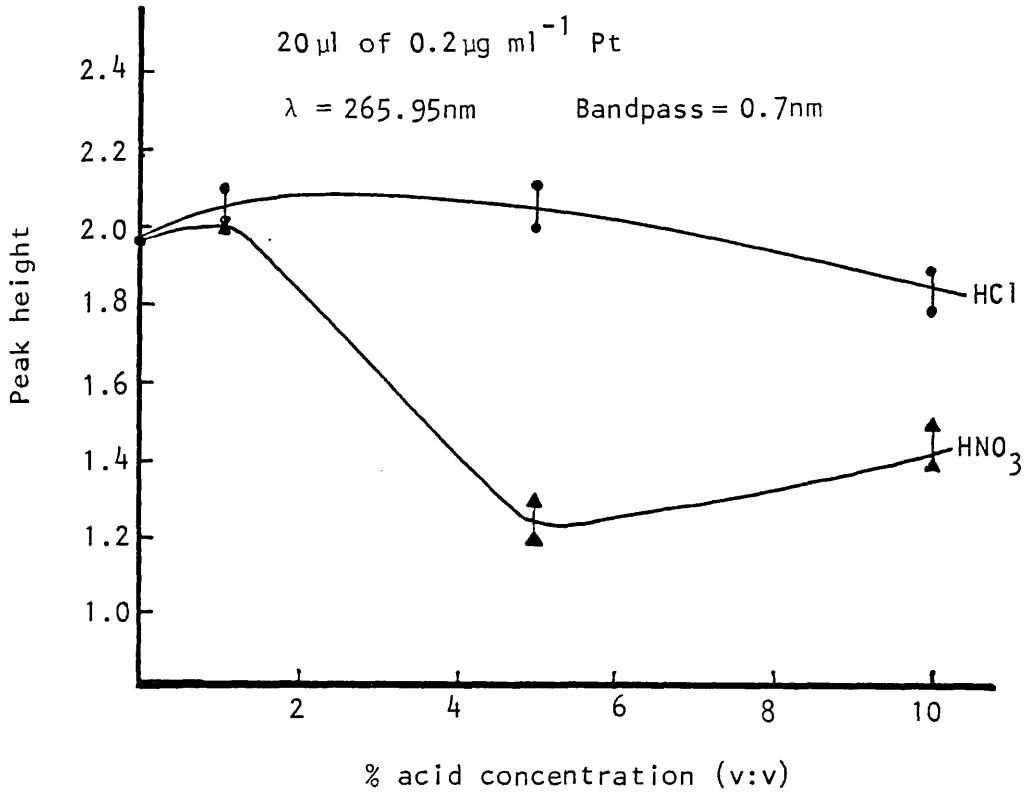


Figure 4.3 Effect of acids on platinum peak heights; P.E. 306 + HGA 74

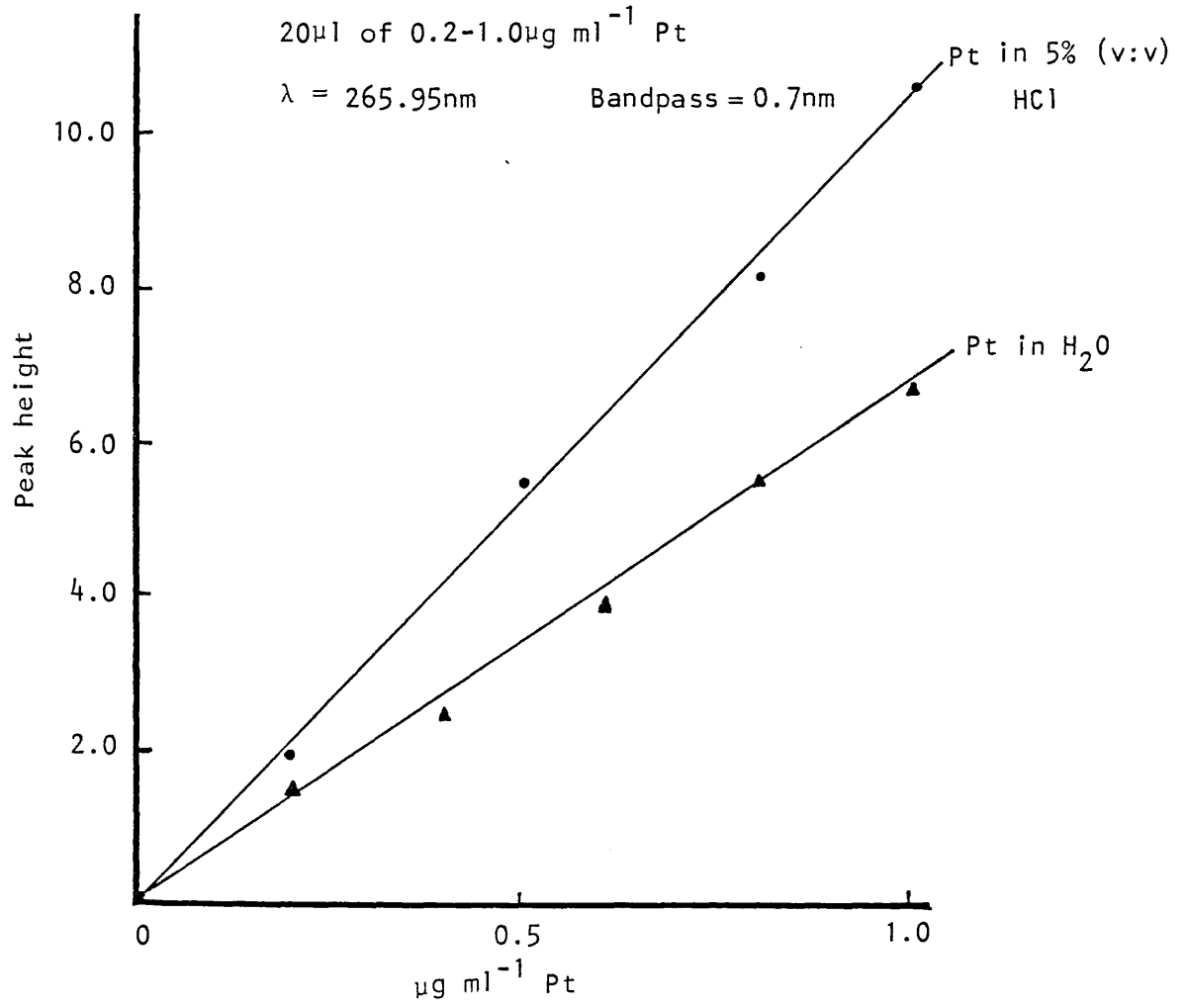


Figure 4.4 Calibration curves for platinum; P.E. 306 + HGA 74

Platinum programme and Acid Effects, SP9 Video Furnace:-

Atomisation curves for platinum were constructed using both temperature control and voltage control facilities (Figure 4.5). With a bandpass of 0.5nm, better sensitivity is found for temperature control than for voltage control, but with a bandpass of 0.2nm little difference is observed. The final programme for the determination of platinum is shown in Table 4.3. The effects of varying concentrations of nitric and hydrochloric acids are shown in Figure 4.6.

4.2.3 Programme Settings for Palladium and Acid Effects

Palladium Stock Solution:- 0.5000g palladium sponge (Johnson Matthey, Specpure) was dissolved in 5ml aqua regia and evaporated to dryness. The residue was dissolved in 2.5ml of concentrated HCl and 12.5ml of water, and finally diluted to 500ml. The solution was stored in a polythene bottle.

Palladium Programme and Acid Effects, SP2900/SP9-01:- Using an injection of 25µl of a palladium solution containing 0.1µg ml^{-1} (2.5ng of Pd), the curves shown in Figure 4.1 were constructed. The final optimum settings for palladium are shown in Table 4.1. The effects of nitric and hydrochloric acids on peak heights are shown in Table 4.4.

An investigation of the sensitivity of the lines at 247.64nm and 340.46nm was made, and some recorder traces for injections of 25µl of a solution of 0.1µg ml^{-1} Pd in 5% HCl are shown in Figure 4.7. The line at 244.79nm is the most intense, but is very noisy, whilst the line at 247.64nm is most sensitive but also noisy. The less sensitive line at 340.46nm appears to be the most suitable for good precision, particularly with plant samples which have high non-specific absorption.

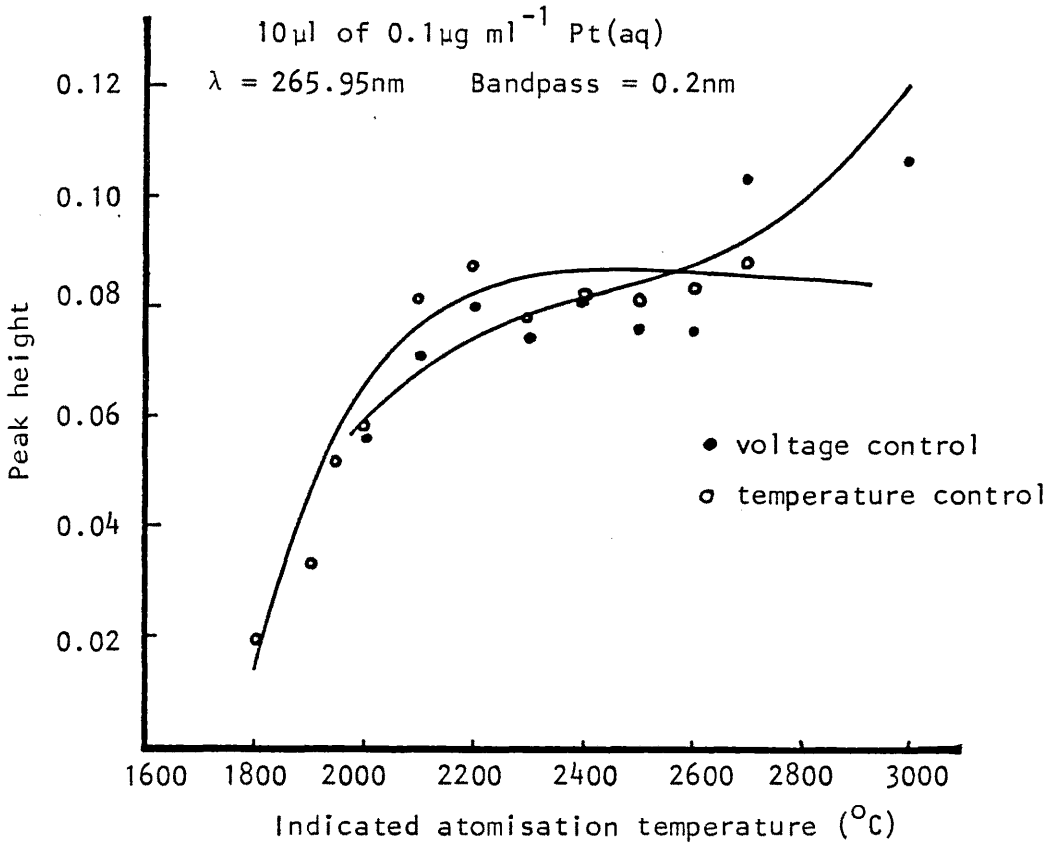


Figure 4.5 Platinum atomisation curves: Pye Unicam SP9 Video Furnace

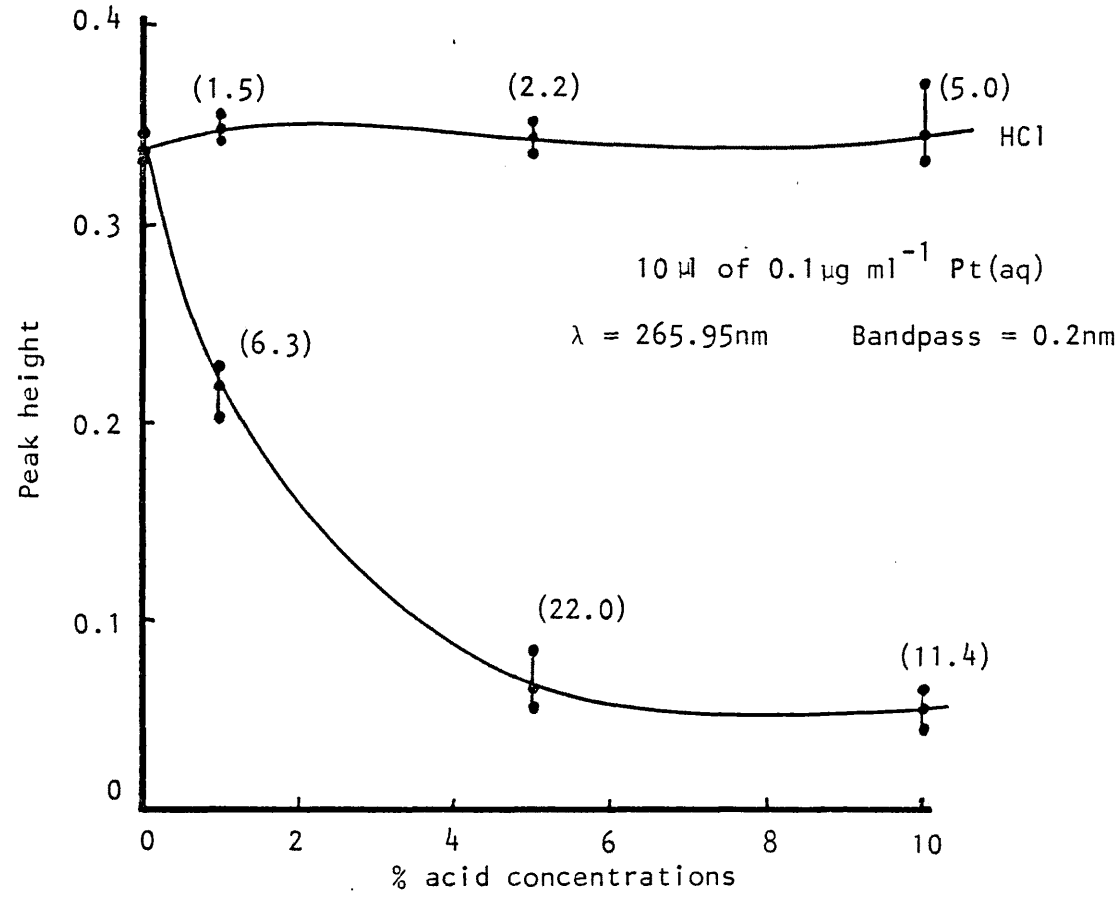


Figure 4.6 Effect of acids on platinum peak heights: Pye Unicam SP9 Video Furnace

Table 4.3 Instrumental guidelines for the determination of Pt, Pd and Rh by ETA AAS using the Pye Unicam SP9 Video Furnace.[a]

			Pt	Pd	Rh
Wavelength, nm			265.95	340.46	343.49
Bandpass, nm			0.2	0.2	0.2
Lamp current, mA			10	8	10
Recorder range, mV			50	50	200
Recorder speed, mm min^{-1}			30	30	30
Furnace Parameters:					
Phase 1:	time, s		35	35	35
	temperature, °C		90	90	90
Phase 2:	time, s		15	15	30
	temperature, °C		1500[b]	1500[b]	1500[b]
Phase 3:[c]	time, s	(TC)	3	3	3
	temperature, °C	(TC)	2700	2600	2600
Phase 3:[c]	time, s	(VC)	5	5	5
	temperature, °C	(VC)	3000	2950	3000
Phase 4:	time, s		10	10	10
Phase 5:	time, s		3	3	3
	temperature, °C		2950	2950	2950

[a]Autosampler programme: volume, 10µl; Resample, 3: Mode, multi.

[b]Ashing temperature dependent on matrix and conditions; for biological samples use of ramping facilities improves analyses.

[c]Phase 3/Atomisation: initiate autozero (AZ), Recorder Control (RC), Peak Timer (PT). TC = temperature control; VC = voltage control.

Table 4.4 Effects of Acids on the Determination of Palladium by ETA AAS

SP9 Video Furnace

0.1 μg ml^{-1} Pd in:	H_2O	1%HCl	5%HCl	10%HCl	1%HNO_3	5%HNO_3	10%HNO_3
Voltage Control; λ = 247.64nm							
Peak Height	-	0.117	0.109	0.139[a]	0.059	0.048	0.092
RSD	-	6.5	6.6	2.6	3.0	8.3	6.7
Peak Area	-	0.1284	0.0606	0.2298	0.0794	-0.057	0.0888
RSD	-	32.1	14.1	21.5	36.7	222.7	44.9
Voltage Control; λ = 247.64nm (repeated)							
Peak Height	0.282	0.400	0.455	0.492	0.333	0.243	0.102
RSD	2.9	4.7	0.3	0.9	3.9	31.9	6.1
Peak Area	0.2877	0.3992	0.4648	0.4797	0.3655	0.2518	0.1670
RSD	6.9	4.2	0.8	2.9	13.0	19.3	11.5
Temperature Control; λ = 340.46nm							
Peak Height	0.217	0.231	0.240	0.222	0.321	0.161	0.136
RSD	1.1	3.6	2.8	2.8	6.4	13.7	3.6
Peak Area	0.1143	0.1214	0.1252	0.1200	0.1486	0.0460	0.0088
RSD	11.9	14.9	8.9	14.0	5.2	64.3	90.8

SP2900/SP9-01

0.1 μg ml^{-1} Pd in:	H_2O	1%HCl	5%HCl	10%HCl	1%HNO_3	5%HNO_3	10%HNO_3
Voltage Control; λ = 247.64nm							
Peak Height	0.384	0.392	0.364	0.375	0.203	0.063	0.084
RSD	1.45	1.03	4.73	2.27	20.06	15.31	28.33

[a]New tube. (this investigation was repeated)

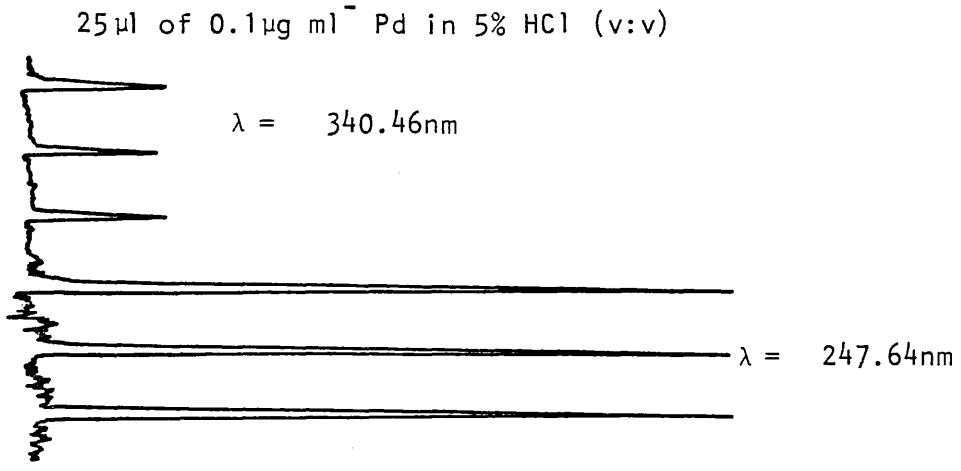


Figure 4.7 Variation in peak height with wavelength for palladium in ETA AAS (Pye Unicam SP2900+SP9-01)

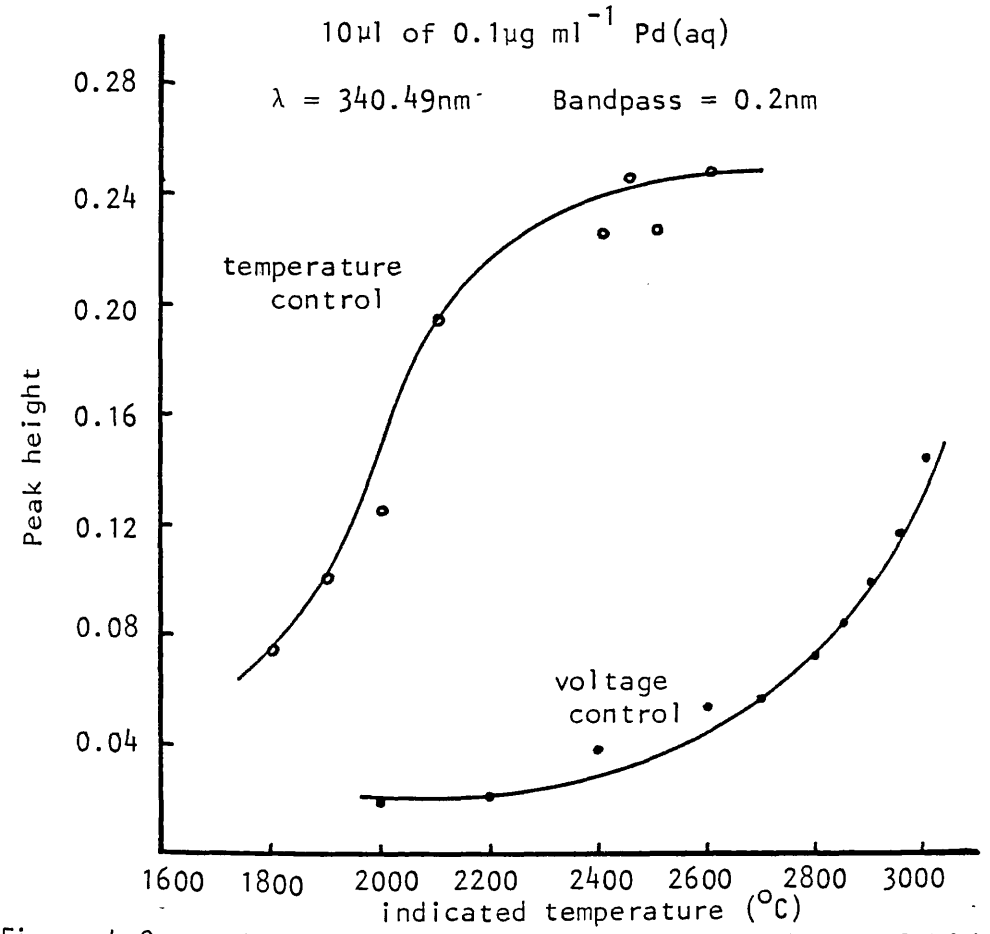


Figure 4.8 Palladium atomisation curves: Pye Unicam SP9 Video Furnace

Palladium Programme and Acid Effects, SP9 Video Furnace:- Palladium atomisation curves constructed using both temperature and voltage control are shown in Figure 4.8. It can be seen that better sensitivity is obtained using the temperature control facility. The final optimum furnace parameters are shown in Table 4.3. The effects of acids on peak heights are shown in Table 4.4.

4.2.4 Programme Settings for Rhodium and Acid Interferences

Rhodium Stock Solution:- 1.1001g rhodium(III)chloride (Johnson Matthey, Specpure) was dissolved in deionised distilled water and diluted to 500ml. The rhodium assay on this sample of rhodium(III)chloride was 41.45% Rh; the solution was thus 1000mg l^{-1} in Rh; the concentration was checked by gravimetric analysis.

Rhodium Programme and Acid Effects, SP2900/SP9-01:- The curves shown in Figure 4.1 were constructed using an injection of 25µl of a rhodium solution containing 0.1µg ml^{-1} Rh. The final optimum settings for rhodium are shown in Table 4.1. The effects of nitric acid and of hydrochloric acid on peak heights are shown in Figure 4.9.

Rhodium Programme SP9 Video Furnace:- Rhodium atomisation curves using both temperature and voltage controls are shown in Figure 4.10.

4.2.5 Programme settings for Iridium, SP9 Video Furnace

Atomisation and ashing curves for iridium were constructed from 10µl injections of 0.5µg ml^{-1} iridium solutions (Johnson Matthey Research Centre) in the SP9 Video furnace. These curves and other iridium details are shown in figure 4.11.

4.3 Discussion

Optimum conditions for the determination of the platinum metals by electrothermal atomisation atomic absorption spectroscopy have been

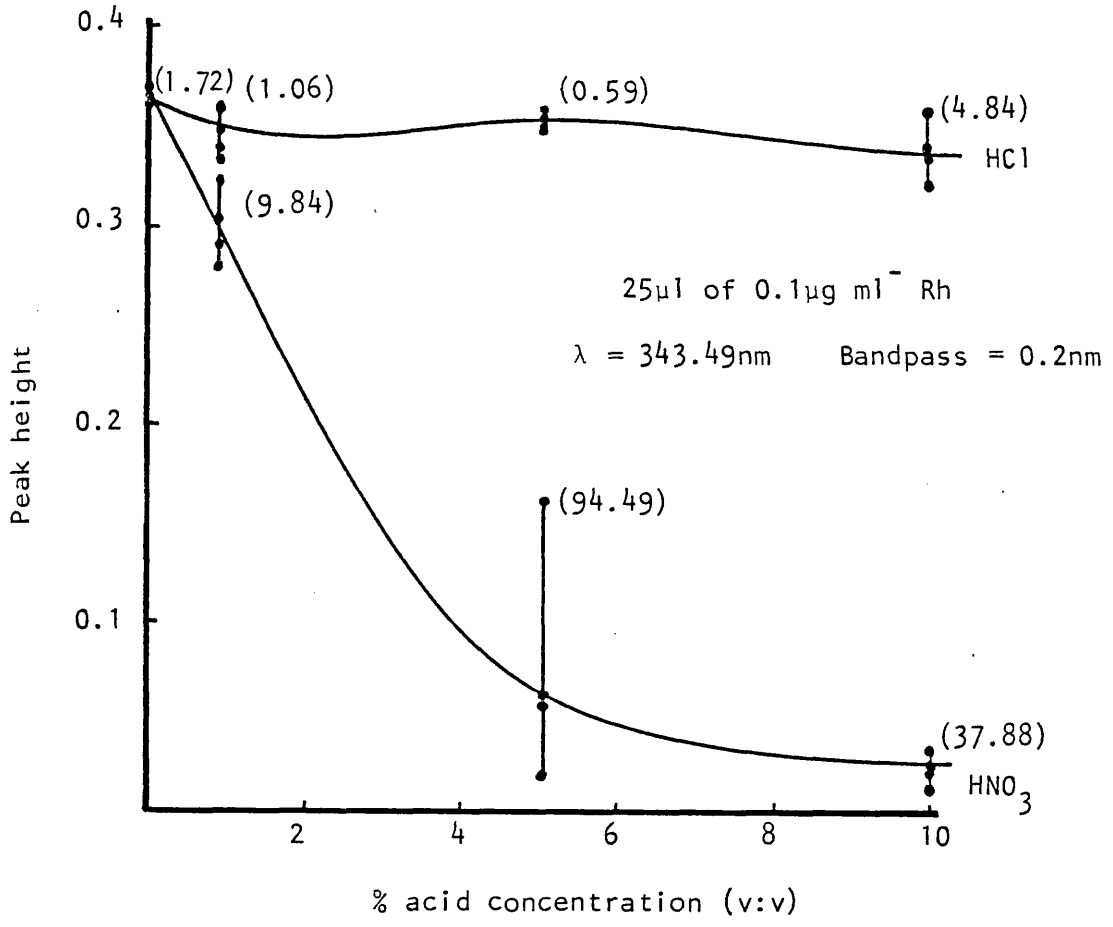


Figure 4.9 Effect of acids on rhodium peak heights; Pye Unicam SP2900 + SP9-01.

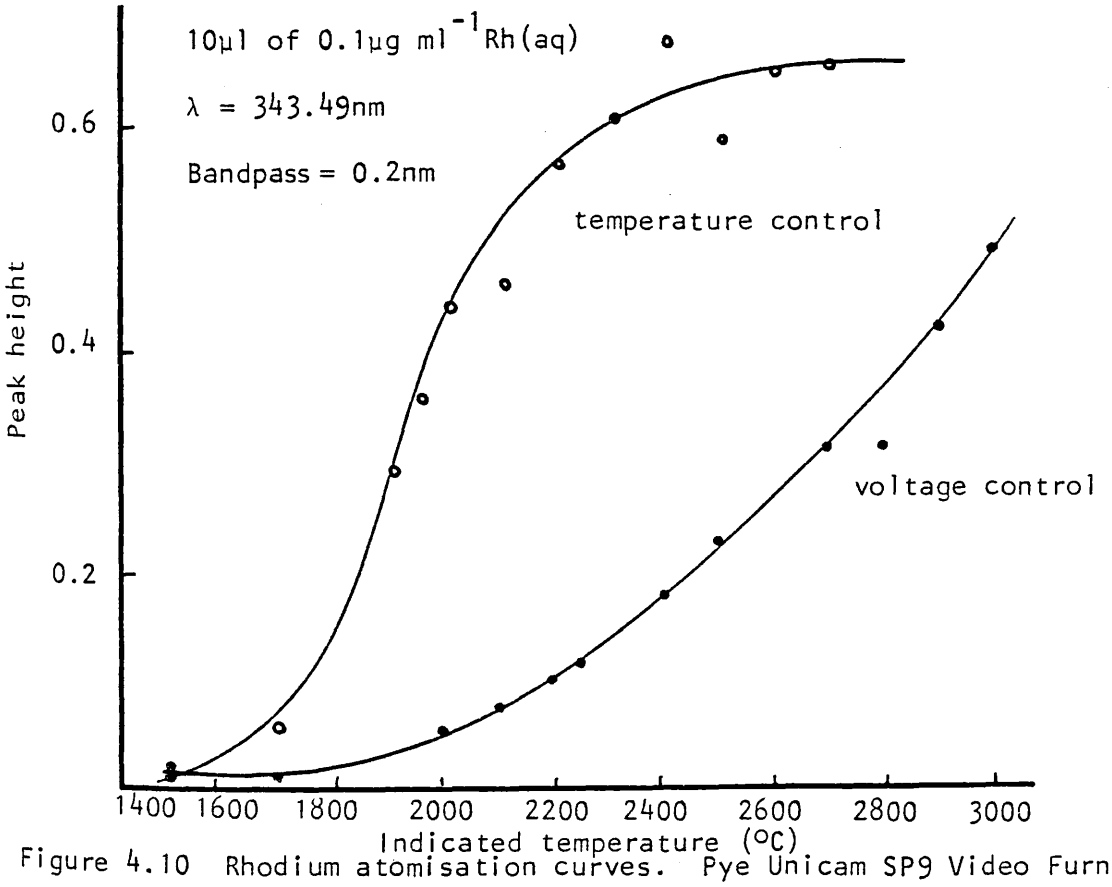
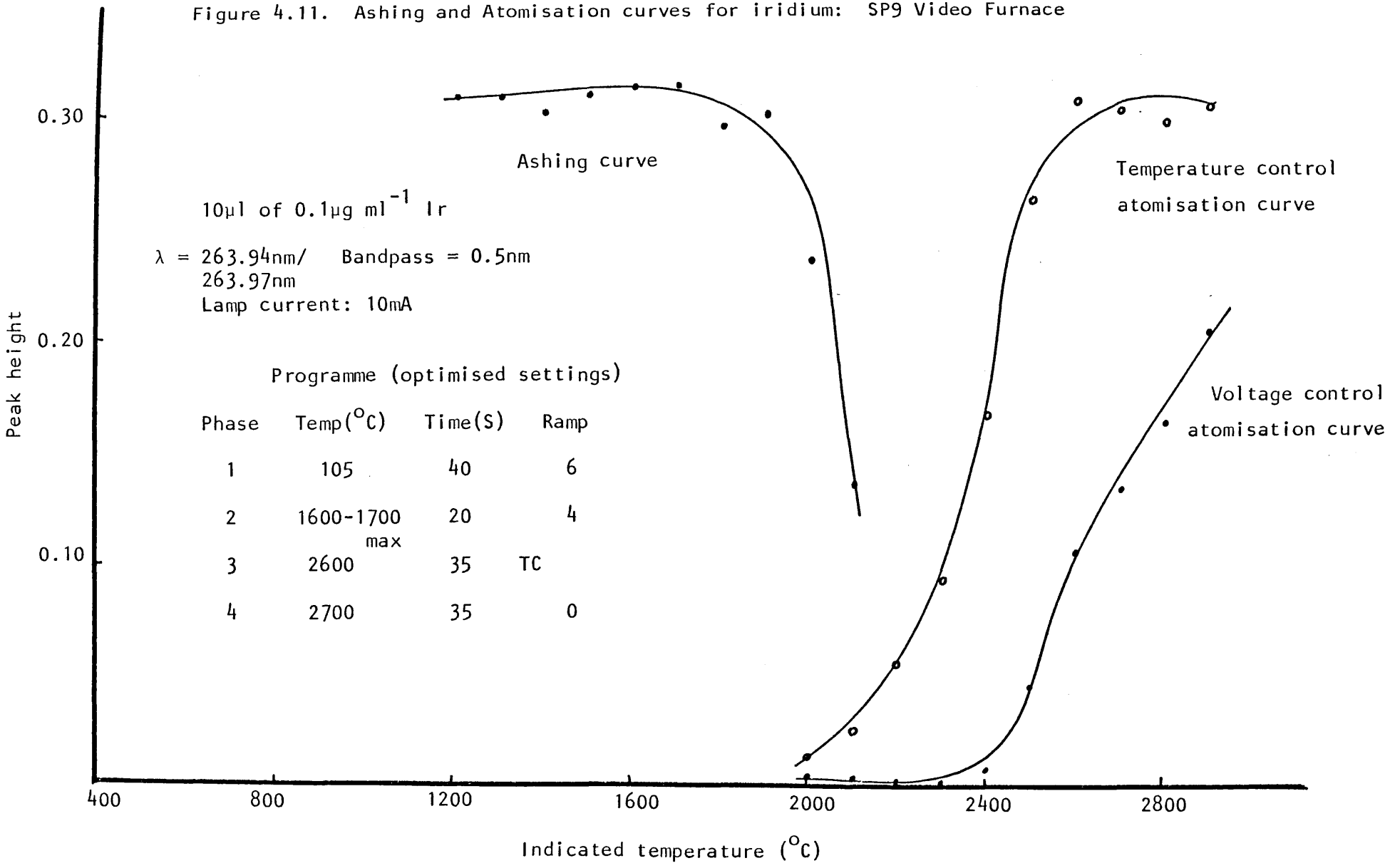


Figure 4.10 Rhodium atomisation curves. Pye Unicam SP9 Video Furnace

Figure 4.11. Ashing and Atomisation curves for iridium: SP9 Video Furnace



reported by many workers, most recently by L'vov et al. (1979), Fishkova and Vilenkin (1978). There is however, some disagreement on methods of sample preparation, furnace parameters and interferences, which appears to result from the variety of instruments used. It would seem that it is the design and structure of the electrothermal atomisation units which are responsible for the differences.

Early investigations of ETA AAS were carried out using a heated graphite rod. Later graphite tubes became more popular, allowing for larger sample volumes. At the same time a number of problems were introduced, some of which appear to depend on tube geometry. Thus the optimum conditions for platinum metal determination for one instrument cannot be adopted directly for another instrument, and in the case of biological samples, for another matrix. Such furnace parameters can serve only as useful guidelines and must be determined for each instrument and each type of matrix.

Incorrect dry phase settings can lead to low precision. The drying time and optimum temperature have been found to vary considerably from sample to sample, and also on the age and condition of the tube (Figure 4.1). The use of pyrolitically coated tubes, whilst improving sensitivity during atomisation for some metals, can result in poor drying characteristics. For example, violent boiling with subsequent analyte loss in the gas stream may occur. Better detection limits have been reported for pyrolitically coated tubes in the determination of platinum metals (L'vov et al., 1979). Since the graphite tubes vary in size and shape, it is not surprising that the deposition and subsequent spread of the sample will vary (Figure 4.12) (Littlejohn and Ottaway, 1977). This will influence the number of atoms released as an atomic vapour into the beam, due to the temperature profile of the tube. The flow of argon within the tube

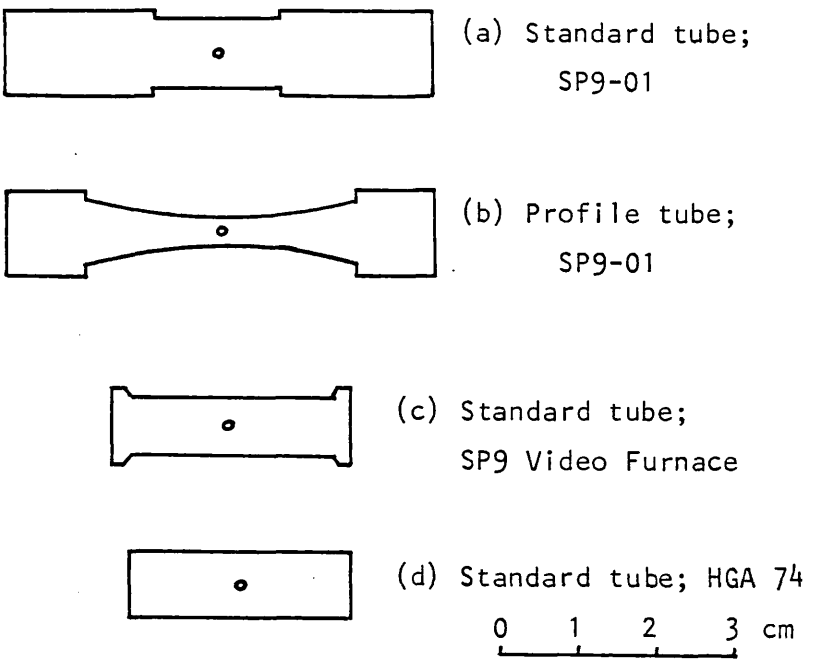


Figure 4.12 Graphite tubes for ETA AAS.

can also affect the transient signal; removal of the cap from the glass chimney enhanced sensitivity for platinum with the SP2900/SP9-01 system.

When using high temperatures for atomisation, non-atomic absorption is considerable. The tube wall emission is reduced by the use of a small bandpass, thus throughout this work, a bandpass of 0.2nm was used. Everett (1976) however, reduced the non-atomic absorption by atomising at a lower temperature, and increasing the bandpass, the lower temperature increasing the life of the carbon rods.

The lifetime of the carbon tubes are also improved by the use of temperature control during atomisation. Unlike voltage control, where atomisation is achieved by the application of a preset voltage across the tube for a preset time, the temperature control facility applies the maximum voltage until a preset temperature is reached. A temperature feedback device is incorporated which can switch off the power once that point is reached. With the SP9 Video Furnace, this was found to increase

the sensitivity for platinum, palladium rhodium and iridium at lower atomisation temperatures, thus bringing about an improvement in tube life. The advantages of temperature control have been discussed by Dymott (1981). For palladium (Figure 4.8) and rhodium (Figure 4.10) this effect is significant, and for platinum using a bandpass of 0.5nm (Morton, 1981) but for a bandpass of 0.2nm no significant difference was observed (Figure 4.5).

For the determination of palladium, there is a choice of lines; that at 244.79nm was very noisy, whilst that at 247.64nm is sensitive, but was noisy also. The palladium spectrum in the region 244-247nm is reported to be complex (Christian and Feldman, 1970) and interferences between lines may occur (Whiteside, 1976). The more stable line at 340.46nm is suitable for samples with high background such as plant digests. This line was also used with the SP9 Video Furnace.

Effects of Mineral Acids

It was found that platinum standards in nitric acid solution when measured using the Pye Unicam SP2900 fitted with the SP9-01 furnace, gave severe depression of peak height and poor precision, especially at the 5% HNO_3 level; with 10% HNO_3 the RSD was better only because of the very severe depression of peak height. The same platinum standards gave a reasonable calibration curve using a Perkin Elmer 360 with HGA 76 graphite furnace. (These last results are not reported in the results section, since only one calibration curve was carried out.) Similarly, although some depression of the signal was encountered, it was possible to use the Perkin Elmer 306 with the HGA 74 furnace (Figure 4.3). It can be seen from Figure 4.6 that there is also severe depression of signal height using the Pye Unicam SP9 Video Furnace with platinum in nitric acid media. Figures 4.2, 4.3 and 4.6 show that 5% hydrochloric

Table 4.5 Absolute Sensitivities[a] of the Platinum Group Metals in ETA AAS

A. Pye Unicam SP2900/SP9-01

	Pt	Pd[b]	Pd[c]	Rh
Concentration of injection, μg ml^{-1}	0.1	0.1	0.1	0.1
Volume of sample injection, μl	25	25	25	25
Voltage Control				
Mean absorbance	0.065	0.071	0.359	0.351
Absolute sensitivity[a], pg	168	154	30	31

B. Pye Unicam SP9 Video Furnace

	Pt[d]	Pt[e]	Pd[b]	Rh
Concentration of injection, μg ml^{-1}	0.1	0.5	0.1	0.1
Volume of sample injection, μl	10	10	10	10
Voltage Control				
Mean absorbance	0.107	0.350	0.146	0.492
Absolute sensitivity[a], pg	41	62	30	9
Temperature Control				
Mean absorbance	0.089	0.444	0.249	0.651
Absolute sensitivity[a], pg	49	49	18	7

[a]The absolute sensitivity is taken as that mass of an element which will give a peak absorbance value of 0.00436A (which is equivalent to peak of 1% absorption); Dymott (1981).

$$\text{i.e.} \quad \text{Sensitivity} = \frac{0.00436 \times \text{injected mass}}{\text{measured absorbance}}$$

For example, for palladium:

$$\text{Sensitivity} = \frac{0.00436 \times (2.5 \times 10^{-9})}{0.362} \text{ g}$$

$$= 30 \times 10^{-12} \text{g} \ (30\text{pg})$$

[b]λ = 340.46nm

[c]λ = 247.64

[d]Bandpass: 0.2nm

[e]Bandpass: 0.5nm

acid is a suitable medium for the determination of platinum using all three instruments. Hydrochloric acid in some cases gives an enhanced signal (Figure 4.4). This pattern of results was repeated for both palladium (Table 4.4) and rhodium (Figure 4.9) using both the SP2900/SP9-01 and the SP9 Video Furnace.

The absolute sensitivities for the determination of the platinum metals by ETA AAS are set out in Table 4.5.

REFERENCES

Adriaenssens, E. and Knoop, P. (1973), Anal. Chim. Acta, 68 37.

Beamish, F.E. and Van Loon, J.C. (1977), Analysis of Noble Metals: Overview and selected methods, Academic Press, London.

Christian, G.D. and Feldman, F.J. (1970), Atomic Absorption Spectroscopy: Applications in Agriculture, Biology and Medicine, Wiley, New York.

Dokiya, Y., Yamamoto, S. and Fuwa, K. (1975), Spect. Lett., 8, 759.

Duffield, F.V.P. (1976), Environ. Health Perspect., 15 131.

Dymott, T.C. (1981), Atomic Absorption with Electrothermal Atomisation, 2nd edn. Pye Unicam Ltd., Cambridge.

Eklund, R.H. and Holcombe, J.A. (1979), Anal. Chim. Acta, 110 29.

Everett, G.L. (1976), Analyst, 101 348.

Hendrikx-Jongerius, C. and De Galan, L. (1976), Anal. Chim. Acta, 87 259.

Janouskova, J., Nehasilova, M. and Sychra, V. (1973), Atomic Abs. Newsl. 12 161.

Jones, A.H. (1976), Anal. Chem., 48 1472.

Kothny, E.L. (1978), Mikrochim. Acta, 1 425.

Le Roy, A.F. (1975), Environ. Health Perspect., 10 73.

Le Roy, A.F., Whehling, M.L., Sponseller, H.L., Friauf, W.S., Solomon, R.E., Dedrick, R.L., Litterst, C.L., Gram, T.E., Guarino, A.M. and Becker, D.A. (1977), Biochem. Med., 18, 194.

Littlejohn, D. and Ottaway, J.M. (1977), Analyst, 102 553.

Lockyer, R. and Hames, G.E. (1959), Analyst, 84 385.

L'vov, B.V., Pelieva, L.A., Mandrazlu, E.K. and Kalinin, S.K. (1979), Zavod. Lab., 45 (12) 1098.

Miller, R.G. and Doerger, J.U. (1975), Atomic Abs. Newsl. 14 66.

Minski, M.J. (1981), private communication.

Morton, S.F. (1981), private communication.

Pera, M.F. and Harder, H.C. (1977), Clin. Chem., 23 1245.

Potter, N.M. and Waldo, R.A. (1979), Anal. Chim. Acta, 110 29.

Sen Gupta, J.G. (1973), Miner. Sci. Eng., 5 (3) 207.

Strasheim, A. and Wessels, G. (1963), J. Appl. Spectrosc., 17 65.

Tello, A. and Sepulveda, N. (1977), Atomic Abs. Newslt., 16 67.

Tillery, J.B. and Johnson, D.E. (1975), Environ. Health Perspect., 12 19.

Towndrow, E.G. (1981), private communication (unpublished results using a Pye Unicam SP1900 with a Massmann-Beckman Furnace).

Van Loon, J.C. (1977), Pure and Appl. Chem., 49 1495.

Vishkova, N.L. and Vilenkin, V.A. (1978), Zh. Anal. Khim., 33 (5) 897.

Whiteside, P.J. (1976), Atomic Absorption Data Book, 2nd ed., Pye Unicam Ltd., Cambridge.

CHAPTER V

THE APPLICATION OF INSTRUMENTAL NEUTRON ACTIVATION ANALYSIS TO PLANT MATERIALS CONTAINING THE PLATINUM METALS

5.1 Introduction (cf. ch.2.3)

Neutron activation methods have been applied successfully to the determination of the platinum metals in a wide range of materials. Many of the methods described in the literature involve radiochemical separation which removes any interfering matrix. The separation may include distillation, solvent extraction or even ion exchange methods (Crocket, *et al.*, 1968) (Ahmad, *et al.*, 1977) (Nadkarni and Morrison, 1974) (Samadi, *et al.*, 1978) (Parry, 1980).

Instrumental neutron activation analysis (INAA) for the determination of iridium in ores has been reported (Le Roux, *et al.*, 1974), but for the determination of platinum metals in biological materials it has been little used. Several environmental studies have reported the determination of platinum metals in natural materials using neutron activation, but with a separation step included (Schutyser, *et al.*, 1977) (Gilbert, *et al.*, 1977).

Caramella-Crespi *et al.*, (1974) reported the platinum and iridium contents of NBS-SRM 1571 Orchard leaves using radiochemical NAA. These were given as: 0.015(±0.003) μg g^{-1} Ir and 1.2(±0.3) μg g^{-1} Pt. Recent attempts have been made to improve the detection limits of the platinum metals in biological materials further still (Zeisler and Greenberg, 1981) (Valente *et al.*, 1981).

In neutron activation methods, the various irradiation conditions are fixed in accordance with the nuclear properties of the various

isotopes of interest. The theoretical principles of this technique have been discussed fully elsewhere (chapter 2.3). Nuclear data concerning the platinum metals and the nuclear reactions utilised for their determination by INAA are presented in Table 5.1.

In this thesis, investigations into the application of INNA to platinum metal determination in plant tissues, were carried out on two sites. Preliminary work was conducted by ICI Radioisotope Services at Billingham. As a follow up to this, more detailed investigations were carried out at the University of London Reactor Centre.

5.2 Preparation of plant materials containing the platinum metals

There is no NBS Biological 'Standard Reference Material' containing certified levels of the platinum metals for comparing analytical techniques. For this reason, a number of plant materials were specially prepared, each of which contained one of the platinum metals. The free floating aquatic plant water hyacinth (*Eichhornia crassipes* (MART) Solms), was grown in half-strength nutrient solution containing one of the platinum metals as a soluble complex. Each metal was supplied at two concentrations, designated as 'HI' and 'LO'. The plants were grown for two weeks and then harvested, washed, dried and homogenised. In this way a number of representative samples, hereafter referred to as Standard Water hyacinths, were obtained. Details of the hydroponic method and the preparation of dried plant samples are reported in chapter 6 where the results of analysis are considered more fully. Preliminary studies were carried out with these samples using the TRIGA nuclear reactor at ICI Billingham.

5.3 Investigations of INAA using the TRIGA reactor

Approximately 50mg of the dried plant material or plant ash was irradiated in the TRIGA reactor, at ICI Billingham, at a thermal neutron

Table 5.1 Nuclear data for the platinum group metals

Element	Nuclide	Isotopic Abundance %	Nuclear reaction utilised	Thermal neutron cross section σ (barns)	Activated Isotope Nuclide	Half Life	Decay mode	γ,keV
Pd	^{108}Pd	26.7	$^{108}Pd(n,\gamma)^{109m}Pd$	0.19	^{109m}Pd	4.75m	IT	188.9
	^{106}Pd	27.3	$^{106}Pd(n,\gamma)^{107m}Pd$	0.013	^{107m}Pd	21.3s	IT	215.0
Rh	^{103}Rh	100.0	$^{103}Rh(n,\gamma)^{104m}Rh$	11	^{104m}Rh	4.34m	IT,β^-	51.5
			$^{103}Rh(n,\gamma)^{104}Rh$	134	^{104}Rh	42.3s	EC,β^-	555.8
Ir	^{191}Ir	37.3	$^{191}Ir(n,\gamma)^{192}Ir$	540	^{192}Ir	74.4d	EC,β^-	316.5
Os	^{190}Os	26.4	$^{190}Os(n,\gamma)^{191}Os$	3.9	^{191}Os	15.4d	β^-	129.4
Ru	^{102}Ru	31.6	$^{102}Ru(n,\gamma)^{103}Ru$	1.3	^{103}Ru	39.4d	β^-	497.1
Pt	^{198}Pt	7.2	$^{198}Pt(n,\gamma)^{199}Pt$	3.7	^{199}Pt	30.8m	β^-	(74...960
			$^{199}Pt \xrightarrow{\beta^-} {}^{199}Au$		^{199}Au	3.15d	β^-	158.37 208.20

after Lederer and Shirley (1978)

flux of 3×10^{12}n $cm^{-2}sec^{-1}$. TRIGA is a 'swimming pool' type research reactor, capable of achieving a steady 250kW as thermal power. The times of irradiation, decay and counting depend on which element is being determined. Table 5.2 gives the conditions used with TRIGA. The activated samples were counted using a Nuclear Data Inc. 6610 multi-channel analyser, together with a lithium drifted germanium detector of resolution 2.00keV/ch at the 1.322 MeV photopeak (Ortec Inc VIP Series, Coaxial Detector). Platinum metal concentrations were calculated by comparing peak areas with those from irradiated chemical standards. The results of the various determinations are given in Table 5.3. A control plant sample grown and prepared under identical conditions, but without any added platinum metals was examined too. Interfering activity, in the control samples, was found at 158keV and was measured over a period of two weeks. The results indicated that the interference was not due to one particular nuclide but possibly several (Table 5.7, pg.135).

5.4 Investigations of INAA using the CONSORT MkII reactor

Further investigations into the use of INAA to determine the platinum metals in plant materials, were carried out at the University of London Reactor Centre. The 'Consort' Mark II is a 'swimming pool' type research reactor which can achieve a maximum thermal power of 100kW. The reactor fuel elements are enriched with uranium - 235 (80%) and are clad in aluminium. Light water acts as both coolant, moderator and reflector. An overall plan of the reactor and its experimental facilities is given in figures 5.1 and 5.2.

Some of the irradiation facilities available together with their respective thermal and epithermal fluxes are given in Table 5.4.

Irradiation Conditions (Reactor Triga)

Nuclide	Half life	γ energy,keV	t irradiation	t decay	t count
^{104m}Rh	4.34m	51.4	0.3m	2-3m	5m
^{109m}Pd	4.69m	188.9	0.3m	1-1.5m	5m
^{192}Ir	74.4d	316.5	30m	3-6w	15h
^{191}Os	14.6d	129.4	30m	2-3w	15h
^{103}Ru	38.9d	497.0	30m	3-6w	15h
^{199}Au[a]	3.15d	158.37	60m	4-5d	20h
		208.20			

[a] from $^{198}Pt(n,\gamma)^{199}Pt$

$\downarrow \beta^-, t_{\frac{1}{2}}$ 30.8m

$^{199}Au \xrightarrow[t_{\frac{1}{2}}\ 3.15d]{\gamma\ 158,208keV} {}^{199}Hg$

Table 5.2

INAA of Platinum Group Metals in Plants (TRIGA)

All ppm ($\mu g\ g^{-1}$) dry wt

Sample	Date of result	Pt	Rh	Os	Ir	Pd	Ru
Ru L0	3.9.80						<0.7 (<0.7,<0.7)
Pd L0	3.9.80					<20 (<20,<20)	
Os L0	3.9.80			5.0 (4.8,5.2)			
Rh L0	3.9.80		5.30 (5.38,5.25)				
Ir L0	3.9.80				1.45 (1.48,1.42)		
[a]Pt $L0_1$	5.6.80	14.2					
[b]Pt $L0_1$	3.9.80	44					
Pt $L0_2$	3.9.80	41					
[c]Pt $L0_1$	5.11.80	19					
Pt $L0_2$	5.11.80	16					

Footnotes

a Plant sample was dry ashed prior to activation; figure given is corrected for % ash and calculated from both 158keV and 208keV photopeaks

b Dried unashed plant sample activated; figure given is calculated from 158keV.

c Dried unashed plant sample; figure given is calculated from 208keV.

Table 5.3

Reactor irradiation facilities (Consort MKII)

Facility	Thermal flux ($n\ cm^{-2}\ sec^{-1}$)	Fast flux ($n\ cm^{-2}\ sec^{-1}$)
In-core irradiation system ICIS (20mm diameter)	2.4×10^{12}	1.0×10^{12}
Cyclic activation system CAS (17mm diameter)	1.3×10^{12}	0.5×10^{12}
Vertical core tubes at side of core (32 and 48mm diameter)	1.4×10^{12} (max)	0.3×10^{12} (max)
Bare face thermal column (910×910mm) containing NISUS fast neutron spectrum assembly	-	1.2×10^{8}

Table 5.4

5.4.1 Preparation of standards

Duplicate standards were prepared in the following way. 5.0000g of dried Bowen's kale was weighed out; enough deionised and distilled water was added to make a slurry. To this, various quantities of the platinum metals were added as aqueous solutions. The slurry was freeze-dried and homogenised using a ball mill (Glen Creston). The final concentration of the platinum group metals in the spiked kale are given in Table 5.5.

Bowen's Kale Standard spiked with the platinum metals

(all values are given as $\mu g\ g^{-1}$ dry weight)

Rhodium	5.00	Palladium	100
Ruthenium	50	Osmium	25
Iridium	5.00	Platinum	25

Table 5.5

5.4.2 Preparation of samples

Initially, the dried plant material was pelleted, as were the standards (ca 150mg) to ensure a homogeneous and reproducible exposure to the neutron flux. The pellets were irradiated in low density polythene capsules. The irradiation of pelleted and non-pelleted plant material was investigated briefly for the palladium content of spiked kale. A comparison of the recorded activity is given in Table 5.6. Counting errors in activation analysis are governed by the Poisson distribution (Bowen and Gibbons, 1963), where the standard deviation is given by

$$\sigma = \sqrt{N}$$

and N is the number of events recorded in a given observation. The

Table 5.6 Activation[a] of pelleted and non-pelleted plant material

Bowen's kale spiked with palladium; non-pelleted

Sample wt g	Nuclide	energy keV	Total Peak Area	σ_1	Background	σ_2	Peak area -bgd.	$\sqrt{(\sigma_1)^2+(\sigma_2)^2}$	Area/g[b] corr.%dt	Detection[c] limit, μg g^{-1}
0.1473	^{109m}Pd	188.8	8581	93	4092	64	4489	113	31577	2.85
0.1436	^{109m}Pd	188.8	7952	89	3806	62	4146	108	29919	2.98
							Mean		30748	2.92

Bowen's kale spiked with palladium; pelleted

Sample wt g	Nuclide	energy keV	Total Peak Area	σ_1	Background	σ_2	Peak area -bgd.	$\sqrt{(\sigma_1)^2+(\sigma_2)^2}$	Area/g[b] corr.%dt	Detection[c] limit, μg g^{-1}
0.1546	^{109m}Pd	188.8	8017	90	4191	65	3826	111	25642	3.38
0.1641	^{109m}Pd	188.8	9173	96	4576	68	4597	117	28880	2.94
							Mean		27216	3.16

[a]Single activation, epithermal: t_i = 180s t_d = 30s t_c = 200s

[b]Student's t = 1.92 for 2df at the 95% confidence limit.

[c]Detection limit defined as 2 $\sqrt{\text{BACKGROUND}}$ in Bowen's kale under the 188.8 keV photopeak (Currie, 1968)

Student's t test, on the area per g, gave an experimental value of 1.92 for 2 degrees of freedom indicating no significant difference at the 95% confidence level, though it must be pointed out that the test itself is suited to a large number of paired observations and therefore the results may be criticised still.

5.4.3 Long irradiations

Platinum, iridium, osmium and ruthenium, have isotopes which lend themselves to long irradiations because of long half lives. These were carried out in the 0^{o} face Core Tube positions in the reactor (figures 5.1 and 5.2) where a maximum thermal flux of 1.4×10^{12}n $cm^{-2}sec^{-1}$ is attainable. The samples and standards were irradiated for a week; this averaged out at 37hrs total flux time, usually. The samples and standards were allowed to cool for two or three days and then counted using a $42cm^{3}$ lithium drifted germanium detector (Princeton-Gamma Tech) of resolution 1.81keV (full width at half maximum) at the 1.322MeV photopeak with a Peak/Compton ratio of 36.3:1 and efficiency of 8.1% linked to a computer based gamma ray spectrometer (Nuclear Data Inc 6620 Multichannel Analyser). A general Neutron Activation Package written in FORTRAN IV was employed to run a peak search, correct for decay and calculate platinum metal concentrations in the plant samples.

The gamma spectrum of irradiated Bowen's kale spiked with the platinum metals was plotted on an X-Y recorder and is shown in figure 5.4. The sample had been irradiated for five days allowed to decay for two days and counted for 30m using a Ge(Li) detector coupled to a 4096-channel analyser (Link Model 290). Some of the possible interfering elements in the determination of platinum (given in Table 5.7), were irradiated too; 5m counts were taken and the gamma spectra plotted as before; these are presented in figure 5.5.

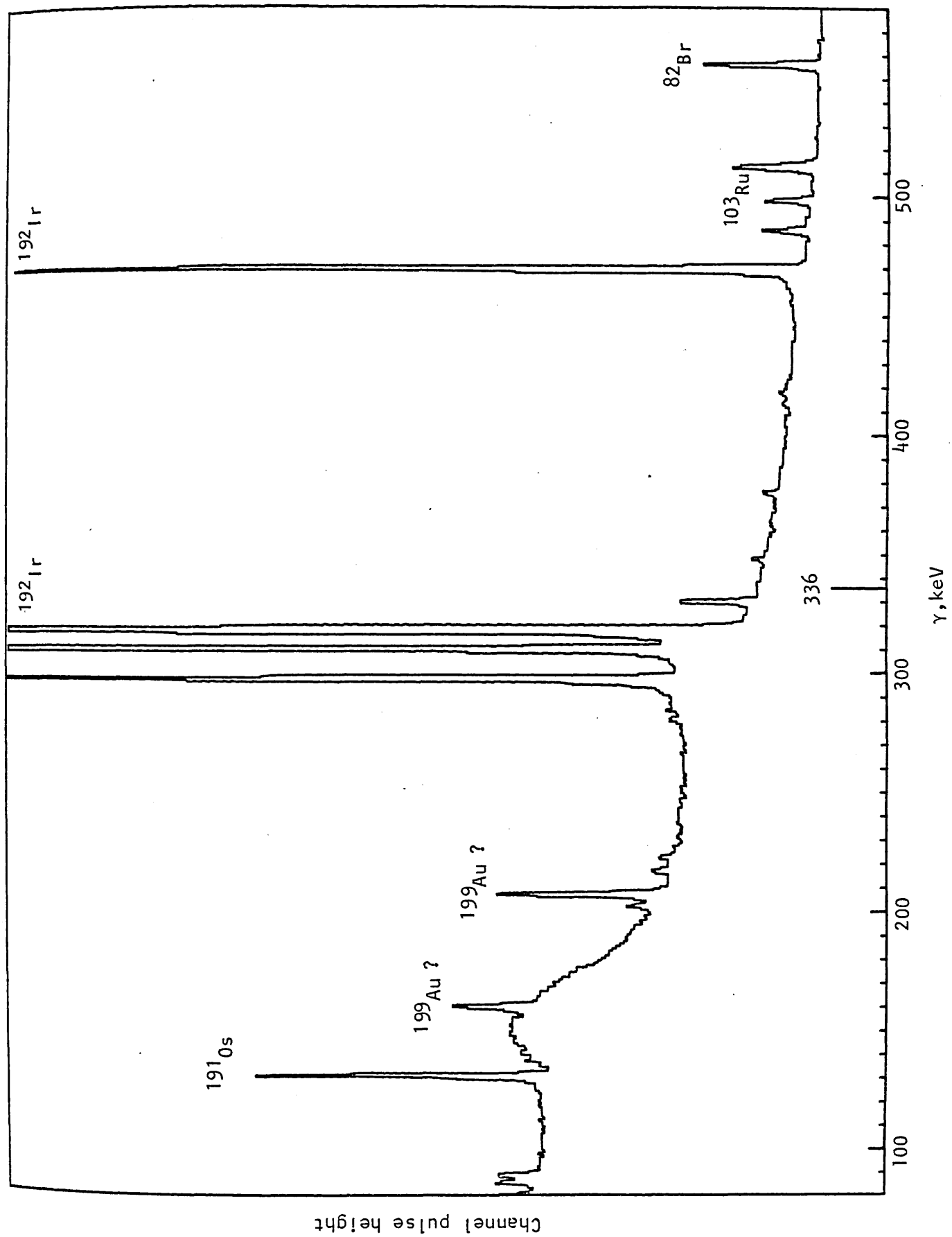


Figure 5.4(a) Gamma spectrum of irradiated Bowen's kale spiked with platinum metals

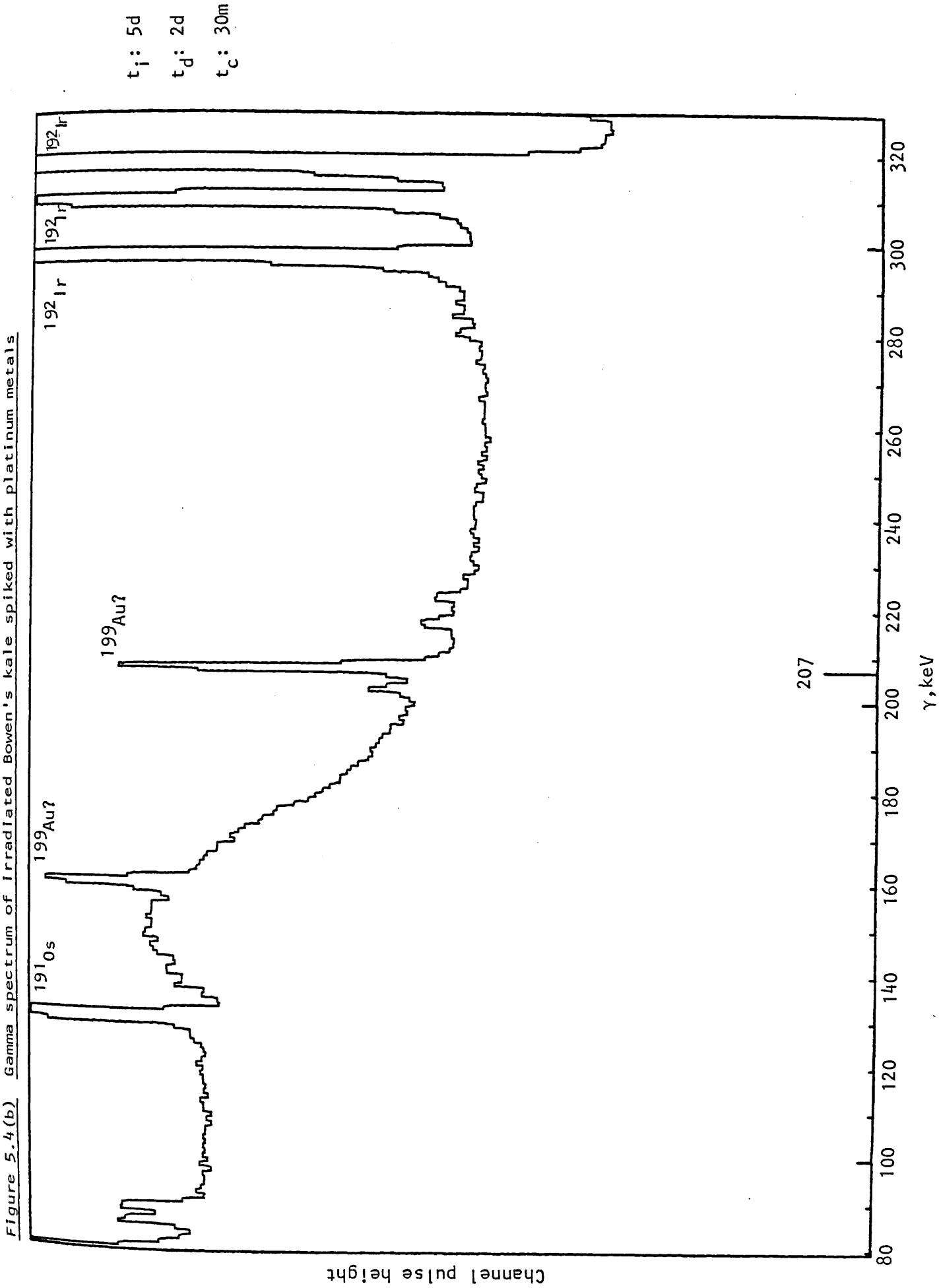


Figure 5.4(b) Gamma spectrum of irradiated Bowen's kale spiked with platinum metals

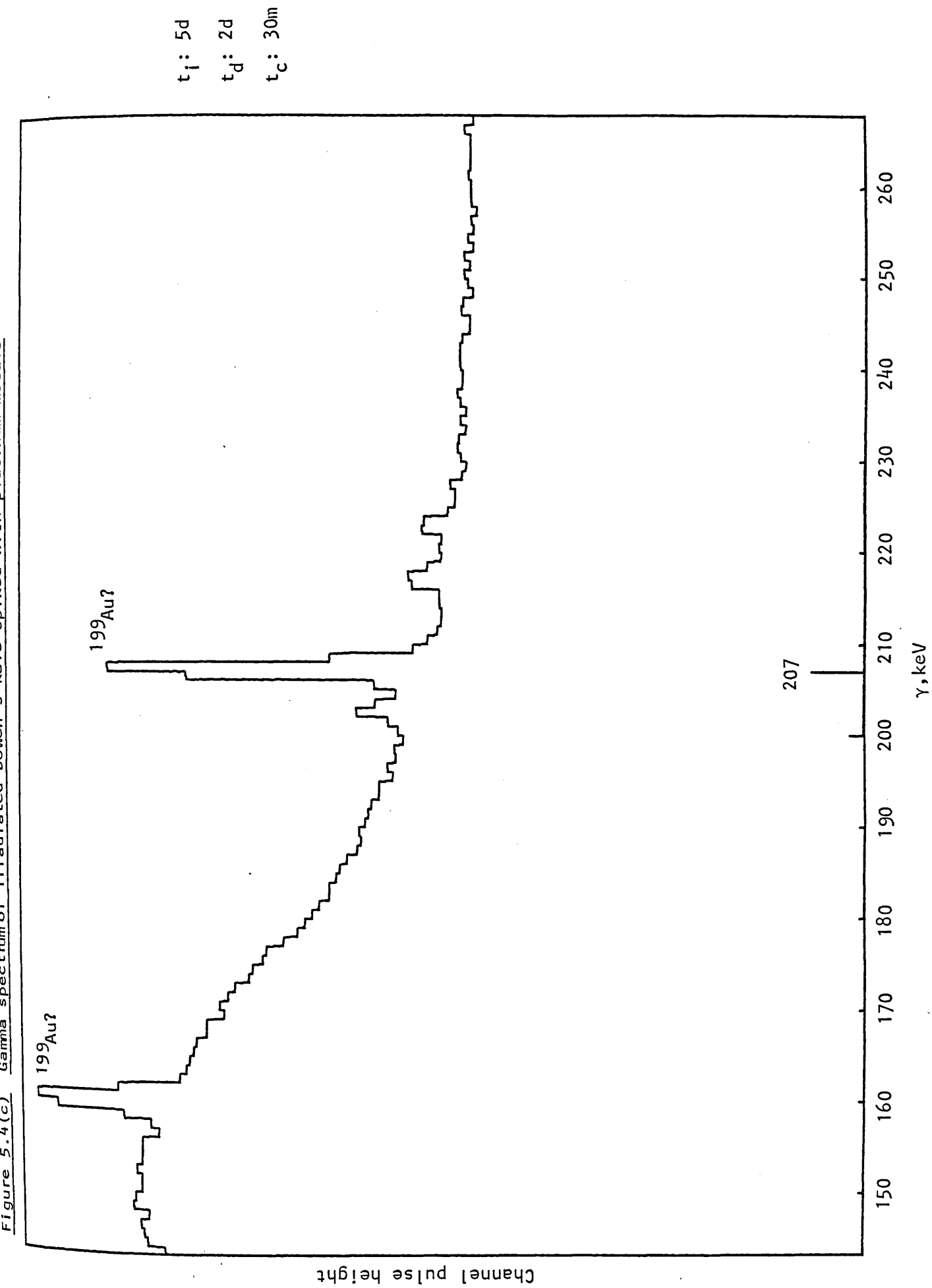


Figure 5.4(c) Gamma spectrum of irradiated Bowen's kale spiked with platinum metals

Table 5.7 Possible interferences in the determination of platinum

Nuclide	Half-life	Gamma energy (keV)	
^{117m}Sn	14.0d	158.40	
^{47}Sc	3.43d	159.40	(via $^{47}Ti(n,p)^{47}Sc$ or from Ca)
^{117m}In	1.9hr	158.40	
^{199m}Hg	42m	158.30	
^{177}Lu	6.74d	208.20	

Figure 5.5(a) Gamma spectrum of irradiated tin standard

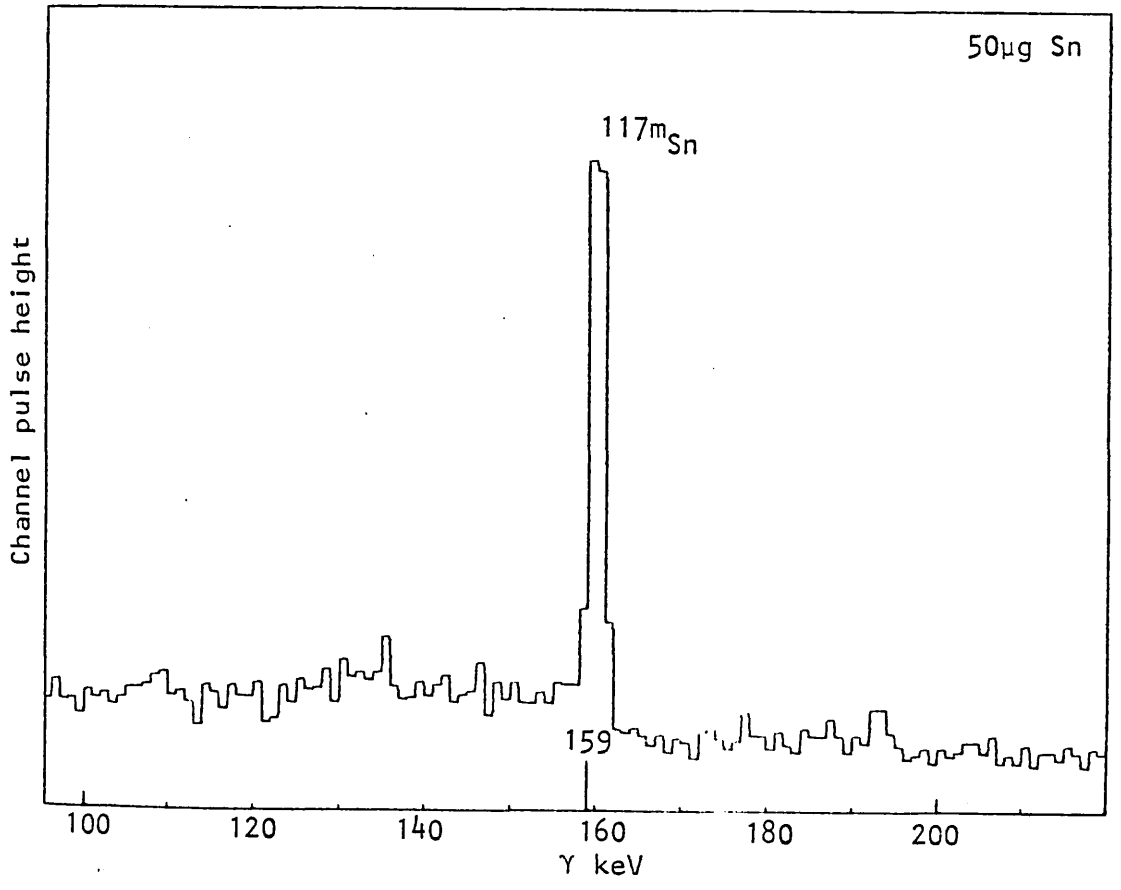


Figure 5.5(b) Gamma spectrum of irradiated calcium standard

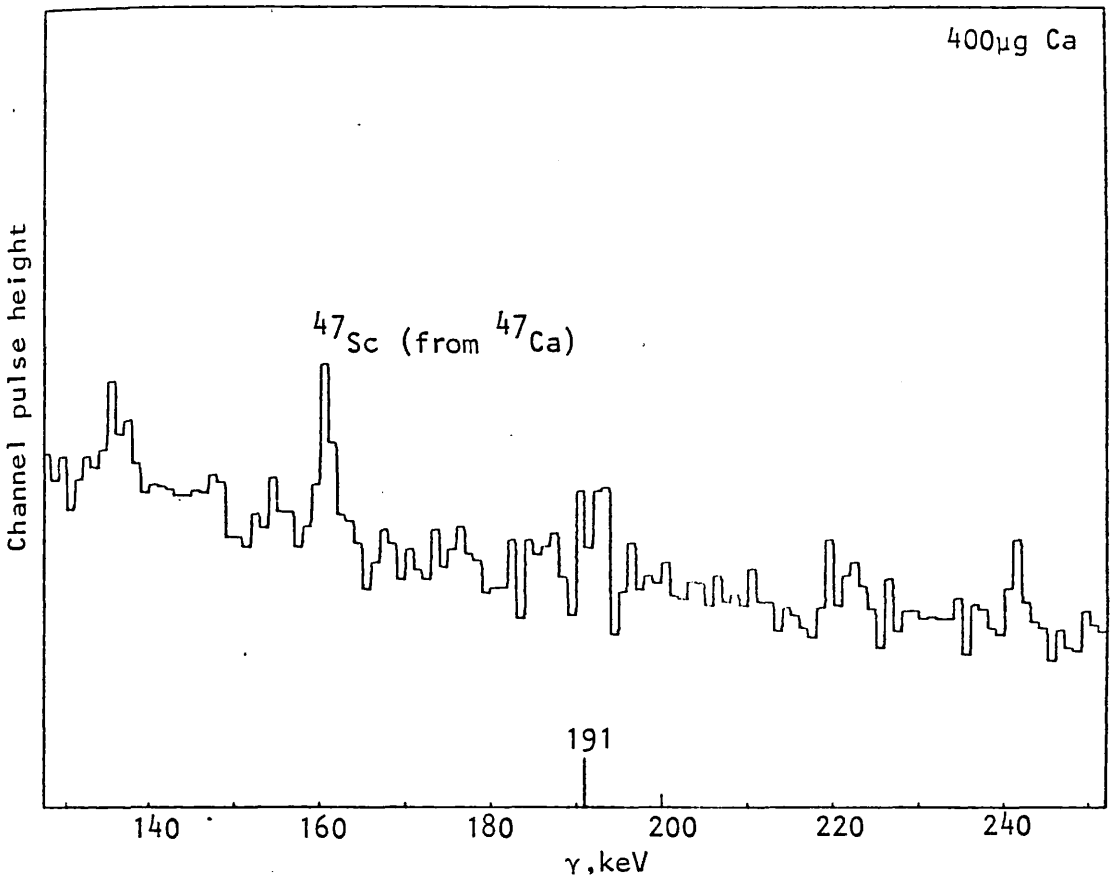


Figure 5.5(c) Gamma spectrum of irradiated titanium standard

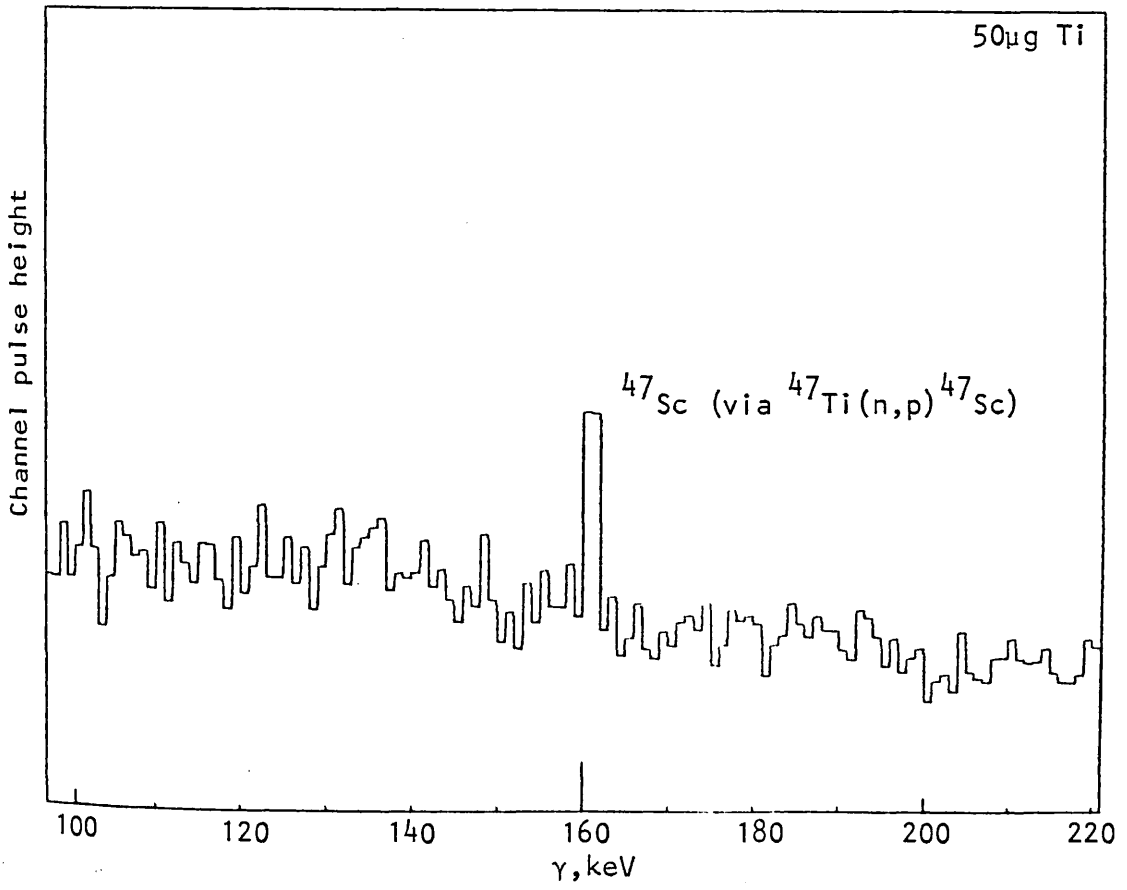


Figure 5.5(d) Gamma spectrum of irradiated indium standard

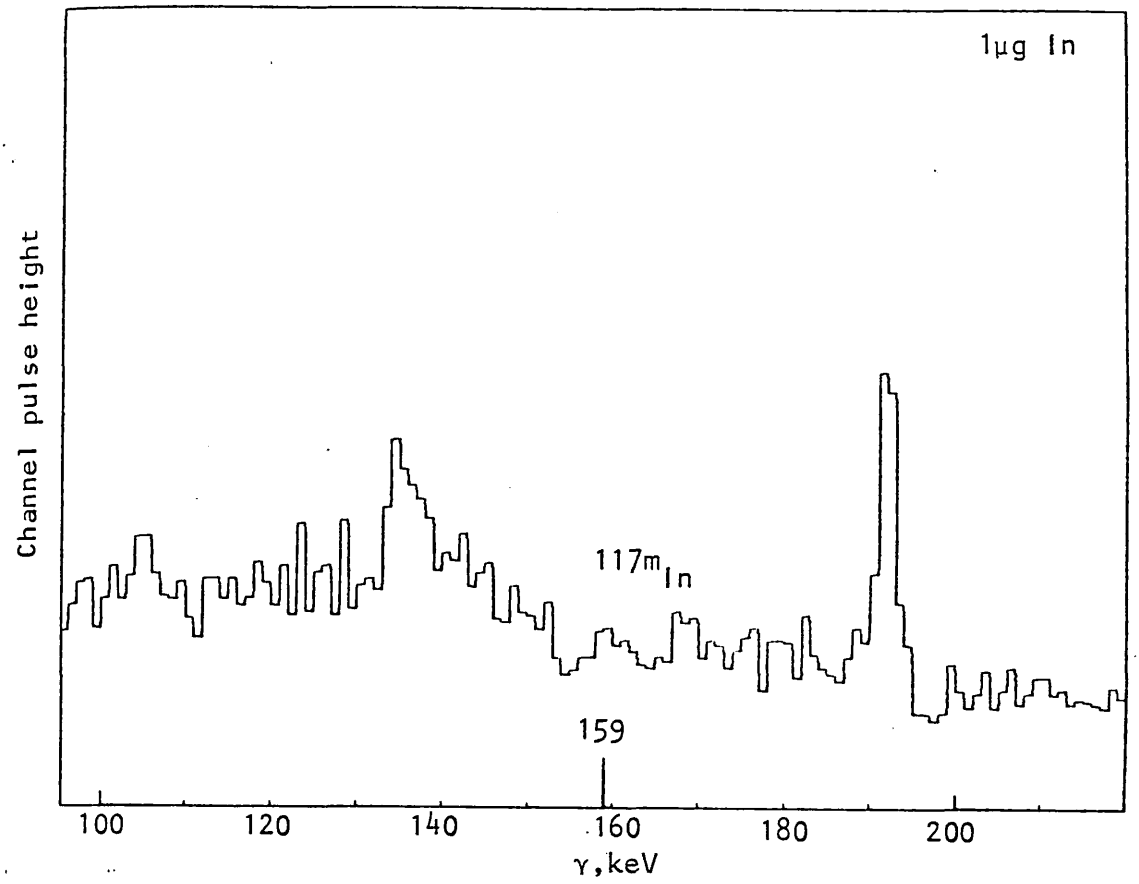


Figure 5.5(e) Gamma spectrum of irradiated mercury standard

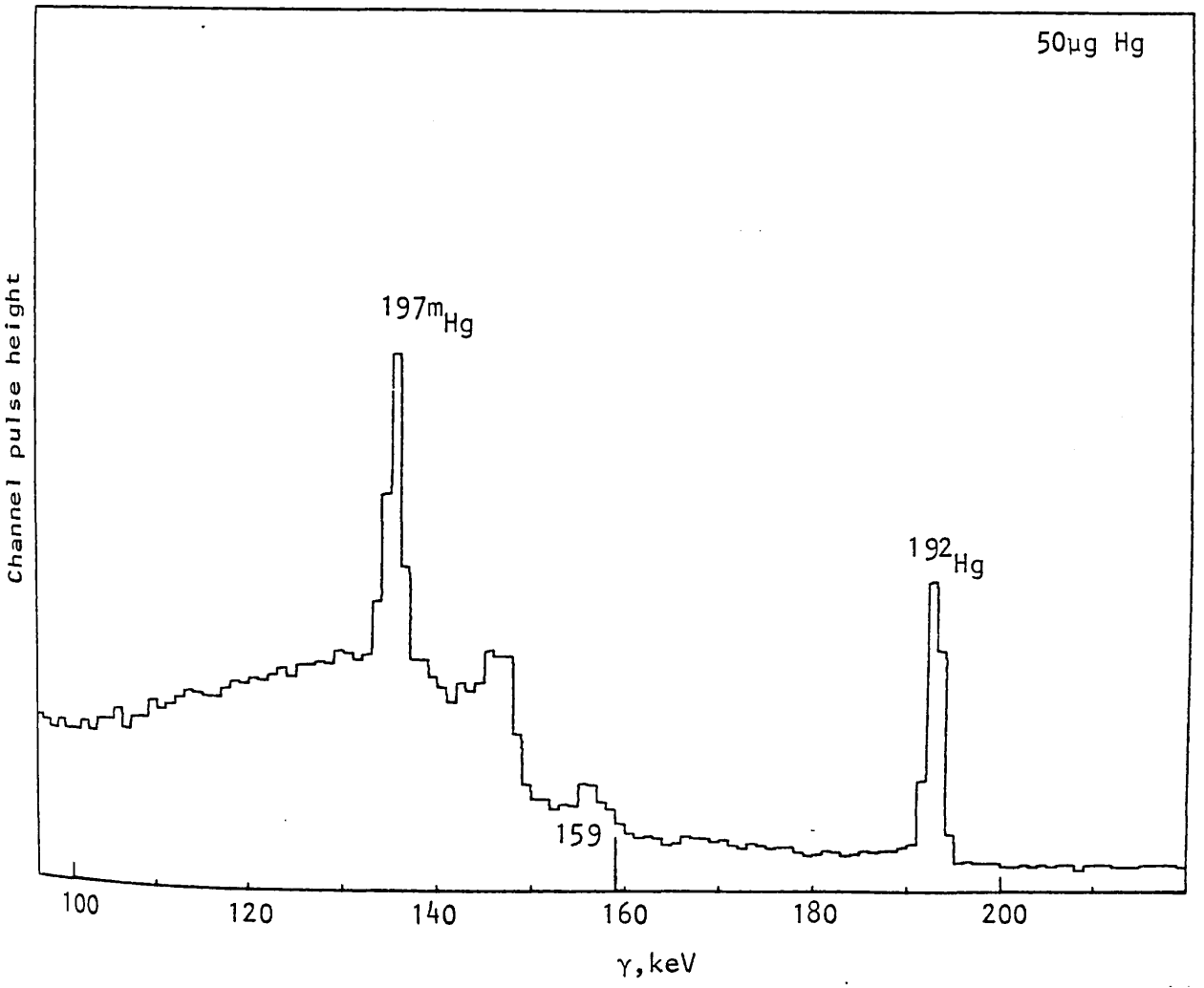


Table 5.8 Instrumental Neutron Activation Analysis of the ash from platinum treated plants

	determined against biological standards[a,b]					determined against chemical standards	
Sample details	Br	Ca	Na	K	Zn	Pt[c]	Pt[d]
all values corrected for % ash and expressed on a dry weight basis	$\mu g\ g^{-1}$	%	$\mu g\ g^{-1}$	%	$\mu g\ g^{-1}$	$\mu g\ g^{-1}$	$\mu g\ g^{-1}$
Pt LO[a]: Water hyacinth	91	4.19	547	3.24	283	30	5.5
Pt LO[b]: Water hyacinth	54	2.39	227	1.52	78	-	-
Pt LO (cp to results from PIXE)	82	3.00	-	2.80	77	-	5.6
Pt LO (cp to results from ICP)	-	4.05	-	-	93	nd	nd
B/15/Floats[a]: Water hyacinth	58	1.31	722	7.06	95	135	129
B/15/Floats[b]: Water hyacinth	34	0.75	300	3.31	26	-	-
Control/V/Tops[a]: Water hyacinth	47	2.57	373	6.73	208	-	-
Control/V/Tops[b]: Water hyacinth	28	1.47	155	3.15	57	-	-

Footnotes:

a: ash of Bowen's kale

b: ash of NBS 1571 Orchard leaves

c: Au-199 at 158keV

d: Au-199 at 208keV

Examination of the irradiated plant ash revealed that some other long lived nuclides were detectable after two day's decay. Results for these elements are given in Table 5.8. A full comparison between INAA and other techniques appears in chapter 6.

Detection limits for platinum, iridium, osmium and ruthenium in plant materials by INAA are given in Table 5.9. Results of the determination of platinum metals in standard water hyacinths are presented in Table 5.10.

Table 5.9 Detection limits for long lived nuclides of the platinum metals

Nuclide	γ energy, keV	detection limit†, μg g^{-1}
^{192}Ir	316.50	0.0049
^{191}Os	129.40	0.533
^{103}Ru	497.00	2.46

†detection limit defined as $2\sqrt{BACKGROUND}$ in dried Bowen's Kale under the peak given (Currie, 1968).

5.4.4 Short irradiations

Both rhodium and palladium undergo (n,γ) reactions which result in short lived decay products. Short irradiation and counting times are required, therefore. In biological samples, matrix elements give rise to an unfavourable background of short lived decay products. Various refinements of INAA can reduce this problem and these are discussed in chapter 2.3.

In this study, single and cyclic irradiations were carried out in the University of London reactor under a thermal neutron flux of

Determination of the platinum metals in Standard Water hyacinths by INAA (Consort Mark II)

Long irradiations: all values in $\mu g\ g^{-1}$ dry weight

Samples	Analytical details	γ,keV	Pt	Ir	Os	Ru
Pt $LO_{(2)}$	dried plant material	158.37	14.1?	-	-	-
Pt $LO_{(2)}$	dried plant material	208.20	nd	-	-	-
Pt $LO_{(2)}$	ashed plant material (dry ashed)	158.37	30?			
Pt $LO_{(2)}$	ashed plant material (dry ashed)	208.20	5.5			
Ir LO	dried plant material	316.50	-	1.29	-	-
Ir LO	plant digest (wet ashed)	316.50	-	1.48	-	-
Os LO	dried plant material	129.40	-	-	1.79	-
Os LO	plant digest (wet ashed)	129.40	-	-	nd	-
Ru LO	dried plant material	497.00	-	-	-	nd
Ru LO	plant digest (wet ashed)	497.00	-	-	-	nd
Pt Hl	dried plant material	158.37	659?	-	-	-
Pt Hl	dried plant material	208.20	155?	-	-	-
Ir Hl	dried plant material	316.50	-	49	-	-
Ir Hl	plant digest (wet ashed)	316.50	-	53	-	-
Os Hl	dried plant material	129.40	-	-	1103	-
Ru Hl	dried plant material	497.00	-	-	-	5141

Table 5.10

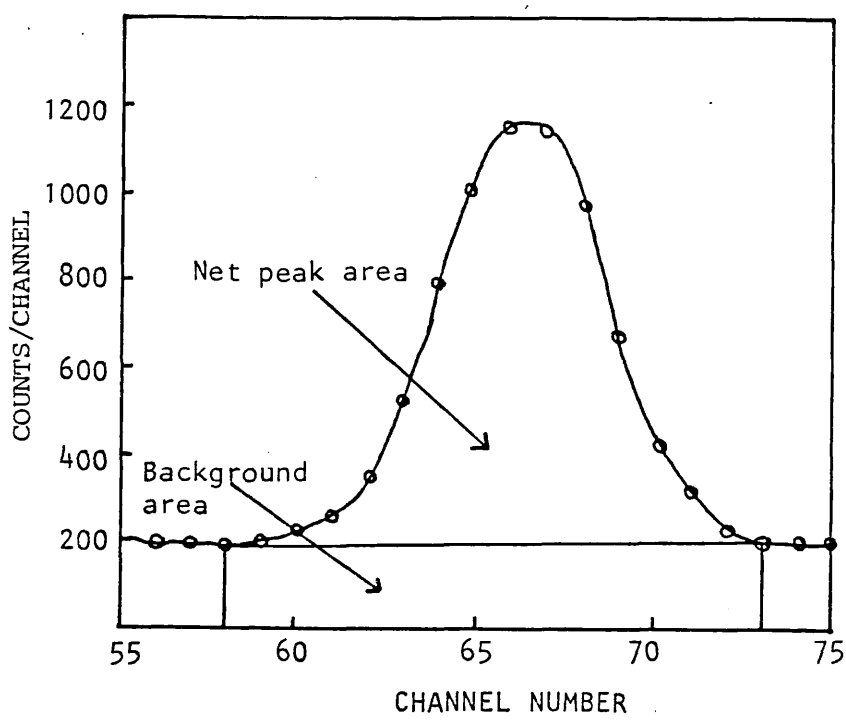


Figure 5.6 An example of the total peak area calculation (after Amiel, 1981).

1.3×10^{12}n cm^{-2}sec^{-1} or an epithermal flux of 0.5×10^{12}n cm^{-2}sec^{-1}. The samples were transferred from the reactor to a Ge(Li) detector, situated close by, by a fast pneumatic system. Gamma spectra were accumulated using a Laben 8000 MCA of 4096 channels. Appropriate regions of each spectrum were printed out in digital form, from which peak areas, corrected for background and analyser dead time, were determined using the Total Peak Area method (Figure 5.6) (Yule, 1966). The signal, S, is computed from equation 5.1.

$$S = \left(\sum_{i=\ell}^{r} R_i\right) - 0.5(r-\ell-1)(R_{\ell-1}+R_{r+1}) \qquad 5.1.$$

where ℓ and r are the channel numbers of the channels bounding the peak on the left and right respectively. The short irradiation conditions used in this study are presented in Table 5.11 (Valente, 1981) and various detection limits for palladium and rhodium in Bowen's Kale in

Short Irradiation Conditions (Reactor Consort II)

Single irradiations

	Thermal flux	Epithermal flux
Neutron flux (n $cm^{-2}s^{-1}$)	1.3×10^{12}	0.5×10^{12}
Irradiation time (s)	30	180
Decay time (s)	120	30
Counting time (s)	300	200
Analyser dead time (%)	16.3	3.6

Cyclic activation system

	Thermal flux	Epithermal flux
Neutron flux (n $cm^{-2}s^{-1}$)	1.3×10^{12}	0.5×10^{12}
Irradiation time (s)	30	60
Decay time (s)	5	1
Couting time (s)	60	60
Number of cycles	5	5
Analyser dead time (%)	56	4.8

Table 5.11

Detection limits for the platinum metals in biological matrices

Short irradiations

1. Cyclic activation system with epithermal flux

Nuclide	γ energy,keV	detection limit[a], μg g^{-1}
^{104}Rh	555.8	0.088 (±0.004)
^{104m}Rh	51.4	0.107 (±0.001)
^{109m}Pd	188.9	2.98 (±0.08)

2. Single irradiation with epithermal flux

Nuclide	γ energy,keV	detection limit[a], μg g^{-1}
^{109m}Pd	188.9	2.92 (±0.07)

3. Single irradiation with thermal flux

Nuclide	γ energy,keV	detection limit , μg g^{-1}
^{109m}Pd	188.9	162 (±4)
^{104}Rh	555.8	0.264 (±0.009)

[a]detection limit defined as 2 $\sqrt{\text{BACKGROUND}}$ in dried Bowen's Kale, under the peak given (Currie, 1968).

Table 5.12

Determination of the platinum metals in Standard Water hyacinths by INAA (Consort Mark II)

Short irradiations: all values in $\mu g\ g^{-1}$ dry weight

Cyclic activation, epithermal

Sample details	Nuclide	γ,keV	found $\mu g\ g^{-1}$
Rh LO	^{104m}Rh	51.4	5.40 (±0.03)
Rh LO	^{104}Rh	555.8	4.31 (±0.03)
Rh Hl	^{104}Rh	555.8	206 (±2)
Pd LO	^{109m}Pd	188.8	4.62 (±1.26)
Pd Hl	^{109m}Pd	188.8	511 (±26)
Single activation, epithermal			
Pd LO	^{109m}Pd	188.8	4.13 (±1.43)
Pd Hl	^{109m}Pd	188.8	551 (±16)
Single activation, thermal			
Rh LO	^{104}Rh	555.8	5.25 (±0.54)
Pd LO	^{109m}Pd	188.8	nd

Table 5.13

Table 5.12. The results of rhodium and palladium determinations under various short irradiation conditions are given in Table 5.13.

5.5 Discussion

Preliminary results obtained from ICI Billingham, with the TRIGA reactor, were very encouraging. Platinum, rhodium, osmium and iridium were detected in plant tissues at low levels; only palladium and ruthenium were not detected. However, the determination of platinum presented a problem.

Values from the determination on the plant ash agreed very well with those from atomic absorption (ETA AAS; preliminary comparison presented in Appendix) but when the dried plant material was activated, a very much higher value was obtained. A cross-check with the 208keV photopeak indicated the presence of an interfering nuclide with a γ energy near to 158keV and with a similar half life to that of ^{199}Au. Activation of control plants confirmed the presence of an interference at 158keV (Table 5.7); a decay plot of this activity in the control plant indicated more than one component to the interference which made the calculation of its half life too difficult. The γ photopeak at 208keV proved unsuitable for the determination of low levels of platinum in plant materials.

These points were investigated further using the Consort MKII reactor of the University of London. From these studies, it seems the most likely origin of the interference is ^{117m}Sn and ^{47}Sc, both of which contribute to the photopeak at 158keV, but other possibilities cannot be ruled out. At present, the only route by which platinum can be determined accurately in plant materials by INAA, is by activation of the ash and measuring the peak area at 208keV, though the detection limit is not good.

Of the other long lived nuclides of the platinum group, iridium is the most sensitive and has the best detection limit; this is in contrast to the poor sensitivity and detection limits for iridium in ETA AAS. INAA has proved useful in the determination of osmium and ruthenium in plants, where the problem of wet ashing can result in the metals being lost as volatile oxides (Holgye, 1979).

With the short lived nuclides of the platinum group, substantial improvements have been made with the determination of palladium, using an epithermal flux, and with rhodium, using the cyclic activation system under epithermal flux. There has been good agreement with other analytical techniques and a detailed comparison is reported in chapter 6.

REFERENCES

Ahmad, I., Ahmad, S. and Morris, D.F.C. (1977), Analyst, 102 17.

Bowen, H.J.M. and Gibbons, D. (1963), Radioactivation Analysis, Clarendon Press, Oxford.

Caramella-Crespi, V., Pisani, U., Ganzerli-Valentini, M.T., Meloni, S. and Maxia, V. (1974), J. Radioanal. Chem., 23 23.

Crocket, J.H., Keays, R.R. and Hsieh, S. (1968), J. Radioanal. Chem., 1 487.

Gilbert, E., Veriovkin, G.V., Mikhailov, V.A., Ankpov, N.I. and Nikitin, V.N. (1977), J. Radioanal. Chem., 38 147.

Holgye, Z. (1979), Radiochem. Radioanal. Lett. 41 (4), 279.

Lederer, C.M. and Shirley, V.S. (1978), eds. Table of Isotopes, 7th ed., Wiley, New York.

Le Roux, H.J., Beukes, P.J.L., Turkstra, J. and De Wet, W.J. (1974), J. S. African Chem. Inst., 27 28.

Nadkarni, R.A. and Morrison, G.H. (1977), J. Radioanal. Chem., 38 435.

Parry, S.J. (1980), Analyst, 105 1157.

Saadia, A. (1981), editor, Nondestructive Activation Analysis: Studies in Analytical Chemistry, Vol.3, Elsevier, Oxford.

Samadi, A.A. and Fedoroff, M. (1978), Analusis, 6 (5) 191.

Schutyser, P., Govaerts, A., Dams, R. and Hoste, J. (1977), J. Radioanal. Chem., 37 651.

Valente, I.M., Minski, M.J. and Peterson, P.J. (1981), 6th International MTAA Conf., Toronto.

Valente, I.M. (1981), private communication.

Yule, H.P. (1966), Anal. Chem., 38 103.

Zeisler, R. and Greenberg, R.R. (1981), 6th International MTAA Conf., Toronto.

CHAPTER VI

A COMPARISON OF ANALYTICAL TECHNIQUES FOR THE DETERMINATION OF THE PLATINUM METALS IN PLANTS

6.1 Introduction

A wide variety of instrumental techniques are available for the analysis of plant materials (figure 6.1). They include (a) atomic absorption spectroscopy, which is the subject of chapter 4, and (b) neutron activation analysis, the subject of chapter 5. Among the techniques investigated in this chapter, are atomic emission spectroscopy and X-ray spectrometry. They are compared to atomic absorption and neutron activation methods.

The use of instrumental techniques for the determination of platinum metals is not new (Beamish and Van Loon, 1977); however, evidence of their use for the determination of platinum metals in biological materials is scarce, and has been reviewed elsewhere (ch.2.1 and ch.3.1).

6.2 Preparation of standard plant materials containing the platinum metals

Plant materials, containing one of each of the six platinum metals, were prepared in order to investigate and compare various analytical methods. This was due to the absence of any biological standard reference material with certified levels of the platinum metals.

The free floating aquatic plant, water hyacinth, (*Eichhornia crassipes* MART Solms), was grown in half strength nutrient solution for two weeks. This was carried out in a Prestcold controlled environment

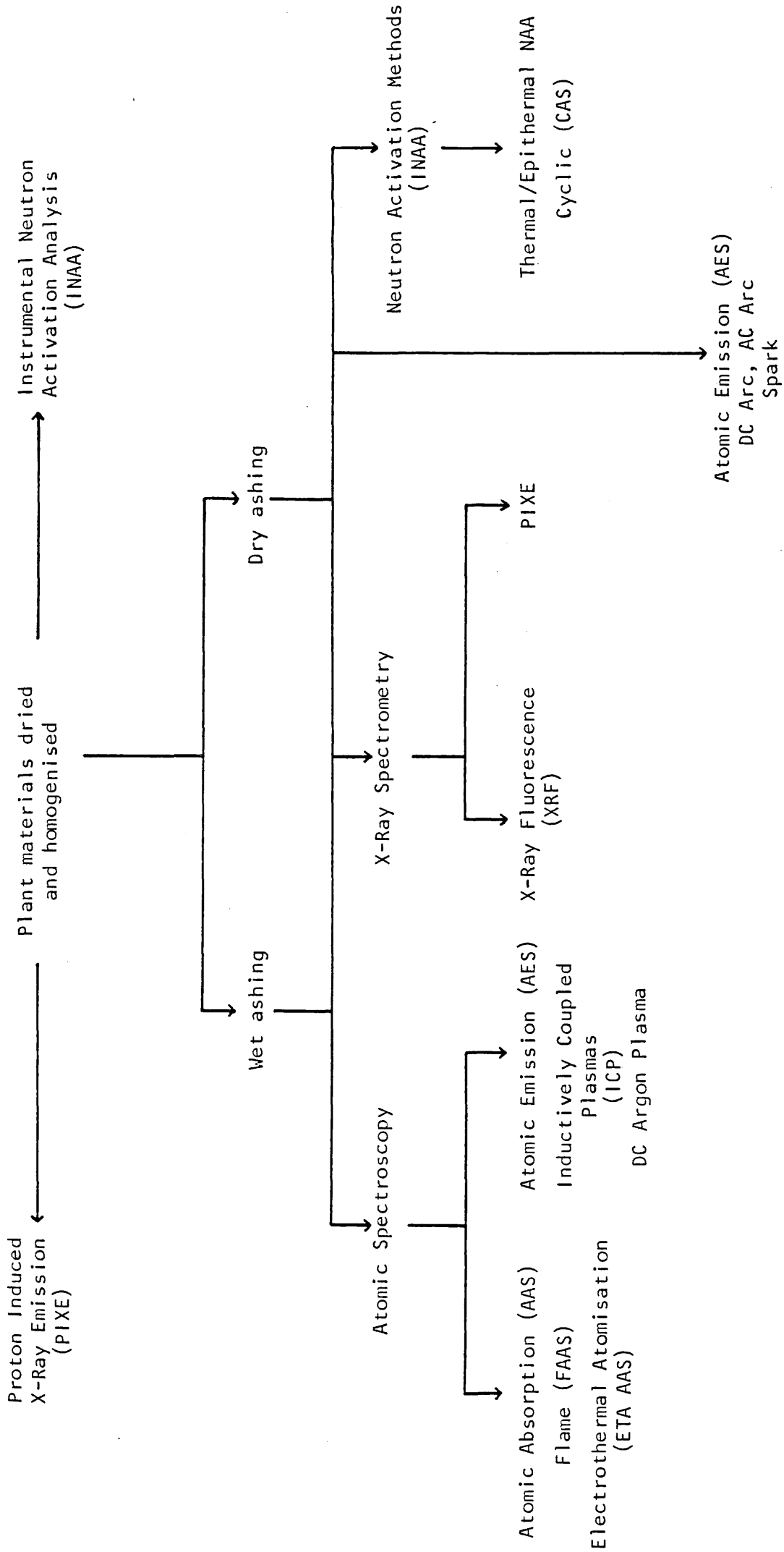


Figure 6.1 Modern Methods for the analysis of plant materials

growth room (ch.3.1). Conditions of growth were: illumination, 10^3ft candles; temperature, $25 \pm 1^{o}C$ (14hr day), $20 \pm 1^{o}C$ (night), relative humidity 70-75%.

Separate water hyacinths were grown in half strength nutrient solutions containing each of the platinum metals. Each metal was supplied as a soluble complex at two concentrations designated as 'HI' and 'LO'; details are given in Table 6.1. After one week, the nutrients and applied platinum metals were replenished. After a second week, the plants were harvested, washed with demineralised water and weighed. Those plants designated as 'LO' were separated into roots and tops, the latter being used as standard material. Plants designated as 'HI' were analysed as whole, roots and tops together. All samples were dried to constant weight at $90^{o}C$ and thoroughly homogenised by grinding in an agate ball mill (Glen Creston). A number of representative samples, now designated Standard Water Hyacinths, 'HI' or 'LO', were prepared for analysis by a range of methods (figure 6.1). Where the quantity of materials permitted, determinations were carried out in duplicate.

The biological effects of platinum group metal complexes are not considered here but are the subject of a detailed investigation in chapter 7.

6.3 D.C. Arc Emission Spectrography[†]

The background and principles of this method have been discussed elsewhere (chapter 2.2). In this study, semi-quantitative spectrographic analysis of plant materials was carried out in the following way. About 500mg of the dried plant materials were placed in silica containers and dry ashed in a muffle furnace at $550^{o}C$ for 14hrs. This ensured the complete removal of the organic matrix without loss of the non volatile

[†]D.C. arc emission facilities were provided by the Analytical Laboratories, Johnson Matthey Chemicals, Royston.

Growth of Standard Water Hyacinths (*Eichhornia crassipes*) containing the platinum metals

Sample	Applied metal complex	Concentration/mg dm^{-3}	Wet Weight/g	Dry weight/g	Wt of H_2O/g	% H_2O	% Ash
Pt LO(1) Tops	$K_2[PtCl_4]$	0.05	23.63	2.14	21.49	90.9	14.9
Pt LO(2) "	$H_2[PtCl_6]$	0.05	43.03	3.78	39.25	91.2	15.8
Pd LO "	$[Pd(NH_3)_2Cl_2]$	0.05	27.18	2.81	24.37	89.7	17.3
Rh LO "	$Na_3[RhCl_6]$	0.05	22.07	1.61	20.46	92.7	17.3
Ir LO "	$Na_3[IrCl_6]$	0.05	27.28	2.81	24.47	89.7	17.4
Ru LO "	$(NH_4)_3[RuCl_6]$	0.05	35.72	3.77	31.95	89.5	16.8
Os LO "	$Na_2[OsCl_6]$	0.05	31.34	2.62	28.72	91.6	—
Control/T/Tops	———	—	24.88	2.01	22.87	91.9	17.3
Pt HI Tops+Roots	$(NH_4)_2[PtCl_6]$	5.00	60.06	4.86	55.20	91.9	16.4
Pd HI "	$H_2[PdCl_4]$	1.00	33.69	2.85	30.84	91.5	12.4
Rh HI "	$Na_3[RhCl_6]$	10.00	45.73	3.43	42.30	92.5	15.8
Ir HI "	$(NH_4)_2[IrCl_6]$	1.00	18.71	1.49	17.22	92.0	12.2
Ru HI "	$(NH_4)_3[RuCl_6]$	5.00	65.72	2.28	63.44	96.5	—
Os HI "	$Na_2[OsCl_6]$	5.00	75.71	3.88	71.83	94.9	—
Pt LO Roots	$K_2[PtCl_4]$	0.05	2.11	0.22	1.89	89.6	—
Pt LO "	$H_2[PtCl_6]$	0.05	25.14	1.59	23.55	93.7	—
Pd LO "	$[Pd(NH_3)Cl_2]$	0.05	13.69	1.06	12.63	92.3	—
Rh LO "	$Na_3[RhCl_6]$	0.05	7.29	0.47	6.82	93.6	—
Ir LO "	$Na_3[IrCl_6]$	0.05	10.63	1.59	9.04	85.0	—
Ru LO "	$(NH_4)_3[RuCl_6)]$	0.05	19.97	1.50	18.47	92.5	—
Os LO "	$Na_2[OsCl_6]$	0.05	13.49	1.05	12.44	92.2	—
Control/T/Roots	———	—	12.06	0.70	11.36	94.2	—

Table 6.1

platinum metals. About 30mg duplicates of the plant ash were accurately homogenised and introduced into graphite electrodes (Ringsdorff). The samples were excited by a direct current discharge in air inside a Spex 9010 water cooled arc box. A Hilger and Watts 3.4m Ebert Spectrograph equipped with Kodak Spectrum Analysis No.1 glass plates was used to record spectra. Details of the apparatus and operating parameters are given in Table 6.2.

Elemental concentrations were calculated on a semi-quantitative basis using a visual comparator with a range of standards containing the platinum metals (Johnson Matthey, Specpure) against (i) a graphite matrix and (ii) a saltcake matrix ($Na_2SO_4.10H_2O$). Results from standard water hyacinths treated with the platinum metals and analysed against both types of standard are presented in Table 6.6. Values from the analysis of Bowen's Kale are given in Table 6.4 where they are compared to the best values obtained from other laboratories. Ashed samples of control water hyacinths, grown under the same conditions as given in section 6.2, were analysed for up to 21 elements by d.c. arc emission. These control plants had been separated into roots, floats and leaves, and were subjected to multielement analysis by a number of other techniques too. Results of d.c. arc emission analysis are presented in Table 6.7, alongside those obtained from ICP emission spectroscopy and PIXE analysis.

6.4 Inductively Coupled Plasma Emission Spectrometry

The plant materials described in section 6.2 were prepared for analysis as follows. Representative samples of the dried and homogenised plants were wet ashed in concentrated nitric acid (ARISTAR). When digestion was complete, the residues were redissolved in concentrated hydrochloric acid (ARISTAR) and diluted to 5% acid with deionised and

Apparatus and operating parameters for d.c. arc emission spectrography†

Spectrograph:	Hilger and Watts 3.4m Ebert grating spectrograph employing a 14600 l.p.i. grating blazed at 530.0nm.
Slit width:	10μm
Angles employed:	9.95^{o} and 14.00^{o}
Wavelength range:	240.0-360.0nm in 2nd order with no filter.
Arc Box:	Spex 9010 water cooled.
Excitation current:	12 amps.
Exposure time:	2-3m for a total energy burn
Electrodes (graphite):	Anode: Ringsdorff RW0055
	Cathode: Ringsdorff RW0083
Analytical gap:	3mm.
Processing:	1. Kodak DG 10 developer (3m at 68^{o}C)
	2. Kodafix fixer (at RT)

Table 6.2

†D.C. arc emission facilities were provided by the Analytical Laboratories, Johnson Matthey Chemicals, Royston.

distilled water in volumetric flasks. Digestion blanks were prepared similarly and corrections made accordingly. Some 18 elements were determined by ICP emission spectrometry†; the apparatus and operating parameters employed are given in Table 6.3. General multielement standards were used to which were added known amounts of the platinum metals (Johnson Matthey, Specpure). The results of analysis of Bowen's Kale and NBS SRM 1571 orchard leaves are presented in Tables 6.4 and 6.5 respectively, along with the certified levels. Control samples of water hyacinth leaves, floats and roots grown under similar conditions, were wet ashed too; ICP results of analysis have been presented with those from other methods in Table 6.7. The standard water hyacinths containing the platinum metals were analysed and these results appear in Table 6.8. Included in this table is the analysis of a sample of Bowen's Kale spiked with the platinum metals which was used as a matrix matched standard in neutron activation work (ch.5).

6.5 Proton Induced X-ray Emission*

The principles and applications of this technique have been reviewed in chapter 2. Several milligrams of the powdered plant material was placed on a target and analysed with respect to an external standard. Table 6.4 gives the results of PIXE analysis of Bowen's Kale samples and are compared to certified values. Control water hyacinths materials were analysed by PIXE; the results of this analysis have been presented, along with similar results from ICP and D.C. arc emission techniques in Table 6.7. Standard water hyacinths containing the platinum metals were

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†ICP facilities were provided by the Analytical Laboratories, Johnson Matthey and Company Limited, Brimsdown.

*PIXE analysis was carried out by the Environmental Science Laboratories at the College of William and Mary, Virgina Associated Research Campus (VARC), Virginia, USA.

Apparatus and operating parameters for ICP†

Emission Spectrometry

R.F. Generator:	Plasma therm 2.5Kw running at 27.12MHz
Torch:	Fassel Design
Nebuliser:	Meinhard
Power settings:	Incident 1300 watts.
:	Reflected < 5 watts.
Argon settings:	Cooling 15 $dm^3 min^{-1}$
	Nebuliser 0.4 $dm^3 min^{-1}$
	Plasma 0.8 $dm^3 min^{-1}$
Spectrometer:	Rank Hilger E1000
Gratings:	2 Holographic
K1111:	Angle 22.21° 2036.3 lines mm^{-1}
:	Dispersion 0.324nm mm^{-1} in the range 188-330nm
K1011:	Angle 30.41° 2036.3 line mm^{-1}
:	Dispersion 0.314nm mm^{-1} in the range 322-461nm
	All first order lines

Table 6.3

†ICP facilities were provided by the Analytical Laboratories, Johnson Matthey and Company Ltd., Brimsdown.

Table 6.4

Analysis of Bowen's Kale by various methods (values in $\mu g\ g^{-1}$, except where shown as percentage)

Element	Best value[a]	DC Arc	PIXE	PIXE[b]	ICP
Au	0.0019 ± 0.0006	nd	<1.2	<0.9	1.7
Ag	0.0155	0.6	<1.4	<1.0	0.28
Al	3.69 ± 7.0	115	<0.4%	<1.1%	158
Ba	5.02 ± 1.0	12	<12	7	-
B	49.9 ± 6.3	76	-	-	-
Br	24.6 ± 2.3	-	27	27	-
Ca	4.1 ± 0.22%	-	4.1%	4.1%	4.4%
Cl	0.36 ± 0.03%	-	0.26%	c	-
Co	0.0624 ± 0.011	-	-	-	1.67
Cr	0.370 ± 0.089	-	-	-	19.8
Cu	4.90 ± 0.65	4	4.5	4.4	4.74
Fe	118 ± 17	76	300[d]	117	104
Ga	0.0267 ± 0.0027	0.8	<0.5	<0.5	-
K	2.42 ± 0.13%	-	2.38%	2.40%	-
Mg	1580 ± 100	>153	-	-	1576
Mn	14.95 ± 1.4	12	14	14	13.38
Mo	2.25 ± 0.37	3	1.6	2.0	12.8
Ni	0.916 ± 0.162	3	<1.4	<0.6	nd
P	4524 ± 0.133	>153	3600	4100	-
Pb	2.48 ± 0.057	0.8	1.7	1.4	-
Pd	0.026?/ 100[e]	0.6	<1.1	119	nd
Pt	0.2?/ 25[e]	nd	<1.1	27	nd
Rb	52.9 ± 4.6	-	52	51	-
Rh	- / 5[e]	0.5	<0.8	5.4	0.14
Ru	0.0045/ 50[e]	nd	0.7	56	0.8
S	1.64 ± 0.24%	-	1.42%	1.47%	-
Si	243 ± 8	153	<700	<1200	-
Sr	87.3 ± 18	-	95	99	-
V	0.39 ± 0.065	0.5	10	5	-
Zn	32.7 ± 23	-	34	31	30
Ti	0.15	2.5	<10	<5	-
Ir	-- / 5[e]	nd	<1.2	3.2	1.5
Os	-- / 25[e]	nd	<1.3	22	-

Footnotes: a Best values (Bowen, 1980); b Bowen's kale spiked with platinum metals; c Extra chloride present in spiked sample; d Possible contamination; e Value($\mu g g^{-1}$) of platinum metal on spiked kale

Analysis of NBS SRM 1571 Orchard Leaves by ICP, (values in $\mu g\ g^{-1}$ except where shown as %)

Element	Certified Value	This work	from (Jones, *et al.*, 1980)
Al	-	292	1.78
Ca	2.09 ± 0.03%	2.01%	1.98%
Cr	2.6 ± 0.3	62	2.4
Co	0.2	1.6	-
Cu	12 ± 1	15.0	12.0
Fe	270,300	285	256, 238
Mg	0.52 ± 0.02%	0.58%	0.6%
Mn	91 ± 4	79	86,51
Mo	0.3 ± 1	39	0.2
Zn	25 ± 3	32	25

Table 6.5

Analysis of plant ash for the platinum group metals by D.C. Arc Emission Spectrography

		$\mu g\ g^{-1}$ ash weight					$\mu g\ g^{-1}$ dry weight				
Sample	Standard matrix	Pt	Pd	Rh	Ir	Ru	Pt	Pd	Rh	Ir	Ru
Pt LO(2)	Graphite	50					8				
	Saltcake	50					8				
Pd LO	Graphite		5					1			
	Saltcake		100					17			
Rh LO	Graphite			200					35		
	Saltcake			200					35		
Ir LO	Graphite				NΔ					NΔ	
	Saltcake				NΔ					NΔ	
Ru LO	Graphite					50					8
	Saltcake					3					0.5
Pt HI	Saltcake	14000					2296				
Pd HI	Graphite		20000					2480			
	Saltcake		20000					2480			
Rh HI	Graphite			500					79		
Ir HI	Graphite				50					6	
	Saltcake				70					9	

NΔ = not detected

Table 6.6

A comparison of results for the analysis of control water hyacinths (*Eichhornia crassipes*) by various methods

Sample and Method		Al	K	Ca	Cr	Co	Cu	Au	Fe	Pb	Mg	Mn	Mo	Ni	Rb	Si	Ag	Sn	Ti	V	Zn	Pt	Pd	Ir	Ru	Rh	B	P	S	Cl	Br
Control/S/leaves:	D.C.Arc	39	-	-	-	-	26	NΔ	65	0.7	>130	65	3	7		130	0.4	1	1	NΔ	-	NΔ	0.7	NΔ	NΔ	NΔ	65	>130	-	-	-
values in $\mu g\ g^{-1}$ dry weight	ICP	249	-	14219	NΔ	NΔ	35	NΔ	106	-	3517	95	28	NΔ	-	-	NΔ	-	-	-	48	NΔ	NΔ	NΔ	NΔ	NΔ	-	-	-	-	-
	PIXE	<8000	26900	10200	<1.2	<0.5	12.8	<0.3	83	3.5	-	65	2.5	2.2	2.8	<1200	<0.3	0.9	<3	<1.6	38	<0.3	<0.2	<0.3	<0.16	<0.20	-	6400	1900	4400	14.9
Control/S/floats:	D.C.Arc	38	-	-	-	-	10	NΔ	38	1	>191	19	2	4	-	38	0.4	1	NΔ	NΔ	-	NΔ	NΔ	NΔ	NΔ	NΔ	19	>191	-	-	-
values in $\mu g\ g^{-1}$ dry weight	ICP	350	-	13763	NΔ	NΔ	44	NΔ	121	-	3782	68	35	NΔ	-	-	NΔ	-	-	-	57	NΔ	NΔ	NΔ	NΔ	NΔ	-	-	-	-	-
	PIXE	<20000	75000	15000	<5	<1.1	23.2	<0.8	140	6.5	-	78	11.4	10.7	8.0	<3000	<0.6	<1.4	8	6	66	<0.8	<0.6	<0.8	<0.5	<0.7	-	9800	1200	13500	43
Control/S/roots:	D.C.Arc	65	-	-	-	-	13	NΔ	65	4	>130	4	3	1	-	65	0.7	1	NΔ	NΔ	-	NΔ	0.4	NΔ	NΔ	NΔ	13	>130	-	-	-
values in $\mu g\ g^{-1}$ dry weight	ICP	249	-	9418	NΔ	NΔ	49	NΔ	915	-	2274	11.5	103	NΔ	-	-	NΔ	-	-	-	165	N	NΔ	NΔ	NΔ	NΔ	-	-	-	-	-
	PIXE	<7000	30000	4800	2.5	<1.6	18.7	<0.4	610	16.3	-	10	53	5.3	3.1	<1000	1.7	3.7	9	<1.8	84	1.3	1.3	<0.4	<0.2	<0.4	-	4900	2500	2400	8.2

Table 6.7

Analysis of standard water hyacinths (*Eichhornia crassipes*) containing the platinum metals by ICP Emission Spectrometry

Sample details	Al	Ca	Cr	Co	Cu	Au	Fe	Mg	Mn	Mo	Ni	Ag	Zn	Pt	Pd	Ir	Ru	Rh
Pt LO(2) found $\mu g\ g^{-1}$ dry wt	403	40485	NΔ	1.66	24	NΔ	267	3230	311	35	NΔ	NΔ	93	NΔ	NΔ	85	NΔ	NΔ
Pd LO " "	264	40569	NΔ	2.01	32	NΔ	436	4631	957	42	NΔ	NΔ	159	NΔ	NΔ	55	NΔ	NΔ
Rh LO " "	400	43677	NΔ	2.53	32	NΔ	338	2944	336	40	NΔ	NΔ	212	NΔ	NΔ	NΔ	NΔ	NΔ
Ir LO " "	192	38644	NΔ	2.17	28	NΔ	310	3168	233	29	NΔ	NΔ	138	NΔ	NΔ	NΔ	NΔ	NΔ
Ru LO " "	225	39033	NΔ	1.81	27	NΔ	342	2942	522	25	NΔ	NΔ	133	NΔ	NΔ	21	NΔ	NΔ
Pt HI found $\mu g\ g^{-1}$ dry wt	2348	28050	NΔ	NΔ	169	NΔ	3519	3001	4246	34	NΔ	NΔ	479	1684	NΔ	310	NΔ	NΔ
Pd HI " "	156	18595	NΔ	NΔ	16	NΔ	489	3715	35	83	NΔ	NΔ	41	NΔ	554	NΔ	NΔ	NΔ
Rh HI " "	216	15462	NΔ	21	58	NΔ	203	3822	62	59	NΔ	NΔ	209	NΔ	NΔ	NΔ	NΔ	212
Ir HI " "	490	18254	NΔ	NΔ	17	NΔ	576	3615	11.5	132	NΔ	NΔ	41	NΔ	NΔ	31	NΔ	NΔ
Bowen's Kale spiked with the platinum metals: found $\mu g\ g^{-1}$ dry wt	237	42890	NΔ	2.33	4.5	NΔ	90	1480	11.5	11.6	NΔ	NΔ	29	6.2	77	NΔ	41	NΔ
(best values[a] and spiked levels)	36.9 ± 7.0	41000 ± 21800	0.370 ± 0.089	0.0624 ± 0.011	4.90 ± 0.65	0.00191 ± 0.00061	118 ± 17	1580 ± 100	14.95 ± 1.40	2.25 ± 0.37	0.916 ± 0.162	0.0155	32.7 ±2.3	(25)	(100)	(5)	(50)	(5)

[a]H.J.M. Bowen (1980) private communication

NΔ = not detected

Table 6.8

Analysis of standard water hyacinths (*Eichhornia crassipes*) containing the platinum metals by PIXE

Sample details	V	Mn	Fe	Zr	Ni	Cu	Zn	Br	Rb	Sr	Mo	Ba	Hg	Pb	S	P	Cl	K	Ca	Pt	Pd	Rh	Ir	Ru	Os	Au	Ag
Pt LO(2) found $\mu g\ g^{-1}$ dry wt	<2.0	280	169	<0.5	1.4	15.6	77	82	3.2	53	8.3	9	7.2	1.3	1800	4600	5400	28000	30000	5.6	<0.3	0.3	<0.6	<0.18	<0.7	<0.7	0.5
Pd LO " "	<7	960	236	<0.9	3.5	24.5	147	84	3.8	55	6.0	12	11.3	3.3	3200	6800	9200	38000	36000	1.2	5.6	<0.6	<0.9	<0.5	<0.9	<1.0	<0.8
Rh LO " "	4	280	230	<0.4	<1.1	23	172	82	3.5	58	16.0	11	8.3	2.2	2800	3800	9500	26000	34000	1.8	<0.4	3.8	<1.0	<0.2	<0.9	<1.0	<0.5
Ir LO " "	<4	134	97	<0.9	1.5	10.8	71	44	2.9	40	4.9	<6	6.9	0.8	1140	1600	2700	14300	17300	<0.5	<0.5	<0.4	<0.6	<0.3	<0.6	<0.6	1.2
Ru LO " "	<3	530	246	<0.5	1.5	15.9	124	106	3.0	67	3.4	8	10.0	1.5	1900	3700	7400	27000	34000	<0.8	<0.3	<0.3	<0.9	1.1	<1.0	<0.9	<0.4
Os LO " "	<2	250	181	<0.7	<0.9	17.8	118	122	4.6	61	6.3	<6	7.4	2.5	2400	3500	8900	27000	33000	<1.0	0.6	<0.3	<1.0	<0.3	<1.2	<1.0	<0.4
Pt HI found $\mu g\ g^{-1}$ dry wt	9	4500	3500	29.3	<8	<12	500	16.5	9.6	68	10.5	46	12	19	2500	4100	9200	15800	26000	1590	<0.8	<0.7	<7	<0.5	<6	<8	<0.9
Pd HI " "	<2	37	480	<0.3	0.6	11.9	49	33.3	4.1	28.3	48	<3	1.1	1.7	2100	6100	11800	34000	17000	<0.6	550	1.6	<0.7	<0.5	<0.6	<0.6	<0.8
Rh HI " "	<9	39	172	<0.9	10.2	15.2	115	17.6	1.7	21.4	14.1	<30	<1.3	6.0	1700	4200	6500	21000	8600	<1.2	13	138	<1.2	3.7	<1.4	<1.4	<0.5
Ir HI " "	<3	15	530	<0.4	<0.6	11.6	27.8	33.9	5.4	28.4	91	<6	2.6	5.1	2800	5700	13800	21800	15500	2.0	<0.5	<0.5	37	0.7	<1.2	<0.7	0.9
Ru HI " "	3	17	740	<0.3	<0.9	23	134	9.5	1.2	7.4	77	<4	3.1	19.8	2900	9300	7400	34000	129000	2.6	2.9	<0.6	<0.6	5900	<0.7	<0.6	10
Os HI " "	<4	76	271	<0.5	<1.3	32	128	10	3.3	11.2	22.3	<7	<6	8	2100	11100	9600	63000	15000	<5	<1.0	<0.7	<13	4.6	1050	<5	<1.3
Control/T/Tops	3	750	164	<0.4	3.3	20	161	77	3.9	41	13.0	14	5.0	3.8	1300	2800	7100	25000	25000	<0.9	<0.3	<0.4	<0.9	<0.3	<1.3	<0.9	<0.3

Table 6.9

A comparison of results for the determination of platinum metals in standard water hyacinths by various techniques

Sample details	Applied metal complex	A.A.S.	I.N.A.A.	I.C.P.	D.C. Arc[a]	P.I.X.E.
Pt LO(2) found µg g^{-1} dry wt	$H_2[PtCl_6]$	4.15	5.51	NΔ	8	5.6
Pd LO " "	$[Pd(NH_3)_2Cl_2]$	3.40	4.62[b]	NΔ	17	5.6
Rh LO " "	$Na_3[RhCl_6]$	4.22	4.31	NΔ	35	3.8
Ir LO " "	$Na_3[IrCl_6]$	-	1.29	NΔ	N	<0.6
Ru LO " "	$(NH_4)_3[RuCl_6]$	-	NΔ	NΔ	0.5	1.1
Os LO " "	$Na_2[OsCl_6]$	-	1.79	-	-	<1.2
Pt HI found µg g^{-1} dry wt	$(NH_4)_2[PtCl_6]$	2386	659?	1805	2296	1590
Pd HI " "	$H_2[PdCl_4]$	-	551	554	2480	550
Rh HI " "	$Na_3[RhCl_6]$	-	206	212	79	138
Ir HI " "	$Na_3[IrCl_6]$	-	49	31	9	37
Ru HI " "	$(NH_4)[RuCl_6]$	-	5141	-	-	5900
Os HI " "	$Na_2[OsCl_6]$	-	1103	-	-	1050

Micc. [c]2419/[d]2060

[a]determined against saltcake standards; [b]determined by CAS

[c]determined by X-ray fluorescence spectrometry (XRF)
details: Philips PW 1410 X-Ray spectrometer cf. Appendix

[d]determined by D.C. Argon Plasma emission analysis
Spectrospan (III) system. cf. Appendix

Table 6.10

submitted for PIXE analysis too, and these results appear in Table 6.9.

6.6 Results and Discussion

Elements other than those of the platinum group

Table 6.4 gives the results of analysis for Bowen's kale by dc arc emission spectrography, PIXE and ICP, together with the 'best' values (Bowen, 1974; 1981) (Wainerdi, 1979). The semi-quantitative method of dc arc emission gives acceptible results for four elements: Cu, Mn, Mo and V. In most cases, however, the results are far from 'best' values. ICP gives better results and in Table 6.5 shows the values found for some elements in NBS SRM 1571 orchard leaves, together with some selected results from another study (Jones *et al.*, 1982). It can be seen from Tables 6.4 and 6.5 that reasonable results from the ICP method are found for Ca, Fe, Mg, Mn, Zn and Cu. However, Al, Cr and Mo are consistently high. Jones *et al.* (1982) have analysed plant materials by ICP after wet ashing in a nitric-perchloric acid mixture. They determined Ca, Fe, Mg, Mn and P directly and other elements: Cd, Cu, Mo, Ni, V, Zn, Al, Co and Pb after concentration on Chelax 100 resin.

Using PIXE, acceptable results for Bowen's kale were obtained for Br, Ca, Cu, Fe, K, Mn, P, Rb, S, Sr and (Ba). Maenhaut *et al.* (1982) suggested that PIXE can be used to estimate Ca, Fe, Cu, Zn and Br in human serum and it has been suggested also that Cu, Fe, Mn, Pb, Sr and Zn can be determined in soils (Baum *et al.*, 1976). Both Jones (1982) and Robberecht *et al.* (1982) have shown that the pre-concentration step in the analysis for trace elements is critical. For example, Si interferes with the determination of Fe, Al and Mn if HF is not used as part of the pre-digestion acid treatment. Bromine may be lost even using low temperature ashing (Robberecht, 1982). In this study PIXE analysis was

carried out on the dried homogenised plant material without pre-digestion. For routine analyses, the elimination of pre-concentration steps reduces the possibility of contamination.

Table 6.7 shows the results of the analysis of control water hyacinths, grown without added platinum metals, by the three analytical methods: dc arc emission, ICP and PIXE. The analysis of Bowen's kale had indicated that all three methods should give reasonable results for Cu and Mn and these are found to be of the same order by all three methods. The ICP results were, however, consistently higher.

Examination of the distribution of trace elements in water hyacinth shows there is a large variation in the levels between controls and plants treated with the platinum metals. In particular, the levels of Fe and Mn seem affected by treatment with platinum metals. Further investigation of these interelement relationships is needed.

Determination of the platinum group metals

Table 6.4 includes the PIXE analysis of a sample of Bowen's kale which had been spiked with the platinum metals. This same spiked sample was used as a matrix matched biological standard in neutron activation analysis. Values ($\mu g\ g^{-1}$) obtained by PIXE analysis were as follows: Pd: (100) found 119; Pt: (25) found 27; Rh: (5) found 5.4; Ru: (50) found 56; Ir: (5) found 3.2; Os: (25) found 22.

A comparison of values for the platinum metals, in standard water hyacinths, determined by various techniques is presented in Table 6.10. None of the platinum metals in the 'LO' samples were detected by ICP; 'HI' samples show a good measure of agreement. PIXE is suitable as a method for the determination of elements in plant samples including those of the platinum group. ICP is satisfactory for the platinum metals in plant samples but only at relatively high concentrations. DC arc emission

spectrography is unsuitable for low level determinations of the platinum metals in plant ash, palladium in particular. However, it is apparent from this work and other studies (Le Roy *et al.*, 1977) that of all the elements in the platinum group, platinum is the most difficult to determine in plants and other biological samples.

REFERENCES

Baum, R., Gutnecht, W.F., Willis, R.D. and Walter, R.L. (1976), Anal. Chim. Acta, 85 323.

Beamish, F.E. and Van Loon, J.C. (1977), Analysis Noble Metals, Academic Press, London.

Bowen, H.J.M. (1974), J. Radioanal. Chem., 19 215.

Bowen, H.J.M. (1981), private communication.

Jones, J.W., Capar, S.G. and O'Haver, T.C.O. (1982), Analyst, 107 353.

Le Roy, A.F., Wheling, M.L., Sponseller, H.O., Friauf, W.S., Solomon, R.E., Dedrick, R.L., Litterest, C.L., Gram, T.E., Guarino, A.M. and Becker, D.A. (1977), Biochem. Med., 18 194.

Maenhaut, M., De Reu, L. and Tomza, U. (1982), Anal. Chim. Acta, 136 301.

Robberecht, R., Van Grieken, R., Shani, J. and Borak, S. (1982), Anal. Chim. Acta, 136 285.

Wainerdi, R.E. (1979), Pure Appl. Chem., 51 1183.

CHAPTER VII

A STUDY OF THE UPTAKE AND ACCUMULATION OF THE PLATINUM METALS BY *EICHHORNIA CRASSIPES* (MART.) SOLMS (WATER HYACINTH)

7.1 Introduction

7.1.1 The distribution of the water hyacinth (*Eichhornia crassipes*)

Eichhornia crassipes (MART.) Solms (water hyacinth) is a free floating aquatic plant which is found in many tropical and sub tropical regions of the world, although it is a true indigene of South America. With its highly prolific, vegetative reproductive system, it can double in number every 8-10 days, particularly in warm nutrient enriched waters. As a consequence, many rivers, ditches and canals in Africa, Asia and the southern U.S. have become infested with this plant. It is not clear just how this plant came to spread so rapidly in the U.S.A., but it is thought to have been introduced during the International Cotton Exposition in 1884. The plants were distributed as souvenirs, prized for their beauty (Plate 7.1), and cultivated in surrounding districts. From there, they found their way into streams and rivers, where the rapid rate of growth caused severe overcrowding (Hildebrand, 1946).

The subsequent spread of the water hyacinth has been both spectacular and disastrous everywhere. Throughout Africa and Asia, the plant has spread, clogging rivers, lakes and streams, sometimes competing for space with other aquatic weeds such as water lettuce (*Pistia stratiotes*) and duckweed (*Lemna minor*) (Sculthorpe, 1967).

Before 1937, water hyacinths were controlled with sodium arsenite, until the toxicity hazard was realised; after this the plant hormone 2,4-dichlorophenoxyacetic acid (2,4-D) was used to control its growth

Plate 7.1 Inflorescences of the water hyacinth (*Eichhornia crassipes*) (MART.) Solms)

Hitchcock *et al.*, 1949). A recent report has suggested that gibberellic acid can enhance the effect of 2,4-D on water hyacinth tenfold (Pieterse, 1980).

7.2.1 The morphology of the water hyacinth (*Eichhornia crassipes*)

A fully grown water hyacinth consists of roots, rhizomes, stolons, leaves, inflorescences and fruit clusters (Figure 7.1) (Plate (7.2). In a dense mat it is very difficult to distinguish between rhizomes and stolons, but both serve distinct and different functions. The 'tops' of water hyacinth possess swollen portions of the petioles, which are called floats. Thus the plant tops consist of a membraneous ligule, with sub-float, float and an isthmus joining the blade or pseudolamina.

The biology of the water hyacinth has been the subject of several comprehensive botanical reviews (Arber, 1918; 1922) (Penfound and Earle,1948) (Sculthorpe, 1967). Anatomical studies have been reported also (Weber, 1950) (Hasman and Inanc, 1957).

7.1.3 The inorganic composition of the water hyacinth (*Eichhornia crassipes*)

It has been suggested that vascular aquatic plants might prove useful for mineral nutrient removal from polluted waters (Boyde, 1970). Water hyacinths have been used successfully to treat anaerobic lagoon effluent (Miner *et al.*, 1971) and sewage waste (Rogers and Davis, 1972) (McDonald and Wolverton, 1980). Laboratory studies on their growth characteristics, yield potential and nutritive content have indicated that the harvested plants could be a useful food supplement for livestock (Knipling *et al.*, 1971). Pilot studies have been conducted into the uptake and accumulation of nitrogen, potassium and phosphorus by water hyacinths, from lakes, ponds and sewage lagoons, for the purpose of producing biogas and fertilizer, and feeding livestock (Boyde, 1970) (Boyde and Scarsbrook, 1975) (Wolverton and McDonald, 1976).

Many recent studies of the water hyacinth have centred on the uptake

Plate 7.2 The water hyacinth (*Eichhornia crassipes MART Solms*)

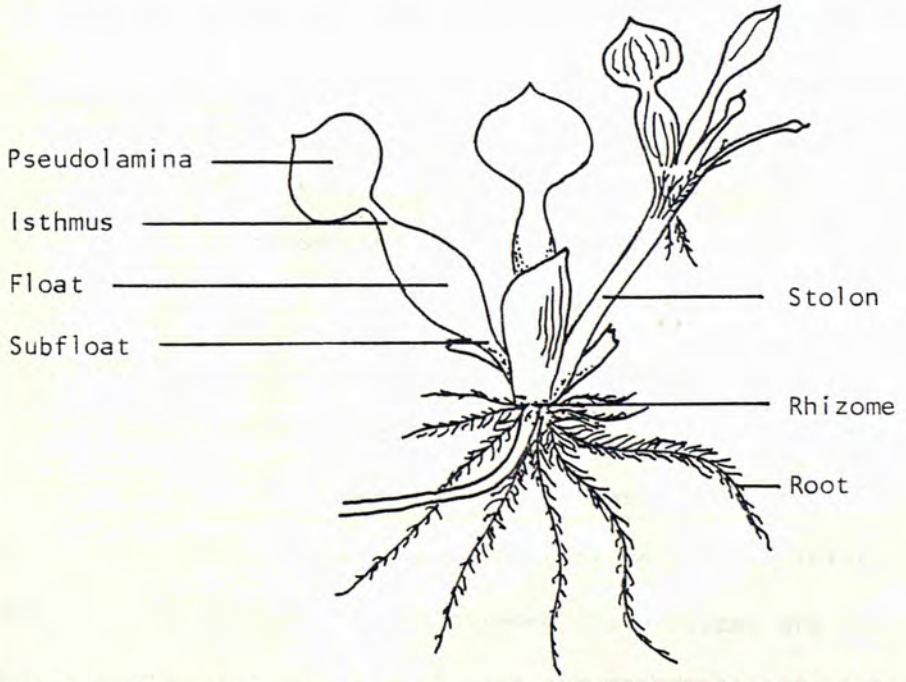


Figure 7.1 Schematic drawing of a water hyacinth (*Eichhornia crassipes MART Solms*) (after Cooley and Martin, 1977)

of trace metals as well as macronutrients. Boyde (1971) has reported that the elemental composition of water hyacinths falls within the range of elemental values reported for other aquatic and terrestial plants. It has been demonstrated that the water hyacinth is able to remove substantial quantities of silver, cobalt and strontium from static water systems (Wolverton and McDonald, 1975).

The trace metal distribution within the water hyacinth has been studied and related to the solubility (log Ksp) of the metal carbonate (Cooley and Martin, 1976; 1977). Laboratory experiments have been conducted, using hydroponic techniques, to investigate the uptake of heavy metals in water hyacinths. These have ranged from the beneficial elements copper, manganese and zinc (Johnson and Sheeham, 1977) to the toxic metals cadmium, lead, arsenic, mercury and nickel (Tatsuyana *et al.*, 1977; 1979) (Chigbo *et al.*, 1979) (Widyanto *et al.*, 1978). A large part of this study is concerned with the uptake, by water hyacinths, of the six heaviest elements in group VII: the platinum metals (platinum group metals). The aims of the uptake experiments reported in this chapter can be summarised: (a) to investigate the relative toxicity of the platinum metals when applied as water soluble chloro-complexes; (b) to ascertain the potential use of water hyacinths in recovering trace quantities of the platinum metals from solution.

Cooley and Martin (1978, 1979) have identified at least three water hyacinth biotypes based on size differences. As well as the more usual small and medium sized plants, they have designated the large water hyacinths with stems rather than floats as "super-hyacinths". Variation in the trace metal distribution between the biotypes are reported also. It would seem that the uptake of iron and manganese are particularly important (Cooley and Martin, 1980).

7.2 Experimental

Supplies of water hyacinths (*Eichhornia crassipes*) were obtained from Adventure Water Gardens Ltd., Bracknell and were maintained as stock throughout the duration of the uptake experiments. The mother plants were kept in polypropylene tanks (Osmaflow, Wavin Plastics Ltd.) containing 68dm^3 of water with a nutrient base of 4-5kg of John Innes No.1 soil. Prior to the commencement of the uptake experiment, young vegetative offspring were removed from the mother plants by severance of the stolon where it joined the rhizome of the daughter. These plants were washed in deionised water and transferred to a large plastic container of nutrient solution at half strength. Details of the full strength nutrient solution used are given in Table 3.1, chapter 3.1. The plants selected for each individual experiment were chosen for uniformity of size and growth, however, it was not possible to obtain the same sized plant for every experiment and consequently some uptake experiments were conducted with medium sized plants, others with small sized plants. This is indicated in the respective tables.

The metal uptake experiments were carried out under controlled conditions in a Prestcold environmental growth room (Chapter 3.1). Cool white fluorescent and tungsten filament tubes provided a complete spectral coverage of 10^3ft candles or 10760 lux. A 14hr day was maintained at a temperature of 25±1°C (day) and 20±1°C (night). Relative humidity was difficult to control and remained in the region 70-75% throughout the experiments. The plants were placed in 2dm^3 plastic containers covered with black adhesive tape. Aeration was carried out by supplying compressed air via plastic tubing to sintered glass filter sticks, which were suspended in the solutions. The tubing was ringed and clamps used for uniform pressure. To reduce the possibility of contamination by splashing

white plastic balls were placed on the surface of the nutrient solution with the plants for the duration of the uptake experiment (Plate 7.3).

At the beginning of each uptake experiment, the $2dm^3$ container was charged with half strength nutrient solution and a specific concentration of a platinum group metal complex. Letters were used as metal concentration codes; these are given in Table 7.1.

Platinum metals applied concentration

mol dm^{-3}

Code	ppm or mg dm^{-3}	Ru	Rh	Pd	Os	Ir	Pt
A	0.05	4.94×10^{-7}	4.85×10^{-7}	4.69×10^{-7}	2.62×10^{-7}	2.60×10^{-7}	2.56×10^{-7}
B	0.5	4.94×10^{-6}	4.85×10^{-6}	4.69×10^{-6}	2.62×10^{-6}	2.60×10^{-6}	2.56×10^{-6}
C	2.5	2.47×10^{-5}	2.42×10^{-5}	2.34×10^{-5}	1.31×10^{-5}	1.30×10^{-5}	1.28×10^{-5}
D	10.0	9.89×10^{-5}	9.71×10^{-5}	9.39×10^{-5}	5.25×10^{-5}	5.20×10^{-5}	5.12×10^{-5}
E	20.0	1.98×10^{-4}	1.94×10^{-4}	1.88×10^{-4}	1.05×10^{-4}	1.04×10^{-4}	1.03×10^{-4}
F	30.0	2.97×10^{-4}	2.92×10^{-4}	2.82×10^{-4}	1.58×10^{-4}	1.56×10^{-4}	1.54×10^{-4}

Table 7.1

7.2.1 The uptake of platinum, applied as various complexes, by the water hyacinth

The uptake of platinum by water hyacinths was studied by applying the metal in various complexed forms in combination with a nutrient solution at half strength. The following platinum concentrations were examined: 0.05, 0.5, 2.5 and 10.0 ppm (mg dm^{-3}). Each uptake experiment was designated by a number; these and the forms in which platinum was applied are given in Table 7.2.

The plants were grown alongside controls (Table 7.15) which were in

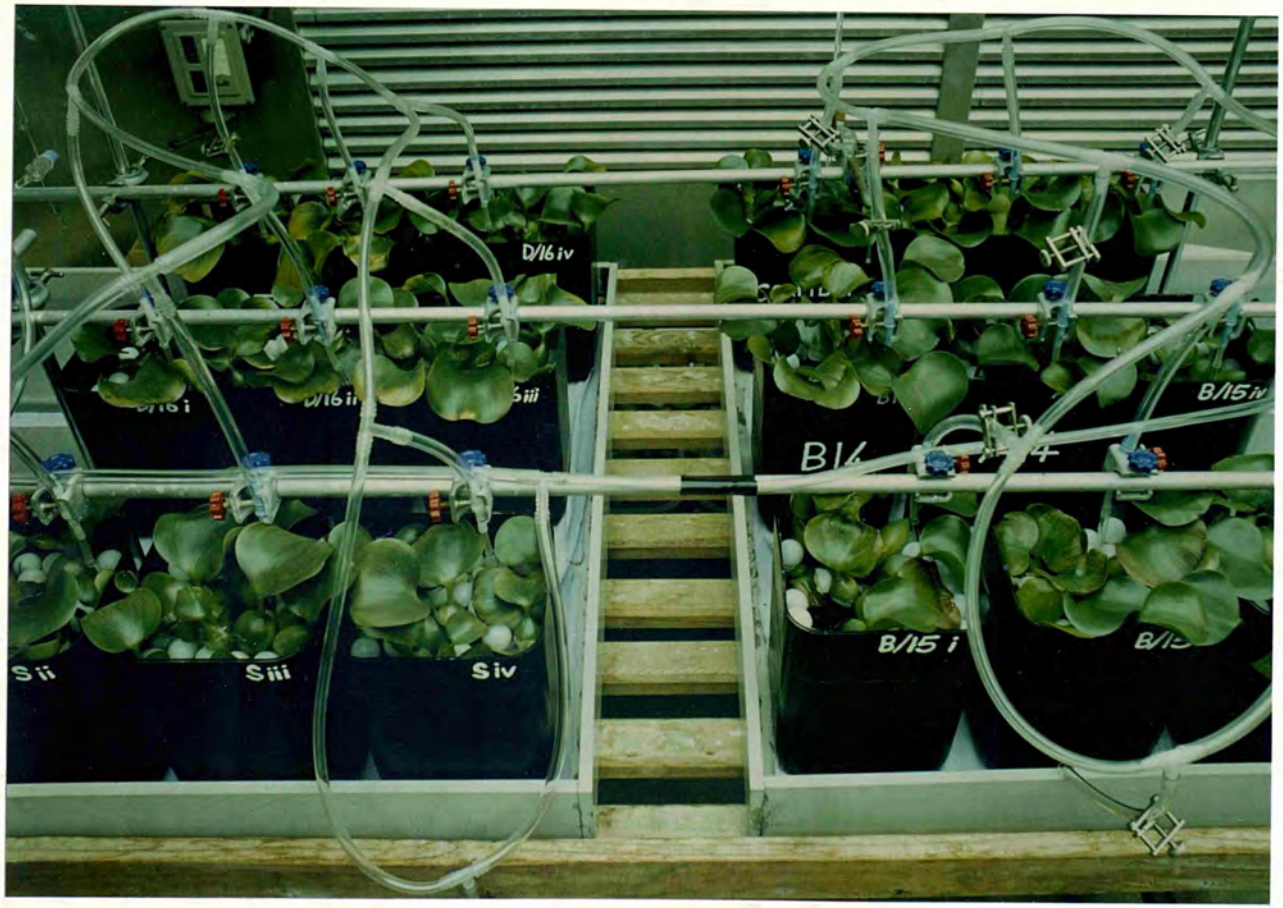


Plate 7.3 A typical experimental set up in the growth room

Expt. No.	Platinum applied as	Pt^{n+}	complex stereochemistry
5	cis $[Pt(NH_3)_2Cl_2]$	Pt^{2+}	neutral complex; square planar
8	trans $[Pt(NH_3)_2Cl_2]$	Pt^{2+}	neutral complex; square planar
10	$K_2[PtCl_4]$	Pt^{2+}	anionic complex; square planar
14	$(NH_4)_2[PtCl_6]$	Pt^{4+}	anionic complex; octahedral

Table 7.2 The uptake of platinum by water hyacinths

in half strength nutrient solution only. All solutions were topped up daily with deionised water. After one week, the nutrient solutions and applied platinum were renewed. The plants were harvested after two weeks growth. Each was washed carefully with distilled deionised water and placed on clean dry blotting paper for surplus water to drain off.

The plants were separated into roots and tops, placed in sealed plastic bags and the wet weights determined as soon as possible. They were oven dried at 90°C for 24hrs, allowed to cool in a desiccator and the dry weights determined. The dried plant material was analysed for platinum by wet ashing in concentrated ARISTAR nitric acid. When ashing was complete, the digest was taken to near dryness and 5ml concentrated ARISTAR hydrochloric acid added. The digests were taken down with hydrochloric acid several times to drive off nitrate fumes. Finally, they were taken up with conc. hydrochloric acid and made up to 5% v:v in acid with deionised and distilled water. Platinum was determined by atomic absorption spectroscopy. High levels were determined in an air-acetylene flame, with lanthanum chloride (0.2% w/v La^{3+}) used as a releasing agent. Details of instrument settings and other parameters are given in Table 7.3. Lower levels of platinum, which were below the detection limit of the

Operating details and data for flame atomic absorption spectroscopy[a]

Element	Ground State of Atom	Wavelength nm	Bandpass nm	Lamp Current, mA	Fuel/Oxidant flow rates, $dm^3\ min^{-1}$	Solution composition	Releasing agent	Sensitivity[b]	Detection[c] limit
Platinum	$^3D_3$	265.95	0.4	10	C_2H_2:1.0/Air:5.0	5% HCl	0.2% w/v La^{3+}	0.59	0.43
Palladium	$^1S_0$	340.46	0.2	10	C_2H_2:1.0/Air:5.0	5% HCl	0.5% w/v La^{3+}	0.30	0.09
Rhodium	$^4F_{9/2}$	343.49	0.4	10	(i) C_2H_2:3.4/N_2O:5.5	0.5% HCl	1% w/v Na_2SO_4	0.29	0.08
					(ii) C_2H_2:1.0/Air:5.0	0.5% HCl	1% w/v Na_2SO_4	0.32	0.16
Calcium	$^1S_0$	422.67	0.8	8	C_2H_2:1.4/Air:5.0	1% H_2SO_4	0.04% w/v La^{3+}	0.09	0.06

[a]Pye Unicam SP2900 Double Beam Spectrophotometer equipped with SP9-10 Gas Control Box and interfaced with the Hewlett Packard Laboratory Data System (HP97)

[b]Sensitivity defined as the concentration of an element in μg cm^{-3} required for 1% absorption

[c]Detection limit defined as the concentration of an element in μg cm^{-3} required to produce a signal twice the standard deviation of the background.

Table 7.3

flame, were determined by electrothermal atomisation atomic absorption (ETA AAS). The details of this method are reported in chapter 2. Calcium was determined in some of the experiments but not all. The yields and analyses of plants treated with platinum appear in Tables 7.4 to 7.7. Values for the recovery of platinum from solution are given in Table 7.16.

Results and Discussion

The plants were photographed at the start and at the conclusion of the uptake experiment. A selection of these appear in plates 7.4 to 7.9. Toxic effects were observed for cis $[Pt(NH_3)_2Cl_2]$ at the 2.5ppm level where some chlorosis was evident and a drop off in yield was recorded. At the 10.0ppm level, the drop off in yield was more marked, with some plant leaves visibly necrotic; chlorosis was much more in evidence also. Roots at this level were stunted and darkened quite considerably. Similar toxic effects were observed for the uptake of trans $[Pt(NH_3)_2Cl_2]$ and $K_2[PtCl_4]$, with the drop off in yield occuring between the 0.5 and 2.5 ppm levels. The effect of platinum as $(NH_4)_2[PtCl_6]$ was much less toxic. Only a slight drop off in yield at the 10.0ppm level was recorded whilst the roots at this level were slightly yellowed; at the highest level of applied platinum,chlorosis of the tops was evident too (Plate 7.9).

The accumulation of platinum in the roots of water hyacinths is quite substantial. In all cases the amount of platinum taken up by the plants increases as the concentration of applied platinum is increased. Some of the platinum taken up by the roots is transported to the plant tops. This is less significant for $[PtCl_6]^{2-}$ where very little platinum was detected in the plant tops; this may account for the absence of toxic effects observed with other forms of platinum. The calcium levels did not appear significant for plant tops but for the plant roots, a clear increase in calcium concentration occurs with the increase in applied platinum.

Plate 7.4

The uptake of platinum applied as cis $[Pt(NH_3)_2Cl_2]$

Plate 7.5

The uptake of platinum applied as trans $[Pt(NH_3)_2Cl_2]$

Plate 7.6

The uptake of platinum applied as $K_2[PtCl_4]$

Plate 7.7 The effect of platinum, applied as trans $[Pt(NH_3)_2Cl_2]$, on the roots of *Eichhornia crassipes*

Plate 7.8 The effect of platinum, applied as $K_2[PtCl_4]$, on the roots of *Eichhornia crassipes*

(a)

(b)

Plate 7.9 The uptake of platinum, applied as $(NH_4)_2[PtCl_6]$, by the water hyacinth (*Eichhornia crassipes*); (a) before platinum application; (b) after 14d growth with applied Pt.

Yields and analysis of *Eichhornia crassipes* treated with cis[$Pt(NH_3)_2Cl_2$]

Plant tops[a]	A5	B5	C5	D5
Applied Pt (ppm)	0.05	0.5	2.5	10.0
Wet weight (g)	20.87	37.24	15.96	21.43
Dry weight (g)	1.47	2.89	0.98	1.47
Weight of Water (g)	19.40	34.35	14.98	19.96
Water present (%)	93.0	92.2	93.9	93.1
Total Pt (µg)	12.5	124	1050	3275
Pt (ppm dry weight)	8.5	43	1068	2225
Total Ca (mg)	29.4	52.0	24.6	42.7
Ca (% dry weight)	2.0	1.8	2.5	2.9

Plant roots[a]	A5	B5	C5	D5
Applied Pt (ppm)	0.05	0.5	2.5	10.0
Wet weight (g)	5.51	7.71	4.44	10.30
Dry weight (g)	0.63	1.10	0.52	0.91
Weight of water (g)	4.88	6.61	3.92	9.39
Water present (%)	88.6	85.7	88.3	91.2
Total Pt(µg)	100	1751	6050	23889
Pt (ppm dry weight)	159	1590	11546	26397
Total Ca (mg)	1.6	3.6	3.0	7.8
Ca (% dry weight)	0.25	0.33	0.58	0.86

[a]Medium sized plants

Table 7.4

Yields and analysis of *Eichhornia crassipes* treated with trans[$Pt(NH_3)_2Cl_2$]-

Plant tops[a]	A8	B8	C8	D8
Applied Pt (ppm)	0.05	0.5	2.5	10.0
Wet weight (g)	33.53	39.65	22.44	16.74
Dry weight (g)	3.07	2.85	2.39	2.63
Weight of water (g)	30.46	36.80	20.00	14.11
Water present (%)	90.8	92.8	89.3	84.3
Total Pt (µg)	8.3	162	946	2580
Pt (ppm dry weight)	2.7	57	396	981
Total Ca (mg)	67.5	51.3	52.6	55.2
Ca (% dry weight)	2.2	1.8	2.2	2.1

Plant roots[a]	A8	B8	C8	D8
Applied Pt (ppm)	0.05	0.5	2.5	10.0
Wet weight (g)	4.29	5.27	3.21	1.89
Dry weight (g)	0.49	0.45	0.48	0.32
Weight of water (g)	3.80	4.82	2.73	1.57
Water present (%)	88.6	91.5	85.0	83.1
Total Pt (µg)	74	1313	1865	4006
Pt (ppm dry weight)	152	2919	3885	12520
Total Ca (mg)	2.0	1.9	2.1	1.6
Ca (% dry weight)	0.41	0.43	0.43	0.49

[a]Medium sized plants

Table 7.5

Yields and analysis of *Eichhornia crassipes* treated with $K_2[PtCl_4]$

Plant tops[a]	A10	B10	C10	D10
Applied Pt (ppm)	0.05	0.5	2.5	10.0
Wet weight (g)	35.06	30.19	19.36	17.99
Dry weight (g)	2.69	2.33	1.72	1.81
Weight of water (g)	32.37	27.86	17.64	16.18
Water present (%)	92.3	92.3	91.1	89.9
Total Pt (µg)	6	56	232	2778
Pt (ppm dry weight)	2.2	24	135	1535
Total Ca (mg)	35.0	28.0	34.4	34.4
Ca (% dry weight)	1.3	1.2	2.0	1.9

Plant roots[a]	A10	B10	C10	D10
Applied Pt (ppm)	0.05	0.5	2.5	10.0
Wet weight (g)	5.99	5.01	3.91	5.76
Dry weight (g)	0.47	0.42	0.31	0.44
Weight of water (g)	5.52	4.59	3.60	5.32
Water present (%)	92.1	91.6	92.1	92.4
Total Pt (µg)	126	730	2276	13069
Pt (ppm dry weight)	268	1739	7341	29702
Total Ca (mg)	1.7	1.7	2.5	4.3
Ca (% dry weight)	0.37	0.41	0.81	0.97

[a]Medium sized plants

Table 7.6

Yields and analysis of *Eichhornia crassipes* treated with $(NH_4)_2[PtCl_6]$

Plant tops[a]	A14	B14	C14	D14
Applied Pt (ppm)	0.05	0.5	2.5	10.0
Wet weight (g)	51.79	62.16	48.86	44.88
Dry weight (g)	3.38	4.11	3.30	3.05
Weight of water (g)	48.41	58.05	43.56	41.83
Water present (%)	93.5	93.4	93.0	93.2
Total Pt (µg)	nd	103	89	549
Pt (ppm dry weight)	nd	25	27	180

Plant roots[a]	A14	B14	C14	D14
Applied Pt (ppm)	0.05	0.5	2.5	10.0
Wet weight (g)	7.96	9.81	7.82	9.02
Dry weight (g)	0.42	0.49	0.50	0.48
Weight of water (g)	7.54	9.32	7.32	8.54
Water present (%)	94.7	95.0	93.6	94.7
Total Pt (µg)	56	522	3127	5484
Pt (ppm dry weight)	133	1066	6253	11424

[a]Medium sized plants

Table 7.7

7.2.2 The uptake of palladium by the water hyacinth

Experimental

The apparatus and conditions used in this experiment were the same as those used for platinum. Palladium was applied in two forms (Johnson Matthey Research Centre) at the following levels: 0.05, 0.5, 2.5 and 10.0ppm Pd.

Expt. No.	Palladium applied as	Pd^{n+}	complex sterochemistry
6	$K_2[PdCl_4]$	Pd^{2+}	anionic complex, square planar
9	$cis[Pd(NH_3)_2Cl_2]$	Pd^{2+}	neutral complex, square planar

The plants were grown for two weeks; palladium and nutrients were renewed after one week. The plants were harvested and prepared for analysis as described before. Palladium was determined by atomic absorption spectroscopy; high levels using the air-acetylene flame (Table 7.3), and low levels using electrothermal atomisation (chapter 4). The results are given in Tables 7.8 and 7.9.

Results and Discussion

After two weeks growth, the plants treated with $[PdCl_4]^{2-}$ displayed toxic effects at the 2.5 and 10.0ppm levels. Chlorosis of the leaves was evident and normal growth had been restricted. The roots were stunted in growth and dark brown in colour, indicating some precipitation may have taken place. These effects were more marked at the 10.0ppm level (Plate 7.10). As with platinum, a drop off in yield was recorded between the 0.5 and 2.5ppm levels. The water content of the plant tops in both palladium experiments decreased with increase in applied palladium concentration.

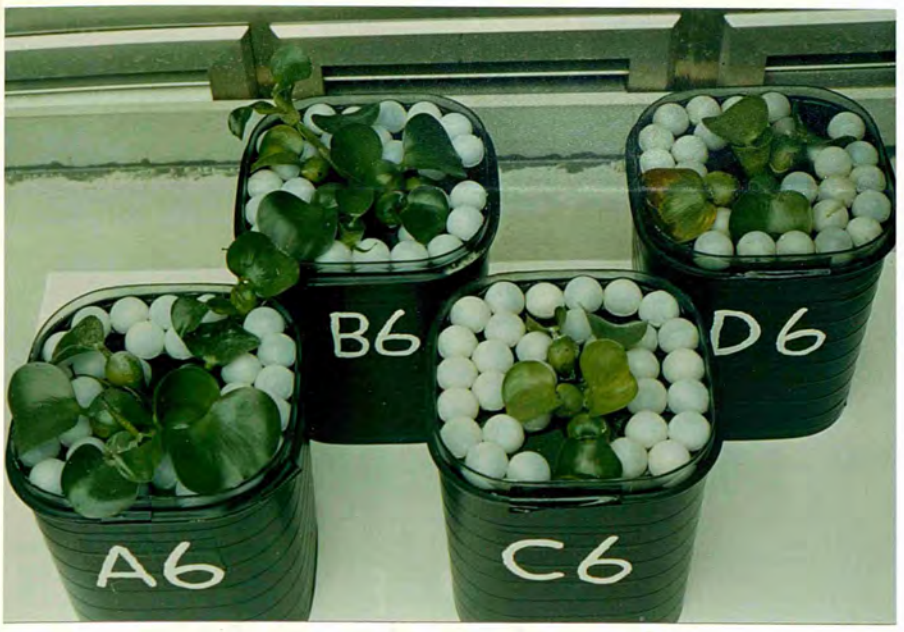


Plate 7.10

The uptake of palladium applied as $K_2[PdCl_4]$

Plate 7.11

The uptake of palladium applied as cis $[Pd(NH_3)_2Cl_2]$

Plate 7.12

The effect of palladium applied as cis $[Pd(NH_3)_2Cl_2]$ on roots of *Eichhornia crassipes*

Yields and analysis of *Eichhornia crassipes* treated with $K_2[PdCl_4]$

Plant tops[a]	A6	B6	C6	D6
Applied Pd (ppm)	0.05	0.5	2.5	10.0
Wet weight (g)	14.09	12.47	5.78	9.73
Dry weight (g)	0.89	0.86	0.53	0.80
Weight of water (g)	13.20	11.61	5.25	8.93
Water present (%)	93.7	93.1	90.8	91.8
Total Pd (µg)	35	87	460	2120
Pd (ppm dry weight)	39	102	876	2664

Plant roots[a]	A6	B6	C6	D6
Applied Pd (ppm)	0.05	0.5	2.5	10.0
Wet weight (g)	1.25	2.10	0.56	1.26
Dry weight (g)	0.13	0.22	0.07	0.10
Weight of water (g)	1.12	1.88	0.49	1.16
Water present (%)	89.6	89.5	87.5	92.1
Total Pd (µg)	42	410	1417	7043
Pd (ppm dry weight)	316	1908	20241	67723

[a]Small sized plants

Table 7.8

Yields and analysis of *Eichhornia crassipes* treated with cis[$Pd(NH_3)_2Cl_2$]

Plant tops[a]	A9	B9	C9	D9
Applied Pd (ppm)	0.05	0.5	2.5	10.0
Wet weight (g)	20.76	20.45	9.60	9.74
Dry weight (g)	1.90	1.95	1.12	1.56
Weight of water (g)	18.86	18.50	8.48	8.18
Water present (%)	90.9	90.5	88.3	84.0
Total Pd (µg)	42	127	1071	4086
Pd (ppm dry weight)	22	65	956	2619

Plant roots[a]	A9	B9	C9	D9
Applied Pd (ppm)	0.05	0.5	2.5	10.0
Wet weight (g)	4.71	6.47	1.57	2.12
Dry weight (g)	0.40	0.34	0.16	0.19
Weight of water (g)	4.31	6.13	1.41	1.93
Water present (%)	91.5	94.8	89.8	91.0
Total Pd (µg)	103	660	2905	9979
Pd (ppm dry weight)	255	1935	17934	52246

[a]Small sized plants

Table 7.9

With palladium applied as cis $[Pd(NH_3)_2Cl_2]$, again a drop off in yield was recorded between the 0.5 and 2.5ppm levels. The toxic effects observed at high levels appeared more severe than those with $[PdCl_4]^{2-}$ (plate 7.11). Necrotic plant tops were evident and growth was very restricted. The roots were stunted and dark brown in colour (Plate 7.12). Both forms of palladium were taken up in substantial quantities by the plant roots; smaller but similar amounts were transported to the plant tops in both cases. Values for the recovery of palladium from dilute solutions are given in Table 7.16.

7.2.3 The uptake of ruthenium by the water hyacinth

Experimental

The uptake of ruthenium was investigated using similar techniques. Two ruthenium complexes (Johnson Matthey Research Centre) were applied at the following levels: 0.05, 0.5, 2.5 and 10.0ppm Ru;

13	$(NH_4)_3[RuCl_6]$	Ru^{3+}	anionic complex, octahedral
18	$[Ru(phen)_3]I_2$	Ru^{2+}	cationic complex, octahedral with three bidentate chelating ligands.

The plants were grown for two weeks, harvested and dried. Ruthenium determinations were carried out on the dried homogenised plant material using Instrumental Neutron Activation Analysis (INAA). Results are given in Tables 7.10 and 7.11. Details of the analytical method are given in chapter 5.

Results and Discussion

After several days, a black precipitate was observed in the nutrient solutions containing the complex anion $[RuCl_6]^{3-}$, which is known to be unstable in aqueous solution; it undergoes aquation in a matter of seconds:

$$[RuCl_6]^{3-} \underset{\longrightarrow}{\overset{\text{fast}}{\longleftarrow}} [RuCl_5(H_2O]^{2-}$$

Plate 7.13 The uptake of ruthenium applied as $(NH_4)_3[RuCl_6]$

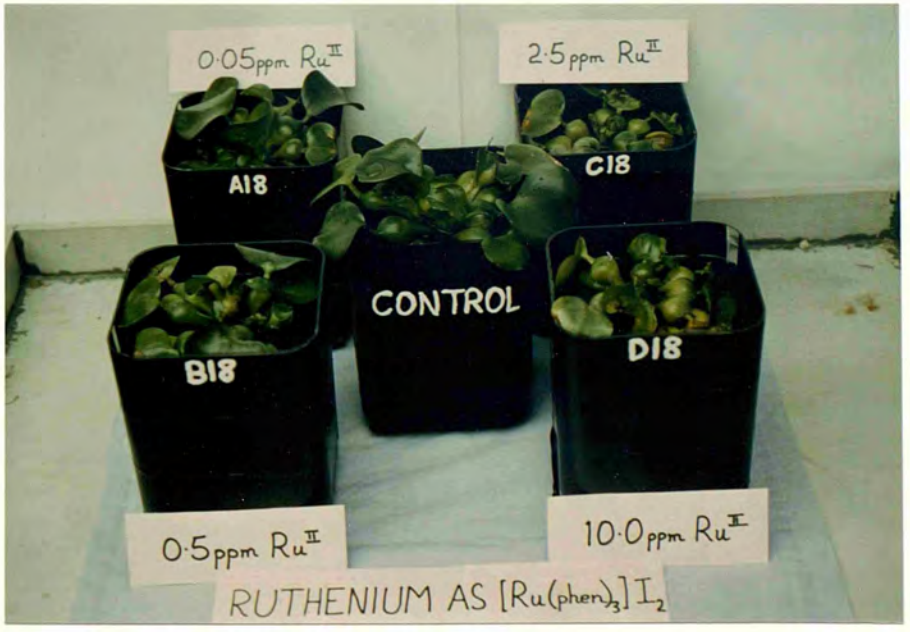


Plate 7.14 The uptake of ruthenium applied as $[Ru(phen)_3]I_2$

Yields and analysis of *Eichhornia crassipes* treated with $(NH_4)_3[RuCl_6]$

Plant tops[a]	A13	B13	C13	D13
Applied Ru (ppm)	0.05	0.5	2.5	10.0
Wet weight (g)	9.39	10.66	10.17	9.13
Dry weight (g)	0.70	0.94	0.74	0.95
Weight of water (g)	8.69	9.72	9.43	8.18
Water present (%)	92.6	91.2	92.7	89.6
Total Ru (µg)	41	258	722	2452
Ru (ppm dry weight)	59	274	975	2581

Plant roots[a]	A13	B13	C13	D13
Applied Ru (ppm)	0.05	0.5	2.5	10.0
Wet weight (g)	1.20	1.32	0.95	0.80
Dry weight (g)	0.10	0.13	0.09	0.15
Weight of water (g)	1.10	1.19	0.86	0.65
Water present (%)	91.7	90.2	90.5	81.3
Total Ru (µg)	16	519	1402	7505
Ru (ppm dry weight)	156	3990	15578	50036

[a]Small sized plants

Table 7.10

Yields and analysis of *Eichhornia crassipes* treated with $[Ru(phen)_3]I_2$

Plant tops[a]	A18	B18	C18	D18
Applied Ru (ppm)	0.05	0.5	2.5	10.0
Wet weight (g)	21.12	25.94	17.06	17.37
Dry weight (g)	1.58	1.96	1.75	1.65
Weight of water (g)	19.54	23.98	15.31	15.72
Water present (%)	92.5	92.4	89.7	90.5
Total Ru (µg)	19	251	2328	1054
Ru (ppm dry weight)	12	128	1330	639

Plant roots[a]	A18	B18	C18	D18
Applied Ru (ppm)	0.05	0.5	2.5	10.0
Wet weight (g)	3.24	3.64	5.25	3.72
Dry weight (g)	0.30	0.32	0.40	0.27
Weight of water (g)	2.94	3.32	4.85	3.45
Water present (%)	90.7	91.2	92.4	92.7
Total Ru (µg)	71	670	4237	1637
Ru (ppm dry weight)	238	2094	10592	6063

[a]Medium sized plants.

Table 7.11

The rate of replacement of Cl^- by H_2O gets slower as the number of chloride ligands decreases, thus the conversion of $[RuCl(H_2O)_5]^{2+}$ has a half reaction time of about a year (Cotton and Wilkinson, 1972).

At the end of the experiment, toxic effects were evident at the 10.0ppm level only. These included some chlorosis and blackened, stunted roots, due to precipitation, possibly of some ruthenium hydroxy species in the outer free spaces of the roots (Plate 7.13). A slight drop off in yield and % water was recorded at this level.

The effects of ruthenium applied as the more stable cationic complex $[Ru(phen)_3]I_2$ were more marked. A drop off in yield was recorded between the 0.5 and 2.5ppm levels, and an increasing yellow-orange colouration was observed in the roots as the concentration of the complex applied was increased. This was due to the intense yellow orange colour of the complex in aqueous solution. At the 10.0ppm level, the leaves were dried and curled; the floats had necrotic patches, the roots were stunted and highly coloured (Plate 7.14).

Results of INAA indicate that substantial amounts of ruthenium are accumulated from solution by the water hyacinth. Most of the ruthenium is deposited in the roots, probably as some chlorohydroxy species in the case of $[RuCl_6]^{2-}$; some ruthenium is transported to the plant tops. The best total recovery values were obtained for the 0.5-2.5ppm levels of applied ruthenium.

7.2.4 The uptake of rhodium, iridium and osmium by the water hyacinth

Experimental

Using the techniques established, water hyacinths were grown in half strength nutrient solutions, to which chloro complexes of the other platinum metals had been added:

7	$Na_3[RhCl_6]$	Rh^{3+}	anionic complex, octahedral
11	$Na_3[IrCl_6]$	Ir^{3+}	anionic complex, octahedral
12	$Na_2[OsCl_6]$	Os^{4+}	anionic complex, octahedral

The plants were harvested, dried and prepared for analysis as before. Rhodium determinations were carried out by atomic absorption techniques, using a nitrous oxide acetylene flame for high levels (Table 7.3) and ETA AAS for the low levels (chapter 4). Iridium samples were wet ashed in concentrated nitric acid, but owing to the poor sensitivity and detection limits of iridium in atomic absorption, representative samples of the digests were spiked on filter papers; these were activated along with iridium chemical standards and iridium content determined by INAA. Osmium determinations were carried out by INAA of the dried and homogenised plant material. The results are presented in Tables 7.12, 7.13 and 7.14 respectively.

Results and Discussion

The effects of rhodium applied as $Na_3[RhCl_6]$ on the water hyacinth was one of growth enhancement rather than toxicity (Plate 7.15). The applied rhodium concentration range was extended to include 20ppm and 30ppm applications. The tonic effect of rhodium is the subject of further detailed investigations elsewhere (chapter 8). The levels of rhodium accumulation are much less than for other platinum metals. The rhodium appears to be distributed evenly between roots and tops and this may be part of the mechanism by which the plant can tolerate such high external concentrations with little toxic effects in evidence. Some mild chlorosis was evident at the very highest level (30ppm Rh).

A slight drop off in yield was recorded for iridium treated plants, between the 0.5 and 2.5ppm levels. Toxic effects included the absence of

Plate 7.15

The uptake of rhodium applied as $Na_3[RhCl_6]$

Plate 7.16

The uptake of iridium applied as $Na_3[IrCl_6]$

Plate 7.17

The uptake of osmium applied as $Na_2[OsCl_6]$

Yields and analysis of *Eichhornia crassipes* treated with $Na_3[RhCl_6]$

Plant tops[a]	A7	B7	C7	D7	E7	F7
Applied Rh (ppm)	0.05	0.5	2.5	10.0	20.0	30.0
Wet weight (g)	24.65	16.75	24.44	39.31	23.71	26.45
Dry weight (g)	1.42	0.94	1.61	2.66	2.03	2.12
Weight of water (g)	23.23	15.81	22.83	36.65	21.68	24.33
Water present (%)	94.2	94.4	93.4	93.2	91.4	92.0
Total Rh (µg)	0.3	15	76	800	975	1250
Rh (ppm dry weight)	0.2	16	47	301	481	590

Plant roots[a]	A7	B7	C7	D7	E7	F7
Applied Rh (ppm)	0.05	0.5	2.5	10.0	20.0	30.0
Wet weight (g)	4.05	3.99	4.82	3.69	1.41	2.46
Dry weight (g)	0.40	0.38	0.47	0.38	0.19	0.26
Weight of water (g)	3.65	3.60	4.35	3.31	1.22	2.20
Water present (%)	90.1	90.2	90.2	89.7	86.5	89.4
Total Rh (µg)	1.0	10	50	125	75	176
Rh (ppm dry weight)	2.5	26	107	326	404	814

[a]Medium sized plants

Table 7.12

Yield and analysis of *Eichhornia crassipes* treated with $Na_3[IrCl_6]$

Plant tops[a]	A11	B11	C11	D11
Applied Ir (ppm)	0.05	0.5	2.5	10.0
Wet weight (g)	26.10	23.50	19.09	18.63
Dry weight (g)	1.84	2.07	2.04	2.03
Weight of water (g)	24.26	21.43	17.05	16.60
Water present (%)	93.0	91.2	89.3	89.1
Total Ir (µg)	2.5	6.9	51	177
Ir (ppm dry weight)	1.33	3.32	25	87

Plant roots[a]	A11	B11	C11	D11
Applied Ir (ppm)	0.05	0.5	2.5	10.0
Wet weight (g)	4.44	3.19	2.42	2.28
Dry weight (g)	0.38	0.40	0.37	0.26
Weight of water (g)	4.06	2.79	2.05	2.02
Water present (%)	91.4	87.5	84.7	88.6
Total Ir (µg)	1.2	6	14	30
Ir (ppm dry weight)	3.06	15	37	114

[a]Medium sized plants

Table 7.13

Yields and analysis of *Eichhornia crassipes* treated with $Na_2[OsCl_6]$

Plant tops[a]	A12	B12	C12	D12
Applied Os (ppm)	0.05	0.5	2.5	10.0
Wet weight (g)	10.71	9.59	13.10	12.79
Dry weight (g)	0.85	0.79	1.04	0.89
Weight of water (g)	9.86	8.80	12.06	11.90
Water present (%)	92.1	91.8	92.1	93.0
Total Os (µg)	10.6	73	581	2021
Os (ppm dry weight)	12.5	92	559	2271

Plant roots[a]	A12	B12	C12	D12
Applied Os (ppm)	0.05	0.5	2.5	10.0
Wet weight (g)	1.44	1.27	1.60	1.25
Dry weight (g)	0.16	0.11	0.17	0.13
Weight of water (g)	1.28	1.16	1.43	1.12
Water present (%)	88.9	91.3	89.4	89.6
Total Os (µg)	48	457	1379	1896
Os (ppm dry weight)	302	4154	8111	14588

[a]Small sized plants

Table 7.14

A comparison of control water hyacinths in uptake experiments

Plant tops

Medium sized plants (10)	Wet wet/g	Dry wt/g	Weight of H_2O	% water
Mean:	27.65	1.80	25.85	93.4
SD:	5.48	0.47	5.30	1.7
RSD	19.8	26.02	20.5	1.79
Small sized plants (7)				
Mean:	10.65	0.71	9.95	93.6
SD:	2.22	0.35	1.89	1.5
RSD:	20.8	48.8	19.0	1.62

Plant roots

Medium sized plants (10)	Wet wt/g	Dry wt/g	Weight of H_2O	% water
Mean:	8.17	0.50	7.68	93.5
SD:	3.86	0.28	3.63	2.2
RSD:	47.2	55.6	47.3	2.38
Small sized plants (7)				
Mean:	1.74	0.10	1.64	93.5
SD:	0.62	0.03	0.60	2.1
RSD	35.4	27.9	36.8	2.25

Table 7.15

Recovery of the platinum metals from solution using water hyacinths (*Eichhornia crassipes*)

Exp.No.	(plant size)	Metal complex	0.05 ppm[a] µg[b]	0.05 ppm[a] %[c]	0.5 ppm[a] µg[b]	0.5 ppm[a] %[c]	2.5 ppm[a] µg[b]	2.5 ppm[a] %[c]	10.0 ppm[a] µg[b]	10.0 ppm[a] %[c]
5	(m)	cis-$[Pt(NH_3)_2Cl_2]$	112	56	1875	94	7100	71	27164	68
8	(m)	trans-$[Pt(NH_3)_2Cl_2]$	82	41	1475	74	2811	28	6586	17
10	(m)	$K_2[PtCl_4]$	132	66	786	39	2508	25	15847	40
14	(m)	$(NH_4)_2[PtCl_6]$	56	28	625	31	3216	32	6033	15
6	(s)	$K_2[PdCl_4]$	77	38	497	25	1877	19	9163	23
9	(s)	cis-$[Pd(NH_3)_2Cl_2]$	145	73	787	39	3980	40	14065	35
13	(s)	$(NH_4)_3[RuCl_6]$	57	29	777	39	2124	21	9957	25
18	(m)	$[Ru(phen)_3]I_2$	90	45	921	46	6565	66	2691	7
7	(m)	$Na_3[RhCl_6]$	1.3	0.7	25	1.3	126	1.3	925	2.3
11	(m)	$Na_3[IrCl_6]$	3.7	1.9	13	0.7	65	0.7	266	0.7
12	(s)	$Na_2[OsCl_6]$	59	30	530	27	1960	20	3917	10

[a]Applied metal concentration in ppm (µg ml^{-1}) [b]Total metal content of plants in µg

[c]Percentage of metal recovered by plant from applied solution

Table 7.16

vegetative daughter plants, whilst young roots were stunted and blackened (Plate 7.16). Similarly, few effects were evident with osmium treated plants, but at the 10.0ppm level, growth was restricted (Plate 7.17). Like rhodium relatively little iridium was accumulated by water hyacinths, the best recovery value being 1.9%. Some iridium was deposited in the roots and smaller amounts detected in the plant tops. Rather more osmium than iridium was recovered from solution by water hyacinth; again, substantial amounts were deposited in the roots, with some osmium finding its way into the tops.

7.3 Limitations of uptake experiments

The aims of these uptake experiments (5-14incl.) have been outlined already. Such experiments suffer from the limitations imposed by the preliminary nature of the investigation. Each individual experiment was carried out in order to identify the level of applied metal (ppm) at which the metal became toxic. The extent of metal toxicity was judged in a qualitative way from the observed damage to plant roots and leaves. This enabled a relative order of toxicity to be established for the platinum metals. At the 10ppm (mg dm^{-3}) level the increasing order of toxicity was found to be:

$$Pt^{2+} \approx Pd^{2+} > Os^{4+} \approx Ru^{3+} > Ir^{3+} > Rh^{3+}$$

$$\longrightarrow$$

toxicity decreases.

Another limitation of these uptake experiments has been the wide variations in wet weight, dry weight and water content. Throughout the experiments some ten medium sized control plants and seven small sized control plants were monitored. The wet weights, dry weights and water contents were calculated for all of them; average values were found and

are presented in Table 7.15. The results indicate large variations in wet weights and dry weights within each group, though good agreement was found for the % water present.

However, the results presented for the uptake experiments (5-14) can be criticised on these grounds. Ideally, such experiments should be conducted with a large number of plants wherever possible. The results presented are still valid but only as a guide to

(i) the extent to which the water hyacinth can recover soluble forms of the platinum metals

(ii) the relative toxicity of the platinum metals

(iii) the biological effects of the platinum metals.

From the results of these experiments, various complexes were selected for further detailed study. Both the antitumour drug, $cis[Pt(NH_3)_2Cl_2]$, and the non-toxic rhodium complex, $Na_3[RhCl_6]$ were investigated at one particular concentration, 0.5 and 10.0 ppm respectively, with a number of water hyacinths in an attempt to quantify the effects reported in this chapter. These investigations are the subject of the next chapter.

REFERENCES

Arber, A. (1918), Ann. Bot., 32 465.

Arber, A. (1922), Botanical Gazette, 74 80.

Boyde, C.E. (1970), Econ. Bot., 24 95.

Boyde, C.E. and Vicker, D.H. (1971), Hydrobiologia, 38 (3-4) 409.

Boyde, C.E. and Scarsbrook, E. (1975), Aquatic Botany, 1 253.

Chigbo, F.E., Clark, M., Thompson, L. and Shore, F. (1979), J. Miss. Acad. Sci., 24 13.

Cooley, T.N. and Martin, D.F. (1976), Fla. Sci., 39 19.

Cooley, T.N. and Martin, D.F. (1977), J. Inorg. Nucl. Chem., 39 1893.

Cooley, T.N. and Martin, D.F. (1978), J. Environ. Sci. Health, A13 469.

Cooley, T.N., Martin, D.F., Durden, W.C. and Perkins, B.D. (1979), Water Res., 13 343.

Cooley, T.N. and Martin, D.F. (1980), J. Inorg. Nucl. Chem., 42 151.

Cooley, T.N. and Martin, D.F. (1980), Fla. Sci., 43 (1) 24.

Cotton, F.A. and Wilkinson, G.I. (1972), Advanced Inorganic Chemistry, 3rd ed., Wiley, London.

Johnson, C.R. and Sheehan, T.J. (1977), Proc. Fla. State Hort. Soc., 90 118.

Knipling, E.B., West, S.H. and Haller, W.T. (1971), Soil and Crop Science Soc. of Fla. Proc., 30 51.

McDonald, R.C. and Wolverton, B.C. (1980), Econ. Bot., 34 (2) 101.

Miner, J.R., Wooten, J.W. and Dodd, J.D. (1971), Livestock Waste Management and Pollution Abatement, Proc. Int. Symp. 170.

Hasman, M. and Inanc, N. (1957), Fen Fakultesi Mecmuasi, (Univ. of Istanbul), 137.

Hildebrand, E.M. (1946), Science N.Y., 103 477.

Hitchcock, A.E., Zimmerman, P.W., Kirkpatrick, H. and Earle, T.T. (1949), Cont. Boyce Thompson Inst. Plant Research, 15 363.

Penfound, W.T. and Earle, T.T. (1948), Ecological Monographs, 18 47.

Pieterse, A.H., Roorda, F.A. and Verhagen, L. (1980), Experientia, 36 (6) 650.

Rogers, H.H. and Davis, D.E. (1972), Weed Sci., 20 423.

Sculthorpe, C.D. (1967), The Biology of Aquatic Vascular Plants, Edward Arnold, London.

Tatsuyama, K., Egawa, H. and Yamagishi, T. (1977), Zasso Kenkyu, 22 (3) 151.

Tatsuyama, K., Egawa, H., Yamamoto, H. and Nakamura, M. (1979), Zasso Kenkyu, 24 (4) 260.

Weber, H. (1950), Abh. Math-Naturw K.L. Akad. Wiss Mainz, 3.

Widyanto, L., Susilo, H. and Sutikno, I. (1978), Pract. Appl. Biochem. Econ. Dev. Countries, Proc. FAOB Symp., 1st, 189.

Wolverton, B.C. and McDonald, R.C. (1975), NASA report no. TM-X72727.

Wolverton, B.C. and McDonald, R.C. (1976), New Scientist, 71 381.

CHAPTER VIII

AN INVESTIGATION OF THE TOXIC AND TONIC EFFECTS OF PLATINUM AND RHODIUM ON *EICHHORNIA CRASSIPES* (WATER HYACINTH)

8.1 Introduction

From the studies of platinum metal uptake by water hyacinths, reported in chapter 7, it became apparent that the effects of platinum and rhodium needed further investigation. The ability of the plant to distinguish between Pt^{4+} and Pt^{2+} was accompanied by visible toxic symptoms to both roots and leaves. Rhodium, in contrast was the least toxic of the metals and appeared to exhibit a tonic effect. This effect has been observed elsewhere with rhodium (Mullen, 1980).

In this chapter, the effects of platinum group metal complexes on the roots of water hyacinth are reported. Measurements of platinum and rhodium tolerance were carried out using a technique based on those used to measure copper and lead tolerance in plants. (Wilkins, 1957) (Jowett, 1964) (McNeilly and Bradshaw, 1968). The index of tolerance given here was calculated from the following relationship.

$$\text{Index of tolerance} = \frac{\text{Mean increase in length of the plants' longest root when treated with platinum metal}}{\text{Mean increase in length of the control plant's longest roots}}$$

The uptake and effect of the antitumour drug cis diamminedichloro-platinum II was investigated further too. Sequential extraction techniques, described in chapter 3 were applied to the dried plant material in order to determine the chemical binding of the platinum. The effect of the platinum drug on the amino acid composition of water hyacinth are reported here but the location and examination of platinum

using analytical electron microscopy is reported in chapter 9.

The distribution, morphology and inorganic composition of water hyacinth have been reviewed in chapter 7.1. Several investigators have examined the trace metal content of these plants in relation to their environment (Boyde and Vickers, 1971) (Wolverton and McDonald, 1976) (Sheehan and Johnson, 1977) (Cooley et al., 1979). Some of their findings are presented in Table 8.1 for comparison with results obtained with control plants in this study.

8.2 Experimental

8.2.1 An investigation of the toxic effect of platinum and tonic effect of rhodium on the water hyacinth

a) The uptake of rhodium by the water hyacinth

Water hyacinths were grown in half-strength nutrient solutions containing rhodium applied as $Na_3[RhCl_6]$ at a concentration of 10.0 ppm (mg dm^{-3}). Seven days prior to the application of rhodium, experimental plants were selected for uniformity of size and growth by separating them from the mother plants as described in chapter 7.2. The plants were settled in half-strength nutrient solution for one week, after which they were transferred to $2dm^3$ plastic containers filled with half-strength nutrient solution and rhodium at 10.0ppm. Four plants (D/16/i-iv) were grown with rhodium and four were grown as controls (Control/S/i-iv). The plant wet weights and root lengths were recorded at the beginning and end of the experiment; results are given in Table 8.2 and 8.3.

The experiments were carried out in a Prestcold environmental growth room (chapter 3.1), with 10^3ft candles of illumination. The conditions were set to a 14hr day with temperatures of 25 $\pm 1^{\circ}C$(day) and 20 $\pm 1^{\circ}C$ (night); relative humidity was 70-75%. Solutions were aerated by

Summary of metal content of water hyacinth for whole plants, dry wt basis

Element ppm

Source	Ca	K	Mg	Co	Cu	Mn	Fe
Lawrence (1968)	10400	24800	4200	0.4	90	680	2900
Boyd (1970)	1000	4250	1050	-	11	3940	250
Boyd and Vickers (1971)	1350	4450	550	-	15	270	3420
Easley and Shirley (1974)	2200	4100	590	-	12	142	1701
Wolverton and McDonald (1976)	13000	25000	3000	-	-	80	500
Cooley, Martin, Durden and Perkins (1979)							
Peace River I (Small or "normal sized")	128	708	1025	2.92	0.46	-	35
Peace River II (Small or "normal sized")	228	1018	848	-	0.38	540	70
Hillsborough River (Small or "normal sized")	286	1211	211	-	0.45	597	48
Medium or "Stunted" sized	1890	1390	211	0.50	0.75	3.9	15
Large or "Super" sized	1490	2123	173	0.50	2.55	2.4	18
Parsons (1982) (this study)[a]	11014	46645	3366	<1.0	18	59	210

[a] results from PIXE analysis except Mg which was by ICP

Table 8.1

Plate 8.1 The uptake of rhodium applied as $Na_3[RhCl_6]$; before rhodium application

Plate 8.2 The uptake of rhodium applied as $Na_3[RhCl_6]$; after 14 days growth.

Plate 8.3 The uptake of rhodium applied as $Na_3[RhCl_6]$; after 14 days growth

Plate 8.4 The effect of rhodium, applied as $Na_3[RhCl_6]$, on the roots of water hyacinth (*Eichhornia crassipes*) right hand plants: rhodium treated; left hand plants: controls.

supplying compressed air via plastic tubing to sintered glass filter sticks, suspended in the containers.

Throughout the experiments, the containers were topped up regularly with deionised water. Samples of each solution were analysed for rhodium content regularly to monitor the uptake. These results are presented in Table 8.4. The nutrients and applied rhodium were renewed after one week; after two weeks, the experiment was stopped and the plants washed and harvested. Plates 8.1 and 8.2 depict the state of growth before and after rhodium treatment. Plates 8.3 and 8.4 show more detailed effects of rhodium treatment. The plants were separated into leaves, floats and roots; some material was removed for histochemical examination (LM, SEM, TEM). The plant material was oven dried at $40^{o}C$ for 24hrs and homogenised using a ball mill (Glen Creston). Rhodium content was determined by INAA and ICP techniques. The latter was used to determine several other elements too, and the results are presented in Table 8.5.

b) The uptake of platinum applied as cis $[Pt(NH_3)_2Cl_2]$ by water hyacinths

The uptake, by water hyacinths, of platinum applied as the anti-tumour complex, cis $[Pt(NH_3)_2Cl_2]$ was investigated. Platinum was applied at a concentration of 0.5 ppm (mg dm^{-3}), since this appeared to be the threshold of severe toxicity. The experimental procedure followed that described previously for the rhodium study. Four plants were treated with the complex and grown for two weeks under controlled conditions. The changes in biomass and root lengths are reported in Tables 8.2 and 8.3 respectively, alongside those measurements from control plants and rhodium treated plants. The change in the platinum content of the nutrient solutions was monitored for the first week of the experiment (Table 8.6)

Plate 8.5 The uptake of platinum applied as cis $[Pt(NH_3)_2Cl_2]$; before platinum application

Plate 8.6 The uptake of platinum applied as cis $[Pt(NH_3)_2Cl_2]$; after 14 days growth

Plate 8.7 The effect of platinum, applied as cis $[Pt(NH_3)_2Cl_2]$, on the water hyacinth (*Eichhornia crassipes*)

Plate 8.8 The effect of platinum on leaves of water hyacinth showing appearance of reddish-brown discolouration

Changes in the biomass of water hyacinths treated the rhodium and platinum

Plant details	Wet wt(start)/g	Wet wt(finish)/g	Wet weight increase/g	No. of daughter plants produced
Uptake of rhodium, applied as $Na_3[RhCl_6]$				
D/16/(i)	15.83	47.44	31.61	3
" (ii)	12.24	37.60	25.36	3
" (iii)	14.06	42.42	28.36	3
" (iv)	11.30	35.62	24.32	3
mean:	13.36	40.77	27.41	
SD:	2.01	5.28	3.28	
RSD:	15.03	12.96	11.97	
Uptake of platinum applied as $cis[Pt(NH_3)_2Cl_2]$				
B/15/(i)	10.61	32.18	21.57	3
" (ii)	14.56	37.97	23.41	2
" (iii)	13.36	37.02	23.67	2
" (iv)	11.75	31.58	19.83	2
mean:	12.75	34.69	22.12	
SD:	1.74	3.28	1.79	
RSD:	13.85	9.45	8.09	
Control plants/S/(i)	14.71	38.02	23.31	3
" (ii)	13.52	37.74	24.22	3
" (iii)	13.64	42.73	29.09	3
" (iv)	10.98	37.13	26.15	3
mean:	13.21	38.91	25.70	
SD:	1.58	2.58	2.56	
RSD:	11.97	6.62	9.95	

Table 8.2

The effects of platinum and rhodium on water hyacingth roots

Plant details	Longest root (start)/mm	Longest root (finish)/mm	Increase in root length/mm
Uptake of rhodium applied as $Na_3[RhCl_6]$			
D/16/(i)	85	154	69
" (ii)	50	162	112
" (iii)	96	150	54
" (iv)	125	152	27
mean:	89	155	66
SD:	31	5	35
RSD:	34.8	3.4	54.3
Uptake of platinum applied as $cis[Pt(NH_3)_2Cl_2]$			
B/15/(i)	113	148	35
" (ii)	100	115	15
" (iii)[a]	76	76	0
" (iv)	64	96	32
mean:	92	120	27
SD:	25	26	11
RSD:	27.5	22.0	39.5
Control plants/S/(i)	64	113	49
" (ii)	84	150	66
" (iii)	75	190	115
" (iv)	100	145	45
mean:	81	150	69
SD:	15	32	32
RSD:	18.8	21.1	46.8

[a]this set of results not used in statistical data

Table 8.3

The uptake of rhodium by water hyacinth over a two week period

Nutrient solutions containing		concentration of rhodium (mg dm^{-3}) in nutrient solutions† Day 0	Day 1	Day 2	Day 3	Day 5	Day 6
rhodium applied as $Na_3[RhCl_6]$							
1st week	D/16/(i)	10.80	10.90	10.36	10.06	9.70	9.98
	" (ii)	10.66	10.14	10.24	9.66	9.56	9.70
	" (iii)	10.74	9.86	9.96	9.48	9.44	9.56
	" (iv)	10.50	10.16	10.22	9.92	9.52	9.68
	Mean	10.68	10.27	10.20	9.78	9.56	9.73
	SD	0.13	0.45	0.17	0.26	0.11	0.18
	RSD	1.2	4.3	1.7	2.7	1.1	1.8
		Day 7	Day 8	Day 9	Day 12	Day 13	Day 14
2nd week	D/16/(i)	10.78	11.26	11.48	11.50	10.76	10.76
Nutrients and applied	" (ii)	10.94	11.48	10.98	10.92	10.30	10.22
rhodium replenished	" (iii)	11.22	11.62	10.80	10.80	10.22	10.26
	" (iv)	11.18	11.50	11.02	10.98	10.68	10.66
	Mean	11.03	11.47	11.07	11.05	10.49	10.48
	SD	0.21	0.15	0.29	0.31	0.27	0.28
	RSD	1.9	1.3	2.6	2.8	2.6	2.6

†rhodium determined by FAAS

Table 8.4

The uptake of rhodium applied as $Na_3[RhCl_6]$ by the water hyacinth

(all values in ppm ($\mu g\ g^{-1}$) dry weight[a])

	Wet wt/g	Dry wt/g	Wt of H_2O/g	% H_2O	Ca	Mg	Cu	Fe	Mn	Zn	Rh	Rh[b]
D/16/leaves from Rh treated plants (i-iv)	35.02	3.02	32.00	91.4	13902	3375	37	38	89	57	189	183
D/16/floats from Rh treated plants (i-iv)	76.07	2.85	73.22	96.2	12126	3609	36	90	54	50	42	59
D/16/roots from Rh treated plants (i-iv)	31.81	1.53	30.28	95.2	8326	1986	126	627	6.8	154	123	118
Control/S/leaves from plants (i-iv)	34.30	2.95	31.35	91.4	14219	3517	35	106	95	48	nd	nd
Control/S/floats from plants (i-iv)	74.47	2.95	71.52	96.0	13763	3782	44	121	68	57	nd	nd
Control/S/roots from plants (i-iv)	30.28	1.47	28.81	95.1	9418	2274	49	915	11.5	165	nd	nd

[a]determined by ICP atomic emission spectroscopy, except where indicated

[b]determined by INAA

Table 8.5

The uptake of platinum by water hyacinth over a one week period

Platinum (mg dm^{-3}) applied as cis$[Pt(NH_3)_2Cl_2]$ at 0.5ppm (mg dm^{-3}) Pt

Nutrient solutions containing platinum	Day 0	Day 1	Day 2	Day 3
B/15/(i)	0.76	0.69	0.68	0.61
" (ii)	0.79	0.78	0.66	0.64
" (iii)	0.64	0.59	0.69	0.64
" (iv)	0.79	0.72	0.66	0.59
Mean	0.75	0.70	0.67	0.62
SD	0.07	0.08	0.02	0.03
RSD	9.6	11.4	2.2	3.0

	Day 4	Day 6	Day 7
B/15/(i)	0.52	0.58	0.55
" (ii)	0.64	0.61	0.58
" (iii)	0.62	0.60	0.57
" (iv)	0.57	0.60	0.56
Mean	0.59	0.60	0.57
SD	0.05	0.01	0.01
RSD	9.2	2.1	2.3

Table 8.6

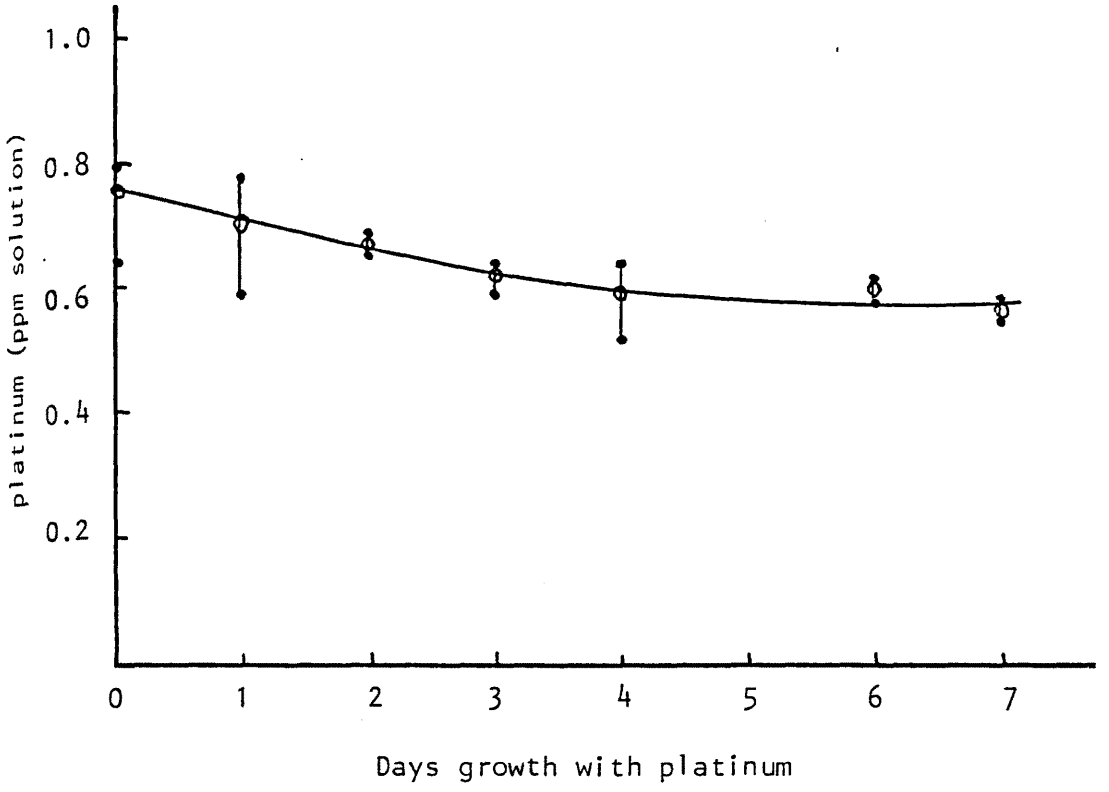


Figure 8.1(a) The uptake of platinum applied as cis $[Pt(NH_3)_2Cl_2]$, from nutrient solution.

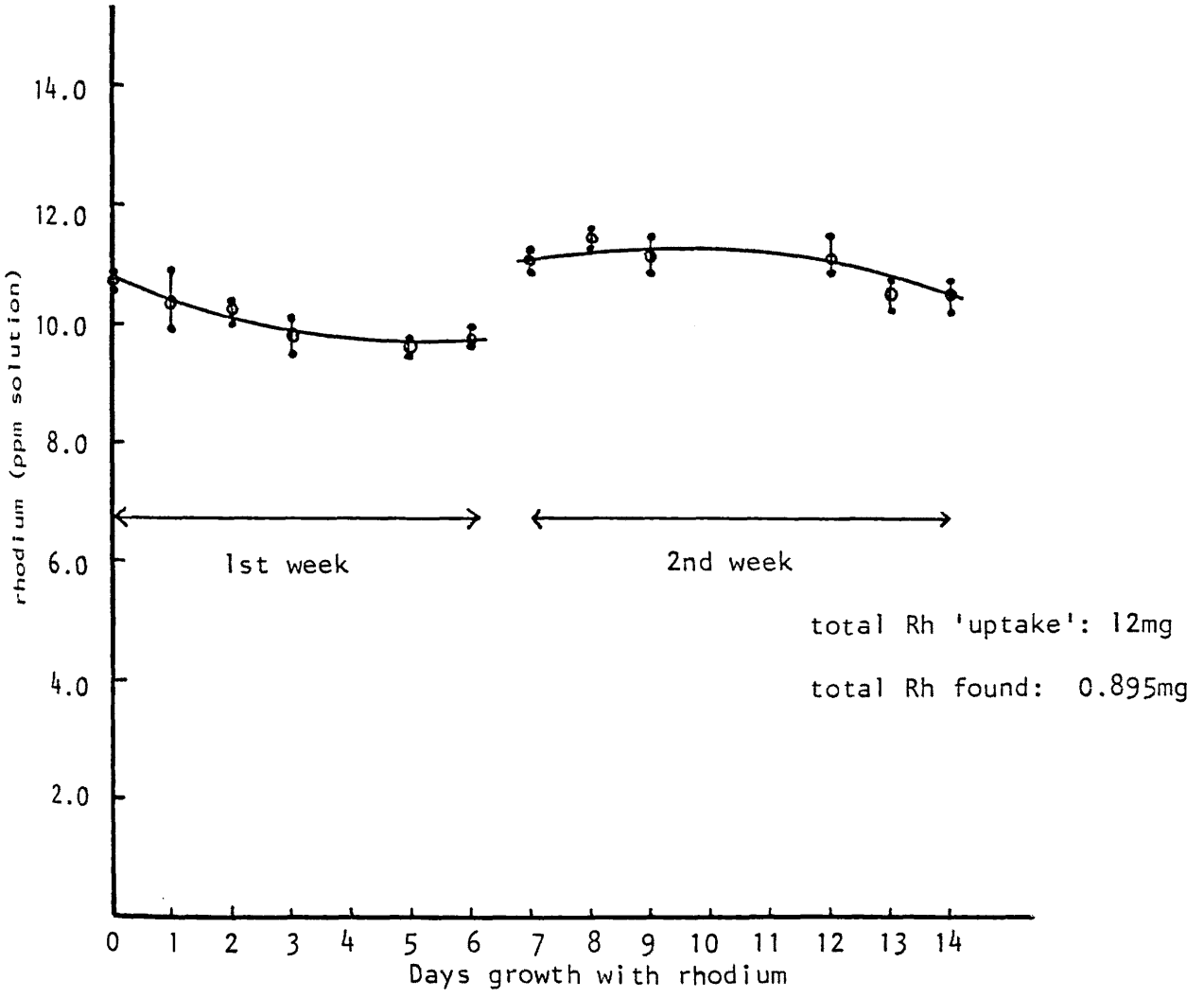


Figure 8.1(b) The uptake of rhodium, applied as $Na_3[RhCl_6]$

after which the nutrients and applied platinum were renewed.

After two weeks growth, the plants were harvested, washed and separated into roots, floats and leaves. Plates 8.5 and 8.6 show the plants before and after treatment with platinum. The effects of platinum on the leaves are shown in Plates 8.7 and 8.8. Specimens of root and leaf were selected for examination by LM, SEM and TEM. These investigations are reported in chapter IX. The remainder of the plant material was dried at 40°C for 24 hrs and homogenised, ready for investigations involving sequential extraction and amino acid analyses (8.2.3 and 8.2.4).

8.2.2 The uptake by water hyacinths of platinum applied in various forms

The effects and uptake of different soluble platinum complexes with water hyacinths were compared. Hydroponic growth experiments were conducted using the same procedures outlined previously in 8.2.1. Platinum in various complex forms was administered to water hyacinth at a concentration of 0.5ppm (mg dm^{-3}). Four plants were selected for each experiment; two small or "normal" sized plants, and two medium or "stunted" plants in accordance with the size biotypes defined by Cooley *et al.* (1979). The forms of platinum applied are given in Table 8.7.

Expt No	Platinum applied as	Pt^{n+}	complex stereochemistry
19	$K_2[PtCl_4]$	Pt^{2+}	anionic complex, square planar
20	$Pt\,IV\,Cl_4$	Pt^{4+}	probably octahedral in aqueous solution $[PtCl_4(H_2O)_2]^{o}$
21	$[Pt(NH_3)_4]Cl_2$	Pt^{2+}	cationic complex, square planar

Table 8.7 The uptake of platinum by water hyacinth

After two weeks' growth, the plants were harvested, washed and

Plate 8.9

The uptake of platinum applied as $K_2[PtCl_4]$

Plate 8.10

The uptake of platinum applied as $Pt(IV)Cl_4$

Plate 8.11

The uptake of platinum applied as $[Pt(NH_3)_4]Cl_2$

Changes in the biomass of water hyacinths treated with various complexes of platinum

Experimental details	Wet wt(start)/g	Wet wt(finish)/g	Wet weight increase/g	ratio of final wt / start wt
Uptake of platinum applied as $K_2[PtCl_4]$				
B/19/(i) medium	6.28	15.19	8.91	2.42
" (ii) small	2.32	6.26	3.94	2.70
" (iii) medium	5.46	13.87	8.41	2.54
" (iv) small	1.68	3.64	1.96	2.17
			Mean	2.46
			SD	0.22
			RSD	9.1
Uptake of platinum applied as $Pt(IV)Cl_4$				
B/20/(i) medium	7.24	20.38	13.14	2.80
" (ii) small	3.14	5.55	2.41	1.77
" (iii) medium	4.00	14.35	10.35	3.59
" (iv) small	0.83	2.88	2.05	3.47
			Mean	2.91
			SD	0.83
			RSD	28.7
Uptake of platinum applied as $[Pt(NH_3)_4]Cl_2$				
B/21/(i) medium	9.47	26.54	17.07	2.80
" (ii) small	4.25	10.36	6.11	2.44
" (iii) medium	5.96	19.69	13.73	3.30
" (iv) small	1.25	2.78	1.53	2.22
			Mean	2.69
			SD	0.47
			RSD	17.5
Control plants grown in half-strength nutrient solution				
Control/22/(i) medium	8.33	23.36	15.03	2.80
" (ii) small	2.86	7.34	4.48	2.57
" (iii) medium	3.77	12.82	9.05	3.40
" (iv) small	1.97	6.67	4.70	3.39
			Mean	3.04
			SD	0.42
			RSD	13.8

Table 8.8

The effects on water hyacinths of platinum applied in various complexed forms

Experimental details	Day 1	Day 7	1st week		Day 14	2nd week	
	root length /mm	root length /mm	increase /mm	daughter plants	total root length /mm	total increase /mm	total daughter plants
Uptake of platinum applied as $K_2[PtCl_4]$							
B/19/(i) medium	81	84	3	1	84	3	3
" (ii) small	30	32	2	0	76	46	1
" (iii) medium	53	53	0	1	71	18	2
" (iv) small	24	25	1	0	36	12	1
Uptake of platinum applied as $Pt(IV)Cl_4$							
B/20/(i) medium	80	93	13	1	98	18	4
" (ii) small	38	40	2	0	43	5	0
" (iii) medium	63	80	13	1	88	25	2
" (iv) small	17	40	23	0	43	26	0
Uptake of platinum applied as $[Pt(NH_3)_4]Cl_2$							
B/21/(i) medium	106	108	2	2	110	4	4
" (ii) small	46	65	19	0	76	30	2
" (iii) medium	79	80	1	1	80	1	3
" (iv) small	17	20	3	0	29	12	0
Control plants grown in half-strength nutrient solution							
Control/22/(i) medium	126	128	2	2	143	17	3
" (ii) small	39	43	4	0	68	29	0
" (iii) medium	90	93	3	0	93	3	2
" (iv) small	21	34	13	0	60	39	0

Table 8.9

The uptake by water hyacinths of platinum applied in various complexed forms

Experimental details	Wet wt/g	Dry wt/g	Wt of H_2O/g	% H_2O	Pt µg g^{-1}	Pt µg (dw)
Platinum applied as $K_2[PtCl_4]$ at 0.5mgdm^{-3}						
B/19/leaves	8.84	0.87	7.97	90.2	62	54
B/19/floats	16.00	0.66	15.34	95.9	112	74
B/19/roots	9.55	0.39	9.16	95.9	1694	661
					Total Pt µg	789
					% recovered	19.7
Platinum applied as $Pt(IV)Cl_4$ at 0.5mgdm^{-3}						
B/20/leaves	10.15	1.02	9.13	90.0	100	102
B/20/floats	21.75	0.83	20.92	96.2	175	145
B/20/roots	8.51	0.39	8.12	95.4	1588	619
					Total Pt µg	866
					% recovered	21.7
Platinum applied as $[Pt(NH_3)_4]Cl_2$ at 0.5mgdm^{-3}						
B/21/leaves	12.28	1.21	11.07	90.1	102	123
B/21/floats	29.57	1.14	28.43	96.2	123	140
B/21/roots	12.59	0.50	12.09	96.0	242	121
					Total Pt µg	384
					% recovered	9.6
Control plants grown in half-strength nutrient solution Control/22/leaves	12.11	1.24	10.87	89.8	nd	nd
Control/22/floats	27.35	1.01	26.34	96.3	nd	nd
Control/22/roots	8.64	0.42	8.22	95.1	nd	nd

Table 8.10

weighed. The biomass and root measurements are presented in Tables 8.8 and 8.9 respectively. Plates 8.9, 8.10 and 8.11 show the visible effects of platinum on water hyacinth. The plants were divided into roots, floats and leaves, dried and homogenised to provide representative samples of the major plant parts from each experiment. The bulked and homogenised leaves, floats and roots were analysed for platinum by wet ashing and ETA AAS. Results are given in Table 8.10.

8.2.3 Sequential extraction of water hyacinths containing platinum applied as cis[$Pt(NH_3)_3Cl_2$]

Introduction

The background to this technique and the particular extraction scheme employed has been reviewed in chapter 3. The extraction was carried out on separately dried and homogenised samples of the leaves, floats and roots of water hyacinths treated with platinum applied as the antitumour complex cis [$Pt(NH_3)_2Cl_2$].

In chapter 7.2.1, the uptake of platinum, applied as cis[$Pt(NH_3)_2Cl_2$], by the water hyacinth, was investigated at a range of concentrations. At high levels, this form of platinum was highly toxic but at low levels, though the plant leaves developed brown streaks, the rate of vegetative reproduction appeared enhanced (Plates 8.7 and 8.8). This effect was investigated further in 8.2.1(b) where the root lengths and vegetative reproduction were measured. The plant material from this investigation was used in the extraction study to give some idea of how the platinum was bound. A replicate analysis could not be performed because of the small amount of dried plant material available, and given the detection limit for platinum by ETA AAS, it was not certain that the relative distribution of platinum between the various fractions could be determined.

The method of sequential extraction decided upon followed that

reported by Farago *et al.* (1980) and Mullen (1980) for the identification of the form of copper within tolerant *Armeria maritima*. The extraction sequence is shown in figure 8.2.

Method

Dried samples of leaves (2.3924g), floats (2.8053g) and roots (1.4400g) from platinum treated water hyacinths had each been homogenised in an agate ball mill (Glen Creston) previously. Each sample was weighed into a soxhlet thimble and placed in a soxhlet extraction apparatus mounted on a 500ml round-bottomed flask containing 150ml of 80% ethanol solution. The ethanol extraction was repeated and the extracts pooled. The residue, A, was oven dried at 40°C.

The 80% ethanol extract, P, was taken to dryness by rotary evaporation at 40°C followed by freeze drying to remove water. The dried extract was redissolved in 300ml water and transferred to a separating flask with 125ml of ether. The aqueous extract P was extracted with ether and the ether extract R rotary evaporated: the residue was wet ashed and the digest analysed for platinum by ETA AAS.

The remaining water extract, Q, was shaken with 30ml of Analar pentan-1-ol and 75ml of Analar trichloromethane for 15 minutes. The lower white trichloromethane-pentanol gel, S, was run off, rotary evaporated and analysed for platinum content. The remaining aqueous layer, T, was rotary evaporated to remove traces of organic solvents and an equal volume of Analar acetone added to form a precipitate, U. The mixture was centrifuged and the supernatant liquor, V, decanted. Extracts U and V were dried and analysed for platinum.

The residue, A, remaining from the ethanol extraction was dried and reweighed in the soxhlet thimble. This residue was extracted with 100ml of water for 10 minutes in the soxhlet apparatus as before. The water

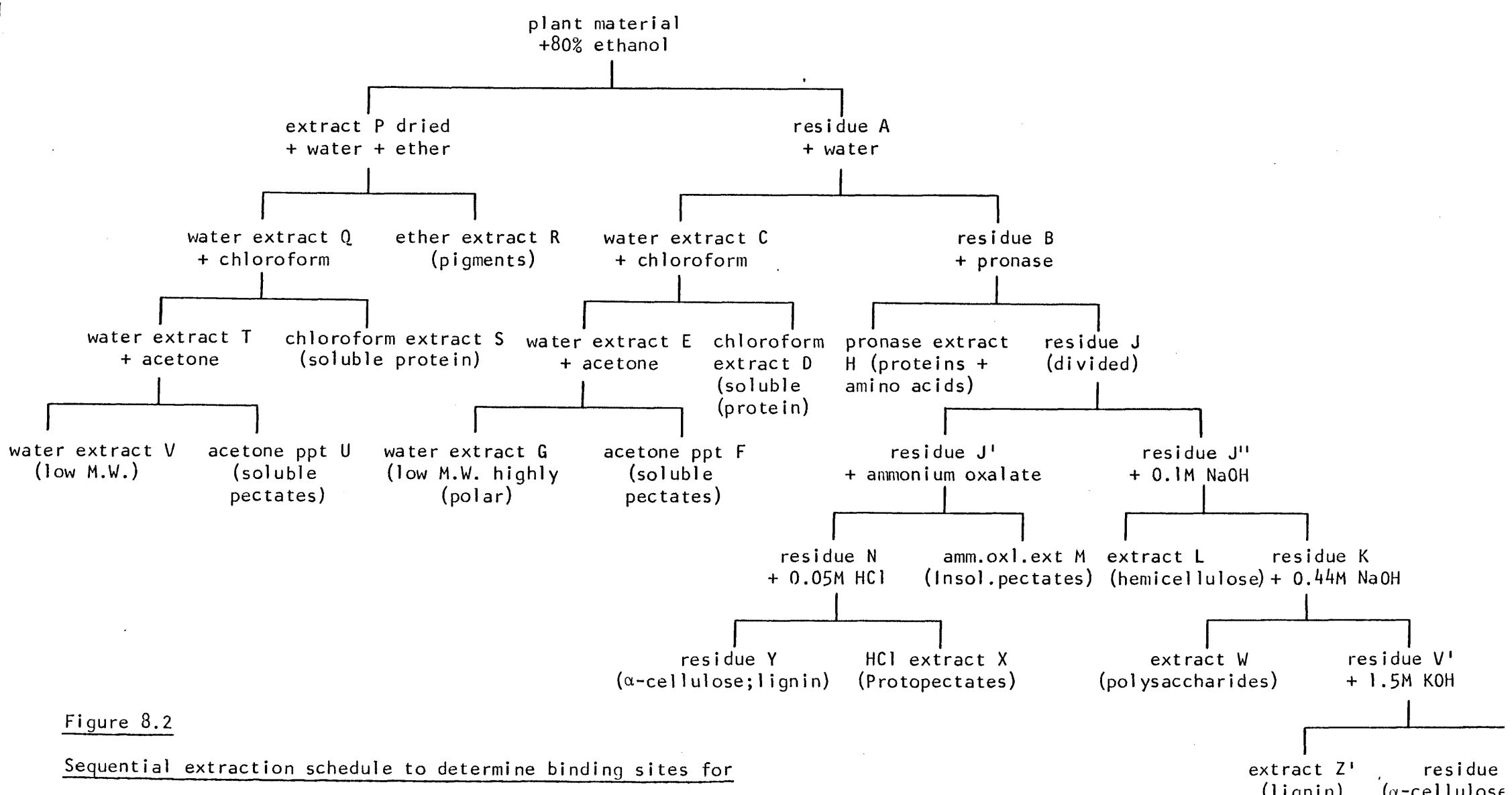


Figure 8.2

Sequential extraction schedule to determine binding sites for platinum within water hyacinths

extract, C, was freeze-dried and redissolved in 50ml of water and the trichloromethane-pentanol and acetone treatments were carried out as before to give samples D, E, G and F.

The residue, B, remaining after water extraction, was dried to constant weight, and shaken with 1g of the proteolytic preparation pronase, together with 10mg of chloroamphenicol and 200ml of phosphate buffer (0.01M, pH7.4), for 40 hours in a 500ml conical flask. The residue was filtered off and the pronase treatment repeated. The pooled extracts, H, were freeze dried and analysed for platinum. The residue, J, from the pronase extraction, was washed, dried to constant weight and divided accurately into two parts, each of which were extracted in different ways.

The first residue, J', was shaken with 100ml of a 2% ammonium oxalate solution for 2 hours. After centrifugation the extract, M, was decanted off carefully, dried and analysed for platinum. The residue, N, was treated similarly with 100ml of 0.05M hydrochloric acid to give extract X and residue Y, which were both analysed for platinum.

The second residue, J'', was extracted successively with 0.1M NaOH, 0.44M NaOH (100ml, 2hrs) to give samples L and W. The residue, V', was extracted further by boiling with 100ml of 1.5M KOH to give sample Z'. These extracts and the final residue Z were wet ashed and analysed for platinum too. The results of these sequential extraction studies are given in Tables 8.12 to 8.14.

8.2.4 Amino acid analysis of water hyacinths treated with platinum applied as cis$[Pt(NH_3)_2Cl_2]$

Introduction

The biological effect of platinum on the water hyacinth (*Eichhornia crassipes*) reported in 8.2.1(b) prompted further investigation of the

Platinum (ppm dry weight) sequentially extracted from the leaves of water hyacinths grown in solutions containing platinum applied as cis[$Pt(NH_3)_2Cl_2$]

Samples			Classes of compounds extracted	Pt(ppm dry wt)	% of total Pt
after 80% ethanol extraction					
Ether extract	R		Pigments	0.44	1.3
Chloroform extract	S		Soluble protein	0.11	0.3
Acetone, ppt	U		Soluble pectates	0.42	1.2
Water solution	V		Low molecular weight materials	nd	-
after water extraction					
Chloroform extract	D		Soluble protein	1.05	3.0
Acetone, ppt	F		Soluble pectates	7.32	20.8
Water solution	G		Polar low molecular weight materials	3.14	8.9
Pronase extract	H		Proteins, amino acids	5.66	16.1
Amm.oxalate extract	M	J'	Insoluble pectates	nd	-
0.05 M HCl	X	J'	Protopectates[a]	0.21	0.6
Residue	Y	J'	α-cellulose, lignin	16.9	47.9
			Total:	35.3	100.1
			Total found[b]:	42.6	

[a] Including polysaccharides

[b] Determined by ETA AAS

Table 8.11

Platinum (ppm dry weight) sequentially extracted from the floats of water hyacinths grown in solutions containing platinum applied as cis$[Pt(NH_3)_2Cl_2]$

Samples			Classes of compounds extracted	Pt(ppm dry wt)	% of total Pt
after 80% ethanol extraction					
Ether extract	R		Pigments	0.36	0.4
Chloroform extract	S		Soluble protein	0.45	0.5
Acetone, ppt	U		Soluble pectates	4.01	4.7
Water solution	V		Low molecular weight materials	0.53	0.6
after water extraction					
Chloroform extract	D		Soluble protein	2.23	2.6
Acetone, ppt	F		Soluble pectates	21.4	24.8
Water solution	G		Polar low molecular weight materials	nd	-
Pronase extract	H		Proteins, amino acids	14.4	16.7
Amm.oxalate extract	M	J'	Insoluble pectates	0.60	0.7
0.05 M HCl	X	J'	Protopectates	0.60	0.7
Residue	Y	J'	α-cellulose, lignin	41.7	48.3
			Total:	86.3	100.0
			Total found:	109.3	

[a] Including polysaccharides

Table 8.12

Platinum (ppm dry weight) sequentially extracted from the roots of water hyacinths grown in solutions containing platinum applied as cis$[Pt(NH_3)_2Cl_2]$

Samples			Classes of compounds extracted	Pt(ppm dry wt)	% of total Pt
after 80% ethanol extraction					
Ether extract	R		Pigments	nd	-
Chloroform extract	S		Soluble protein	1.04	0.2
Acetone, ppt	U		Soluble pectates	20.8	3.9
Water solution	V		Low molecular weight materials	124	23.1
after water extraction					
Chloroform extract	D		Soluble protein	4.5	0.8
Acetone, ppt	F		Soluble pectates	76.4	14.2
Water solution	G		Polar low molecular weight materials	64.6	12.0
Pronase extract	H		Proteins, amino acids	51.0	9.5
Amm.oxalate extract	M	J'	Insoluble pectates	3.5	0.7
0.05 M HCl	X	J'	Protopectates[a]	3.1	0.6
Residue	Y	J'	α-cellulose, lignin	188	35.0
			Total:	537	100.0

[a]Including polysaccharides

Table 8.13

Sequential extraction of dried water hyacinths

treated with platinum

Plant material	Percentage material removed by		Percentage material remaining prior to pronase extract
	80% aq. ethanol	water extract	
Leaves	27.3	10.4	62.3
Floats	31.7	10.9	57.4
Roots	30.2	11.3	58.6

Table 8.14

plant tissues. A complete amino acid analysis of leaf and root extracts from platinum treated and control plants was undertaken. The LKB 4101 amino acid analyser (LKB Biochrom Ltd) operates on the principle of liquid chromatography and subsequent detection of the separated components using ninhydrin with a spectroscopic photocell.

Method

Homogenised plant material (ca.30mg) was shaken with 50ml water for three hours. The water extract was filtered and treated with trichloromethane and pentan-1-ol to precipitate water soluble proteins (Sevag *et al.*, 1938). The water extraction was repeated and the pooled extracts freeze dried. Samples of the dried extracts were redissolved in 2ml of water for analysis.

An aliquot of 0.5ml of this sample solution was added to 2.5ml of a sodium citrate loading buffer (pH2.2); 0.5ml of this buffered solution was passed on to the column for analysis.

Another aliquot of 0.5ml of the sample solution was hydrolysed in 2M hydrochloric acid for 4 hours at 110°C, evaporated and dissolved in 6ml of the loading buffer (pH2.2). Again, 0.5ml of this hydrolysed buffered solution was analysed.

Analyses were performed by passing 0.5ml of the loading buffer solution onto a 35cm single column packed with Ultrapac II, a sulphonated polystyrene cation exchange resin. The column had been regenerated with 0.4M sodium hydroxide for 10 minutes, followed by equilibration with a citrate buffer (pH3.25) for 45 minutes. The solutions containing the buffered extracts were eluted with three buffers: 10 minutes, buffer A (pH3.25); 40 minutes, buffer B (pH4.25); 66 minutes, buffer C (pH6.45). The elution programme also included a temperature change: initial temperature 50°C for 42 minutes followed by 70°C for 74 minutes.

Amino acid composition of various *Eichhornia crassipes* extracts

Free amino acids in aqueous extracts	% of total amino acids present			
	Control plants		Platinum treated[a] (B/15)	
	leaves	roots	leaves	roots
Alanine	13.1	7.1	11.2	12.0
4-aminoisobutyric acid	5.3	4.9	6.1	3.2
Arginine	0.1	1.7	0.8	4.6
Asparagine	10.2	18.0	20.9	27.3
Aspartic acid	15.5	20.1	19.1	27.0
Glutamic acid	1.3	2.9	3.3	3.1
Glutamine	5.3	18.4	5.3	4.1
Glycine	5.6	1.9	2.9	3.9
Histidine	3.7	0.2	1.3	1.0
Isoleucine	4.2	2.8	2.0	1.1
Leucine	2.6	3.5	1.6	0.6
Lysine	1.0	0.6	0.8	1.1
Phenylalanine	5.7	1.7	2.9	0.2
Serine	7.8	10.5	7.9	5.9
Threonine	9.8	1.4	7.1	3.3
Tyrosine	0.5	1.2	0.6	0.4
Valine	8.2	3.1	6.4	1.3

[a]Platinum applied as cis$[Pt(NH_3)_2Cl_2]$, 0.5 ppm Pt.

Table 8.15

The absorbance of the eluted fractions, mixed with ninhydrin, was measured at 440 and 570nm; a mixture of standard amino acids (25 nanomoles) was treated similarly and used to calculate the amounts of amino acids present in the extracts.

Various amino acids present in the root and leaf extracts were identified from charts of peaks from the standard mixture. The results of the percentage composition of amino acids in various extracts are presented in Table 8.15.

8.3 Discussion

The effects of treating water hyacinths with complexes of the platinum metals were recorded over a growth period of two weeks. During that period, vegetative reproduction took place. The same number of daughter plants appeared on rhodium treated plants as on controls, but not for those plants treated with cis $[Pt(NH_3)_2Cl_2]$. The average increase in biomass was 6.7% more for rhodium treated plants than for controls whereas it was 13.9% less for plants treated with cis $[Pt(NH_3)_2Cl_2]$. This confirmed earlier indications of growth stimulation by rhodium (pg.194). The effect on plant roots was similar too: rhodium treated roots were little affected (index of tolerance = 0.96) while roots from plants treated with the antitumour drug were restricted in growth (index of tolerance = 0.36).

The values presented in Tables 8.2, 8.3, 8.8 and 8.9 represent an attempt to quantify the effects of various metal complexes on aquatic plant growth which can vary at random. For this reason, average changes in biomass and root length must be interpreted carefully since they are usually accompanied by a high SD and RSD. Nonetheless the values reported can be interpreted in a semi-quantitative manner.

Plate 8.4 shows a distinct difference in the colour of roots from the daughter of a rhodium treated plant when compared to controls. This red colouration may be due to the presence of a rhodium complex or to some enhanced production of a natural pigment, but this has yet to be identified. The flavanoid pigment anthocyanin has been reported to be present in the roots of *Eichhornia crassipes* (Hasman and Inanc, 1957).

Plates 8.7 and 8.8 show the toxic effects of platinum applied as cis $[Pt(NH_3)_2Cl_2]$, on the leaves of water hyacinth. Longitudinal brown streaks are visible on the pseudolaminae. This was particularly noticeable with newer leaves and with the leaves of daughter plants. Similar toxic effects to plants have been reported for other metals such as Cd, Co, Ni and Zn. (Rauser, 1973; 1978) (Robb, Busch and Rauser, 1980). These effects were also observed when water hyacinths were treated with platinum applied in other forms: Plate 8.9 $K_2[PtCl_4]$; Plate 8.11 $[Pt(NH_3)_4]Cl_2$.

The assimilation of rhodium and platinum from solution was monitored (Tables 8.4 and 8.6; figure 8.1). In the case of rhodium the amount of uptake was small resulting in little change in the rhodium concentration of the applied nutrient solution (figure 8.1(b)). This is reflected in the low concentrations of rhodium found in both roots and tops of treated plants when analysed. Good agreement was found between ICP and INAA for the determination of rhodium, where similar levels of Rh were found in the roots and leaves and rather less in the floats.

The uptake of platinum (Table 8.6) was monitored for the first week only. The results are presented as a graph of platinum concentration in the nutrient solution against days growth in platinum (figure 8.1(a)). A steady decrease in the level of platinum is shown for the first four days, levelling off towards the end of the first week. A similar trend is shown

for the first week of rhodium uptake (figure 8.1(b)), though in the second week of rhodium treatment the concentration appears to vary little. Given the limits of error involved in measuring such small changes in metal concentration and the variation of uptake between individual plants, it is impossible to measure the total rhodium uptake, in all plants, by analysing for rhodium in the nutrient solutions. An estimate of 7600 μg Rh for the first week does not agree with values for the total found by analysis of 895 μg Rh. The decrease in rhodium concentration (and platinum too) is affected by evaporation, transpiration, adsorption effects as well as by other sources of error mentioned above. All that may be concluded is that much of the uptake almost certainly takes place within the first few days of growth.

The toxic effects of different forms of platinum on the water hyacinth show that there is little difference between $K_2[PtCl_4]$ and $[Pt(NH_3)_4]Cl_2$ but $Pt(IV)Cl_4$ appears to be less toxic than complexes of Pt^{2+}. This confirms the results of investigations in chapter 7.2.1 where Pt^{4+}, as $(NH_4)_2[PtCl_6]$, was found to be much less toxic than complexes of Pt^{2+}. Values for the increase in biomass support this, since complexes of Pt^{2+} restrict the weight increase ratio to 2.46 for $K_2[PtCl_4]$, 2.69 for $[Pt(NH_3)_4]Cl_2$ and 2.91 for $Pt(IV)Cl_4$ compared to 3.04 for control plants. The effects on root lengths (Table 8.9) were inconclusive. Vegetative reproduction did appear to be affected by Pt^{2+} though this is difficult to quantify. Platinum was taken up by the plant roots mostly, with lesser amounts detected in the leaves and floats.

The results of sequential extraction of the dried plant material treated with platinum applied as the anti-tumour drug show that in the dried plant leaves, almost half the platinum present (47.9%) is insoluble and associated with α-cellulose and lignin. Lesser amounts are associated

with soluble pectates (20.8% after water extraction) and proteins/amino acids (16.1%). A similar distribution is found in float material. In the plant roots, rather less is associated with α-cellulose and lignin (35%), Proteins, amino acids (9.5%) and soluble pectates (14.2%). More platinum in root material was found with water soluble low molecular weight materials after both ethanol (23.1%) and water extraction (12.0%). It would seem that most of the platinum is bound by α-cellulose of cell walls with more bound by amino acids, proteins and soluble pectates in plant tops. In the extraction schedule (figure 8.2), the material (J'') extracted with caustic alkali resulted in extracts which overloaded the background correction system in ETA AAS during the determination of platinum. Further problems were experienced in an attempt to use solvent extraction techniques to separate the platinum from the interfering matrix and thus, these results do not appear in the respective tables. It is evident that in the +2 oxidation state, platinum can affect the growth pattern of water hyacinths; thus the mode of chemical binding in plant tissues and the chemical form of platinum within the plant both need to be investigated further.

The results of sequential extraction prompted further investigation of the distribution of free amino acids in aqueous extracts. The results from control plant material indicate the presence of relatively large amounts of the common amino acids, asparagine and aspartic acid. The control roots contained high levels of glutamine and serine too, whilst leaf material contained a higher proportion of alamine, threonine and valine compared to roots. In platinum treated plants both root and leaf material contained a much higher proportion of asparagine and aspartic acid.

REFERENCES

Boyde, C.E. (1970), Econ. Bot., 24 95.

Boyde, C.E. and Vickers, D.G. (1971), Hydrobiologia, 38 (3-4) 409.

Cooley, T.N., Martin, D.F., Durden, W.C. and Perkins, B.D. (1979), Water Res., 13 343.

Easley, J.F. and Shirley, R.L. (1974), Hyacinth Contr. J, 12 82. Cited in Cooley *et al.*, (1979).

Farago, M.E., Mullen, W.A., Cole, M.M. and Smith, R.F. (1980), Environ. Pollut. Ser. A., 21 225.

Hasman, M. and Inanc, N. (1957), Fen Fakultesi Mecmuasi, University of Istanbul, 137.

Jowett, D. (1964), Evolution, Lancaster, Pa., 18 70.

Lawrence, J.M. (1968), OWRR Final Project, B-005-ALA, 1. Cited in Cooley *et al.*, (1979).

McNeilly, T. and Bradshaw, A.D. (1968), Evolution, Lancaster, Pa., 22 108.

Mullen, W.A. (1980), Ph.D. Thesis, University of London.

Rauser, W.E. (1973), Can. J. Bot., 51 301.

Rauser, W.E. (1978), Can. J. Bot., 56 1744.

Robb, J., Busch, L. and Rauser, W.E. (1980), Ann. Bot., 46 43.

Sevag, M., Lackman, D.B. and Smolens, J. (1938), J. Biol. Chem., 124 425.

Sheehan, T.J. and Johnson, C.R. (1977), Proc. Fla. State Hort. Soc., 90 118.

Wilkins, D.A. (1957), Nature, 180 37.

Wolverton, B.C. and McDonald, R.C. (1976), New Sci., 71 381.

CHAPTER IX

DETECTION AND LOCATION OF PLATINUM METALS IN *EICHHORNIA CRASSIPES* USING ELECTRON MICROSCOPY WITH X-RAY MICROANALYSIS

9.1 Background

The classical technique of histochemistry has been of little use in the study of the distribution of heavy metals in plant tissues, except in those cases where the metal concentration is unusually high. Histochemical staining of sections for examination by light microscopy, (LM), has been described for some metals, e.g.: Al, Fe, As, Ca, Mg and K (Gurr, 1958) but the concentration of most micronutrients is well below the detection limits of such staining techniques. The advent of electron microscopy, however, has led to increasing interest in the use of X-ray microanalysis for the study of metal distribution in plant tissues.

The development of electron microscopy in the 1930's, with its ability to extend resolution beyond that of light microscopy has had far reaching consequences in biological disciplines. Much of current thinking on the chemistry of plant cells comes from studies which have used electron microscopy as a tool to investigate cell structure.

Basically, there are two types of electron microscopy: Transmission Electron Microscopy, (TEM), and Scanning Electron Microscopy (SEM). TEM is the older of the two techniques and involves the passage of a homogeneous beam of electrons through a thin section of plant tissue. Typical section thickness would be of the order of 100nm giving a maximum resolution of between 0.2-0.3nm in practice.

The events which take place in an electron microscope when the

sample is bombarded with a beam of high velocity electrons have been discussed in chapter 2.4 (figure 2.9). The electron beam causes the emission of secondary electrons, backscattered electrons, characteristic X-rays and other types of radiation (Hess, 1979). The theory of X-ray production and subsequent microanalysis has been explained previously (chapter 2.4.2). In SEM, the sample, which is not sectioned (as it is for TEM) is scanned by a narrow beam of electrons. The secondary electrons emitted are collected and used to construct an image which is displayed on a CRT screen. The resolution is much poorer, (5-10nm), than for TEM but SEM is useful in the study of surface detail. Other instruments, which are variations on the two basic techniques, are available; these include the Scanning Transmission Electron Microscope (STEM) and the dedicated Electron Microprobe Analyser (EMPA).

Since the electron microprobe X-ray analyser was introduced (Castaing and Guinier, 1949) (Castaing, 1951) it has been used in many fields, ranging from metallurgy to medicine. The progress of microanalysis in SEM was the subject of a recent review (Newbury, 1979). The study of heavy metal distribution in plant tissues by EMPA has been restricted because the practical detection limits in bulk materials are between 0.1-0.01%; most micronutrient concentrations are well below this. Some studies have been conducted, but mainly on the distribution of the macronutrients Ca, K, P and S (Laüchli and Schwander, 1966) (Laüchli, 1967). Rasmussen, (1968), has used electron microprobe analysis to locate aluminium in the roots of corn plants.

Various problems in EMPA are associated with biological specimens particularly. These are: (a) the distortion and burning of the tissues by the electron beam; (b) tissue preparation techniques which can result in the leaching out of weakly bound elements of interest (Morgan, 1979);

(c) non-conductivity of biological samples. Freeze drying and cryostatic techniques have been developed to reduce the movement of elements during preparation (Morgan, 1979) (Rasmussen, *et al.*, 1968).

Recent trends in microprobe techniques with biological samples have moved towards examination of ultrathin specimens with TEM or STEM instruments equipped with energy dispersive analysis (EDXA). The examination of thin sections enables quantitative as well as qualitative analysis to be carried out. Ophus and Gullvag (1974) used TEM and X-ray microanalysis to locate lead within the leaf cells of *Rhytidiadelphus squarrosus*. Applications of EMPA in biology have been reviewed recently (Erasmus, 1978).

In this chapter, root and leaf samples from *Eichhornia crassipes* (water hyacinth) containing the platinum metals were examined in the electron microscope. As a guide to anatomical detail, sections of root and leaf were prepared by conventional techniques for examination under the light microscope (LM). Typical examples of leaf and root were examined then by SEM. Specimens containing the platinum metals were examined further in an SEM equipped with EDXA facilities. Several specimens which gave positive results with EDXA were selected for brief investigations (i) with the Electron Microprobe Analyser (EMPA) (ii) by STEM with EDXA, and (iii) by X-ray Photoelectron Spectroscopy (XPS).

9.2 Light microscopy (LM)

Method

Samples of leaf and root tissues were taken from water hyacinths, grown in solutions containing platinum and rhodium, and fixed in FAA (formaldehyde-acetic acid-alcohol). Samples from control plants were treated similarly. The tissues were dehydrated in a graded series of

alcohol-water mixtures ending with tertiary-butyl alcohol (TBA), followed by infiltration with paraffin wax; they were sectioned at 10μm using a microtome. The resulting sections were mounted on glass slides using Haupt's adhesive.

The sections were double stained with safranine and fast-green FCF to show up the various cell types and display the root and leaf anatomy of water hyacinth. Safranine is a basic stain of red colouration which is specific for lignified and cutinised cell walls. Fast-green, FCF, is specific for cellulose cell walls and cytoplasm. Plates 9.1 to 9.6 represent transverse sections of leaf and root from a typical control and treated water hyacinth.

Results

The structure of the leaf of *Eichhornia* can be divided conveniently into three distinct regions: the epidermis, mesophyll and conducting system (Hasman and Inanç, 1957). The epidermal cells are nearly all isodiametric in shape, with a thin cuticle layer. The mesophyll is well differentiated into palisade and spongy parenchyma which contains large air spaces partitioned by diaphragms. The conducting system is clearly visible, consisting of irregularly spaced vascular bundles. Each bundle contains the xylem and phloem elements, surrounded by a sheath of collenchymatic inner and parenchymatic outer cell layers. The transverse section of the platinum treated plant leaf included that part of the leaf displaying a brown streak described in chapter 8; (Plate 8.8). The palisade mesophyll here appears distorted in contrast to that of the control or rhodium treated plant leaves, which have characteristic elongated cells of the palisade mesophyll behind the epidermis. However, further histochemical staining would be required to ascertain the nature

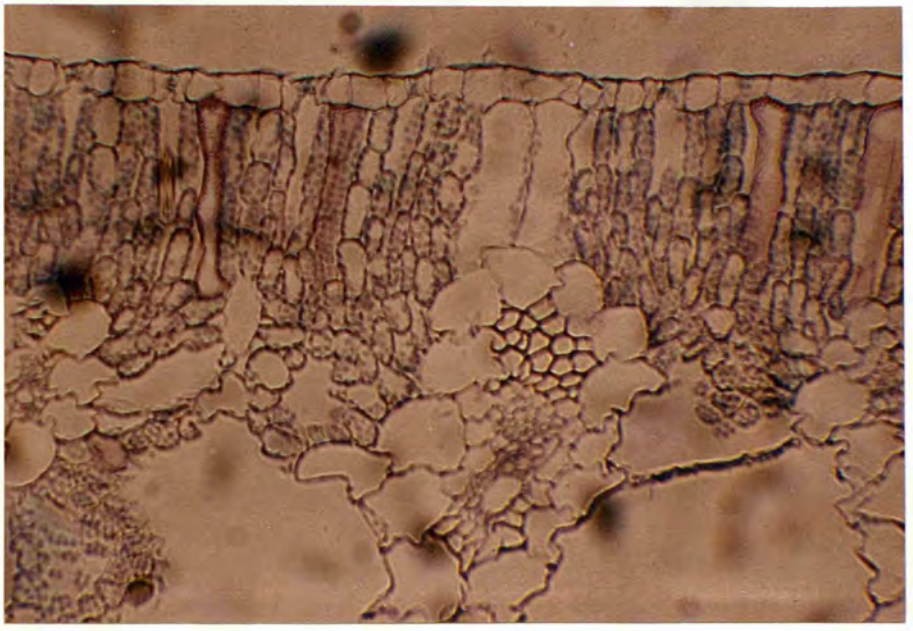


Plate 9.1 Leaf t.s. from control water hyacinth (×70) double stained with safranine and fast-green (FCF)

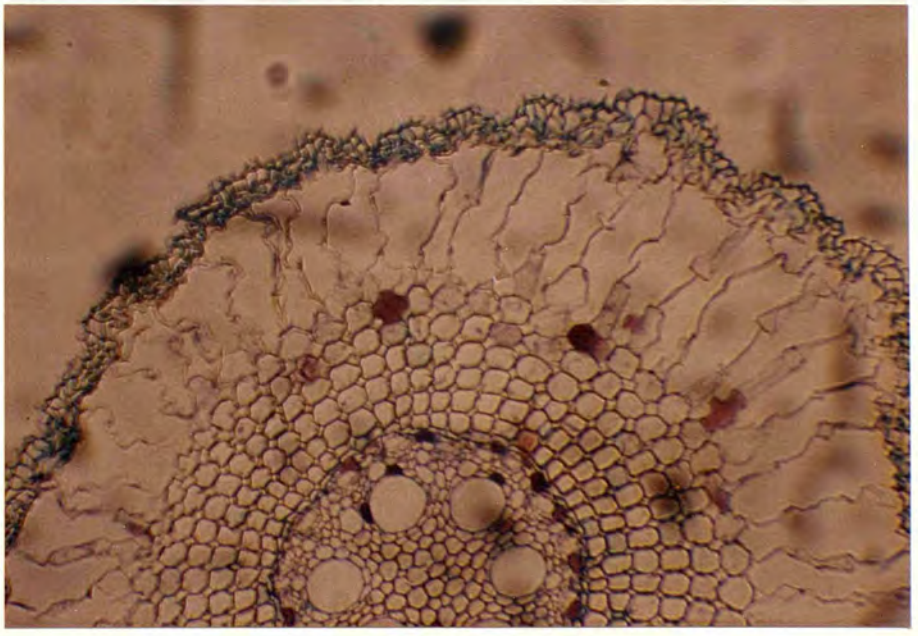


Plate 9.2 Root t.s. from control water hyacinth (×70) double stained with safranine and fast-green (FCF)

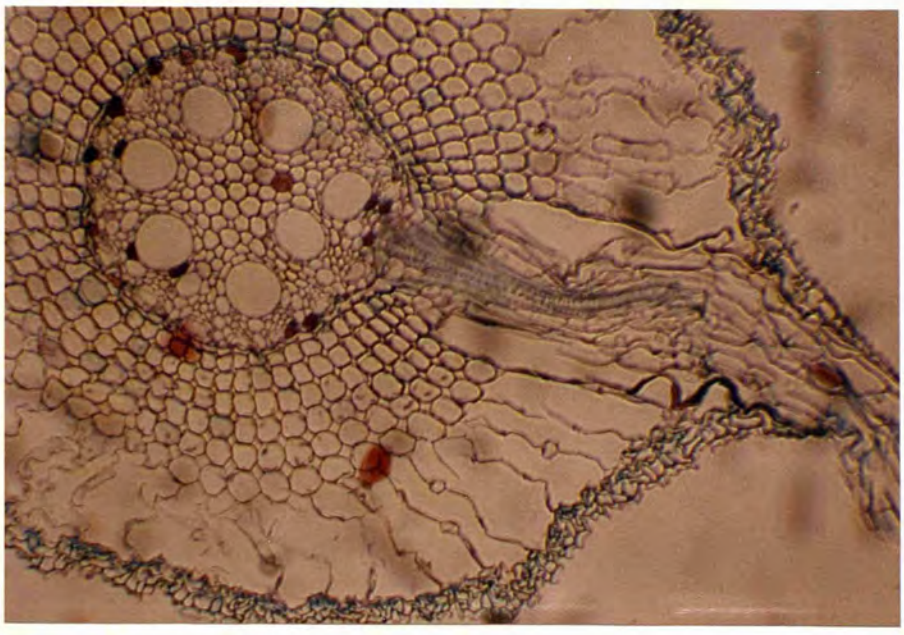


Plate 9.3 Root t.s. from control water hyacinth (×70) double stained with safranine and fast-green (FCF)

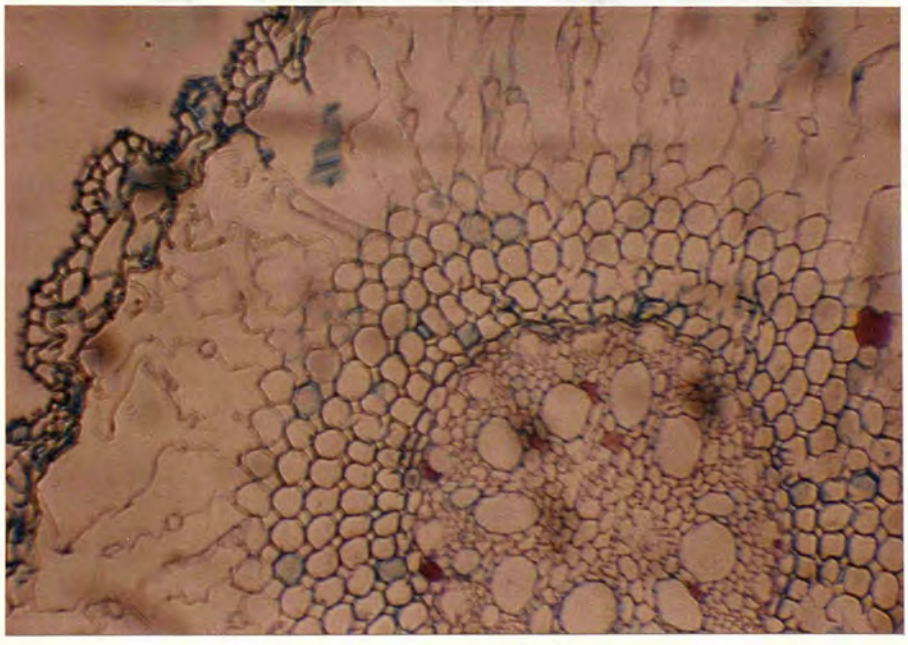


Plate 9.4 Root t.s. from platinum treated water hyacinth (×70) double stained with safranine and fast-green (FCF)

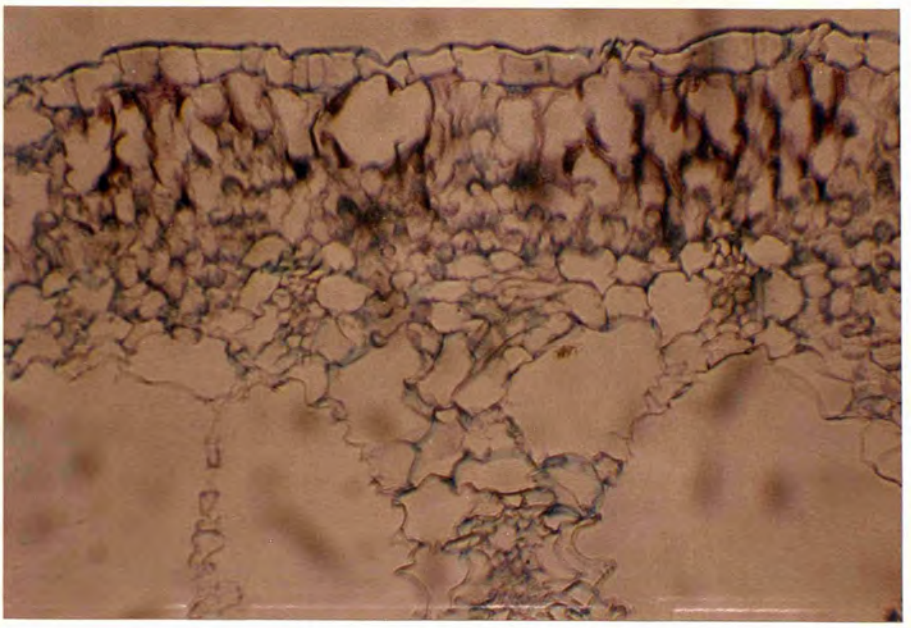


Plate 9.5 Leaf t.s. from platinum treated water hyacinth (×70) double stained with safranine and fast-green (FCF)

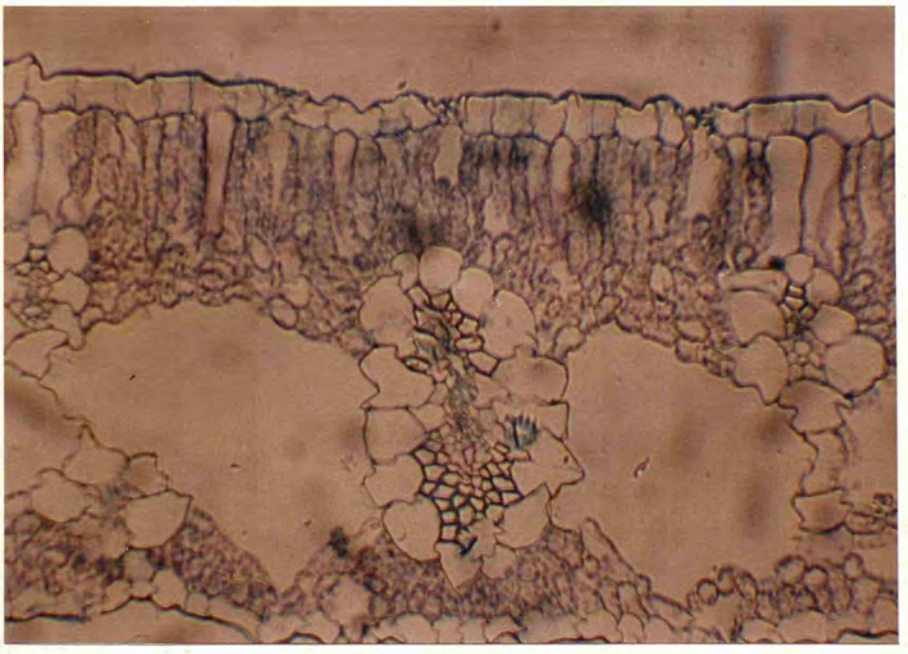


Plate 9.6 Leaf t.s. from rhodium treated water hyacinth (×70) double stained with safranine and fast-green (FCF)

of this distortion. Similar toxic effects have been reported for zinc in beans, soybeans and corn (Rauser, 1973), cadmium, cobalt, nickel and zinc in white beans (Rauser, 1978).

Eichhornia has a well developed root system which can be divided conveniently into epidermis, cortex and central cylinder. The epidermal cells are smaller in size than those of the leaf; the cell walls contain anthocyanin: a flavonoid pigment. The cortex consists of three distinct regions stretching from the epidermis to the stele. Below the epidermis is a layer of thick walled cells packed closely together. A region of aerenchymatic tissue follows, consisting of a number of thin walled cells arranged radially. A third region approaching the stele consists of a number of regularly arranged cells, of very similar shape. A weakly developed endodermis is visible, separating the cortex from the stele. The central cylinder contains the xylem and phloem elements, along with compactly arranged parenchymatic tissue.

9.3 Scanning electron microscopy (SEM)

Method

Samples of leaf and root from water hyacinths were removed and fixed in 3% glutaraldehyde in 0.1M phosphate buffer (pH7) for 1 hour. The tissue samples were dehydrated in a graded series of ethanol-water mixtures. Specimens were cut after removal from 95% ethanol and critical point dried from liquid carbon dioxide for 1½ hours. They were mounted on self adhesive aluminium stubs, sputter-coated with carbon (8.0nm) and examined in the scanning electron microscope (Cambridge Instruments S4-10 Stereoscan). Electron micrographs were taken at gun potential of 10kV and some typical examples of leaf surfaces, and root cross sections are presented in plates 9.7 to 9.12.

(a) ×30 (b) ×60 (c) ×120

Plate 9.7 Control water hyacinth: leaf surface

(a) ×300 (b) ×600 (c) ×1200

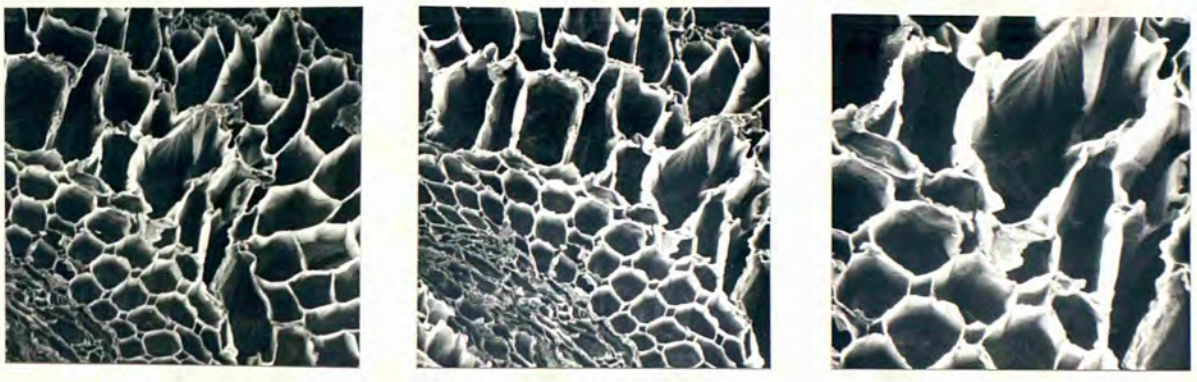
Plate 9.8 Control water hyacinth: leaf surface

(a) ×120 (b) ×63 (c) ×126

Plate 9.9 Water hyacinth leaf surface: (a) control; (b) and (c) platinum treated (B/15).

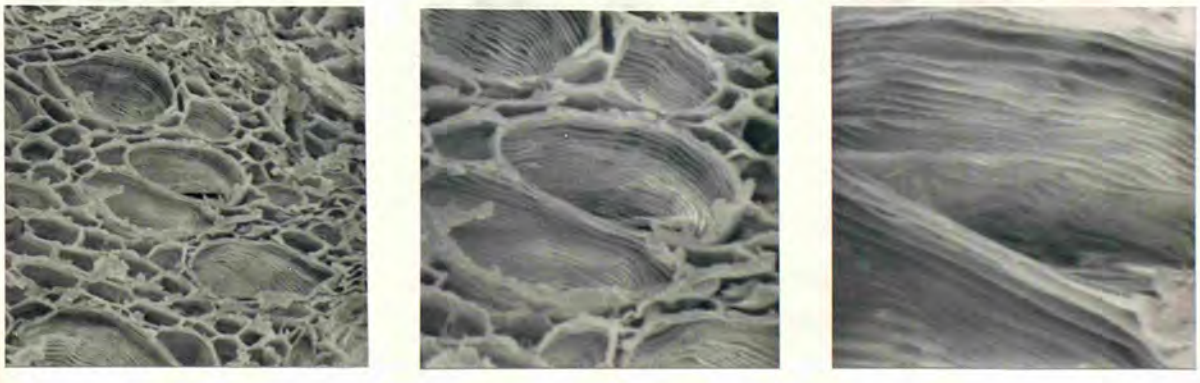
(a) ×126 (b) ×315 (c) ×630

Plate 9.10 Water hyacinth leaf surface: platinum treated (B/15)



(a) ×320 (b) ×330 (c) ×650

Plate 9.11 Control water hyacinth: root (ts)



(a) ×630 (b) ×1260 (c) ×3150

Plate 9.12 Control water hyacinth: root (ts)

Results

Plates 9.7 and 9.8 represent a typical leaf upper surface of *Eichhornia* at various magnifications. The leaf has amaryllis type stomata (fig.9.8c) on both upper and lower surface; it is therefore designated amphistomatic (Hasman and Inanc, 1957). There is no detectable difference between the leaf surface of the platinum treated plant and control plant as seen in the electron micrographs (Plate 9.10).

Electron micrographs of the root, shown in Plates 9.11;9.12, complement the photo micrographs of root transverse sections shown in Plates 9.2;9.3. The aerenchymatic tissues are clearly visible as are the xylem elements in the central cylinder.

9.4 Energy dispersive X-ray analysis (EDXA) with SEM

Method

The specimens of leaf and root, from water hyacinths, described in 9.3 were examined further in the SEM with EDXA[a] (Cambridge Instruments S2 A). All electron micrographs were taken at a gun potential of 2kV and a gamma setting of 2. EDXA was carried out on the whole field at a magnification of X625. The elements of interest and their corresponding X-ray lines are given in Table 9.1. Two spectra were recorded: 0-10 keV and 0-5 keV at 1K fsd. Specimens which gave positive results for platinum were examined in the 6-16 keV range. Results are presented in Plates 9.13 to 9.23.

Results

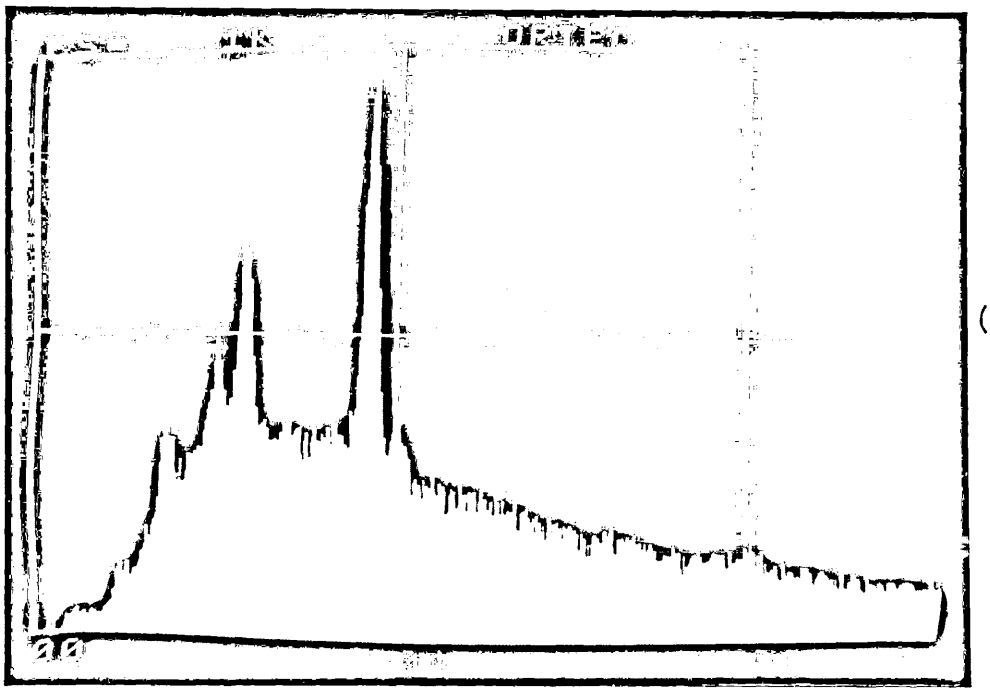
The spectra of control leaf and root specimens show a high concentration of calcium and smaller amounts of phosphorus and sulphur;

[a]SEM-EDXA investigations were carried out by the Electron Optics Unit at the Johnson Matthey Research Centre.

X-ray peaks (keV) for microanalysis

Element	K_α	K_β	L_α	L_β	L_γ	M_α
Platinum	66.82		9.44	11.07	12.94	2.05
Rhodium	20.20		2.70	2.83	3.14	
Ruthenium	19.28		2.56			
Aluminium	1.49					
Silicon	1.74					
Phosphorus	2.02					
Sulphur	2.31					
Chlorine	2.64					
Potassium	3.31	3.59				
Calcium	3.69	4.01				
Iron	6.40	7.06	0.70			

Table 9.1

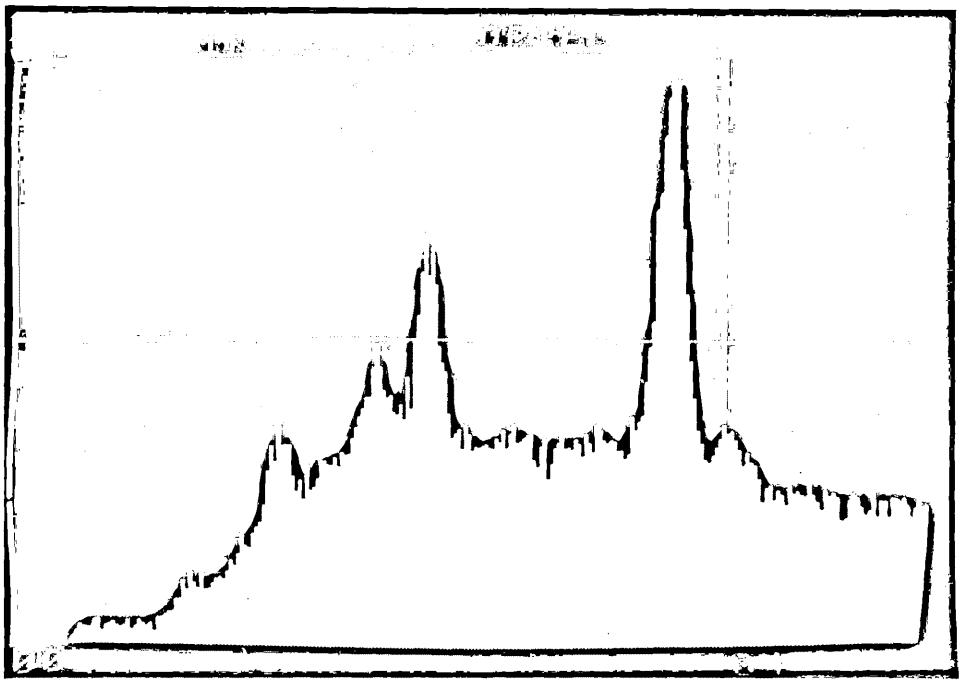


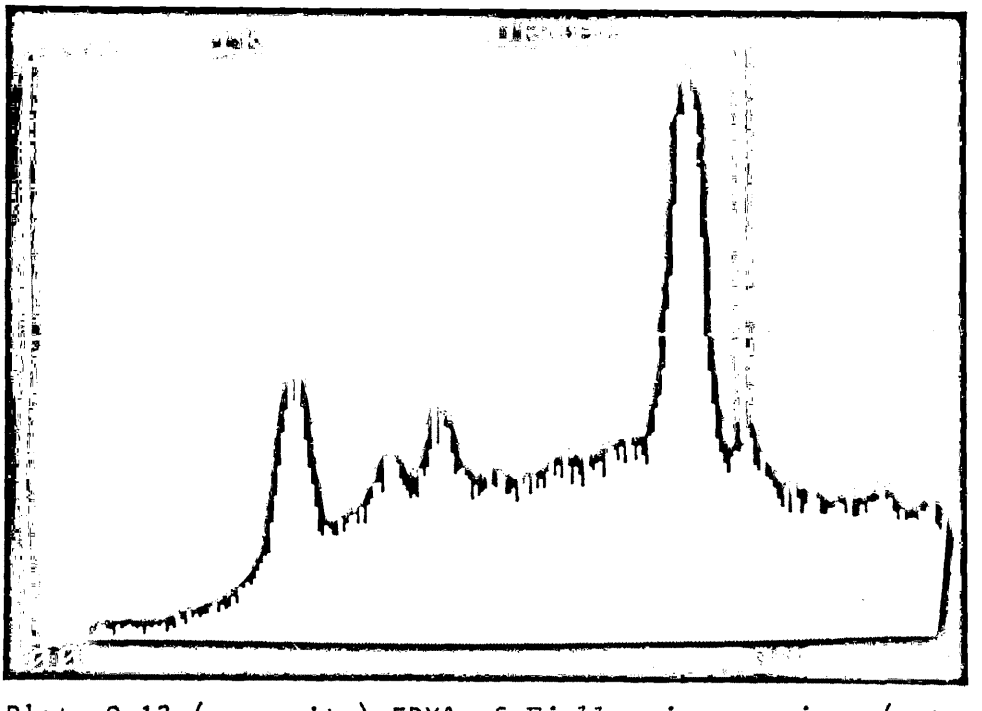
(a) leaf surface

(0-10keV)

(b) leaf surface

(0-5keV)

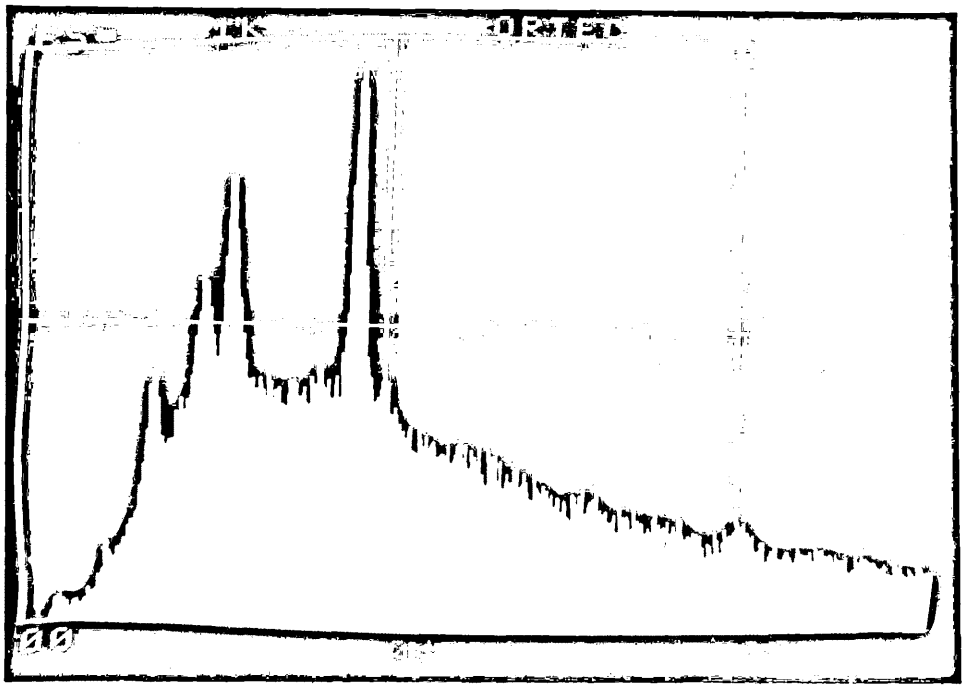




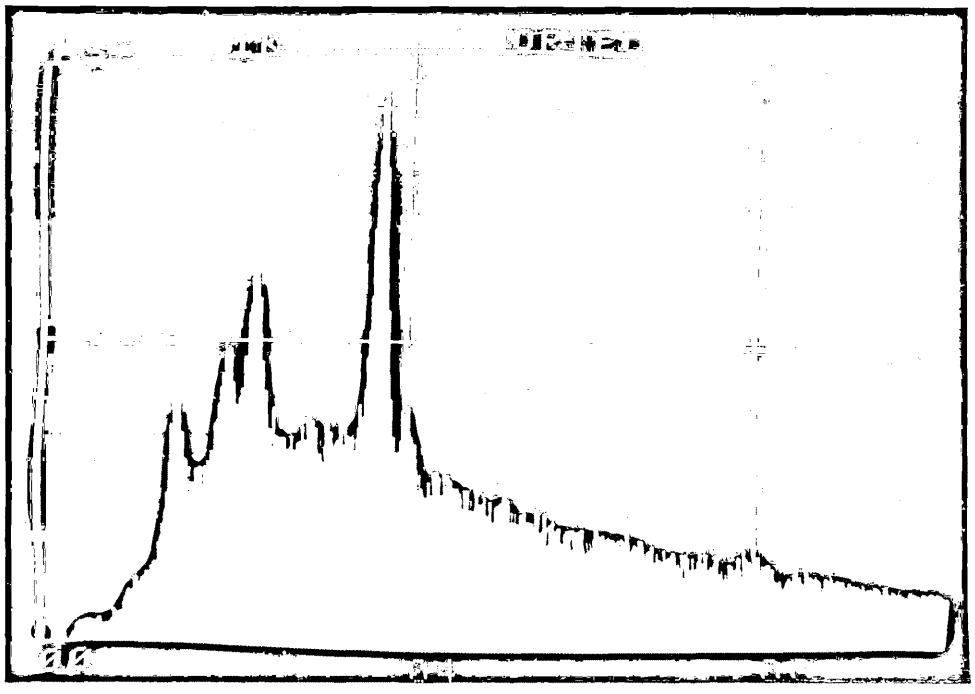
(c) root surface

(0-5keV)

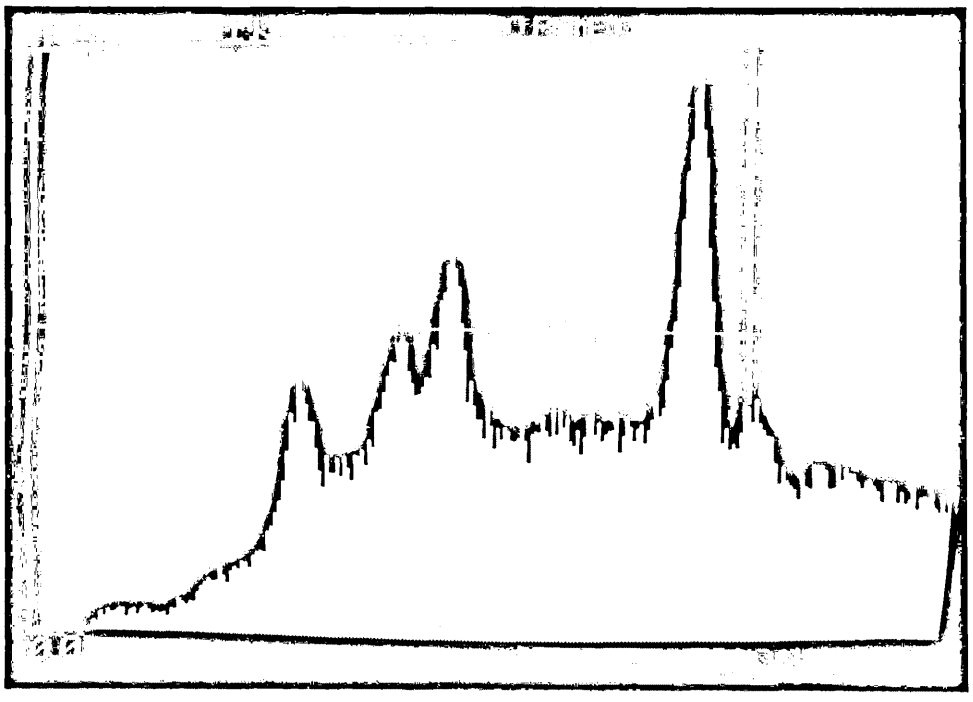
Plate 9.13 (composite) EDXA of *Eichhornia crassipes* (untreated control)



(a) leaf from plant treated with $(NH_4)_2[PtCl_6]$ (0-10keV)



(b) leaf from a plant treated with cis $[Pt(NH_3)_2Cl_2]$ (0-10keV)



(c) leaf from a plant treated with cis $[Pt(NH_3)_2Cl_2]$ (0-5keV)

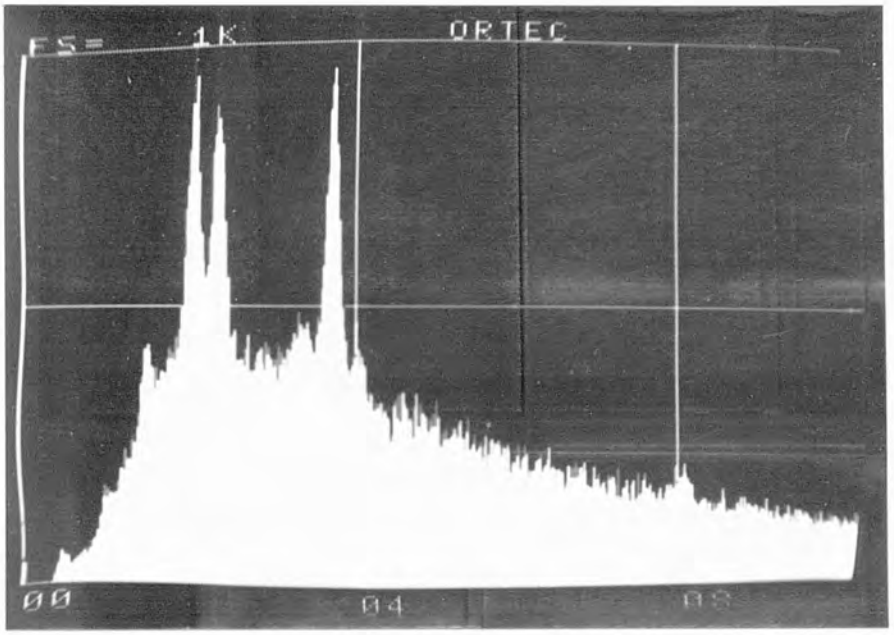
Plate 9.14 (composite) EDXA of leaf surfaces from *Eichhornia crassipes* treated with platinum

(a) ×180

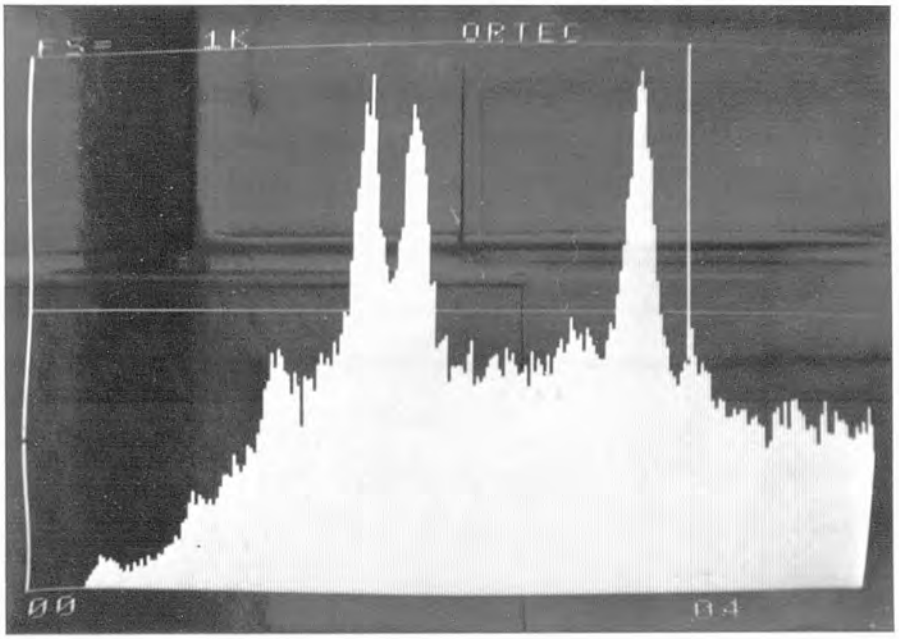
(b) ×450

(c) ×900

Plate 9.15 (composite) Leaf surface of *Eichhornia crassipes* treated with $Na_3[RhCl_6]$



(a) 0-10keV

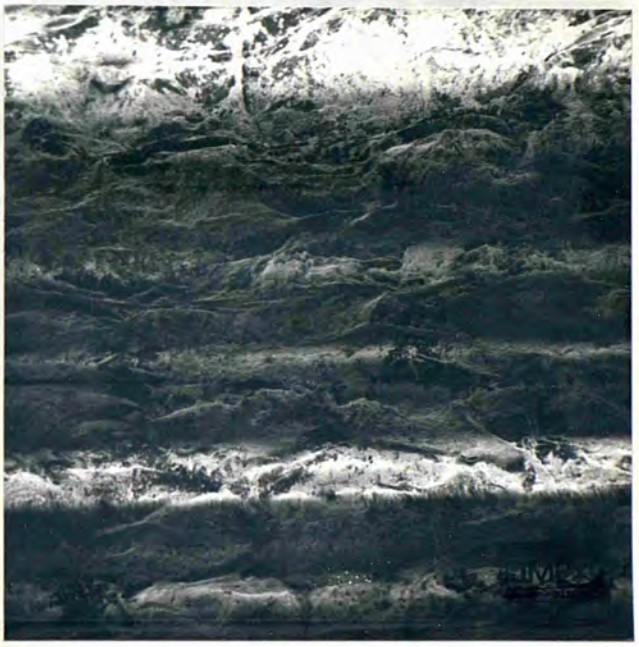


(b) 0-5keV

Plate 9.16 (composite) EDXA of leaf surface from *Eichhornia crassipes* treated with $Na_3[RhCl_6]$

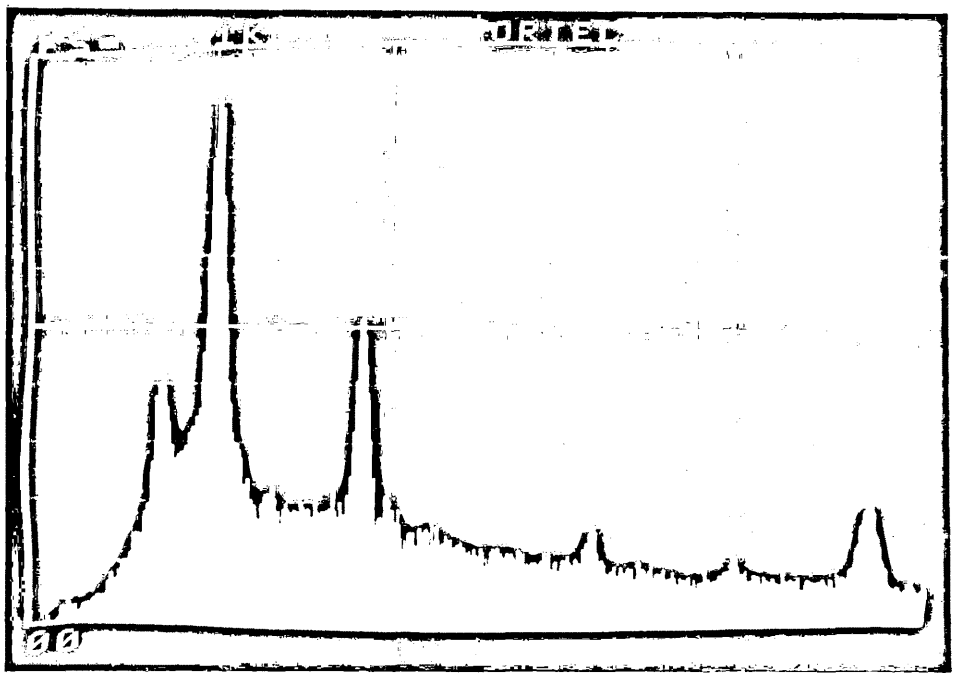
(a) ×180

(b) ×450

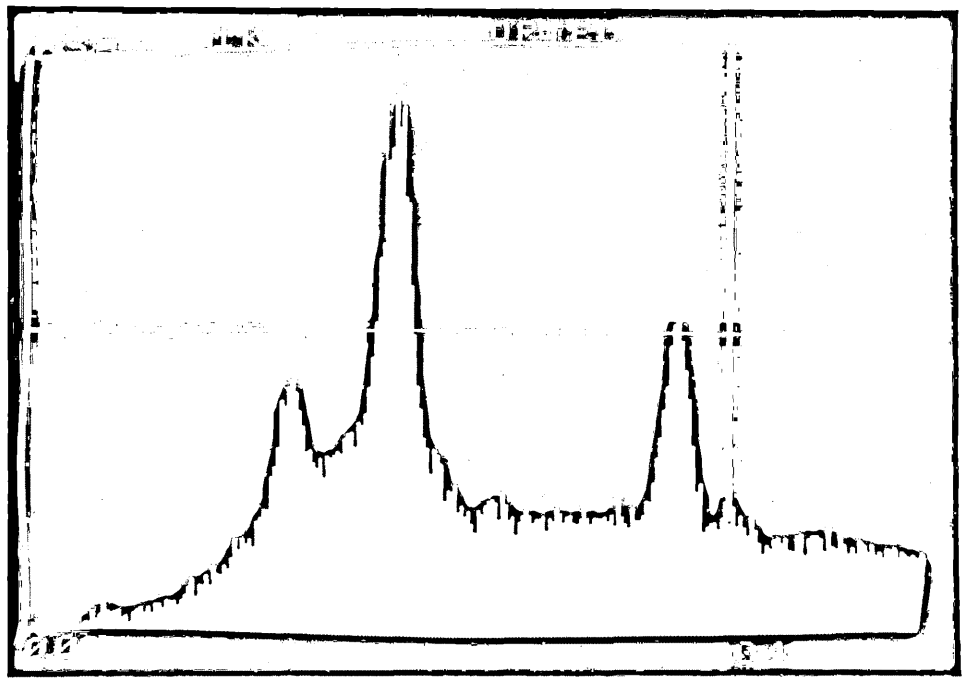


(c) ×900

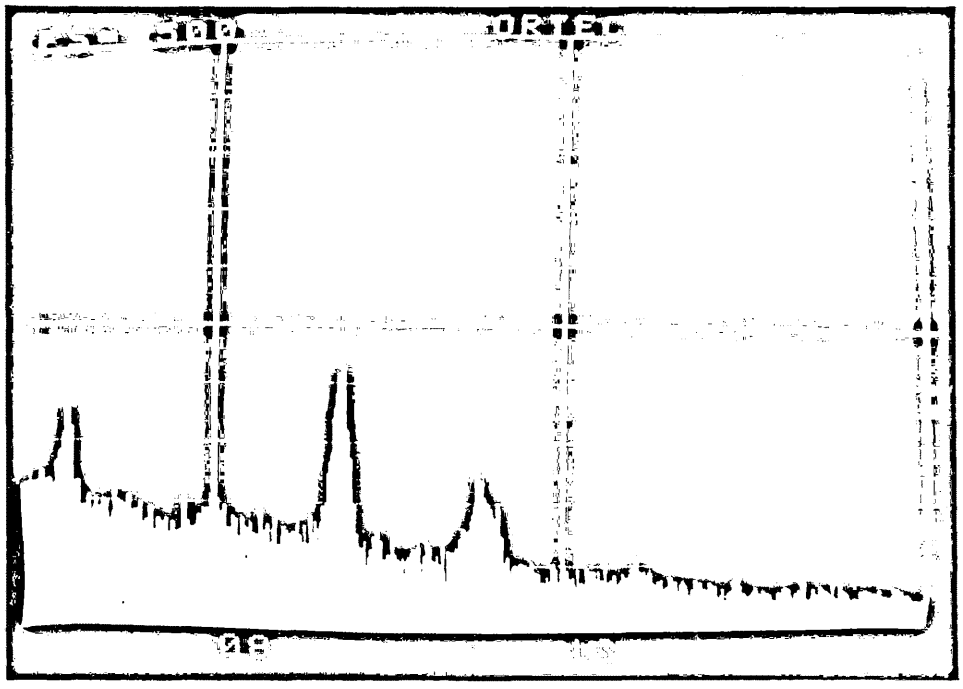
Plate 9.17 (composite) Root surface of *Eichhornia crassipes* treated with $(NH_4)_2[PtCl_6]$



(a) 0-10keV

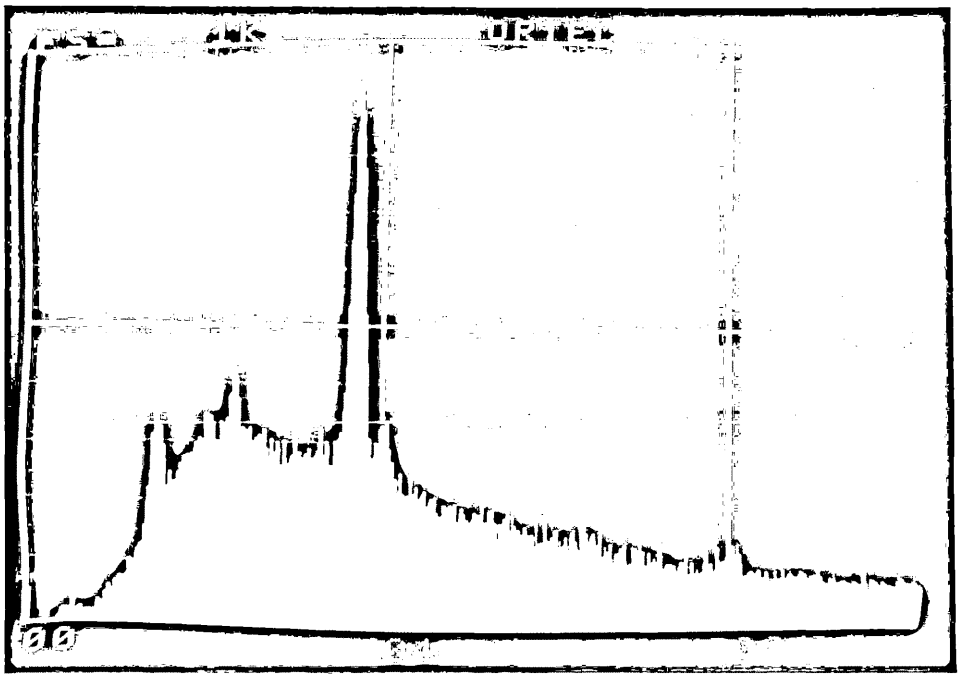


(b) 0-5keV

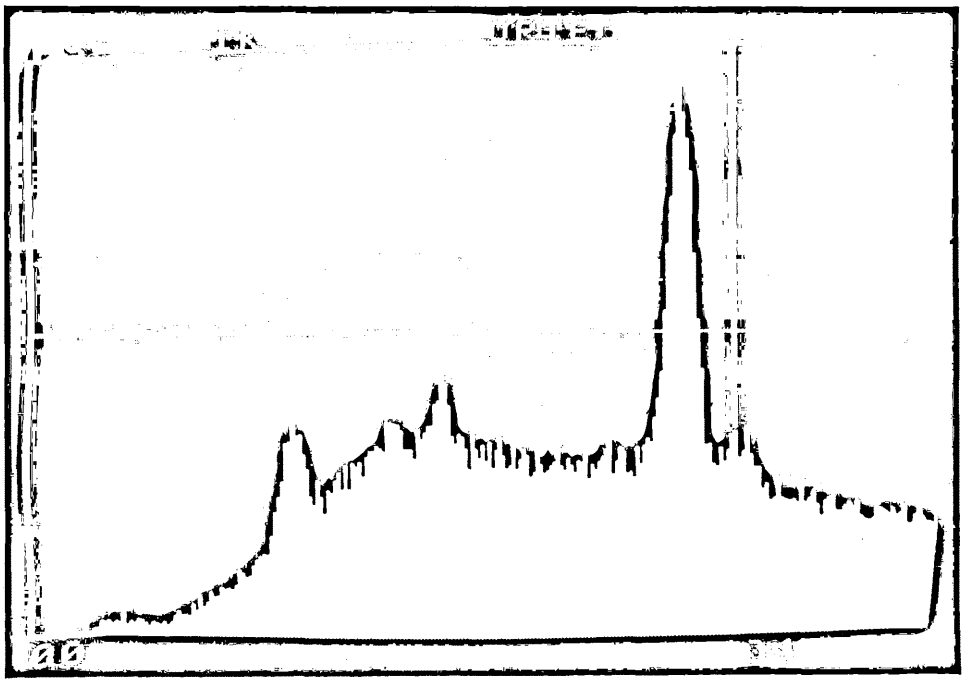


(c) 6-16keV

Plate 9.18 (composite) EDXA of root surface from *Eichhornia crassipes* treated with $(NH_4)_2[PtCl_6]$

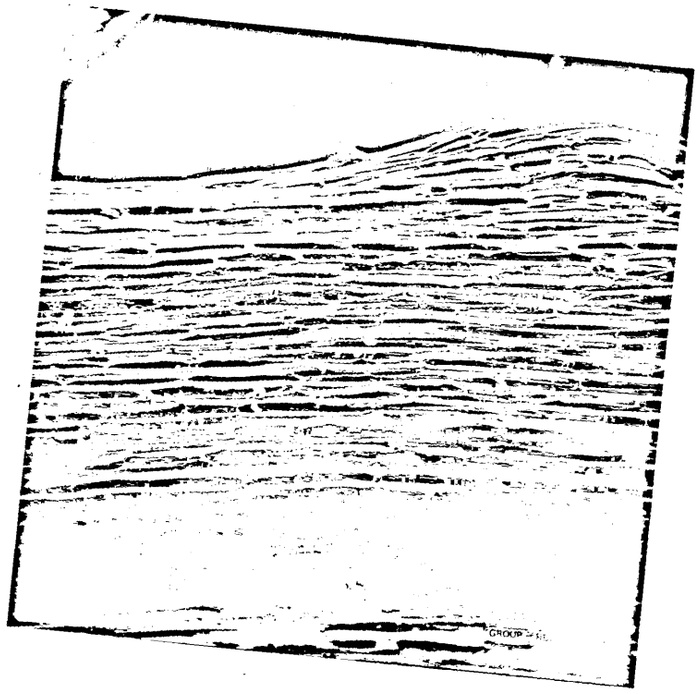


(a) 0-10keV

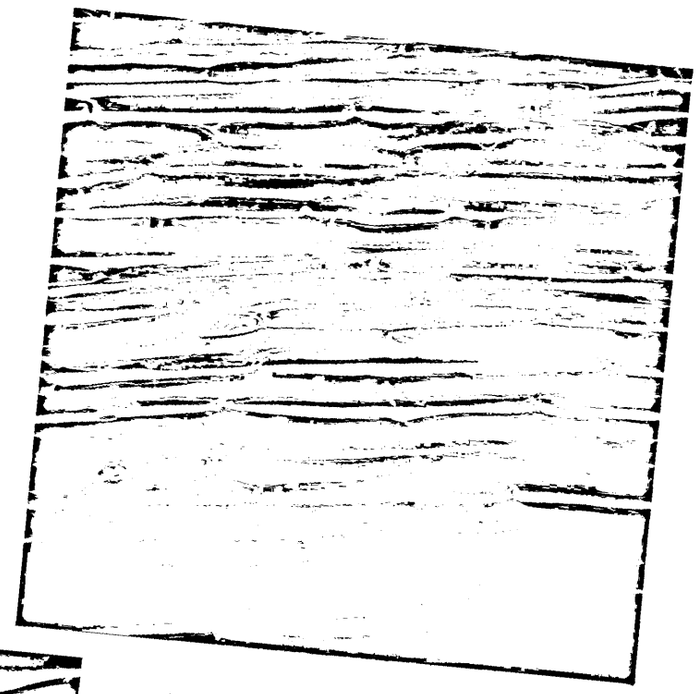


(b) 0-5keV

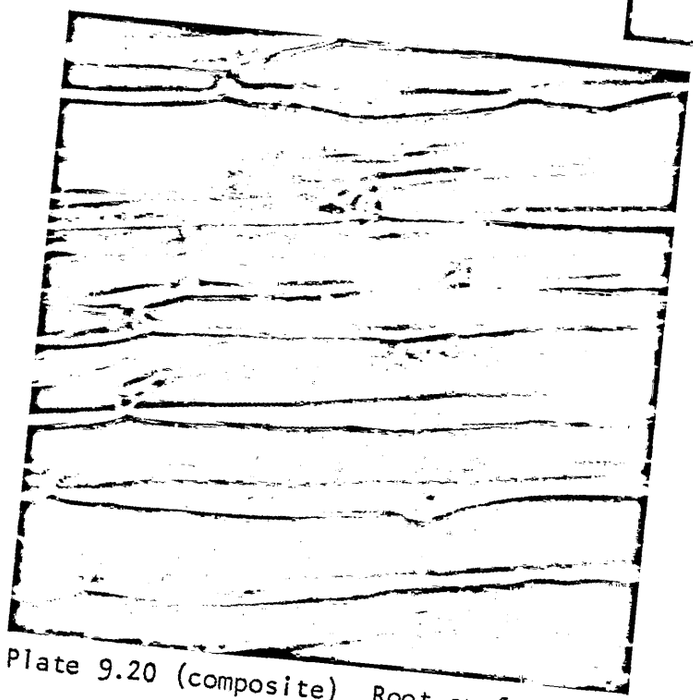
Plate 9.19 EDXA of root surface from *Eichhornia crassipes* treated with cis $[Pt(NH_3)_2Cl_2]$



(a) ×180

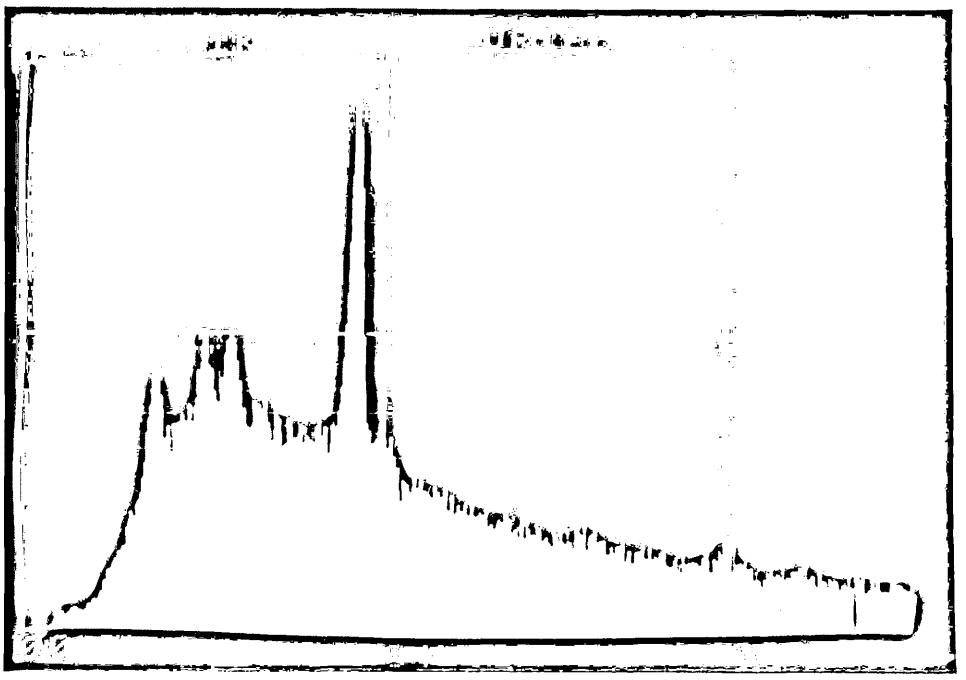


(b) ×450

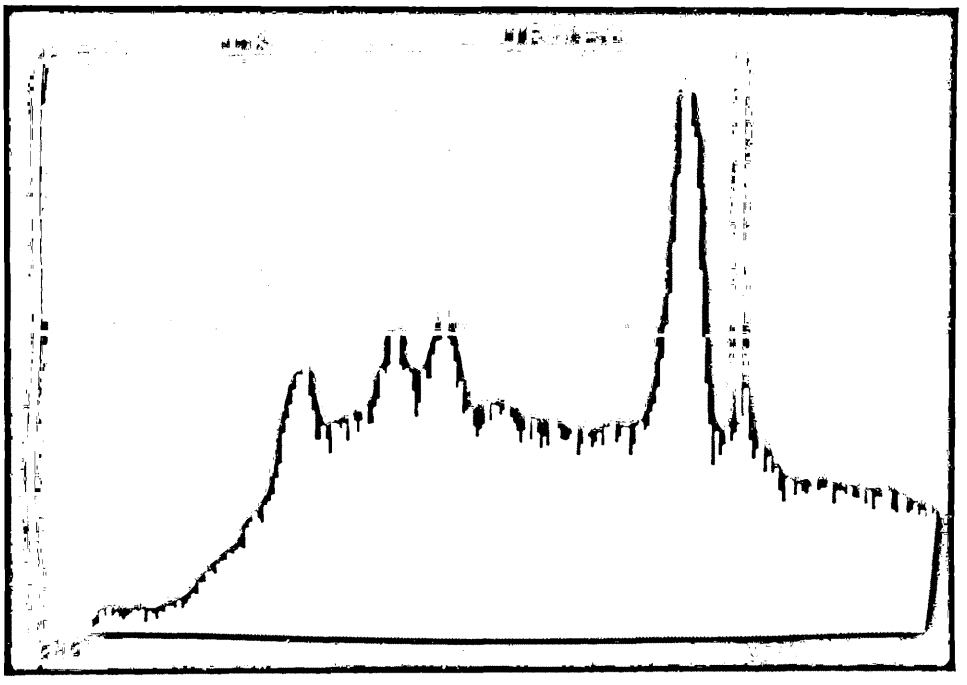


(c) ×900

Plate 9.20 (composite) Root surface of *Eichhornia crassipes* treated with $Na_3[RhCl_6]$



(a) 0-10 keV



(b) 0-5 keV

Plate 9.21 EDXA of root surface from *Eichhornia crassipes* treated with $Na_3[RhCl_6]$

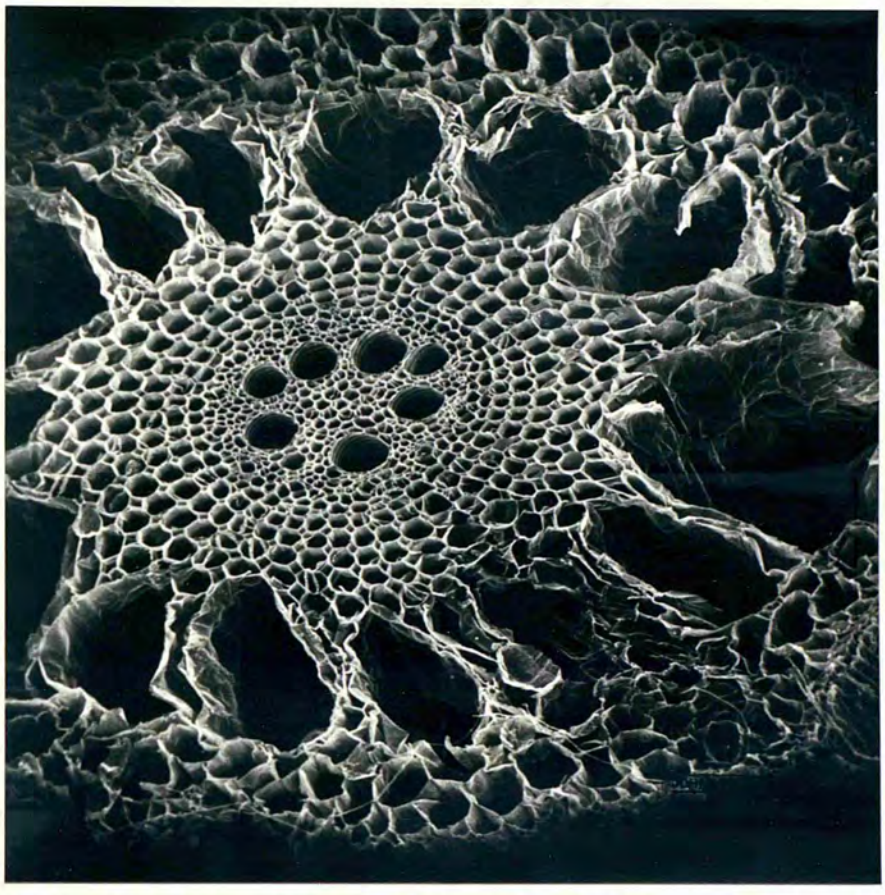


Plate 9.22 *Eichhornia crassipes* root cross section, ruthenium treated; (×250)

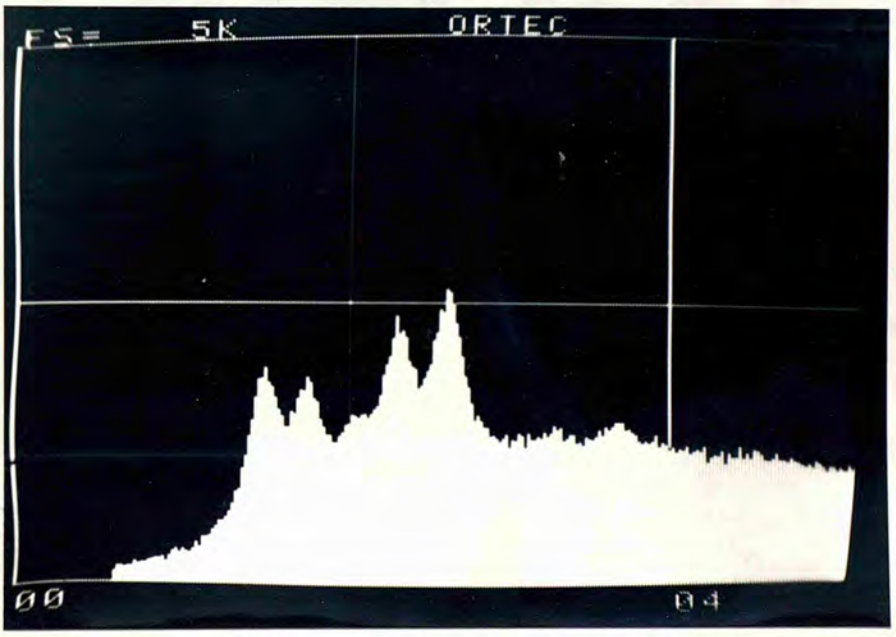


Plate 9.23 EDXA of root from *Eichhornia crassipes* treated with $(NH_4)_3[RuCl_6]$; (0-5keV)

aluminium from the stub is detectable too. The root treated with 10ppm platinum, applied as $(NH_4)_2[PtCl_6]$, appears covered in electron dense deposits (Plate 9.17). EDXA of these samples (Plate 9.18) show calcium K_α at 3.69 keV, and a distinctly large peak at the platinum M_α line at 2.05 keV, which completely masks the phosphorus line at 2.02 keV. The platinum L_α line at 9.44 keV and L_β line at 11.07 keV are visible also (Plate 9.18(c)).

Platinum was not detected in leaf material from any plant, nor was it detected in any material from plants treated with the antitumour drug (Plates 9.14(c) and 9.19). Rhodium was not detected in any sample (Plates 9.16 and 9.21), although there is only one area to scan i.e. the L lines whereas both the L and M lines are visible with platinum. EDXA of the root from the plant treated with 5ppm ruthenium, applied as $(NH_4)_3[RuCl_6]$ (Plates 9.22;9.23), show a peak at about 2.56 keV but this may be due to either ruthenium L_α or chlorine K_α, or both.

On uneven samples such as these, the amount of "bounce" is very high, leading to high aluminium (from the stub) and high iron (from the pole piece of the microscope) signals. Even when the signal is distinct and not obscured by background, the topographical relief was found to make mapping with such low levels of metal impossible with this system.

9.5 Electron microprobe analysis (EMPA)

Method

Selected specimens of root from *Eichhornia crassipes*, treated with the platinum metals, and described in 9.3 were examined in the electron microprobe analyser (EMPA)[a]. The instrument used was a Camebax MBX

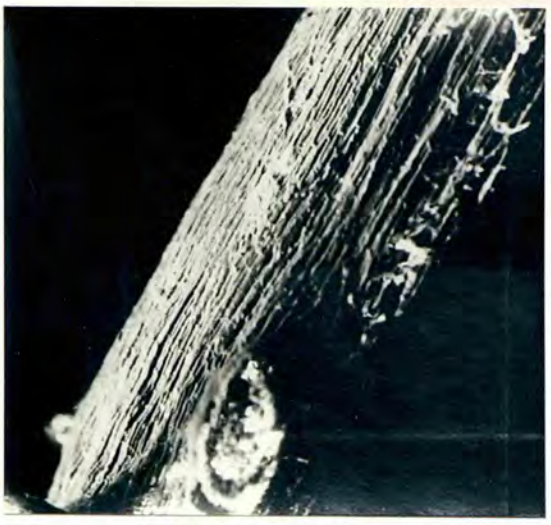
[a]EMPA carried out by the Electron Optics Unit at the Johnson Matthey Research Centre.

Electron Probe micro X-ray analyser, fully computerised and programmable, supported by a Tracor Northern TN 2000 main frame computer and PDP 1104 with a TN 1310 interface. Initially, wavelength dispersive analysis was employed for mapping out platinum distribution but improved software enabled successful energy dispersive mapping to be carried out. The limitations of traditional EDXA stem from the use of the standard raster scan of the electron beam which scans too quickly. The use of an X-Ray Imaging Program, XIP, (Tracor Northern) overcomes these limitations and improves resolution significantly.

Linescans and distribution maps were obtained for platinum and ruthenium in root samples of treated plants. Initially, specimens were prepared in resin blocks (as for TEM) and polished flat, but platinum was not detected; the samples prepared for SEM were recoated either with carbon or metal (e.g.: Al; Ni/Cr) and examined by EMPA. The accelerating voltage was 35kV to allow the K_α line of Ru to be used ot map the elemental distribution across the energy band 19.2-19.3keV. The same voltage was used to map the platinum distribution using the L_α line of platinum across the energy band 9.3-9.5keV.

Results

The results of these investigations are presented in Plates 9.24 to 9.28. The surface of a root taken from a platinum treated water hyacinth (D/14/R) is shown in Plate 9.24(a); a map of the platinum distribution in the same field is given in (b) of the same plate. A longitudinal section through a root tip from the same plant and the corresponding Pt distribution is shown in Plate 9.24(c) and (d). Another root tip (l.s.) from the same plant was examined by wavelength dispersive analysis (WDXA) and compared to EDXA using the XIP facility (Plate 9.25). Transverse sections were examined also (Plates 9.26 and 9.27). The results indicate quite



(a) root surface (×100)

(b) platinum distribution in (a); wavelength dispersive X-ray analysis

(c) l.s. of root tip from sample D/14/R; (×100)

(d) platinum distribution in (c); energy dispersive X-ray analysis using XIP.

Plate 9.24 Electron microprobe X-ray analysis of roots from water hyacinth treated with platinum applied as $(NH_4)_2[PtCl_6]$

(a) root rip of D/14/R

(×100)

(b) platinum distribution in (a); WDXA

(c) platinum distribution in (a); EDXA using XIP

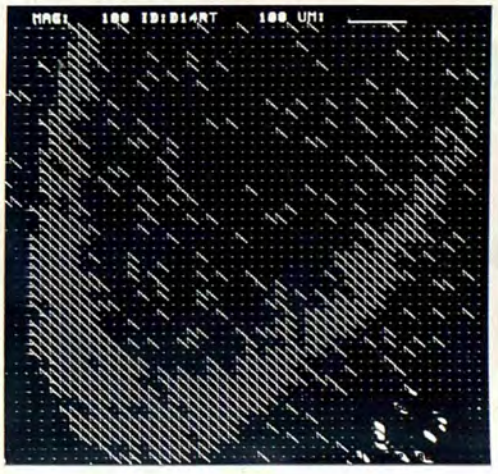
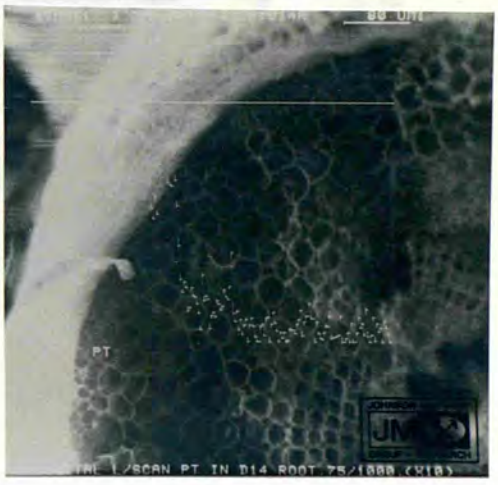
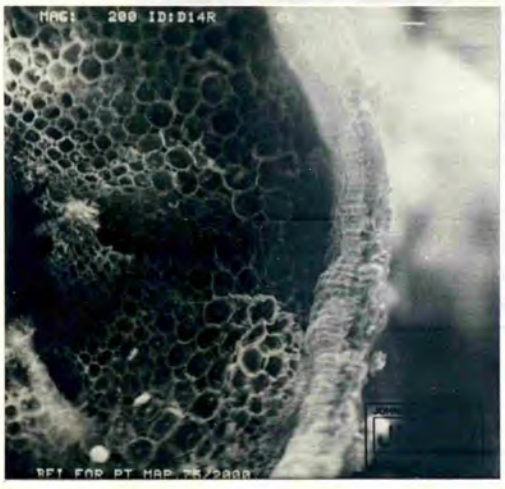


Plate 9.25 Comparison of WDXA and EDXA facilities for the location ot platinum in the root tip of water hyacinths treated with $(NH_4)_2[PtCl_6]$

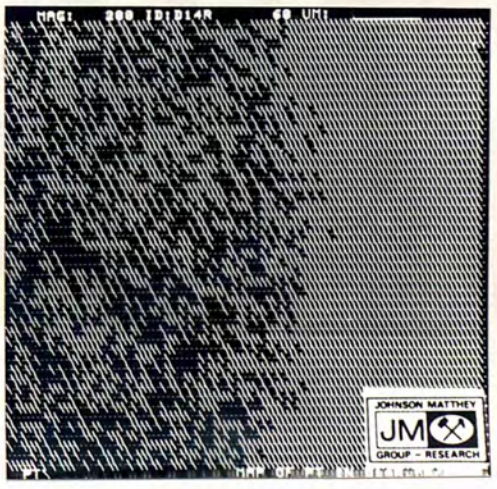
(a) platinum linescan across root t.s. (×200)



(b) platinum linescan across root t.s. (×200)



(c) root t.s. (×200)



(d) platinum distribution in (c); EDXA using XIP

Plate 9.26 Platinum distribution in roots from water hyacinth treated with $(NH_4)_2[PtCl_6]$

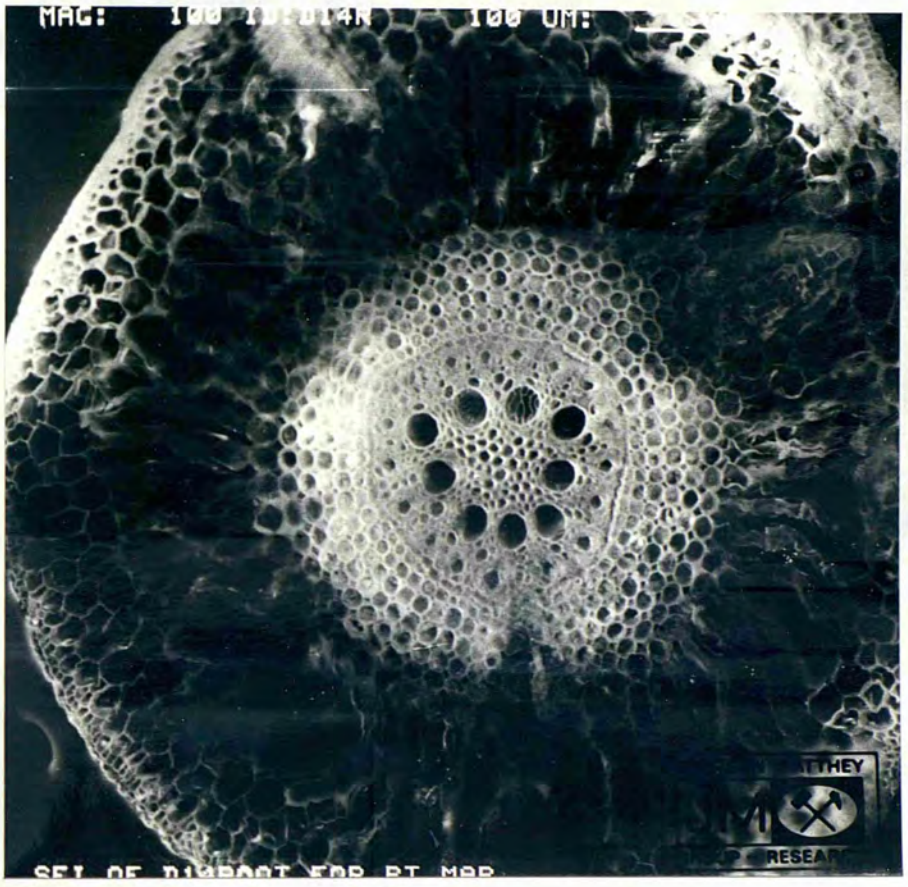


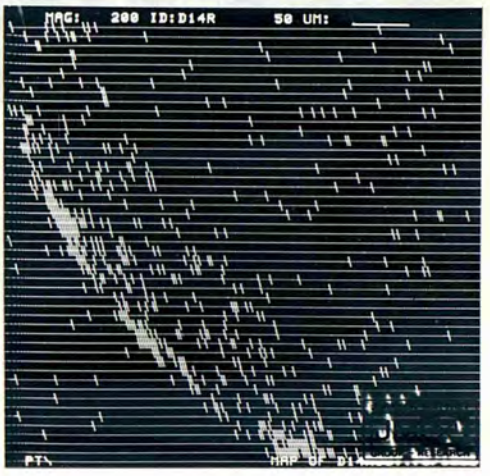
Plate 9.27 (composite) (a) Root (t.s.) taken from a water hyacinth treated with 10mg dm^{-3} platinum applied as $(NH_4)_2[PtCl_6]$; (×182); this electron micrograph represents the complete t.s. which is examined for platinum distribution in more detail in b, c, d and e.

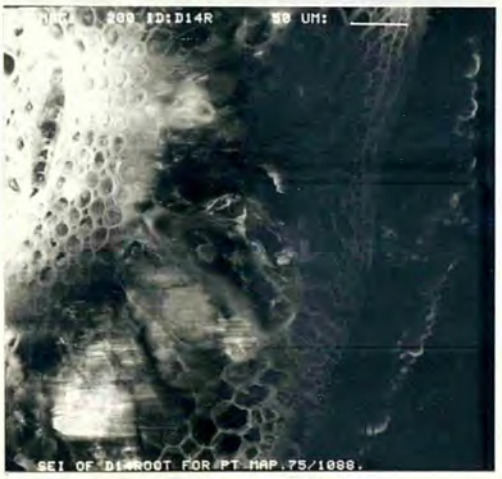
(b) root t.s. of D14/R

(×200)

(c) platinum distribution in (b)

EDXA with XIP





(d) root t.s. of D14/R

(×200)

(e) platinum distribution in (d)

EDXA with XIP

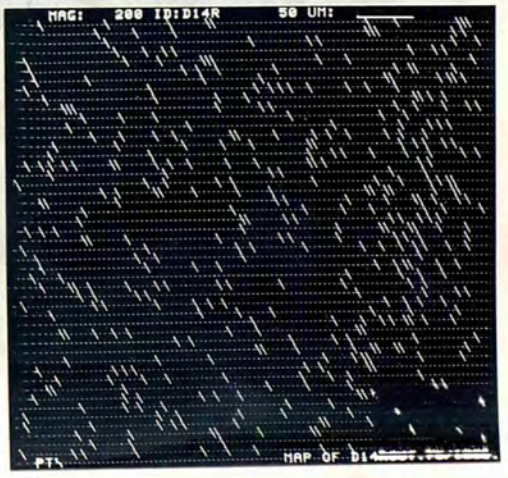
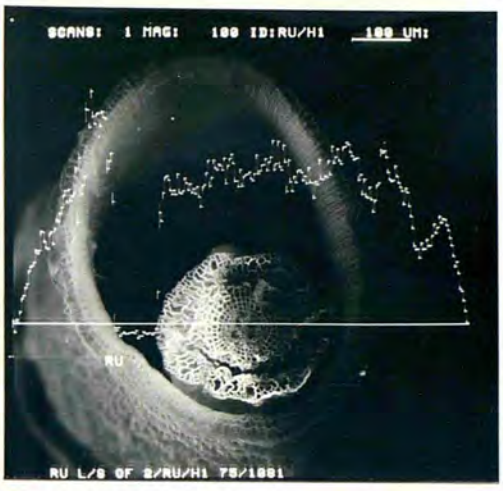
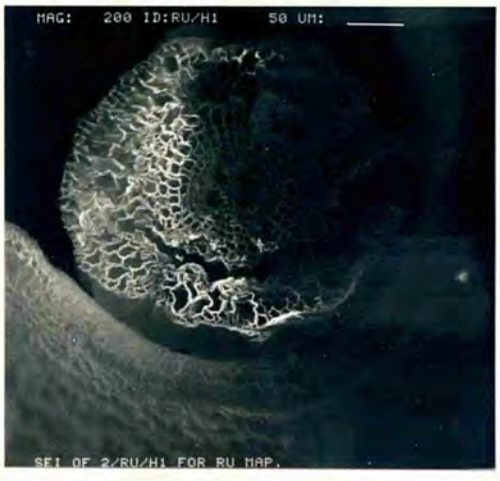


Plate 9.27 (b,c,d,e) Platinum distribution in root sections of water hyacinth

(a) linescan of root (t.s.) showing Ru distribution (×100)





(b) root (t.s.) of water hyacinth (×200)

(c) ruthenium distribution in (b)

Plate 9.28 Ruthenium distribution in the roots of water hyacinth treated with $(NH_4)_3[RuCl_6]$

clearly that much of the platinum present is located in the epidermal region of the root; lesser amounts were found in the cortex.

Similar investigations with roots from ruthenium treated water hyacinths show Ru distributed rather more evenly across the root (Plate 9.28). However, several problems emerged in these studies using EMPA and these impose limitations on the conclusions which may be drawn. Specimen charging was difficult to avoid and thick metal coatings would have impeded microanalysis, thus, the image quality is degraded. Specimens were found to be distorted, sometimes by preparation or by the electron beam itself; this is seen in Plate 9.28, where the cortex has become detached from the epidermis. As described previously (pg.261), the topography of these samples can lead to artificial signal enhancement, though large areas of localisation can be identified.

9.6 Scanning Transmission Electron Microscopy (STEM) with analysis Method

Segments of root from *Eichhornia crassipes* treated with $(NH_4)_2[PtCl_6]$ were fixed in 3% glutaraldehyde at 4^oC, in a 0.1M phosphate buffer, for 1hr. After treatment in 0.1M phosphate buffer (30min) and deionised water (30min) the specimens were post-fixed in 1% OsO_4 for 1hr at 4^oC. After further treatment with deionised water (30min) the specimens were dehydrated in a graded series of water/ethanol and brought to room temperature. The specimens were transferred from absolute ethanol to a 1:1 solution of epoxy resin in ethanol and left overnight. This was followed by infiltration with undiluted epoxy resin (5hrs) which was then polymerised for 8hrs.

Resin blocks were trimmed and the root segments orientated for the collection of transverse sections. Ultrathin sections (<100nm) were cut

with glass knives on an ultramicrotome (Huxley) and mounted on copper grids. Sections were examined by Scanning Transmission Electron Microscopy[a] (STEM) using a Jeol 100CX Temscan equipped with EDXA (Link Systems Ltd.). Electron micrographs were taken at a gun potential of 40keV and EDXA carried out on sections from both platinum treated and untreated plants; results are presented in Plates 9.29; 9.30 and figures 9.1 and 9.2.

Results

Plate 9.29 shows details of the ultrastructure of control roots. Cell walls are clearly visible as are vacuoles and intercellular spaces. Sections were viewed unstained in order to avoid interfering with the location of platinum by EDXA, hence exact cytological detail is absent. Problems arose with the examination of platinum treated specimens which showed widespread electron dense deposits (Plate 9.30). EDXA of these deposits confirmed the presence of platinum (figure 9.2), but ultrastructural detail was obscured. The deposits were randomly distributed throughout the section which appeared to have 'holes' in those areas where there should have been an intercellular space. It is possible that the platinum deposits are located here but loosely bound and are dislodged during sectioning. EDXA spectra displayed Cu K_α and Cu L_α peaks (from the grid), Os L_α (from post-fixing). The Pt M_α and Pt L_α peaks were present in sections from Pt treated plants. In the control sections, EDXA showed an anomalous peak at 2.36keV which may be due to contamination from lead (Pb M_α has a peak at this energy) (figure 9.1).

[a] STEM facilities were provided by the Chemical Crystallography Laboratory, University of Oxford.

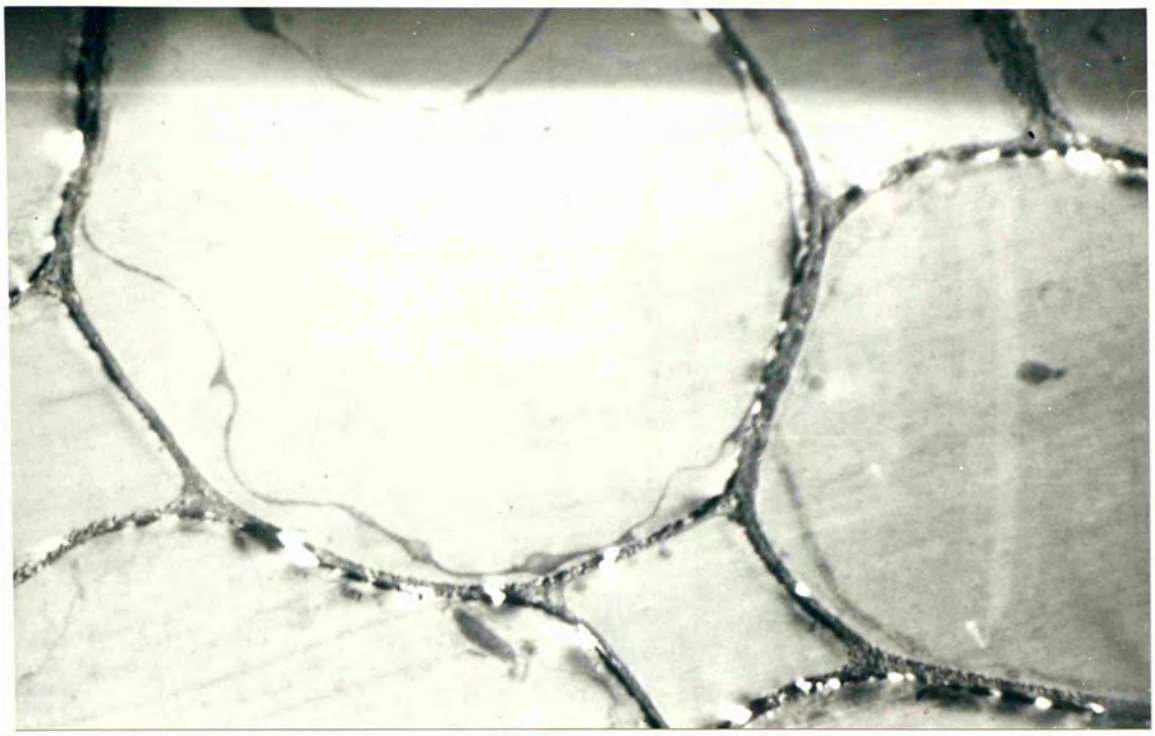


Plate 9.29 STEM of root (t.s.) from control water hyacinth (×5000)

Plate 9.30 STEM of root from platinum treated water hyacinth (×2800)

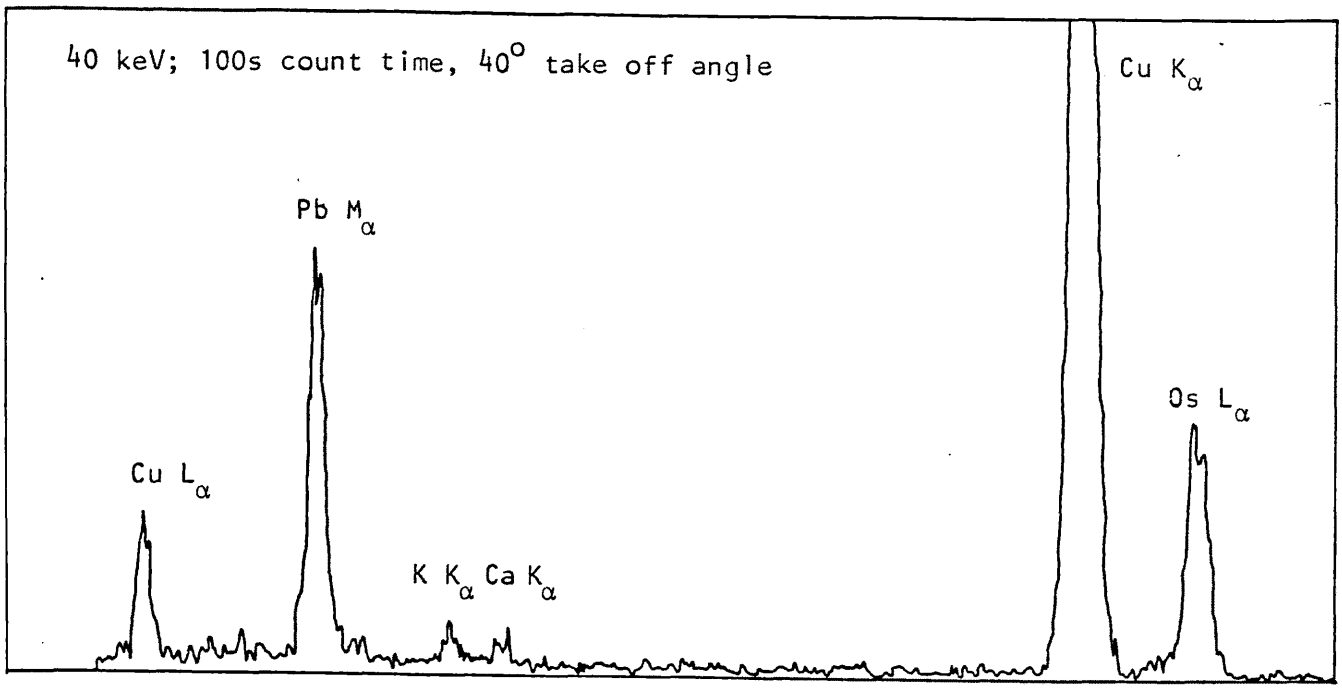


Figure 9.1 EDXA spectrum of t.s. from control water hyacinth root sample as viewed by STEM

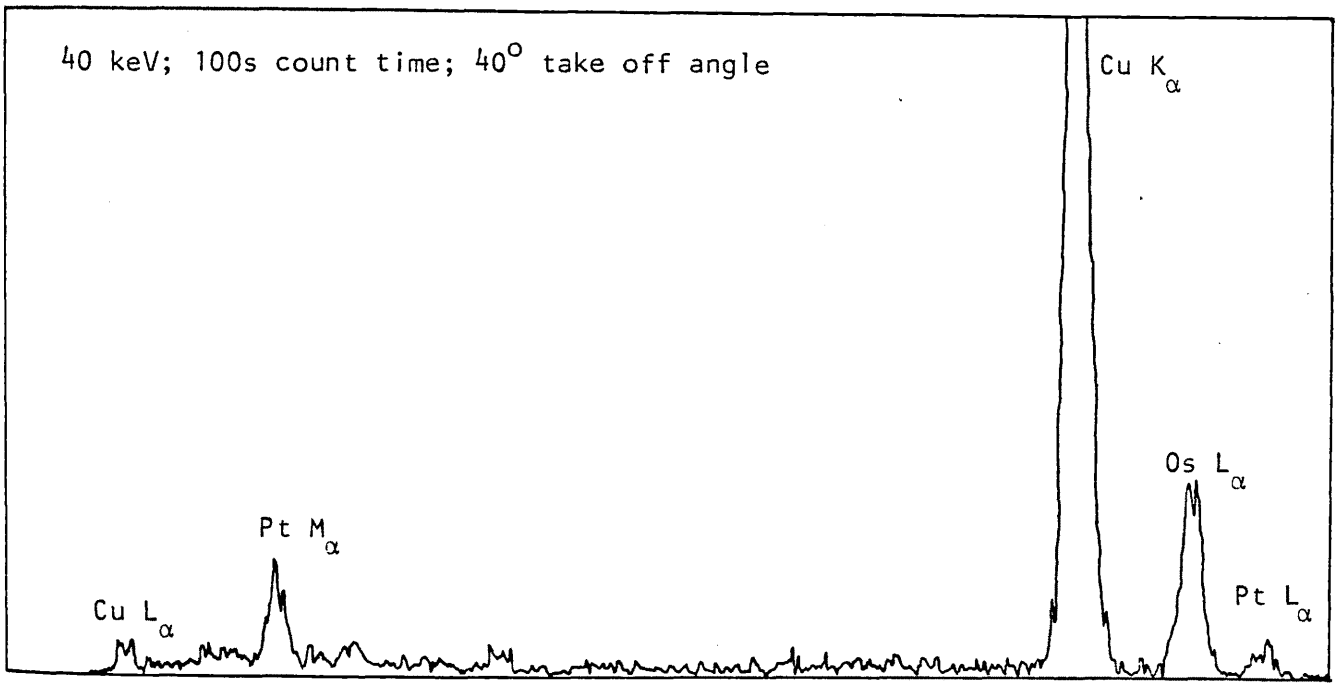


Figure 9.2 EDXA spectrum of t.s. from platinum treated water hyacinth root sample as viewed by STEM

9.7 X-ray photoelectron spectroscopy (XPS)

Background

When a photon of energy $h\nu$ strikes a solid the ejected photoelectrons have a kinetic energy distribution composed of a series of bands reflecting the electronic structure of the sample (Roberts, 1981). If the kinetic energy E_K of the photoelectrons can be determined experimentally then the binding energy E_B of the photoelectron can be calculated from

$$E_K = h\nu - E_B \qquad 9.1$$

Thus the photoelectron spectrum consists a number of peaks at discrete values of E_K corresponding to values of E_B. The value of E_B can identify the atom involved in the ionisation process and through small changes in E_B, reveal the chemical environment of a given atom.

X-ray photoelectron spectroscopy (XPS) involves the photoejection of electrons from core levels, which can lead to valuable information concerning the mode of chemical bonding arising from the relationship between the effective charge on the atom and the binding energy of an inner shell electron.

Method

Specimen pieces of root from *Eichhornia crassipes* treated with $(NH_4)_2[PtCl_6]$ were prepared as for SEM. The specimens were mounted together on a single holder using double sided adhesive tape and examined by XPS (Vacuum Generators Scientific Ltd. ESCA3 Mkl). They were illuminated with Al K_α radiation and the emitted electron energies analysed. The results are presented in figures 9.3; 9.4 and 9.5.

Results

A wide scan shows the presence of C, O, and Si (figure 9.3). A narrow scan of the Pt 4f region shows emission at an apparent binding energy of 79.7eV (figure 9.5). Taking into account the specimen charging

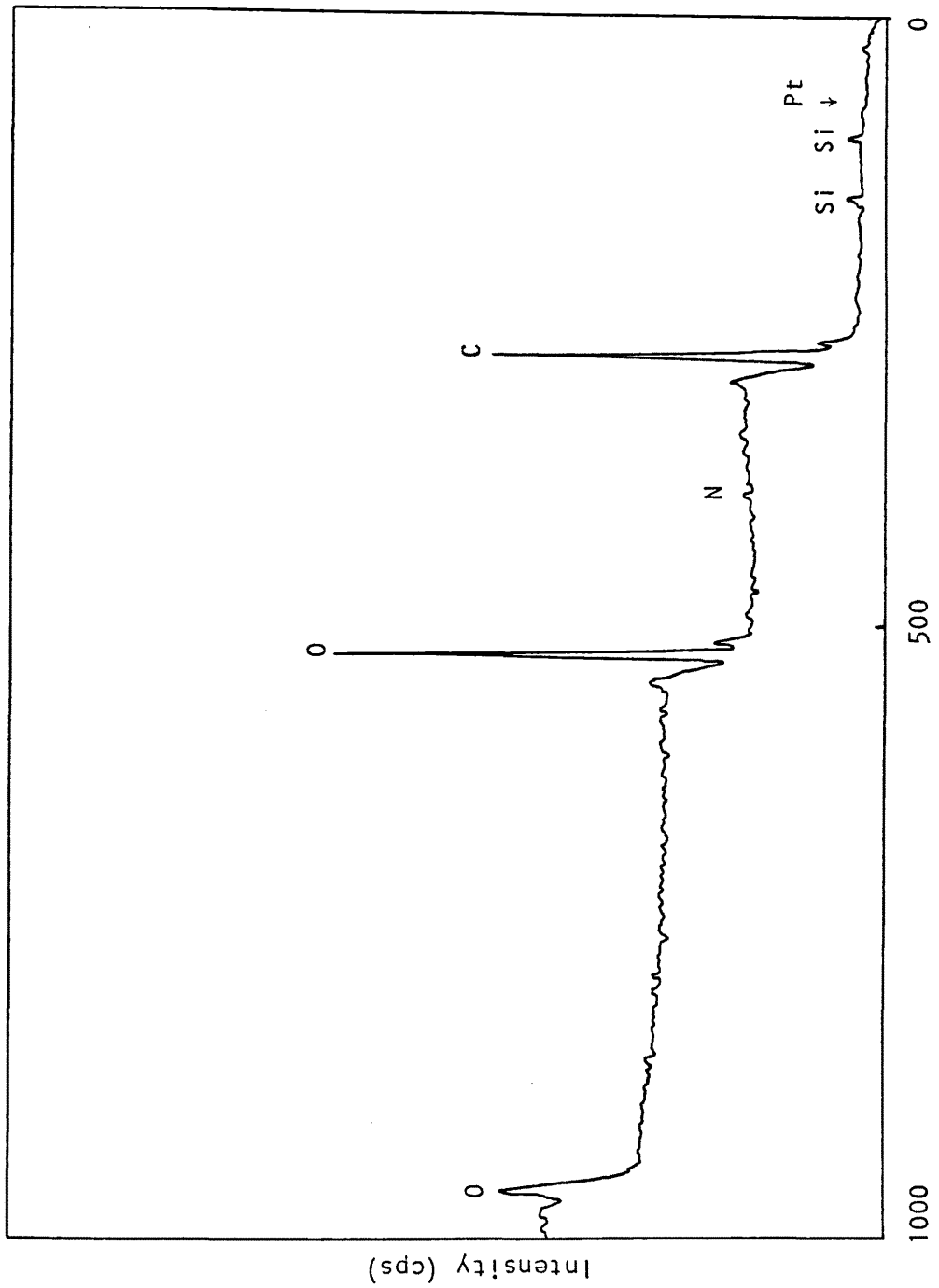


Figure 9.3 X-ray photoelectron spectrum of roots from platinum treated water hyacinth

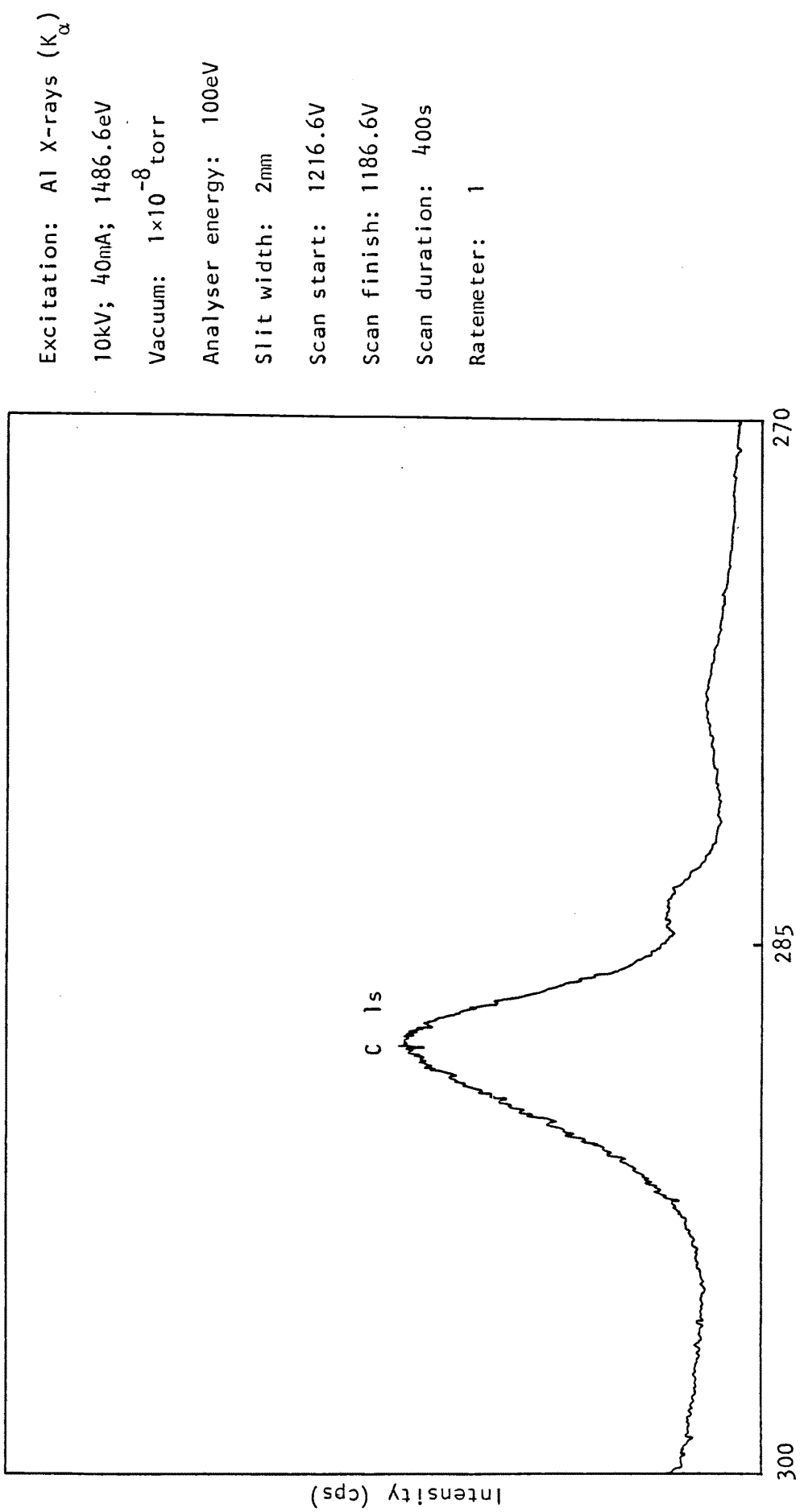


Figure 9.4 X-ray photoelectron spectrum of roots from platinum treated water hyacinth

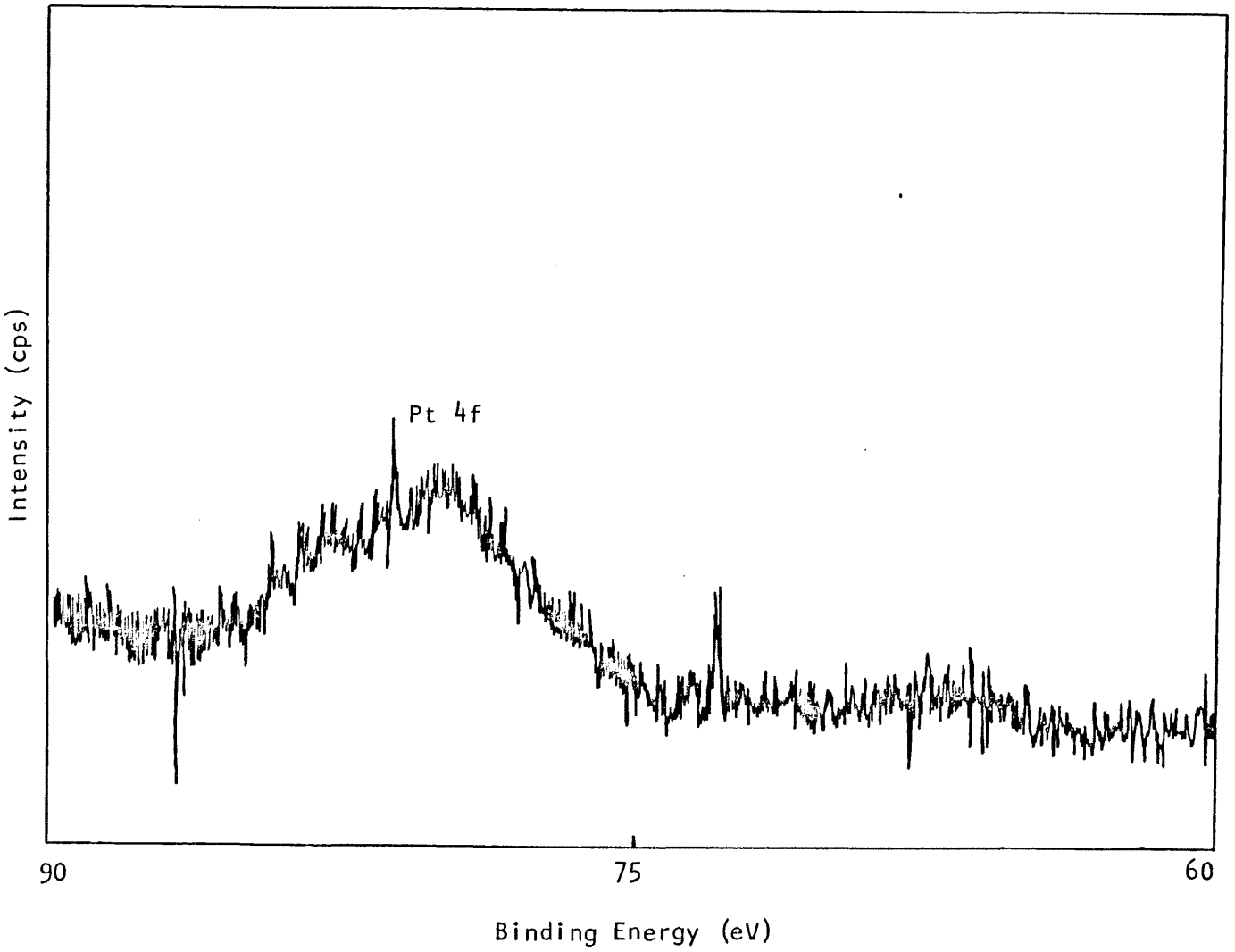


Figure 9.5 X-ray photoelectron spectrum of roots from platinum treated water hyacinth

of about 3-4eV, this emission could well be due to the oxidised Pt present. The corrected Pt 4f binding energy of 76.2±0.5eV agrees with values found for Pt^{4+} as $K_2[PtCl_6]$ of 75.5±0.5eV (Leigh and Bremser, 1972) (Escard, *et al.*, 1975). On the root surface, the apparent Pt to C ratio is 0.001.

REFERENCES

Benedict, W.G. (1970), Can. J. Bot., 48 (1) 91.

Castaing, R. and Guinier, A. (1949), Proc. Int. Congr. for Electron Micros., Delft, 60. Cited in Rasmussen (1968).

Castaing, R. (1951), Thesis, University of Paris, ONERA Publ., No.55. Cited in Erasmus (1978).

Erasmus, D.A. (1978), editor, Electron Probe Microanalysis in Biology, Chapman and Hall, London.

Escard, J., Pontvianne, B. and Contour, J. (1975), J. Elect. Spectros., 6 17.

Gurr, E.G. (1958), Methods of Analytical Histology and Histochemistry, Leonard Hill Ltd., London.

Hasman, M. and Inanc, N. (1957), Fen Fakültesi Mecmuasi (University of Istanbul), 137.

Laüchli, A. and Schwander, H. (1966), Experientia, 22 503.

Laüchli, A. (1967), Planta, 75 185.

Leigh, G.J. and Bremser, W. (1972), J. Chem. Soc. (Dalton), 1217.

Morgan, A.J. (1979), Scan. Electron Micros., 11 635.

Newbury, D.E. (1979), Scan. Electron Micros., 11 1.

Ophus, E.M. and Gullvag, B.M. (1974), Cytobios, 10 45.

Rasmussen, H.P. (1968), Planta (Berl.), 81 28.

Rasmussen, H.P., Shull, V.E. and Dryer, H.T. (1968), Develop. in Appl. Spectros., 6 29.

Rauser, W.E. (1973), Can. J. Bot., 51 301.

Rauser, W.E. (1978), Can. J. Bot., 56 1744.

Robb, J., Busch, L. and Rauser, W.E. (1980), Ann. Bot., 46 43.

Roberts, M.W. (1981), Chem. Brit., 17 (11) 510.

CHAPTER X

A STUDY OF THE ACCUMULATION OF PLATINUM BY THE SOUTH AFRICAN GRASS *SETARIA VERTICILLATA*

10.1 Background

A discussion of the availability of the platinum group metals with special reference to the Merensky Reef in South Africa has been presented in chapter I (pg. 36). The aims of the investigations described in this chapter can be summarised as follows:

(i) to identify plant species, indigenous to South Africa, which might be used to colonise ore tailings.

(ii) to investigate whether such species might accumulate significant levels of platinum from ore tailings.

Flotation tailings are the final waste product from the mining techniques employed to extract the platinum metals from their ore. The deposits worked by Rustenburg Platinum Mines Ltd. form part of the Merensky Reef, which is approximately 50cm thick and some 8 ppm total in platinum metals. The mined ore is crushed in a series of jaw crushers and fed into ball mills. The technique of gravity concentration is used to remove the coarse particles of platinum bearing mineral, yielding a final high grade concentrate. The tailings are returned to the mill circuit for further treatment to recover the remaining small amounts of the platinum group metals as a flotation concentrate. The final flotation tailings are discarded; they consist mainly of the elements Al, Ca, Cr, Mg and Si, and minor amounts of Fe and Na; trace amounts of Mn, Ti, Cu, Pb, Ni, Ag, W, V and Zn are present also, as well as traces of the platinum group metals. (Hunt, 1971) (Hunt and Lever, 1971).

A variety of South African grass seeds were collected† from plants growing in the vicinity of the Matthey Rustenburg Refiners plant in South Africa. The seeds were dried, packed and sent to the U.K. for investigation. Unfortunately, the species collected had not been identified, and preliminary attempts to germinate the seeds indicated that most were not viable. However, one species which did germinate, was selected for further investigation. This species was presented for identification, to the Royal Botanic Gardens, Kew and was named as *Setaria verticillata* (L) P. Beauv.

10.2 The colonisation of Rustenburg Flotation Tailings by *Setaria verticillata*

Rustenburg Flotation tailings (297kg) were obtained from the Waterval Reduction Works of Rustenburg Platinum Mines Ltd., for the investigation of its possible colonisation by South African grasses. The tailings had been analysed by wet chemical methods, for the individual precious metals present, by Rustenburg Platinum Mines Ltd.; these values are given in Table 10.1.

Seeds of the South African grass *Setaria verticillata* were germinated by sand culture methods; the seeds were sown in plastic pots containing acid washed Loch Aline sand, moistened with full strength nutrient solution (Epstein, 1972). The pots were covered with polyethylene film to aid germination, and transferred to a controlled environment growth room (Prestcold Central). Conditions in the growth room have been described elsewhere (ch.3.1). After five days, the young seedlings were transplanted into three trays (355×217mm) containing flotation tailings (+deionised water), flotation tailings (+half strength

†South African grass seeds were collected by Dr. M.E. Chalkley, Matthey Rustenburg Refiners Ltd., Rustenburg, South Africa.

Precious Metal Content of Rustenburg Flotation Tailings

	values in ppm (mg kg^{-1})	% of total
Platinum	0.612	50.00
Palladium	0.230	18.78
Gold	0.075	6.12
Ruthenium	0.215	17.55
Rhodium	0.045	3.67
Iridium	0.027	2.20
Osmium	0.021	1.71
Total	1.225	

Table 10.1

nutrient solution) and John Innes No.3 potting compost, respectively. The latter served as a control.

Three rows of approximately 10-11 plants were planted in each tray, all of which were irrigated with deionised water for 15 minutes each day, using the automatic irrigation system. The second tray containing flotation tailings was treated periodically with half-strength nutrient solution to compare growth patterns with those plants grown on tailings without additional nutrients. One row of plants from each of the three trays was harvested at various intervals (32, 44 and 60 days) during the course of two months, to determine the uptake of any platinum, which accounts for some 50% of the total precious metals in tailings. The plants were photographed at these points in the course of the experiment; they are presented in plates 10.1, 10.2 and 10.3 respectively.

The plants were harvested in the following way: each was separated into leaves, stems and root stock. The roots were washed by gentle agitation in deionised water, which removed almost all tailing debris. The leaves, stems and roots from each crop were weighed, oven dried at $90^{o}C$ for 24 hours and reweighed. The dried plant material was analysed by wet ashing in concentrated Aristar nitric acid and platinum determined by ETA AAS. The results of the three harvests are presented in Table 10.2.

10.3 The uptake of platinum applied as $K_2[Pt\ Cl_4]$ by *Setaria verticillata*

Seeds of the South African grass *Setaria verticillata* were germinated by sand culture methods as described previously. Young plants were selected for uniformity of size and growth, and transferred to 2 dm^3 black plastic boxes. Half-strength nutrient solution was added to three plastic boxes each containing four plants. When the plants were well

Colonisation of Rustenberg Flotation Tailings

by *Setaria verticillata*

(No platinum detected† in any plant digest)

Days growth	Sample details	Wet wt/g	Dry wt/g	Water content %
32 days	SV/T/leaves	0.45	0.089	80.2
	SV/T/stems	0.37	0.052	85.9
	SV/T/roots	0.15	0.061	59.3
	SV/TN/leaves	1.89	0.279	85.2
	SV/TN/stems	1.68	0.155	90.8
	SV/TN/roots	0.69	0.262	62.0
	SV/Control/leaves	11.11	1.201	89.2
	SV/Control/stems	9.46	0.633	93.3
	SV/Control/roots	1.58	0.203	87.2
44 days	SV/T/leaves	0.69	0.146	78.8
	SV/T/stems	0.76	0.110	85.5
	SV/T/roots	0.26	0.053	79.6
	SV/TN/leaves	5.05	0.752	85.1
	SV/TN/stems	4.99	0.556	88.9
	SV/TN/roots	3.72	0.581	84.4
	SV/Control/leaves	9.07	1.303	85.6
	SV/Control/stems	9.31	1.390	85.1
	SV/Control/roots	6.14	0.983	84.0
60 days	SV/T/leaves	0.36	0.109	83.7
	SV/T/stems	0.42	0.065	84.2
	SV/T/roots	0.61	0.123	79.8
	SV/TN/leaves	3.27	0.674	79.4
	SV/TN/stems	4.21	0.692	83.6
	SV/TN/roots	4.20	0.848	79.8
	SV/Control/leaves	8.52	0.788	90.8
	SV/Control/stems	5.19	0.531	89.8
	SV/Control/roots	4.02	0.384	90.4

†Determinations by ETA AAS

Table 10.2

Plate 10.1 The growth of *S. verticillata* on tailings (32 days)

Plate 10.2 The growth of *S. verticillata* on tailings (44 days)

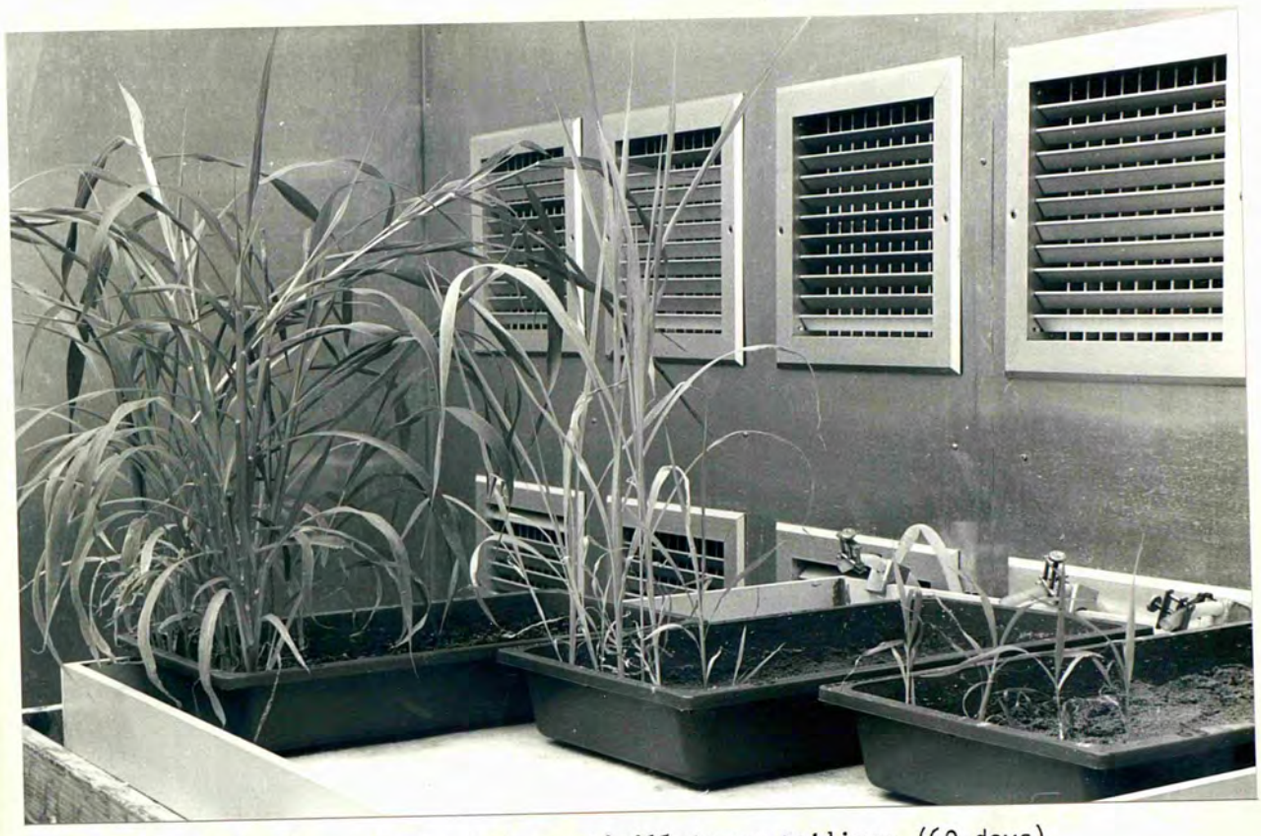


Plate 10.3 The growth of *S. verticillata* on tailings (60 days)

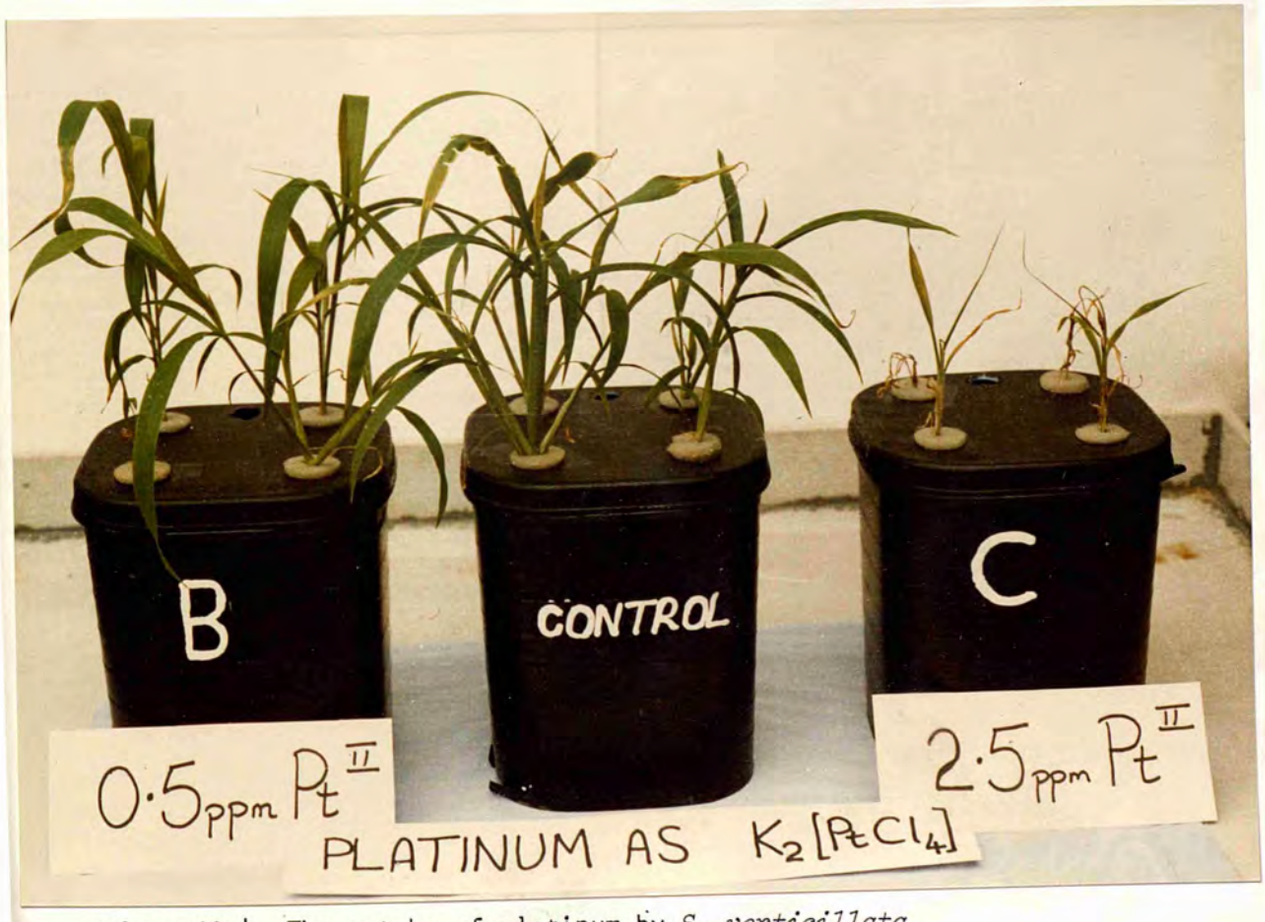


Plate 10.4 The uptake of platinum by *S. verticillata*

established, the boxes were treated with full strength nutrient solution containing the following concentrations of platinum: 0.5 (Box B) and 2.5 ppm (mg dm^{-3}) (Box C). The third box contained just full strength nutrient solution and served as a control (Box A).

The conditions of the growth room have been described elsewhere (ch.3.1). The nutrients and applied platinum were renewed after 7 days and the plants harvested after 14 days. The plant roots were measured before, after and during the experiment; shoot heights were measured after the experiment too. These results are presented in Table 10.3. Plate 10.4 shows the state of growth after 14 days.

The harvested plants were divided into roots and tops, weighed, dried and reweighed. The dried plant material was wet ashed and the platinum content determined by ETA AAS. Results are presented in Table 10.4.

10.4 Discussion

The growth of *S. verticillata* on untreated flotation tailings proved very difficult, since the substrate lacks the essential macronutrients: K, P, NO_3^-, NH_4^+. Plants grown on this medium showed a very poor growth yield and suffered from a low water content compared to controls, even though irrigation was provided. Adding nutrients to the tailings improved the yield but it was still less than control yields. In control plants, grown on John Innes No.3, the water content was similar for leaves, stems and roots, but when under stress (growing on tailings) the root system suffered from lower water content compared to leaves and stems.

Platinum was not detected in any of the digests from these plant samples. This shows that this particular species is unable to concentrate or recover platinum, the most abundant PGM in tailings even after

Table 1. Effect of platinum applied as $K_2[PtCl_4]$ on *Setaria verticillata*

Plant ref. No.	Longest root start/mm	Longest root 1 wk/mm	Increase in root length/mm	Longest root 2 wks/mm	Total increase in root length/mm	Shoot height 2 wks/mm
A/17/i	82	243	161	265	183	138
A/17/ii*	70	73	3	135	65	78
A/17/iii	90	238	148	380	290	168
A/17/iv	85	218	133	330	245	128
$\bar{n}$	86	233	147	325	239	145
SD	4	13	14	58	54	21
%RSD	4.7	5.7	9.5	17.7	22.4	14.4
B/17/i	101	273	172	520	419	158
B/17/ii	80	253	173	480	400	158
B/17/iii*	55	63	8	70	15	48
B/17/iv	77	218	141	440	363	158
$\bar{n}$	86	248	162	480	394	158
SD	13	28	18	40	28	0
%RSD	15.2	11.2	11.2	8.3	7.2	0
C/17/i	83	88	5	90	7	43
C/17/ii*	60	60	0	60	0	68
C/17/iii	80	81	1	85	5	103
C/17/iv	62	68	4	65	3	20
$\bar{n}$	75	79	3	80	5	55
SD	11	10	2	13	2	43
%RSD	15.1	12.8	62.5	16.5	40.0	77.5

Key: Box A, control; Box B, 0.5 $\mu g\ ml^{-1}$ Pt; Box C, 2.5 $\mu g\ ml^{-1}$ Pt.

*Plants which failed to take to hydroponic growth, these data are omitted from statistical calculations.

Table 10.3

The uptake of platinum applied as $K_2[PtCl_4]$ by *Setaria verticillata*

Plant tops	Applied Pt/ppm	Wet wt/g	Dry wt/g	Water content/%	Pt(dry wt)/ppm*	Total Pt µg
A/17/SV	0.00	5.92	0.713	88.0	nd	nd
B/17/SV	0.50	4.29	0.712	83.4	nd	nd
C/17/SV	2.50	0.35	0.145	58.6	275	40
Plant roots	**Applied Pt/ppm**	**Wet wt/g**	**Dry wt/g**	**Water content/%**	**Pt(dry wt)/ppm***	**Total Pt µg**
A/17/SV	0.00	0.92	0.194	78.9	nd	nd
B/17/SV	0.50	0.73	0.252	65.5	902	227
C/17/SV	2.50	0.07	0.019	72.9	2500	48

*Pt determined by ETA AAS.

Table 10.4

60 days growth.

When grown hydroponically, *S. verticillata* was stimulated at low levels of platinum applied as $K_2[PtCl_4]$. Table 10.3 and figure 10.4 show that when the grass was grown with 0.5 ppm (mg dm^{-3}) platinum, growth of both roots and shoots were stimulated, however, at the 2.5 ppm level, growth was stunted and chlorosis was evident. For those plants treated with 0.5 ppm Pt, the index of tolerance was 1.10 after one week's growth and 1.65 after two week's growth. A 65% average increase in the lengths of the roots over the average increase in root length of the control plants demonstrated the growth stimulated. Similarly, the average shoot heights were 9% greater than the average heights of control plants.

Plants grown in 2.5ppm Pt were stunted in growth. An index of tolerance of 0.02 was found after both the first and second week of growth. The average root lengths were 98% less than the control and the shoot heights were 38% less.

Determination of platinum by ETA AAS of the plant digests show that at the 0.5 ppm Pt level, no platinum was detected in plant tops though some platinum was deposited in the roots. At the phytotoxic level, platinum was deposited in the root and some transported to the tops. In comparison to uptake by aquatic plants, the accumulation reported here is relatively poor even though the platinum was presented to the root in a soluble form.

REFERENCES

Epstein, E. (1972), Mineral Nutrition of Plants: Principles and Perspectives, Wiley, New York.

Hunt, L.B. (1971), Platinum Metal Reviews, 15 102.

Hunt, L.B. and Lever, L. (1971), Platinum Metal Reviews, 15 126.

CHAPTER XI

GENERAL DISCUSSION

Little is known about the inorganic biochemistry of the platinum group metals or to what extent they can alter the growth patterns of plants. With the recent development of platinum drugs for the treatment of a number of cancers, the biomedical significance of platinum and other related metals has increased markedly. In addition to this, the introduction of car exhaust systems containing a platinum/palladium catalyst has raised the possibility of these metals finding their way into the environment.

One of the primary aims of this study was the development of sensitive analytical techniques for the determination of platinum group metals in plant tissues. However, there is no NBS Biological Standard Reference material containing certified levels of the platinum group metals for comparing analytical techniques. Therefore, plant materials were specially prepared, each of which contained one of the platinum group metals. A number of instrumental techniques were investigated, two in detail: electrothermal atomisation atomic absorption spectroscopy (ETA AAS) and instrumental neutron activation analysis (INAA). A comparison with other techniques, including ICP, dc arc emission and PIXE was undertaken. The results of the comparison show no one particular technique fulfills all analytical requirements, though PIXE came close to satisfying most criteria.

INAA is most suitable for the determination of Os and Ru since most other methods require the oxidation of organic matter prior to determination; this may result in Os and Ru being lost as volatile oxides. However,

PIXE does offer relatively good detection limits for a wide range of elements determined simultaneously. This is advantageous since the uptake of non-essential elements can replace or enhance uptake of essential elements. As a routine technique, ETA AAS suffers from matrix effects when platinum metals are at low levels, but the results obtained agree well with other techniques. It would seem that of all the elements in the platinum group, platinum itself is the most difficult to determine in biological samples (Le Roy *et al.*, 1977).

The biological effects of the platinum group metals on plants were studied by treating plants with chlorocomplexes of each metal at a range of concentrations. The vascular aquatic plant *Eichhornia crassipes* (water hyacinth) was selected for detailed study because of its remarkable ability to assimilate high levels of transition elements from solution (Wolverton and McDonald, 1975) (Tatsuyana *et al.*, 1977, 1979). Uptake experiments with water hyacinth show it is capable of recovering platinum group metals even from dilute solution though to varying degrees depending on the metal:

$$Pt^{2+} > Pd^{2+} > Os^{4+} \approx Ru^{3+} > Ir^{3+} \approx Rh^{3+}$$

$\longrightarrow$

decreasing % recovery at the 0.05ppm level

When this is compared to the relative order of toxicity at the 10ppm level for each metal, some similarities emerge:

$$Pt^{2+}(5d^8) \approx Pd^{2+}(4d^8) > Os^{4+}(5d^4) \approx Ru^{3+}(4d^5) > Ir^{3+}(5d^6) > Pt^{4+}(5d^6) >> Rh^{3+}(4d^6)$$

$\longrightarrow$

decreasing order of toxicity at the 10.0ppm level, with the 'd' electron configuration shown in parenthesis

The relationship between phytotoxicity and position in the periodic table is tenuous but appears to be linked with the oxidation state and hence electron configuration of the metal ion. The two least toxic ions, Ir^{3+} and Rh^{3+}, have the same 'd' electron configuration (d^6); it is significant too that $Pt^{4+}(d^6)$ is far less toxic than $Pt^{2+}(d^8)$. A similar relationship has been found for the phytotoxicity of 1st row elements (Rauser, 1978):

$$Cd^{2+}(4d^{10}) >> Zn^{2+}(3d^{10}) > Ni^{2+}(3d^8) > Co^{2+}(3d^7)$$

$$\xrightarrow{\text{decreasing relative toxicity}}$$

Some of these findings can be explained in terms of the principles of hard and soft acids and bases (HSAB) (Pearson, 1963).

Williams (1971) has summarised these principles (Table 11.1). When a metal ion bonds to a ligand one species is considered an acid (Lewis) and the other a base (Lewis). The bond strength is related to two factors: (i) intrinsic strength (s), (ii) a softness parameter (σ). Softness is said to arise from the electron mobility or polarizability of a species. The species is soft if electrons are easily moved; if firmly held, the species is hard. A hard acid is usually of small size with high positive charge denisty and does not contain unpaired electrons usually.

Those metal ions normally considered toxic e.g.: Cd^{2+}, Hg^{2+} are classified as soft acids. They form strong bonds with soft bases particularly those containing sulphur groups (e.g. R_2S, RHS and RS^-) which are important constituents of proteins containing cysteine residues. Included in the soft acid classification is Pt^{2+} and Pd^{2+} whilst Rh^{3+} and Ir^{3+} are considered borderline between hard and soft, along with Fe^{2+}, Co^{2+}, Cu^{2+} and Zn^{2+}. This approach goes some way to explaining the relative toxicity

ACID (Electron acceptor)			BASE (Electron donor)		
Property			Property		
Hard		Soft	Hard		Soft
Low	Polarizability	High	Low	Polarizability	High
High	Electropositivity	Low	High	Electropositivity	Low
Large	Positive charge or oxidation state	Small	Large	Negative charge	Small
Small	Size	Large	Small	Size	Large
Ionic, electrostatic	Types of bond usually associated with the acid	Covalent, π	Ionic, electrostatic	Types of bond usually associated with the base	Covalent, π
Few and not easily excited	Outer electrons on donor atom	Several, easily excited	High energy and inaccessible	Available empty orbitals on donor atom	low lying and accessible

Table 11.1 Hard and soft acids; guidelines to properties (after Williams, 1971)

of the platinum metals. The only anomaly is Pt^{4+} which is classified as a soft acid but appears relatively non-toxic. However, the hardness of an element increases with oxidation state (Williams, 1971) and therefore some softness is lost when Pt^{2+} is oxidised to Pt^{4+}.

Many of the techniques used in this study to investigate the chemical binding and localisation of platinum metals in plant tissues suffer from a number of limitations some of which have been outlined previously. Foremost among these is the possibility that in preparing plant tissues, e.g.: for examination in the electron microscope or prior to chemical extraction, metal species may be relocated or change character substantially, leading to erroneous results. To a certain extent, this is more of a problem where the metal is known to form labile complexes e.g.: Cu^{2+}; however, the platinum group is renowned for the formation of kinetically inert complexes. Though some ligand exchange is likely prior to uptake, the rate is likely to be slow. The nutrient solution contains a large number of potentially good binding ligands: PO_4^{3-}, NO_3^-, NH_4^+ and SO_4^{2-}, though aquation is the most likely reaction of platinum group metal complexes.

In his investigation of the filamentation of *E. coli*, Rosenberg *et al.*, (1967) discovered that a series of photochemical reactions occurred in an aqueous solution of $(NH_4)_2[PtCl_6]$ resulting in the formation of several mixed chlorammine complexes:

$$[PtCl_6]^{2-} + nNH_4^+ \xrightarrow{h\nu} [PtCl_{(6-n)}(NH_3)_n]^{(2-n)^-}$$

Elding (1970) reported that in water, $[PtCl_4]^{2-}$ undergoes extensive solvolysis though the rate is slow:

$$[PtCl_4]^{2-} + H_2O \rightleftharpoons [PtCl_3H_2O]^- + Cl^-$$

$$[PtCl_3H_2O]^- + H_2O \rightleftharpoons [PtCl_2(H_2O)_2] + Cl^-$$

It is also known, that NH_3 ligands can displace Cl^- ligands and that PO_4^{3-} ligands will displace Cl^- ligands in Pt^{2+} complexes (Sigler and Blow, 1965). Little is known about the interaction of platinum complexes with organic matter though binding has been reported (i) between cationic complexes and carboxylate groups and (ii) between all other kinds of platinum species and proteins, probably at the R-S group (Blundell and Jenkins, 1977). Recent studies on the pharmacokinetic behaviour of the antitumour drug cis $[Pt(NH_3)_2Cl_2]$ after administration indicate the presence of a large number of platinum species originating from the drug (Le Roy *et al.*, 1979) (Le Roy *et al.*, 1980).

A variety of Pt^{2+} complexes, neutral, anionic and cationic, were applied to the nutrient solutions of water hyacinths but no significant differences in biological behaviour of treated plants was observed. The most prominent toxic symptom at low levels was the appearance of reddish-brown streaks in the leaves of *Eichhornia crassipes*. Such phytotoxic symptoms have been observed in beans (*Phaseolus vulgaris*) and soybeans (*Glycine max*) treated with high quantities of zinc (Rauser, 1973). A red-brown pigment was found deposited in parenchyma cells of the cortex, xylem and phloem of veins, petioles and stems. Other transition metals are reported to cause similar symptoms: Cd^{2+}, Co^{2+} and Ni^{2+} (Rauser, 1978). Ultrastructural examination of leaf veins bearing this discolouration revealed modification of the vessel walls and the deposition of electron dense material in the secondary vessel walls (Robb *et al.*, 1980). In this study, the reddish-brown streaks which appeared in leaves of platinum treated water hyacinths did not appear to be located around vascular bundles (as seen in the leaf t.s. in LM) but in the upper palisade which was extensively disorganised in the area of pigmentation. It has been suggested that this pigmentation is most likely a polymerisation

product of phenolic acids which may have originated in the plant roots (Rauser, 1981).

In contrast to the toxic effects of Pt^{2+}, Rh^{3+} appears to exhibit a tonic effect. When treated with 10ppm Rh^{3+} applied as $Na_3[RhCl_6]$, water hyacinth increased its biomass some 6.7% more than control plants, grown under the same conditions. When the South African grass *Setaria verticllata* was treated with 0.5ppm Pt^{2+} (as $K_2[PtCl_4]$), vascular discolouration was absent and the roots were growth stimulated some 65% more than controls. This was in marked contrast to the effects observed for water hyacinth (*Eichhornia crassipes*). It would seem that phytotoxic symptoms of platinum vary according to which species is treated, though with water hyacinth, some stimulation of vegetative reproduction was apparent with platinum complexes.

When applied as the antitumour complex cis $[Pt(NH_3)_2Cl_2]$ at low levels, some 47.9% of the platinum found in the leaves of water hyacinth was associated with α-cellulose and lignin; 16.1% was removed by the proteolytic enzyme pronase and 20.8% found with water soluble pectates. A similar distribution of platinum was found in the floats of water hyacinth. In the roots of treated plants, the values were 35%, 9.5% and 14.2% respectively; in addition to this, a further 23.1% was removed with low molecular weight alcohol soluble materials and 12.0% with polar water soluble materials. Thus in water hyacinth, the cell wall acts as an ion exchange column trapping most of the platinum, though some is found bound to water soluble pectates. Together, this accounts for 49.2% of the platinum found in the roots and this figure rises to 68.7% in the leaves. The platinum released by pronase may represent that which is bound to protein from a number of sources including organelle protein, membrane protein and cell wall glycoprotein.

The amino acid profile of control water hyacinth extract shows the presence of large amounts of asparagine and aspartic acid. Extract of root material contained high levels of glutamine and serine too, whilst extract of leaf material contained a higher proportion of alanine, threonine and valine. The shoot extract of water hyacinth has been reported to possess gibberellin-like substances which have been characterised (Sircar *et al.*, 1973). Further studies on the root extract of water hyacinth have shown that it can increase the root and shoot lengths of rice seedlings (*Oryza sativa L*) significantly after ten days growth (Gopalakrishnan and Sircar, 1973). The free amino acid constituents of the rice seedlings were also affected by treatment with the water hyacinth root extract. After ten days growth, treated seedlings had considerably less asparagine, suggesting its rapid utilisation for growth promotion. In this work, platinum treated water hyacinths contained a much higher proportion of asparagine and aspartic acid compared to control plants.

When applied as $(NH_4)_2[PtCl_6]$, Pt^{4+} is relatively non-toxic, though some yellow discolouration of roots does occur, probably due to the precipitation of platinum hydroxy species in the porous outer free spaces of the root. In the SEM these platinum treated roots appear covered in electron dense deposits and EDXA confirms the presence of platinum. Electron microprobe investigations reveal that the platinum deposits are concentrated in the epidermis with lesser amounts extending up to the endodermis. X-ray photoelectron spectroscopy of these roots gives a distinct platinum signal with a binding energy which is close to that expected for Pt^{4+}. Ruthenium treated roots show a similar localisation of the metal when viewed in the microprobe.

The emphasis throughout this thesis, has been towards the analytical techniques employed and the various problems associated with them. In

some cases, the investigations described here are just the beginning and a great deal more research is needed if the problems described in this study are to be solved.

REFERENCES

Blundell, T.L. and Jenkins, J.A. (1977), Chem. Soc. Rev., 6 139.

Elding, L.I. (1970), Acta Chem. Scand., 24 1331; 1341; 1527.

Gopalakrishnan, S. and Sircar, S.M. (1973), Indian J. Agric. Sci., 43 (11) 1012.

Le Roy, A.F., Wheling, M.L., Sponseller, H.L., Friauf, W.S., Solomon, R.E., Dedrick, R.L., Litterst, C.L., Gram, T.E., Guarino, A.M. and Becker, D.A. (1977), Biochem. Med., 18 194.

Le Roy, A.F., Lutz, R.J., Dedrick, R.L., Litterst, C.L. and Guarino, A.M. (1979), Cancer Treat. Rep., 63 (1) 59.

Le Roy, A.F., Wehling, M., Gormley, P., Egorin, M., Ostrow, S., Bachur, N. and Wiernik, P. (1980), Cancer Treat. Rep., 64 (1) 123.

Pearson, R.G. (1963), J. Chem. Educ., 45 581; 643.

Rauser, W.E. (1973), Can. J. Bot. 51 301.

Rauser, W.E. (1978), Can. J. Bot., 56 1744.

Rauser, W.E. (1981), private communication.

Robb, J., Busch, L. and Rauser, W.E. (1980), Ann. Bot., 46 43.

Rosenberg, B., Van Camp, L., Grimley, E.B. and Thompson, A.J. (1967), J. Biol. Chem., 242 (6) 1347.

Sircar, P.K., Banerjee, S. and Sircar, S.M. (1973), Indian J. Agric. Sci., 43 (1) 1.

Williams, D.R. (1971), The Metals of Life, Van Nostrand Reinhold Co., London.

Wolverton, B.C. and McDonald, R.C. (1975), NASA-TM-X-72727, 13 (15).

APPENDIX

A preliminary comparison of instrumental techniques for the determination of platinum at low levels in plants

The first water hyacinth prepared in chapter 6.2 and designated 'Pt $LO_{(1)}$ tops', was used to examine the suitability of a variety of instrumental techniques. Included, but later abandoned, were the techniques X-ray fluorescence spectrometry (XRF) and D.C. Argon plasma atomic emission spectroscopy (Spectrospan III system). The details of instrumental settings for XRF are given in Table 6.11.

The dried plant material was analysed for platinum only by ICP, ETA AAS and INAA (TRIGA) also; results are presented in Table 6.12. A second plant was prepared containing platinum at low levels and designated 'Pt $LO_{(2)}$ tops'. This was analysed along with plants containing other members of the platinum group and results of the full comparison are given in chapter 6.

Instrumental Conditions for the determination of platinum by X-Ray Fluorescence Spectrometry

Philips PW 1410 X-Ray Spectrometer.

Collinator:	Fine	Filter: Out		
Crystal:	LiF_{200}	Order: 1		
Counter:	F+S (Flow + Scintillation)			
Vacuum:	No	Tube:	Chromium	50kV 50mA
Window:	500	Mode:	PT	Gain: 128
Time count:	10s	Peak angle 2θ 38.00^{o} (PtL_{α})		
		Background angle 39.60^{o}		
Standards:	matrix matched (platinum spiked plant ash)			

Table 6.11

Preliminary comparison of instrumental techniques for the determination of platinum in standard water hyacinth Pt LO(I)

Technique	$\mu g\ g^{-1}$ Pt, dry weight
ETA AAS	14.7
DC Argon Plasma (Spectrospan III)	23.1
ICP AES	7.7
XRF	29.0
INAA (TRIGA):	
(i) plant ash, (corrected for % ash) 158/208keV	14.1
(ii) dried plant material ^{199}Au γ 158keV:	44
(iii) " " " ^{199}Au γ 208keV:	16

Table 6.12

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Determination of Platinum, Palladium and Rhodium by Atomic-absorption Spectroscopy with Electrothermal Atomisation

M. E. Farago and P. J. Parsons

Chemistry Department, Bedford College, Regent's Park, London, NW1 4NS

Instrumental conditions for the determination of platinum, palladium and rhodium by atomic-absorption spectroscopy with electrothermal atomisation are described for two different instruments. Platinum determinations were investigated further using a third instrument. The effects of acid concentration on peak heights were found to be instrument dependent. This instrument dependence is discussed in terms of the geometries of the various tubes employed in the furnaces. A comparison is made of voltage control and temperature control facilities for the atomisation of the three metals.

Keywords: Platinum, palladium and rhodium determination; atomic-absorption spectroscopy; electrothermal atomisation; acid effects

Recent interest in the medical and industrial significance of platinum and to a lesser extent palladium and rhodium has been accompanied by an increasing interest in their determination at low levels. Most studies have been concerned with animal tissues[1–3] and body fluids[4–6]; studies of plant material have been few. Kothny[7] has developed a novel spectrophotometric method for the determination of palladium in plants and soils. Because of the poor detection limits for the determination of platinum by flame atomic-absorption spectroscopy (FAAS),[8] atomic-absorption spectroscopy with electrothermal atomisation (ETA-AAS) is often used for the determination of platinum present in low concentrations.[1–4,9–12]

One problem with ETA-AAS has been non-atomic-absorption from matrix salts volatilised in the graphite furnace. Simultaneous deuterium background correction is now the most widely adopted method for the removal of such errors.[10] Signal depression when nitrate and sulphate ions are present is another source of errors.[11] This last point has some bearing on the preparation of biological samples by the wet ashing method: Dokiya *et al.*[13] compared the effects of mineral acids used in the wet digestion of plant material for atomic-absorption spectroscopy. They used a Varian Techtron AA1000 instrument equipped with the CRA 63 unit for electrothermal atomisation work, and came to the conclusion that nitric acid was the most suitable medium. Pera and Harder[1] carried out both wet ashing and analysis of animal tissues in nitric acid solutions. Measurements were carried out on a Perkin-Elmer 360 spectrometer fitted with an HGA 2100 furnace, and these workers reported that they found no interference from nitrate on signal height when concentrations of nitric acid between 0.031 2 and 7.8 mol l^{-1} were investigated. Miller and Doerger[2] also investigated the analysis of tissue samples for platinum, but converted their nitric acid digests into chloride for analysis, by treatment with sodium chloride and hydrochloric acid. The instrument used for the measurements was a Perkin-Elmer 503 with an HGA 2000 furnace, and with this system Miller and Doerger stated, "Several additions of hydrochloric acid are necessary to drive off the nitrate fumes which seem to reduce the sensitivity of the metals." LeRoy *et al.*[9] completed wet ashing of animal tissue in a mixture of nitric and perchloric acids, and converted digests into chloride for analysis, although they did not report nitrate interferences in the determination of platinum.

Optimum conditions for the ETA-AAS of platinum-group metals have been reported previously.[14,15] Effects of acids on signal heights were investigated using a Perkin-Elmer 403 - HGA 70 system,[14] and those of nitric acid were found to be more pronounced than those of hydrochloric acid with regard to both sensitivity and reproducibility. Everett[15] detailed the furnace parameters for the determination of platinum metals using a carbon rod atomiser with a Varian Techtron AA-5 spectrometer.

In this paper we report methods for the determination of platinum, palladium and rhodium by ETA-AAS as the final step in the analysis of plant material for these metals. Programme

settings have been developed for the Pye Unicam SP2900 double-beam instrument fitted with an SP9-01 electrothermal atomiser accessory, and for the Pye Unicam video furnace. The SP2900 - SP9-01 instrument utilises classical voltage control for atomisation. The SP9 video furnace head incorporates an optical temperature feedback facility. The sensitivity of the two methods was investigated using the latter instrument. Acid interferences were investigated using these two systems, and briefly using a Perkin-Elmer 306 together with an HGA 74 furnace.

Subsequent papers will deal with the determination of platinum metals in some specially grown and homogenised plant material by AAS, by instrumental neutron-activation analysis (INAA) and by other techniques. LeRoy *et al.*[9] have compared the results of the analysis of canine heart, liver and muscle by both ETA-AAS and INAA. It was found that the results for platinum using INAA were higher in all instances. Similarly, we and other workers[16] have found that in the analysis of plant material for platinum by INAA, there are interferences that can lead to errors. This point will be discussed in a later paper.

Experimental and Results

Instrumentation

The instruments used were as follows: (*i*) Pye Unicam SP2900 double-beam spectrophotometer fitted with deuterium background correction and an SP9-01 electrothermal atomiser; the system was interfaced with a Hewlett-Packard laboratory data system (HP 97), which was used to record peak heights, correct for blanks and calculate relative standard deviations and absolute sensitivities; (*ii*) Pye Unicam SP9 video furnace, equipped with autosampler (FASI); and (*iii*) Perkin-Elmer 306 spectrometer fitted with an HGA 74 furnace and equipped also with a furnace autosampler injection facility. Manual injections (SP2900 - SP9-01 system) were made with a 25-μl micropipette (Clinipette; Labora Mannheim). All acids were of Aristar grade; solutions were made up with de-ionised, distilled water.

Programme Settings for Platinum and Acid Interferences

Platinum stock solution

Platinum sponge (Johnson Matthey, Specpure) (0.5000 g) was dissolved in 50 ml of aqua regia and evaporated to dryness. A 25-ml volume of concentrated hydrochloric acid and 0.5 g of sodium chloride were added to the residue, and the mixture was again evaporated to dryness. The residue was dissolved in 100 ml of 50% hydrochloric acid and then diluted to 500 ml with water. This stock solution was stored in a polythene bottle.

Construction of atomisation and ashing curves, SP2900 - SP9-01

Using an injection of 25 μl of a platinum solution (1.0 μg ml^{-1}), *i.e.*, 25 ng of platinum, a suitable drying time of about 60 s at a temperature setting of about 25 (*ca.* 240 °C) was established. This was achieved by watching the evaporation of the sample with a dental mirror (**Caution**—ultraviolet radiation). The atomisation curve shown in Fig. I was then constructed; similarly, the ashing curve (Fig. 1) was obtained (see footnotes || and **, Table I). The final settings are shown in Table I.

Effects of acids on peak heights, SP2900 - SP9-01

The effects of varying concentrations of nitric, sulphuric and hydrochloric acids on peak heights were investigated. The results are shown in Fig. 2. It can be seen that there is a severe depression of peak height and very poor precision with nitric acid. Sulphuric acid produced a considerable amount of matrix smoke and condensed on the instrument baffles, causing damage; it was therefore abandoned. Hydrochloric acid was found to be the best medium for the determination of platinum using the SP2900 - SP9-01 system. A calibration graph for platinum in the range 0.2–2.0 μg ml^{-1} is shown in Fig. 3.

Platinum programme and acid effects, PE 306 - HGA 74

The programme settings are shown in Table II. The effects of acids on peak heights were investigated briefly for platinum only. Fig. 4 shows the effects of varying concentrations of

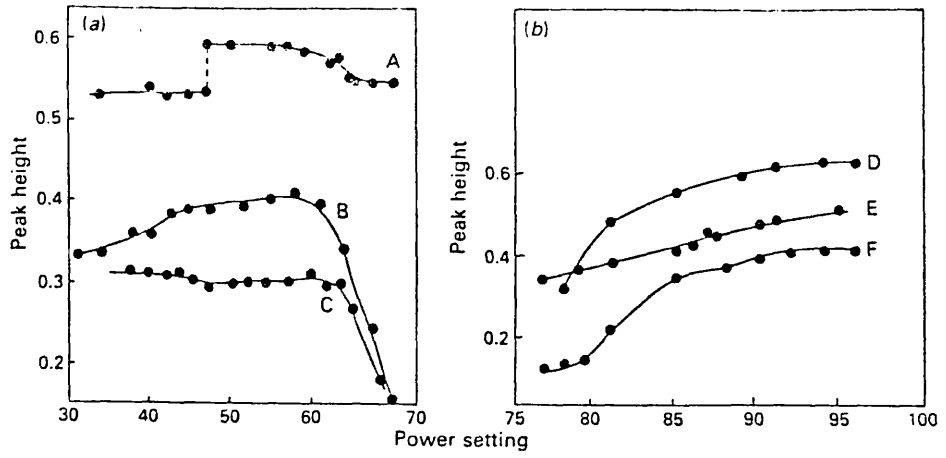

Fig. 1. (*a*) Ashing curves and (*b*) atomisation curves. Pye Unicam SP1900 - SP9-01. A and D: platinum; injection, 25 μl, 1.0 μg ml^{-1} (aqueous); band pass, 0.2 nm; λ, 265.95 nm. B and E: palladium; injection 25 μl, 0.1 μg ml^{-1} (aqueous); λ, 247.64 nm; band pass, 0.2 nm. C and F: rhodium; injection, 25 μl, 0.1 μg ml^{-1} (aqueous); λ, 343.49 nm; band pass, 0.2 nm. The ashing curve for platinum (A) shows typically the effect of changing the tube. The broken line represents the tube replacement, showing that different peak heights are obtained with the new tube for the same power setting. Similarly, Table IV shows that in the analysis of palladium the introduction of a new tube reduced the standard deviations in one set of measurements.

TABLE I

INSTRUMENTAL SETTINGS FOR THE DETERMINATION OF PLATINUM, PALLADIUM AND RHODIUM USING THE PYE UNICAM SP2900 - SP9-01 (ETA-AAS)

Parameter	Pt	Pd	Rh
Wavelength/nm	265.95	340.46, 247.64*	343.49
Band pass/nm	0.2	0.2	0.2
Lamp current/mA	10	5.5	10
Recorder range/mV†	10	10	10
Recorder speed/mm min^{-1}	20	20	20
Furnace parameters‡,§:			
Dry: Time/s	65	60	65
Indicated temperature/°C	300	300	300
Digital setting	28	28	28
Ash: Time/s‖	45	30	30
Indicated temperature/°C	1375	1375	1800
Digital setting	50	50	58
Delay/s¶	10	10	10
Atomisation: Time/s**	5	5	5
Indicated temperature/°C	3025	2950	3000
Digital setting	93	90	92
Delay/s	10	10	10
Tube clean: Time/s	5	5	5
Indicated temperature/°C	3175	3175	3175
Digital setting	99	99	99
Delay/s	10	10	10
Tube blank: Time/s	5	5	5
Indicated temperature/°C	3025	2950	3000
Digital setting	93	90	92

* The line at 340.46 nm is more suitable for samples with high background, and was used subsequently for the analysis of plant samples.

† Philips PM 8251 pen recorder.

‡ Argon flow-rate, 3 l min^{-1}.

§ Isothermal heating.

‖ Optimum ash times and temperatures are matrix dependent; values given were determined empirically for control plant samples by ensuring the removal of matrix smoke prior to atomisation. This gave an acceptable blank signal during atomisation.

¶ Peak height read initiated (time, 15 s).

** Atomisation times were determined by observing the peak profile with a fast response recorder and calculating the time taken for the peak to form and decay.

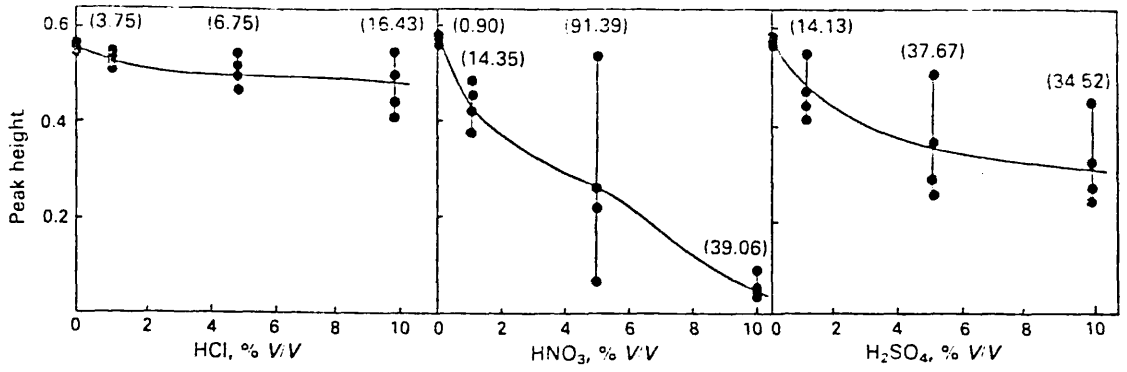

Fig. 2. Effect of acids on peak height. Pye Unicam SP2900 - SP9-01. Platinum; injection, 25 μl, 1.0 μg ml^{-1}; λ, 265.95 nm; band pass, 0.2 nm. RSD values in parentheses.

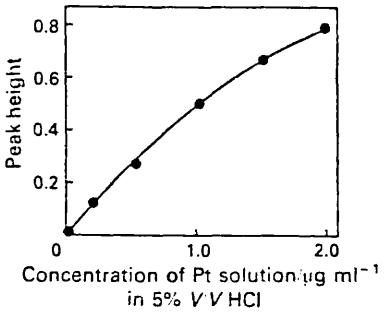

Fig. 3. Calibration graph for platinum. Pye Unicam SP2900 - SP9-01. Injection, 25 μl, 0.2–2.0 μg ml^{-1} (hydrochloric acid, 5% V/V); λ, 265.95 nm; band pass 0.2 nm.

TABLE II

PARAMETERS FOR THE INVESTIGATION OF EFFECTS OF ACIDS ON THE DETERMINATION OF PLATINUM BY ETA-AAS USING THE PERKIN-ELMER 306 - HGA 74, WITH AUTOSAMPLER AS-1

Injection, 20 μl.

Parameter	Value
Wavelength/nm	265.95
Band pass/nm	0.7
Lamp current/mA	10
Recorder range/mV	10
Recorder speed/cm s^{-1}	10
Furnace parameters*:	
Dry: Time/s	60
Indicated temperature/°C	90
Ash: Time/s	26
Indicated temperature/°C	1630
Atomisation†: Time/s	8
Indicated temperature/°C	2700
Tube clean: Time/s	10
Indicated temperature/°C	2700

* Adapted from refs. 1 and 2.
† With gas stop facility.

hydrochloric and nitric acids on peak heights. The depression produced by nitric acid is not as severe as with the Pye Unicam SP2900 - SP9-01 system, in agreement with previous observations.[9,11] Hydrochloric acid in fact gives an enhanced signal with this system (Fig. 5).

Platinum programme and acid effects, SP9 video furnace

Atomisation curves for platinum were constructed using both temperature control and voltage control facilities (Fig. 6). With a band pass of 0.5 nm, better sensitivity is found for temperature control than for voltage control, but with a band pass of 0.2 nm, little difference is observed. The final programme for the analysis of platinum is shown in Table III. The effects of varying concentrations of nitric and hydrochloric acids are shown in Fig. 7.

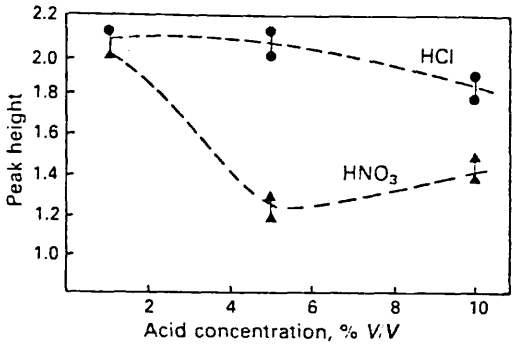

Fig. 4. Effect of acids on platinum peak heights. Perkin-Elmer 306 - HGA 74. Injection, 20 μl, 0.2 μg ml^{-1}; λ, 265.95; band pass, 0.7 nm.

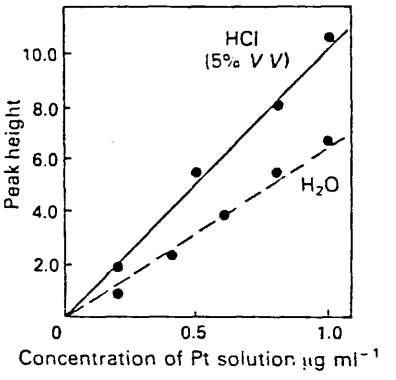

Fig. 5. Calibration graphs for platinum. Perkin-Elmer 306 - HGA 74. Injection, 2 μl, 0.2–1.0 μg ml^{-1}; λ, 265.95 nm; band pass, 0.7 nm.

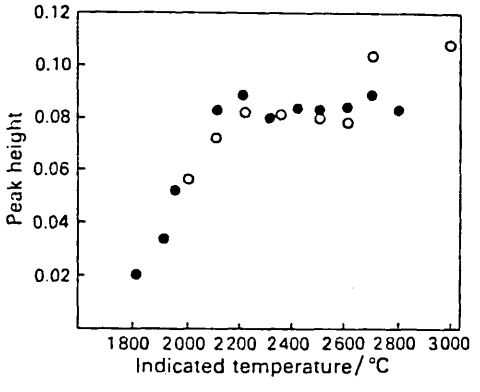

Fig. 6. Platinum atomisation curves. Pye Unicam SP9 video furnace. Injection, 10 μl, 0.1 μg ml^{-1} (aqueous); λ, 265.95 nm; band pass, 0.2 nm. Open circles, voltage control; and closed circles, temperature control.

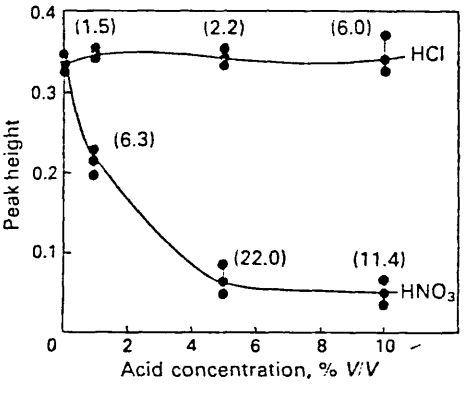

Fig. 7. Effect of acids on peak height. Pye Unicam SP9 video furnace. Platinum; injection, 10 μl, 0.1 μg ml^{-1}; λ, 265.95 nm; band pass, 0.2 nm. RSD values in parentheses.

Programme Settings for Palladium and Acid Effects

Palladium stock solution

Palladium sponge (Johnson Matthey, Specpure) (0.5000 g) was dissolved in 5 ml of aqua regia and evaporated to dryness. The residue was dissolved in 2.5 ml of concentrated hydrochloric acid and 12.5 ml of water, and finally diluted to 500 ml. The solution was stored in a polythene bottle.

TABLE III

INSTRUMENTAL SETTINGS FOR THE DETERMINATION OF PLATINUM, PALLADIUM AND RHODIUM BY ETA-AAS USING THE PYE UNICAM SP9 VIDEO FURNACE

Autosampler programme: volume, 10 μl; re-sample, 3; mode, multi.

Parameter	Pt	Pd	Rh
Wavelength/nm	265.95	340.46	343.49
Band pass/nm	0.2	0.2	0.2
Lamp current/mA	10	8	10
Recorder range/mV	50	50	200
Recorder speed/mm min^{-1}	30	30	30
Furnace parameters:			
Phase 1: Time/s	35	35	35
Temperature/°C	90	90	90
Phase 2: Time/s	15	15	30
Temperature/°C	1500*	1500*	1500*
Phase 3 (TC)†: Time/s	3	3	3
Temperature/°C	2700	2600	2600
Phase 3 (VC)†: Time/s	5	5	5
Temperature/°C	3000	2950	3000
Phase 4: Time/s	10	10	10
Phase 5: Time/s	3	3	3
Temperature/°C	2950	2950	2950

* Ashing temperature dependent on matrix and conditions; for biological samples use of ramping facilities improves ashing.

† Phase 3/atomisation: initiate autozero (AZ), recorder control (RC), peak timer (PT). TC = temperature control; VC = voltage control.

Palladium programme and acid effects, SP2900 - SP9-01

Using an injection of 25 μl of a palladium solution containing 0.1 μg ml^{-1} (2.5 ng of palladium), the curves shown in Fig. 1 were constructed. The final optimum settings for palladium are shown in Table I. The effects of nitric and hydrochloric acids on peak heights are shown in Table IV.

An investigation of the sensitivity of the lines at 247.64 and 340.46 nm was made, and some recorder traces for injections of 25 μl of a solution of 0.1 μg ml^{-1} of palladium in 5% hydrochloric acid are shown in Fig. 8. The line at 244.79 nm is the most intense but is

TABLE IV

EFFECTS OF ACIDS ON THE DETERMINATION OF PALLADIUM BY ETA-AAS

SP9 video furnace

0.1 μg ml^{-1} Pd in:	H_2O	1% HCl	5% HCl	10% HCl	1% HNO_3	5% HNO_3	10% HNO_3
Voltage control; λ = 247.64 nm—							
Peak height	—	0.117	0.109	0.139*	0.059	0.048	0.092
RSD	—	6.5	6.6	2.6	3.0	8.3	6.7
Peak area	—	0.1284	0.0606	0.2298	0.0794	−0.057	0.0888
RSD	—	32.1	14.1	21.5	36.7	222.7	44.9
Voltage control; λ = 247.64 nm (*repeated*)—							
Peak height	0.282	0.400	0.455	0.492	0.333	0.243	0.102
RSD	2.9	4.7	0.3	0.9	3.9	31.9	6.1
Peak area	0.2877	0.3992	0.4648	0.4797	0.3655	0.2518	0.1670
RSD	6.9	4.2	0.8	2.9	13.0	19.3	11.5
Temperature control; λ = 340.46 nm—							
Peak height	0.217	0.231	0.240	0.222	0.321	0.161	0.136
RSD	1.1	3.6	2.8	2.8	6.4	13.7	3.6
Peak area	0.1143	0.1214	0.1252	0.1200	0.1486	0.0460	0.0088
RSD	11.9	14.9	8.9	14.0	5.2	64.3	90.8

SP2900 - SP9-01 system

0.1 μg ml^{-1} Pd in:	H_2O	1% HCl	5% HCl	10% HCl	1% HNO_3	5% HNO_3	10% HNO_3
Voltage control; λ = 247.64 nm							
Peak height	0.384	0.332	0.364	0.375	0.203	0.063	0.084
RSD	1.45	1.03	4.73	2.27	20.06	15.31	28.33

* New tube (this investigation was repeated).

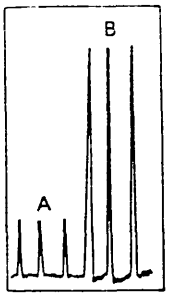

Fig. 8. Effect of wavelength on peak height. Pye Unicam SP2900 - SP9-01. Palladium; injection, 25 μl, 0.1 μg ml^{-1} (hydrochloric acid, 5% *V/V*). A, 340.46 nm; and B, 247.64 nm.

very noisy, and that at 247.64 nm is the most sensitive but is also noisy. The less sensitive line at 340.46 nm appears to be the most suitable, particularly with plant samples that have high non-specific absorption.

Palladium programme and acid effects, SP9 video furnace

Palladium atomisation curves constructed using both temperature and voltage control are shown in Fig. 9. It can be seen that better sensitivity is obtained using the temperature control facility. The final optimum furnace parameters are shown in Table III. The effects of acids on peak heights are shown in Table IV.

Programme Settings for Rhodium and Acid Interferences

Rhodium stock solution

A 1.100 1-g amount of rhodium(III) chloride (Johnson Matthey, Specpure) was dissolved in de-ionised, distilled water and diluted to 500 ml. The rhodium assay on this sample of rhodium(III) chloride was 41.45%, the solution thus being 1000 mg l^{-1} in rhodium; the concentration was checked by gravimetric analysis.

Rhodium programme and acid effects, SP2900 - SP9-01

The curves shown in Fig. 1 were constructed using an injection of 25 μl of a rhodium solution containing 0.1 μg ml^{-1} of rhodium. The final optimum settings for rhodium are shown in Table I. The effects of nitric and of hydrochloric acids on peak heights are shown in Fig. 10.

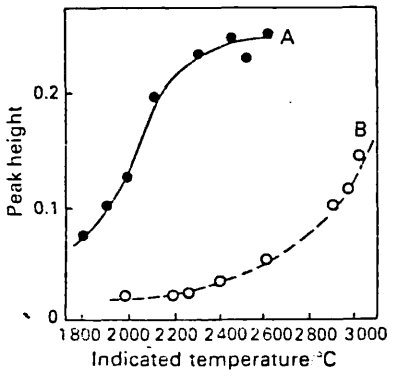

Fig. 9. Palladium atomisation curves. Pye Unicam SP9 video furnace. Injection, 10 μl, 0.1 μg ml^{-1} (aqueous); λ, 340.46 nm; band pass, 0.2 nm. A, Temperature control; and B, voltage control.

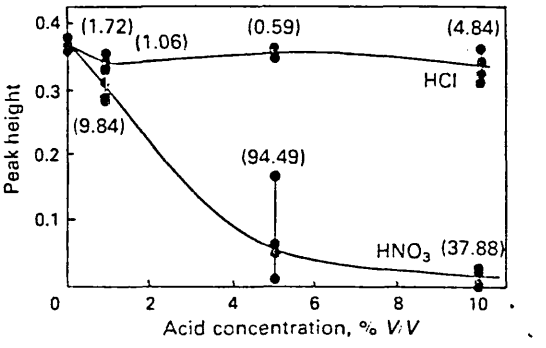

Fig. 10. Effect of acids on peak height. Pye Unicam SP2900 - SP9-01. Rhodium; injection 25 μl, 0.1 μg ml^{-1}; λ, 343.49 nm; band pass, 0.2 nm. RSD values in parentheses.

Rhodium programme, SP9 video furnace

Rhodium atomisation curves using both temperature and voltage controls are shown in Fig. 11.

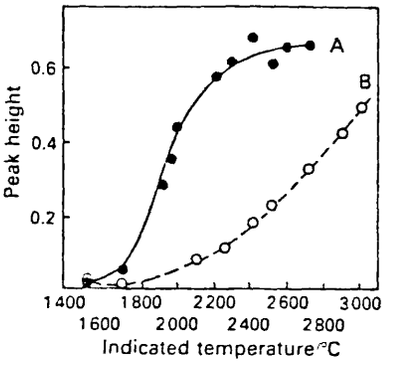

Fig. 11. Rhodium atomisation curves. Pye Unicam SP9 video furnace. Injection, 10 μl, 0.1 μg ml^{-1}; λ, 343.49 nm; band pass, 0.2 nm. A, Temperature control; and B, voltage control.

Discussion

Optimum conditions for the determination of platinum group metals by ETA-AAS have been reported by many workers.[1–4,9–15,17–19] There is, however, some disagreement on methods of sample preparation, furnace parameters and interferences, which appears to result from the variety of instruments used. It would seem that it is the design and structure of the electrothermal atomisation unit that are responsible for the differences.

Early investigations of electrothermal atomisation were carried out using a heated graphite rod. Later, graphite tubes became more popular, allowing for larger sample volumes. At the same time a number of problems arose from this, some of which appear to depend on tube geometry. Thus the optimum conditions for the determination of platinum metal for one instrument cannot be adopted directly for another instrument or, for biological samples, for another matrix. Such furnace parameters can serve only as useful guidelines and must be determined for each instrument and each type of matrix.

Incorrect dry phase settings can lead to low precision. The drying time and optimum temperature have been found to vary considerably from sample to sample, and also on the age and conditions of the tube (Fig. 1). The use of pyrolytically coated tubes, although improving sensitivity during atomisation for some metals, generally results in poor drying characteristics. For example, violent boiling with subsequent analyte loss in the gas stream may occur. A small dental mirror is ideal with the observation of the controlled evaporation of the sample in the tube (**Caution**—ultraviolet radiation), and this was used to set the drying times. As the graphite tubes vary in size and shape it is not surprising that the deposition and subsequent spread of the sample will vary (Fig. 12).[20] This will influence the number of atoms released as an atomic vapour into the beam, owing to the temperature profile of the tube. The flow of argon within the tube can also affect the transient signal; thus, removal of the cap from the glass chimney enhances sensitivity in the SP2900 - SP-01 system.

Throughout this work a band pass of 0.2 nm was used. When using high temperatures for atomisation, non-atomic absorption is considerable. The tube-wall emission is reduced by the use of a small band pass in the region of 0.2 nm. Everett,[15] however, reduced the non-atomic absorption by atomising at a lower temperature, and increasing the band pass, the lower atomisation temperatures increasing the life of the carbon rods.

The lifetimes of the carbon tubes are also improved by the use of temperature control for atomisation. Unlike voltage control, where atomisation is achieved by the application of a pre-set voltage across the tube for a pre-set time, the temperature control facility applies

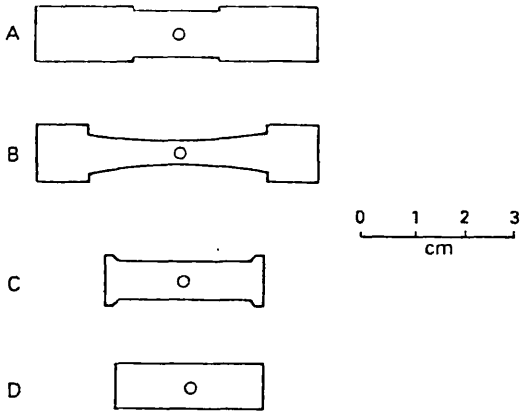

Fig. 12. Cross-sections of various types of graphite furnace tubes: A, standard tube for Pye Unicam SP9-01; B, "profile" tube for Pye Unicam SP9-01; C, standard tube for Pye Unicam SP9; and D, standard tube for Perkin-Elmer HGA 74.

the maximum voltage until a pre-set temperature is reached. A temperature feedback device is incorporated, which can switch off the power once that point is reached. With the SP9 video furnace, this was found to increase the sensitivity for platinum, palladium and rhodium at lower atomisation temperatures, thus bringing about the improvement in tube life. The advantages of temperature control have been discussed by Dymott.[21] For palladium (Fig. 9) and rhodium (Fig. 11) this effect is significant, and for platinum using a band pass of 0.5 nm,[22] but for a band pass of 0.2 nm no significant difference was observed (Fig. 6).

For the determination of palladium, there is a choice of lines; that at 244.79 nm is very noisy, whilst that at 247.64 nm is sensitive, but is noisy also. The palladium spectrum in the region 244–247 nm is reported to be complex[23] and interferences between lines may occur.[24] The more stable line at 340.46 nm is suitable for samples with high backgrounds, such as plant samples. This line was also used with the SP9 video furnace.

Effects of Mineral Acids

It was found that platinum standards in nitric acid solution, when measured using the Pye Unicam SP2900 fitted with the SP9-01 furnace, gave severe depression of peak height and poor precision, especially at the 5% nitric acid level; with 10% nitric acid the relative standard deviation (RSD) was better only because of the very severe depression of peak height. The same standards gave a reasonable calibration graph using a Perkin-Elmer 360 with the HGA 76 graphite furnace (these last results are not reported in the results section, as only one calibration graph was constructed). Similarly, although some depression of the signal was encountered, it was possible to use the Perkin-Elmer 306 with the HGA 74 furnace (Fig. 4). It can be seen from Fig. 7 that there is also severe depression of signal height using the Pye Unicam SP9 video furnace with platinum in nitric acid media. Figs. 2, 4 and 7 show that 5% hydrochloric acid is a suitable medium for the determination of platinum using all three instruments. Hydrochloric acid in some instances gives an enhanced signal (Fig. 5). This pattern of results was repeated for both palladium (Table IV) and rhodium (Fig. 10) using both the SP2900 - SP9-01 and the SP9 video furnace.

Conclusion

It is concluded that for the determination of platinum, palladium and rhodium in biological samples the following method should be adopted. After pre-digestion in concentrated nitric acid, all nitrate must be removed from digests by treating the dried residues with concentrated hydrochloric acid several times, the mixture being taken to dryness each time. The final

chloride residue is resolubilised with concentrated hydrochloric acid, and then diluted to give a solution that is approximately 5% V/V in hydrochloric acid. Standards should be made up similarly in 5% hydrochloric acid. Furnace parameters should be determined for the particular instrument and matrix involved.

The use of the temperature control facility improves sensitivity and prolongs tube life. Although the ashing parameters will depend on the individual matrix, too high a temperature results in analyte loss and shortens the life of the tube. Incomplete ashing occurs if the temperature is too low, resulting in overloading of the background corrector.

The absolute sensitivities for the determination of platinum, palladium and rhodium by ETA-AAS are given in Table V.

TABLE V

ABSOLUTE SENSITIVITIES OF THE PLATINUM GROUP METALS IN ETA-AAS

The absolute sensitivity is taken as that mass of an element that will give a peak absorbance value of 0.00436 absorbance unit (which is equivalent to a peak of 1% absorption),[21] *i.e.*,

$$\text{Sensitivity} = \frac{0.00436 \times \text{injected mass}}{\text{measured absorbance}}$$

For example, for palladium:

$$\text{Sensitivity} = \frac{0.00436 \times (2.5 \times 10^{-9})}{0.362}\ \text{g}$$

$$= 30 \times 10^{-12}\ \text{g (30 pg)}$$

	Pye Unicam SP2900 - SP9-01			
	Pt	Pd*	Pd†	Rh
Concentration of injection/μg ml^{-1} ..	0.1	0.1	0.1	0.1
Volume of sample injection/μl	25	25	25	25
Voltage control—				
Mean absorbance	0.065	0.071	0.359	0.351
Absolute sensitivity/pg	168	154	30	31

	Pye Unicam SP9 video furnace			
	Pt‡	Pt§	Pd*	Rh
Concentration of injection/μg ml^{-1} ..	0.1	0.5	0.1	0.1
Volume of sample injection/μl	10	10	10	10
Voltage control—				
Mean absorbance	0.107	0.350	0.146	0.492
Absolute sensitivity/pg	41	62	30	9
Temperature control—				
Mean absorbance	0.089	0.444	0.249	0.651
Absolute sensitivity/pg	49	49	18	7

* λ = 340.46 nm.
† λ = 247.64 nm.
‡ Band pass: 0.2 nm.
§ Band pass: 0.5 nm.

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References

1. Pera, M. F., and Harder, H. C., *Clin. Chem.*, 1977, **23**, 1245.
2. Miller, R. G., and Doerger, J. U., *At. Absorpt. Newsl.*, 1975, **14**, 66.
3. LeRoy, A. F., *Environ. Health Perspect.*, 1975, **10**, 73.
4. Tillery, J. B., and Johnson, D. E., *Environ. Health Perspect.*, 1975, **12**, 19.

5. Jones, A. H., *Anal. Chem.*, 1976, **48**, 1472.
6. Duffield, F. V. P., *Environ. Health Perspect.*, 1976, **15**, 131.
7. Kothny, E. L., *Mikrochim. Acta*, 1978, **1**, 425.
8. Sen Gupta, J. G., *Miner. Sci. Eng.*, 1977, **5**, 207.
9. LeRoy, A. F., Whehling, M. L., Sponseller, H. L., Friauf, W. S., Solomon, R. E., Dedrick, R. L., Litterst, C. L., Gram, T. E., Guarino, A. M., and Becker, D. A., *Biomed. Med.*, 1977, **18**, 194.
10. Hendrikx-Jangerius, C., and De Galan, L., *Anal. Chim. Acta*, 1976, **87**, 259.
11. Eklund, R. H., and Holcombe, J. A., *Anal. Chim. Acta*, 1979, **109**, 97.
12. Potter, N. M., and Waldo, R. A., *Anal. Chim. Acta*, 1979, **110**, 29.
13. Dokiya, Y., Yamamoto, S., and Fuwa, K., *Spectrosc. Lett.*, 1975, **8**, 759.
14. Adriaenssens, E., and Knoop, P., *Anal. Chim. Acta*, 1973, **68**, 37.
15. Everett, G. L., *Analyst*, 1976, **101**, 348.
16. Minski, M. J., personal communication, 1981.
17. Tello, A., and Sepulveda, N., *At. Absorpt. Newsl.*, 1977, **16**, 67.
18. Janouskova, J., Nehasilova, M., and Sychra, V., *At. Absorpt. Newsl.*, 1973, **12**, 161.
19. Towndrow, E. G., personal communication, 1981 (unpublished results using a Pye Unicam SP1900 with a Massmann - Beckman furnace).
20. Littlejohn, D., and Ottaway, J. M., *Analyst*, 1977, **102**, 553.
21. Dymott, T. C., "Atomic Absorption with Electrothermal Atomisation," Second Edition, Pye Unicam Ltd., Cambridge, 1981.
22. Morton, S. F., personal communication, 1981.
23. Christian, G. D., and Feldman, F. J., "Atomic Absorption Spectroscopy: Applications in Agriculture, Biology and Medicine," Wiley-Interscience, New York, 1970.
24. Whiteside, P. J., "Atomic Absorption Data Book," Second Edition, Pye Unicam Ltd., Cambridge, 1976.

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