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Errata

Contents pages: Numbering out by one unit after page 14 which should be page 13. All subsequent pages should be one less. Hence also Table 4 is on page 30 not 31.

Page 2 line 6: "its" not "it's".

Page 12: last sentence should read "The permissible values may be different for various countries or states depending on individual regulation and land use."

Page 20 line 6: the part of the equation that shows "π^2/mc" should read as "π^2e^2/mc".

References:

Page 19: "Patel and Agarwal 1991" should read as "Patel and Agarwal 1992".
"Cleven and Fokkert 1994" should read as "Cleven and Fokkert 1993".
Page 36 line 14: "Stockey" should read as "Stockley".
Page 61: Nicks and Chambers reference: "phytominig" should read as "phytomining".
Page 63 at top: The Stockley (1980) ref. is missing and should read as follows:
Stockley,E. 1980. Biogeochemical Studies on the Nickel Complex Contained in the Nickel-accumulating Legume *Pearsonia metallifera* from the Great Dyke Area, Zimbabwe. BSc (Hons.) Thesis, Massey University, Palmerston North, New Zealand.
Page 63 line 3: The date of the Tremel et al. ref. is 1997 not 1996.

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Thallium Phytoextraction and its Economic Significance

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Abstract

Thallium is a volatile toxic metal which has many industrial uses. The sources of thallium include natural geochemical sources and by-products of industrial processes. One of the problems in assessing thallium pollution potential is the lack of an accurate method of quantification of this element. Flame atomic absorption spectrometry (FAAS) is a good method of analysis but only down to the 1.0 mg/kg (ppm) level. For lower concentrations, analytical techniques have been difficult and inaccurate. A newly described method using graphite furnace atomic absorption spectrometry (GFAAS) has been found useful for low Tl concentrations. A method was designed to detect low concentrations of thallium in plant and soil samples. The graphite furnace technique worked well for the data presented in this paper. However, samples of different origin could have more severe matrix effects such as high iron contents.

Thallium has been found to be readily available for plant uptake. The extractability of thallium was significantly higher then other metals such as cadmium and zinc. This property is ideal when considering a possible phytoextraction operation. Two plant species were found to accumulate thallium to levels up to 411mg/kg in *Iberis intermedia* and 504mg/kg in *Biscutella laevigata*. These high values indicate potential for phytoremediation and phytomining in areas of sufficient contamination. The properties of which make thallium an ideal candidate for phytoremediation also make the metal a high risk for biota.

Experiments were also conducted to determine the partitioning and sequestration of thallium within plant organelles. This work was limited to the two hyperaccumulating species but future work could compare non-tolerant species. Evidence from this research might give a better understanding to the mechanism involved in plant uptake and storage of thallium. Agricultural crops used for human consumption or animal grazing may cause deleterious health effects. Thallium is extremely toxic, effecting the nervous system and impairing heart function at low levels. Higher concentrations will cause death. It is possible that some of the illness symptoms observed in humans may be derived from low levels of

thallium in foodstuffs. Aside from a few economic mineral deposits, there is no information of a world-wide distribution of thallium, so health effects can not be accurately assessed.

This thesis describes experiments carried out on common vegetable and their uptake of thallium to determine safe levels of this element within the soil. This information will also be useful to farmers growing crops on contaminated soil will advise them on which plants would uptake less thallium. Thallium has not been studied as extensively as many other heavy metals that are more common in the environment. Although the crustal abundance of thallium is low (0.49-0.7 mg/kg average range), the toxicity of this element is very high, and it is readily available for plant uptake. The toxic effects on animals and plants should be monitored closely.

Chapter 1: General Introduction

Discovery and General Properties

Thallium and other rare Group IIIa elements were first described as "scattered" elements because they are not found naturally in large deposits, and they form minerals almost exclusively with other elements (Songina 1970). Most concentrated thallium deposits are found when roasting of pyrite ores or the smelting of copper lead and zinc are carried out. Thallium was discovered in the 1860's by a chemist named Sir William Crooks (Merck Index 1987). Using optical spectroscopy, he detected unusual green emission lines produced from samples of sulfuric acid slurry, hence the name thallium from the Greek word *thallos* meaning green twig (Leonard and Gerber 1997). Thallium has two naturally occurring isotopes, 203 and 205 as well as several which have been synthesized radiochemically. The element is a soft ductile metal, greyish-white in color that dulls when exposed to air. Thallium forms (+1) monovalent, and trivalent (+3) cations. Unlike other Group IIIA elements, the resistance of the 6s electrons to covalent bonding is the reason for the stability of the Tl^+ ion (Smith and Carson 1977). Compounds formed are of importance industrially and may have negative environmental effects. The compounds include the acetate, bromide, carbonate, chloride, cyanide, fluoride, and hydroxide as well as several nitrates and selenates. All of these are considered poisonous even at low concentrations (Merck Index 1987).

Sources of Pollution and the Geochemistry of Thallium

Typical thallium concentrations average 0.49 mg/kg and 0.013 mg/kg in the continental and lower oceanic crusts respectively. Traces have also been detected in meteorites ranging from 0.001-0.2 mg/kg (Leonard and Gerber 1997). Thallium minerals are quite rare and are found almost exclusively in the realgar (arsenic) deposits of Allchar (Alsar) in Macedonia. High concentrations of thallium have been found in plants and domestic animals in the Allchar region (Zyka 1970).

Anomalous levels of thallium in soils are derived from three main sources. The first of these is natural and is related to the geochemistry of the local rocks (Tremel 1996; Tremel *et al.* 1997). The second source is anthropogenic as a result of mining sulfide mineralisations containing a significant level of thallium. An example of this is the mining district of Les Malines along the southern margin of the Massif Central near Montpellier in France (Leblanc *et al.* 1999). A third significant source is the thallium derived from burning of coal at cement works in various countries and most significantly in Germany (Kurz *et al.* 1995). The nature of thallium allows it to pass through conventional emission control equipment such as cement scrubbers allow release into the atmosphere (Kaplan *et al.* 1990). The production of sulfuric acid is also a major source of thallium contamination (Lee 1971).

Geochemically, thallium minerals are found in four major groups: crooksite $(CuTlAg)_2Se$ is found in Sweden and Brazil, hutchisonite $PbS(TlAg)_2S_2As_2S_3$ is a sulfide found in Switzerland, lorandite $TlAsS_2$ is found in Greece and Macedonia, and vrbaite $Tl_2S_3(AsSb)_2S_3$ occurs at several locations (Green 1972). Natural radionuclides of Tl^{208} have been found in Saskatchewan, Canada (Kiss *et al.* 1988). Thallium can substitute lead in galena and because of its similarity to the alkali metals it has been known to substitute potassium in some biological functions. Thallium is normally in the +1 state in fresh water and in the +3 state in sea water. Levels are typically very low in sea water because of fixation within the sediment to form Tl_2S (Leonard and Gerber 1997; Calmano *et al.* 1990).

Uses

There are increasing uses of thallium since the advent of technology and computers. Initial use of thallium in rodenticides, began in the early 1900's. The lack of colour and taste was ideal bait for ants and rats (Smith and Carson 1977). Use was discontinued in the US in 1972 and many other countries classified it as a banned chemical for residential use. Medically, thallium has been used in treatment of skin infections, ringworm and syphilis. Due to a few isolated incidences of poisoning, this form of treatment is rarely used (Leonard and Gerber 1997). Electrical conductivity changes when thallium is exposed to infrared light which is an ideal property for photocells. Glasses can also be made when mixed with sulphur or selenium creating a dark opaque high density glass with a high refractive index. Organic synthesis is one of the largest industrial uses of thallium chloride (Merck Index 1987). The monovalent form of the cation can be used to catalyse alloys and for the production of jewellery and dye (Galvan-Arzate and Santamaria 1998). Annual world production is approximately 35 tonnes for use in photography, semiconductors and electrolytic plating. The current world price of thallium is about $US300/kg.

Toxicology

Animals

Thallium toxicity is comparable to that of cyanide and is even more toxic than mercury. The LD_{50} of rats treated with mercury is 27 mg/kg compared with thallium, 13-19 mg/kg. The only compound with approximately the same toxicity is methyl mercury (LD_{50} = 13 mg/kg). A summary of toxicity levels of various metals can be found in Zitko (1975).

In humans, thallium is absorbed through skin and mucous membranes and is widely distributed throughout the body. It can be accumulated in the central nervous system and is able to cross the placental membrane and mammary glands. Initial symptoms of thallium poisoning include hypertension and paralysis. This is usually followed by, coma, lethargy pain in the abdomen and severe cardiac problems. Thallium is a monovalent cation and could substitute for potassium in several enzymatic reactions (Zitko 1975). The affinity of thallium binding to enzymes is 10 times higher than for potassium. The direct toxic effect of thallium is likely the oxidation of Tl^{1+} to Tl^{3+}. This reaction inhibits ATPase and interferes with catecholamine metabolism in animals (Galvan-Arzante and Santamaria 1998). First aid involves the administration of Prussian blue or activated charcoal which can be effective if used in time.

Plants

One method suggested to determine thallium toxicity in plants is the measurement of root elongation by quantification of radicle length. Other easily observable effects include chlorosis, inhibition of germination and low biomass production (Allus *et al.* 1988). Very small concentrations of thallium can have negative effects on plants. Concentrations as low as 0.04 mg/L can be phytotoxic to tobacco plants (Carlson 1991). Effects on plants will be discussed in detail in Chapter 5.

Micro-organisms

Microbes have adapted specific structures to deal with heavy metal stress. Two methods of adaptation include extracellular polymeric substances, binding or precipitation within the cell or extracellular material. Several other inducible mechanisms include metallothioneins that are analogous to plant phytochelatins. Difficulties arise when comparing heavy metal tolerance between different organisms and site locations. Microbial environments such as cation exchange capacity, organic matter, pH, bulk density are all difficult to quantify for their impacts on heavy metal environments. Metallothioneins are beneficial to eukaryotic cells and to growth of cyanobacteria. Heterotrophic bacteria may be able to cope with small levels of metal with plasmid resistance. Weurtz and Margeay (1997) suggest that insertional mutants are available found in *Alcaligenes eutrophus* CH34 that can allow for resistance to thallium.

The lack of information available for thallium resistance and tolerance is difficult in ascertaining environmental risk from pollution and natural sources. Plant uptake and remediation strategies could be better evaluated if more studies on microbial thallium interactions were accomplished. Detailed microbial studies will not be presented in this paper but future studies may focus on possible plant microbial interactions which could facilitate uptake.

Regulation of Thallium in the Environment

World Wide limits of thallium has been determined by the United States Environmental Protection Agency (USEPA) and the World Health Organization for legislation and environmental risk assessments. The permissible values may be different for various or states depending on individual regulation and land use (Table 1).

Table 1. Permitted concentrations (mg/kg) of thallium in soils.

Country or State	Residential	Agricultural	Industrial
Canada	-	1	-
Germany	.5	2	10
Arizona (USA)	8.2	-	-
Massachusetts (USA)	8	-	150
Michigan(USA)	22	-	75
New Jersey(USA)	2	-	2
New York (USA)	6	-	-
Washington (USA)	5.6	-	-

*Source: World Wide Limits for Toxic and Hazardous Chemicals (1997)

The above differences in regulation are alarming due to the toxic nature of thallium (Merck Index 1987). Limits such as 150 mg/kg in Massachusetts are high considering that a dose of 8 mg/kg is considered lethal to humans (Leonard and Gerber 1997). Massachusetts poses limits within an industrial setting while limits on drinking water are set at 2 ug/L. Thallium is relatively mobile; Martin and Kaplan (1998) showed in field studies that 15% of the thallium moved down the soil profile compared to <3% of cadmium. An industrial setting containing the maximum permissible levels of 150 mg/kg thallium would be likely to have values in the water table well above the 2 ug/L (Schoer 1984). Under more acidic conditions (pH 3.9-4.3), leaching through to lower soil horizons would occur even faster and would concentrate thallium in the water table.

The toxic nature of thallium is evidence for the need for strict regulation and effective remediation strategies in the case of accidental pollution or naturally high levels of this element due to the local geology. Guidelines set out (Table 1) are very inconsistent and should be re-examined by the relevant agencies

Thallium Accumulation by Plants

The first studies on thallium uptake by plants were performed by Zyka (1975) who found up to 1.7% (dry mass) in *Galium* sp. growing near the Alsar mine. Other work has been conducted on *Hyalella azteca* and the environmental impact of thallium in the ecosystem (Borgman *et al.* 1998) Leblanc *et al.*, (1999) reported extraordinary levels of thallium in the brassicaeous herb *Iberis intermedia.* This is the first report of a thallium *hyperaccumulator* plant.

Hyperaccumulator plants were defined by Brooks *et al.* (1977) as those containing >1000 mg/kg nickel in dry mass (d.m.). Since this initial definition, this terminology has been extended to include uptake of several other elements by many other plants. There are about 500 known hyperaccumulator species accumulating up to eight different heavy metals. Most of the known hyperaccumulators take up nickel, though the reason for this may be that most of the research hitherto has been concentrated on plants from ultramafic soils that are high in nickel. The most recent definition (Reeves *et al.* 1981) of hyperaccumulation has a threshold set at >1000mg/kg or 0.1% metal in plant biomass for all metals except zinc, manganese (10,000 mg/kg) and cadmium (100 mg/kg). These limits represent levels that are approximately 100 times more then the average concentrations found in non-accumulating plants.

Most hyperaccumulation studies have been conducted in areas with high concentrations of metals in the soils. Research into thallium has been concentrated in France (Tremel *et al.* 1996; Tremel and Mench 1997): Macedonia (Zyka 1970) and in newly published work in New Zealand (Anderson *et al.* 1999; LaCoste *et al.* 1999) involving pot trials and species already collected in France and Italy.

Brooks (1998) suggested several reasons for hyperaccumulation. These suggestions have been interpreted to possible mechanisms for thallium uptake.

1) Inadvertent uptake. Thallium and potassium have similar charges and ionic radii. It is possible that thallium is inadvertently taken up by a plant through the same pathway as potassium which is required for plant function. Thallium-potassium relationship in soils is important to understand uptake pathways (Frantz and Carlson 1987).

2) Metal tolerance. Thallium could be accumulated and stored in a place where it would not interfere with normal plant function, such as in vacuoles (Welch 1995). To prove or disprove this method the location of metal within the plant must be determined (Chapter 4).

3) Disposal from plant body. This is an extension of the tolerance mechanism whereby the metal is stored in a location of the plant that is easily abscised from the plant. For thallium, it would be important to determine the exact location of concentrated metal and whether this portion of the plant could be removed from the plant in a process such as leaf abscission.

4) Drought resistance. This is a possible mechanism for which there is little information.

5) Alleopathy. This strategy involves the interference of other plants by metal accumulation.

6) Pathogen / herbivore defence. The relatively high toxicity of thallium is a factor that should be considered when the defence strategy is implicated. This mechanism has been proposed by Boyd *et al*. (1994) using nickel hyperaccumulator plants.

The raison d'être for hyperaccumulation will undoubtedly differ between species and little research has been conducted on thallium hyperaccumulators so no one mechanism is favoured.

There is no known biological function of thallium within plants so the reason for hyperaccumulation might be more complicated then that for essential elements. To avoid enzyme interference, the metal must be complexed and inactivated before damage occurs within the cell (Zenk 1996). Proteins that complex metals in animals are called metallothioneins. The plant equivalents are known as phytochelatins although these compounds are not necessarily proteins. Metal binding polypeptides have a structure typically 'Y-Glu-Cys)$_n$-Gly $n = 2\text{-}11$, that can be induced in plant tissues in the presence of a heavy metal (Steffens 1990; Bae *et al.* 1997).

Rauser (1990) suggests that the overproduction of phytochelatins could account for the increased metal tolerance and sequestration observed in some hyperaccumulating species. Zenk (1996) proposed that phytochelatins for metals such as copper or zinc require inactivation and storage in the vacuole as well as transferring the metal to enzymes or other metabolic functions which require it. Currently no research exists with specific reference to thallium phytochelatins, but it is possible that a mechanism exists that is similar to those described for other well studied metals.

Phytoremediation

In recent years, there has been interest in remediation technologies for the growing problem of heavy-metal contaminated soils (Baker and Brooks 1989). Traditional methods of cleanup may involve removal of the soil from the site to storage in an area where it is considered safe such as a clay- bottomed garbage dump. Alternatively, the soil may be treated by using acid leaching. However, these strategies may cause other environmental problems such as downstream pollution or simply shifting the contamination from one site to another. Other techniques such as physical separation are very costly and not always completely effective (Cunningham 1996).

Phytoremediation (Chaney 1983; Brooks 1998) has emerged as a potentially effective method for the *in-situ* decontamination of soils weakly polluted with some heavy metals. In a phytoremediation operation, a crop of plants is grown over a metal-polluted soil with the aim of removing the toxic elements. The plants used for phytoremediation accumulate (either naturally or by addition of a chemical to the soil) the target metal in their stems and leaves. The plants can then be harvested and burnt to reduce the volume. The resulting ash may be stored in a 'safe area', or, if the metal is of sufficient value, be smelted and sold to recoup some of the cost of the operation.

This method is simpler then many of the traditional remediation strategies and is usually more cost effective. There are some drawbacks with this system including the possible introduction of exotic species. Many hyperaccumulators have been found in localised areas of the world, probably because of genetic pressure on these plants growing in their native soils. Other drawbacks include the fact that many polluted soils do not contain solely one metal but rather a mixture of several. In this case, it would be important to determine the primary target for removal; for example, cadmium or zinc.

The plants used in a phytoremediation operation need to accumulate large quantities of the target metal in the aerial portions such as hyperaccumulators (Brooks 1998). Other non-hyperaccumulating species can be induced to accumulate metals by the addition of chemicals to the soil by using chelators such as EDTA and citric acid. Ideally, the plants used in a phytoremediation operation should have a high biomass production, a high metal content, and be easily propagated. Studies should be made to ensure that species will not compete with native vegetation.

Phytomining

Phytomining is fundamentally analogous to phytoremediation. The basic difference is that the phytomining operation (Brooks *et al.* 1998) involves growing a crop of a metal for monetary return. The field of phytomining was first suggested by Chaney (1983), since that time more literature has been published including field studies (Nicks and Chambers 1995; 1998).

The subject of phytomining for thallium has been discussed by Leblanc *et al.* (1999) and Anderson *et al.* (1999) and will also be presented in Chapter 3. Phytomining is the use of hyperaccumulators to grow and concentrate a metal. After this stage the above ground biomass is harvested and burned at a high temperature. The remaining ash contains a concentrated "bio-ore" that can be processed economically (Brooks 1998). The high world price of thallium makes it an ideal candidate for phytomining. The limiting factor may be lack of a sufficient area of contaminated soil to exploit the new technology.

Aims of Study

The broad aims of this study were to:

(1) to develop an accurate method for the quantification of thallium in plants and soils using graphite furnace atomic absorption spectrophotometry (GFAAS),

(2) to investigate the uptake of thallium by vegetables and hyperaccumulator plants and factors affecting uptake and,

(3) to determine the potential of some plants for thallium phytoremediation/ phytomining.

Chapter 2: Development of a Method for Thallium Quantification using Graphite Furnace Atomic Absorption Spectrophotometry

Abstract

Thallium quantification at low levels can be problematic because of the volatility of most thallium compounds coupled with the relatively poor sensitivity of flame atomic absorption spectrometry (FAAS) for analysis of this element. A method was developed for quantification of thallium at the μg/kg (ppb) level using nitric acid digestions and graphite furnace atomic absorption spectrometry (GFAAS). Of paramount importance is the avoidance of chloride during analysis and employment of a three-tier heating regime during quantification. The maximum charring temperature for plant or soil samples digested with nitric acid was around 600°C, At the atomisation stage, a temperature of 1200°C gave maximum sensitivity during atomisation and the remainder of the matrix was cleaned at 2000°C.

Introduction

An investigation into the distribution of thallium in the environment and its uptake by plants requires an accurate method of quantification. Factors considered when selecting an appropriate method included: (1) sensitivity, (2) sample size required, (3) instrument availability and (4) cost. Thallium quantification presents some difficulty because of the volatility of this element. At present, there is little demand for thallium analyses. This combined with its obscure nature, means that little has been reported on analytical techniques and few laboratories are equipped for accurate thallium analysis. Some techniques are well described for medical analysis (Collett and Jones 1990), but the heterogeneity of the soil matrix is inherently difficult to quantify.

Analytical Techniques Available

Colorimetric methods involve the acidification of Tl(III) and subsequent extraction into toluene. The extract is then reacted with crystal violet and is analysed in a spectrophotometer (Patel and Agarwal 1991). Detection methods for analysis in the field are described by Chikhalikar *et al.* (1995). Potentiometric stripping analysis is a method available to determine thallium concentration in water samples (Cleven and Fokkert 1994).

Recently developed techniques such as modifications of GFAAS, require less sample preparation time, thus lowering the risk of contamination. This technique involves suspending the soil in water, mixing with hydrofluoric acid and injection into the graphite furnace (Lopez-Garcia *et al.* 1996; Masson *et al.* 1996).

The main benefits of inductively coupled plasma emission spectroscopy (ICPES), and inductively coupled plasma mass spectroscopy (ICPMS) are their ability to measure a large number of elements at one time. This study, however, required the analysis of a maximum of 4 elements. The cost of ICP was also very high compared with other more available machinery. In addition most ICPES machines are not set up for thallium analyses.

Another method suggested by the United States Environmental Protection Agency (US EPA) for quantification of priority pollutant metals is HPLC which has good detection limits but a complicated extraction phase is required, proscribing a large number of quantifications in the time span available in this study (Shofstahl and Hardy 1990

Analytical Techniques Chosen

Atomic absorption spectrometry

This method has been used in work done on hyperaccumulators and is well described in several papers (Robinson *et al.* 1998; 1997a; 1997b; Helnrichs and Keltscn 1982; Tsakovski *et al.* 1994). Its basic properties include radiation from a light source passing through a population of ground-state atoms, through a monochromator and finally a detector. Each element absorbs light at characteristic wavelengths as predicted by the quantum theory. The degree of absorption is proportional to the concentration in the sample as stated in the

Beer-Lambert Law. A linear relationship of absorbance vs. concentration the law states:

$$A=ecl$$

A=absorbance e=absorption coefficient c=concentration and l=pathlength.

However this relationship requires sample homogeneity, so a more accurate measurement is used to explain the absorption. The relationship is:

$$\int Kvdv= \pi^2/mc*Nf$$

Kv=absorption coefficient
e=charge of electron
m=mass of electron
c=speed of light
N=# of free absorbing atoms in light path
f=oscillator strength of absorbance line

The procedure uses a hollow-cathode tube containing an inert gas such as neon which is ionized by an anode (Cantle 1982). The sample solution is drawn through a nebuliser and injected into an air/acetylene or nitrous-oxide/acetylene flame depending on the element being analysed. This method was used for samples with thallium levels above 1.0 mg/L (ppm). The parameters chosen for the measurement of metal in these experiments were 276.5nm wavelength, three sample replicates of three seconds at a lamp current of 10 mA.

FAAS works well for thallium analyses at the mg/L level. However, sensitivity is insufficient for accurate quantification below about 1 mg/L thallium. This thesis describes the principles of graphite furnace atomic absorption spectrometry (GFAAS) and the parameters which were selected in obtaining a maximum signal response.

Current graphite furnace techniques

Interference effects from halide ions and solutions of hydrochloric and perchloric acid have been reported for thallium, probably due to the formation of volatile thallium chlorides (Hamid *et al.* 1991). Negligible interference was found by use of nitric and sulfuric acids (Xio-Quan *et al.* 1984). Matrix modifiers have been used in thallium quantification; such as tungsten wires, and palladium and/or platinum modifiers. The main purpose of the modifiers was to increase the charring temperature to 600°C.

Materials and Methods

Plant and soil samples were analysed when concentrations were below detection by FAAS (i.e.<1.0mg/L). The samples were accurately weighed and hot-digested in 10 mL of nitric acid until the volume was decreased to 2-3mL. The volumes were then adjusted to 10 mL with distilled water. The parameters chosen for the measure of thallium are shown in Table 2.

Table 2. Parameters used for the quantification of thallium by GFAAS.

Step	Final Temp C°	Ramp Time (s)	Hold Time(s)	Gas Type	Read	Signal Graphics
Step 1	70	5.0	2.0	Inert	Off	Off
Step 2	120	20.0	5.0	Inert	Off	Off
Step 3	150	5.0	3.0	Inert	Off	Off
Step 4	600	1.1	6.0	Inert	Off	On
Step 5	1200	1.0	8.0	None	On	On
Step 6*	2000	1.0	2.0	Inert	Off	On

*The purpose of this step is to clean the tube after thallium atomisation.

Results and Discussion

The final temperatures and the ramp times were experimented with until a maximum signal was obtained. Charring occurred at 600°C allowing the organic material to burn off and the thallium reading was performed at 1200°C. The final stage at 2000°C was selected to volatilise any inorganic residue such as iron. The R^2 value was acceptable in the absorbance versus concentration curve when running thallium standard solutions ranging from 0 to 1 mg/L. An absorbance curve is shown for the standard solutions prepared.

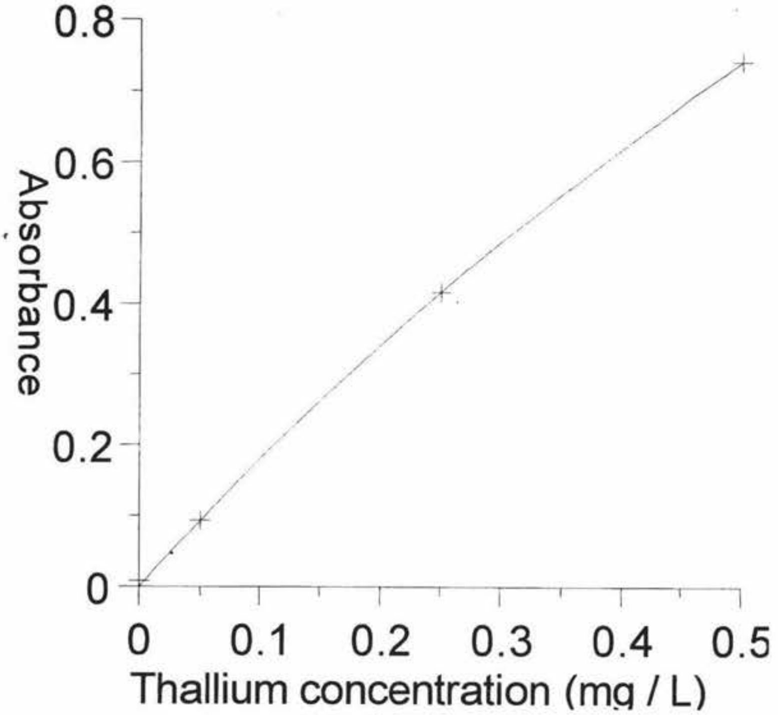

Figure 1. Absorbance curve of standard Tl solutions (mg/L).

The thallium cathode lamp was set at 5.0 mA with 276.8 nm wavelength and with background correction. The injection speed was 2 µL/s and was programmed to re-scale after every 10 samples. The peak for a 1 mg/L standard is shown graphically in Figure 2.

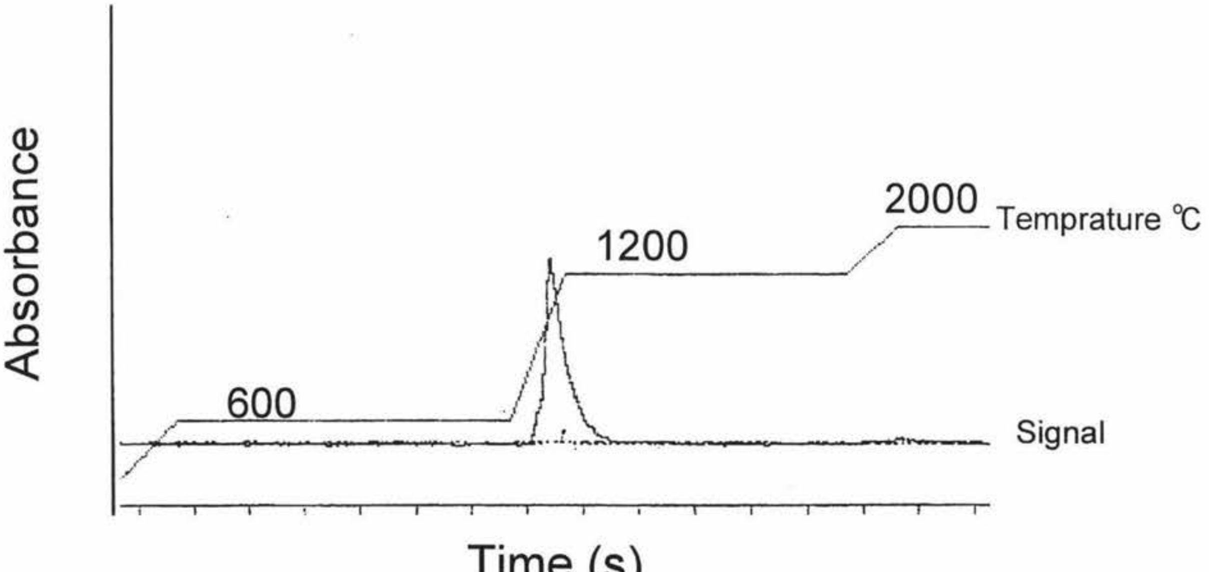

Figure 2. Absorbance peak for 1mg/L thallium standard solution.

Conclusions

The limit of detection was 100 times better using the GFAAS technique described then FAAS because the atoms remain in the ground state longer in the graphite tube. Other advantages of this analytical method include smaller sample sizes. This technique gave consistently good results and was less time-consuming then using several matrix modifiers.

Chapter 3: Thallium Accumulation by *Iberis* and *Biscutella* Species and the Potential for Thallium Phytoextraction

Abstract

Biscutella laevigata and *Iberis intermedia* were sampled from sites near St Laurent le Minier, Southern France and *B. laevigata* was also sampled from Rocca San Silvestro, Tuscany, Italy. Soils associated with the rhizosphere of each plant were also sampled. Both *Biscutella laevigata* and *Iberis intermedia* accumulate inordinately high concentrations of thallium (1.94% and 0.4% respectively) in their above-ground dry tissue. The levels of thallium accumulated by both species were strongly correlated with both the total and extractable concentrations of thallium in the soils. Concentrations of zinc, cadmium and lead were below the threshold for hyperaccumulation. It is proposed that *B. laevigata* and/or *I. intermedia* could be used for phytoremediation or phytomining of thallium-contaminated soils. Such an operation would involve the repeated cropping of either species, until an acceptable level of thallium in the soils was reached. Additionally, the harvested plant material could be burnt and the resulting ash smelted to produce an economically viable 'crop' of thallium.

Introduction

In a preliminary survey of thallium concentrations in plants growing over thallium-rich mine tailings at Les Avininières from southern France, Leblanc *et al.* (1999) recently reported up to 2810 mg/kg (0.28%) thallium in dry leaves of *Iberis intermedia.* This species is now classified as a hyperaccumulator of thallium. Lower concentrations of thallium were also reported in B*iscutella laevigata* by these same authors, though not at the level of hyperaccumulation. Both species are found growing wild in many locations throughout Europe, their growth is not inhibited by high levels of thallium. and they pose a potential hazard to grazing animals.

Biomass experiments by Leblanc *et al.* (1999) indicated up to 15 t/ha of *Iberis intermedia* could be harvested. This degree of accumulation could pay for remediation costs if an economic method of recovery were to be employed. This also has ramifications for the related field of phytomining (growing an economic crop of a metal) because of the high value of thallium ($US300,000/t). Estimates of the feasibility of thallium phytomining have been given by (Anderson *et al.* 1999).

French agrosystems typically have higher concentrations of thallium than those found in other countries. Tremel (1996) found a mean value of 1.513 mg/kg thallium in French soils, compared with Austria (0.30 mg/kg) and China (0.58 mg/kg). These high levels of thallium have prompted my studies on phytoremediation/phytomining by use of native French species.

Recently it was discovered that thallium concentrations in *Biscutella laevigata* are even higher than those previously reported for *Iberis intermedia.* The aims of the present study were therefore to:

(1) extend the investigation of the uptake of thallium and other heavy-metals in *Iberis intermedia* and *Biscutella laevigata* growing on soils with varying heavy-metal contamination in France and Italy;

(2) determine the relationship between the soil thallium concentrations (both total and extractable) and plant uptake;

(3) investigate the solubility of thallium in these soils in relation to its total concentration;

(4) investigate the potential of *I. intermedia* and *B. laevigata* for thallium phytoremediation and phytomining.

Materials and Methods

Site descriptions

Iberis intermedia, and *Biscutella laevigata* were collected from sites around St Laurent le Minier, Southern France, and Rocca San Silvestro, Tuscany, Italy (Figure's 3a/b). The region around St Laurent le Minier, known as Les Malines, is the location of one of the largest base metal mines in Europe that has been exploited since Roman times and only ceased operations about 5 years ago. The mineralisation consists of zinc/lead sulphides and oxides associated with barite. The mine tips typically contain several percent of both lead and zinc with associated thallium and cadmium. The pH of the tailings is 7.3 (average of 60 samples) with a range of 6.4-7.7. The mine waste has been colonised by a base metal flora dominated by *Minuartia verna* (L.) Hiern. and *Thlaspi caerulescens* J.C. & R. Presl with associated *Iberis intermedia* Guersent, *Biscutella laevigata* L. and *Armeria maritima* (Miller) Wild.

The site near Rocca San Silvestro, Tuscany, Italy was an ancient mining town where lead, copper and silver have been mined since the 10th Century. Geologically, the area consists of a limestone massif with parallel veins of porphyritic rocks containing mixtures of sulphides (galena argentite, chalcopyrite, and blende) running in a south-east to north-west direction. The average pH of the soils is 7.3 (15 samples) with a range of 7.1 to 7.5.

Sample collection

Iberis intermedia (15 plants) and *Biscutella laevigata* (37 plants) were sampled at various sites within Les Malines. *Biscutella laevigata* (15 plants) was also sampled from around Rocca San Silvestro. The rhizosphere soil was also sampled in addition to the above-ground biomass.

Plant digestion

Plants were rinsed thoroughly in distilled water and dried at 80°C. Approximately 0.5g of material from each plant was accurately weighed into 50 mL Erlenmeyer flasks. Concentrated nitric acid (10 mL) was added to each tube and the mixtures were heated on a heating block until a final volume of 2-3 mL was reached. The samples were then diluted to 10 mL using distilled water and stored in polythene containers.

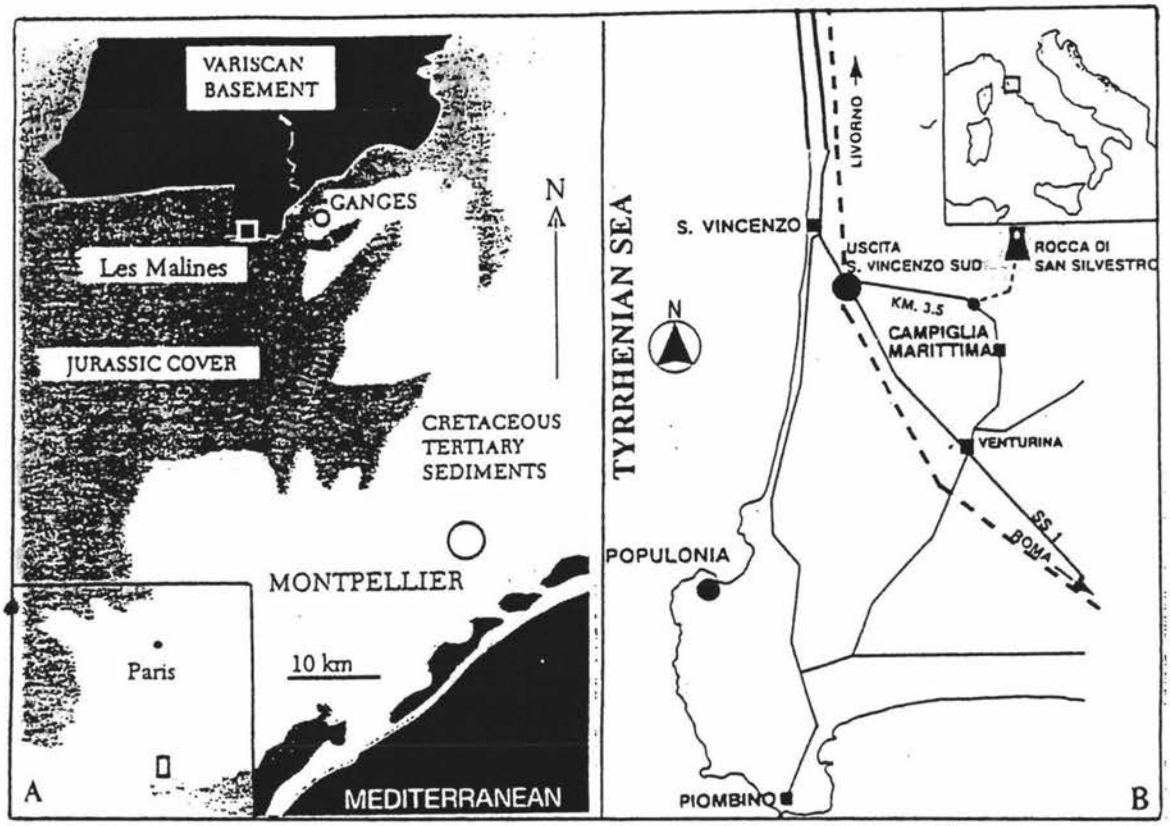

Figure 3. Location maps of collection sites for *Iberis intermedia* and *Biscutella laevigata* in (a) France and (b) Italy.

Soil digestion

Soil samples were dried at 80°C and sieved to <1 mm size using a nylon sieve. About 0.2 g quantities of sieved soil were accurately weighed into boiling tubes. Ten mL of concentrated nitric acid was then added and the mixtures boiled until a final volume of 2-3mL was reached. A further 10 mL of concentrated hydrochloric acid was then added and the mixtures again evaporated to 2-3 mL. After dilution to a final volume of 10 mL with distilled water, the solutions were filtered.

Estimation of the pH and plant-available elemental fractions in the soils and mine waste

Sieved soil (2g) was weighed accurately into 50 mL centrifuge tubes. Then 20 mL of 1*M* ammonium acetate was added to each container. Samples were shaken in an end-over-end mixer (75 rpm) for 24 h, filtered, and stored in polythene containers. Ammonium acetate was chosen for the experiments because of its common use as an approximate measure of the plant-available(extractable) fraction of soils (Ernst 1996).

The pH measurements were made by weighing 1 g samples of soils or mine waste into polythene containers, then adding 2.5 mL of distilled water and shaking. After being allowed to settle for 24 hours, the samples were again shaken and the pH measured.

Chemical analysis

Thallium, cadmium, copper, lead and zinc were quantified in the plant and soil solutions using a GBC 904 flame atomic absorption spectrometer.

Results and Discussion

Accumulation of metals by *Iberis intermedia* and *Biscutella laevigata*

The metal contents of plants and associated soils from St Laurent le Minier and Rocca San Silvestro are shown in Table 3. Since the data were lognormally distributed, geometric means are reported with the corresponding standard deviation ranges. Except for copper, values of all the metals measured are high relative to plants growing over non-metaliferous soils. However, values for lead, cadmium and zinc are well below the threshold for hyperaccumulation {1000, 100 and 10,000 mg/kg respectively as stated by Brooks (1998)}. The concentration of thallium in most plants was above the threshold of 500 mg/kg dry matter set by Leblanc *et al.* (1998) for *Iberis intermedia* from St Laurent le Minier. *Biscutella* specimens from Rocca San Silvestro were below this limit but were well in excess of it (up to 1.96%) at the French location. The lower values could be attributed to the lower soil thallium concentrations at the Italian site. The high levels of lead in most of the plants probably indicate some degree of soil contamination. However, since the thallium concentration in the plants was actually higher than the corresponding soil values, soil contamination will actually lower the measured thallium concentration.

TABLE 3. Mean (geometric) elemental concentrations (mg/kg dry mass) in *Iberis intermedia* and *Biscutella laevigata* from St. Laurent le Minier (France) and Rocca San Silvestro (Italy).

	Thallium	Lead	Cadmium	Copper	Zinc
FRANCE					
I. intermedia	411 (141-1200)	111 (45-278)	9 (3-33)	8 (5-12)	630 (232-1710)
Soil	14 (5-43)	6106 (1860-20100)	122 (46-311)	55 (25-126)	25500 (9810-66100)
B. laevigata	504 (65-3920)	191 (76-470)	27 (6-121)	7 (4-13)	2290 (805-6530)
Soil	25 (11-57)	14600 (3960-54000)	273 (71-1050)	430 (29-6210)	56900 (17300-188000)
ITALY					
B. laevigata	4 92-6)	45 (15-130)	4 (1-17)	133 (15-130)	432 (266-699)
Soil	1 (<1-3)	3791 (2732-5259)	46 (36-60)	683 (34-13808)	19730 (14337-27151)
Normal plants*	<1	5	0.1	5	50

*Mean elemental concentrations expected to be found in plants not growing over mineralisation.

NB - Values in parentheses are standard deviation ranges since data were lognormally distributed.

Relationships between plant-metal concentrations and soil properties for *Biscutella laevigata*.

Table 4. shows a matrix of correlations for elemental concentrations in soils and specimens of *Biscutella laevigata* growing around St Laurent le Minier, southern France. Analogous data have already been presented for *Iberis intermedia* by Leblanc *et al.* (1999). The total elemental concentrations in the soils were all correlated with each other and with

their respective extractable fractions. This is expected as all four elements are geochemically related, and indicate the degree of metal-bearing mineral contamination in the soil.

TABLE 4. Correlation matrix for heavy metals in plants and associated soils for *Biscutella laevigata* from metal-contaminated Soils at St. Laurent le Minier, southern France.

	pTl	PZn	pPb	PCd	PH	STl	sZn	sPb	sCd	eTl	eZn	EPb
PZn	NS											
PPb	NS	S**										
PCd	NS	S**	S**									
PH	S	NS	NS	NS								
STl	S*	S*	S*	NS	NS							
SZn	NS	S*	S**	S*	NS	S**						
SPb	NS	S*	S**	S**	NS	S**	S**					
SCd	NS	S*	S**	S*	NS	S**	S**	S**				
ETl	S**	PS	NS	NS	NS	S**	S	NS	NS			
EZn	S*	S*	S*	S*	NS	S**	S**	S**	S**	S*		
EPb	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	S*	
ECd	NS	NS	S*	NS	NS	S*	S**	S*	S**	NS	S*	NS

p - [plant], s - total [soil], e - extractable concentration in soil. S** - very highly significant ($P<0.001$), S* - highly significant ($0.001\ P<0.01$), S - significant ($0.01\ P<0.05$), PS- possibly significant ($0.05\ P<0.10$), NS - not significant ($P\ 0.10$).

Thallium concentrations in plants were significantly correlated with pH and with the total thallium concentration in the soil. The correlation between plant thallium and the extractable soil fraction of this element was much stronger than its correlation with the total thallium in soils. This reinforces the theory that it is the extractable, rather than the total fraction of a metal which determines plant uptake. Figure 4. shows the relationship between the thallium concentration in *B. laevigata* (from both French and Italian sites) and the extractable thallium concentration in the soil. There was no significant relationship between the levels of lead in the plants (indicating soil contamination because lead is not usually accumulated in plants via root systems) and the plant thallium concentration. This is further evidence that the high measured thallium values are not the result of wind-borne contamination.

Surprisingly, there was a significant positive correlation between plant thallium uptake and soil pH. Previous studies (Robinson *et al.* 1998; Hayes and Swift 1985) have shown that the uptake of other heavy metals was negatively correlated with pH presumably due to the greater solubility of these metals under more acidic conditions.

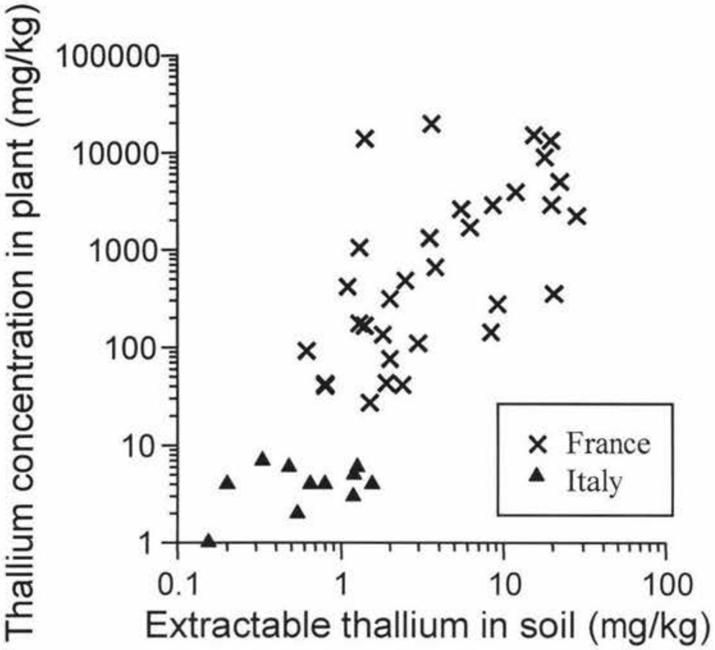

Figure 4. Uptake of thallium by *Biscutella laevigata* as a function of the extractable (1M ammonium acetate) Tl concentration in the soil.

The relationship between extractable and total thallium concentrations in soils

Figure 5. shows the percentage of the total metal concentration that is extractable using 1M ammonium acetate. As a percentage of its total concentration, thallium was significantly ($P<0.005$) more soluble than all the other metals tested and had the lowest total concentrations in soils. This is in contrast to numerous studies (Waitz and Percival 1993) that have shown in soils, the percentage of a metal that is soluble increases as its total concentration increases. This relatively high solubility of thallium indicates that it could have a greater impact on biota than the other metals tested at low total concentrations.

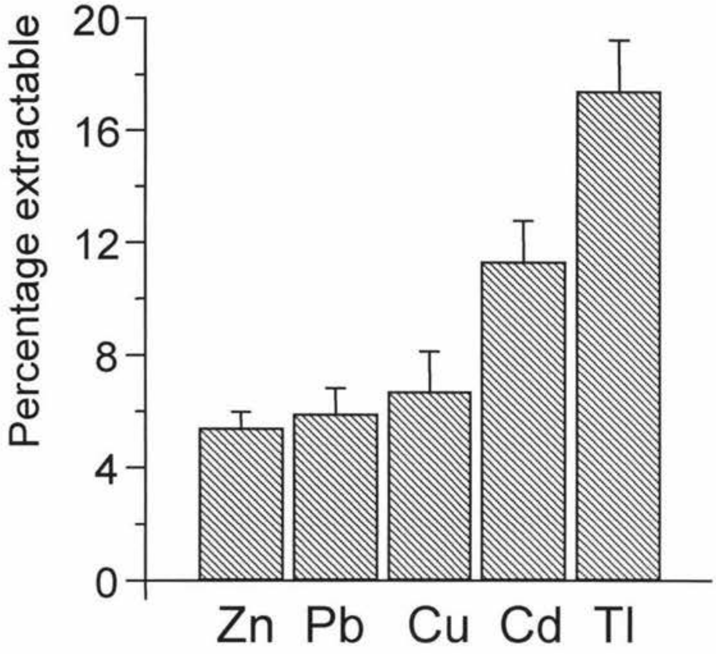

Figure 5. Percentage of the total metal concentration in soil that is extractable with 1M ammonium acetate.

The potential use of *Biscutella laevigata* and *Iberis intermedia* for phytoremediation or phytomining of thallium-rich soils.

The greater solubility of thallium in soils means that a significant fraction is available for plant uptake in phytoremediation/phytomining operations. The high bioaccumulation coefficients (BAC = plant/soil concentration quotients) for thallium in *B. laevigata* and *I. intermedia* (respectively 20.2 and 29.4) make them ideal candidates for the phytoremediation of thallium-contaminated soils. Experiments by Leblanc *et al.* (1999) demonstrated that *I. intermedia* had a fertilised biomass production of around 15 tonnes per hectare per annum. A single crop of this grown on a soil with 14 mg/kg thallium would have a concentration of 411 mg/kg (from Table 3.), and would thus remove 6.165 kg of thallium per hectare worth US$1850 at today's prices. Assuming a root depth of 10cm and a soil density of 1.1, the thallium burden in the top 10 cm of soil would be reduced to 9.2 mg/kg in one year.

At present, some sites around the town of St Laurent le Minier are being used for vegetable production. Crop growth is poor, presumably due to the high soil metal burden. Additionally, the produce may pose a health risk due to heavy metal contamination both from plant uptake and wind-borne contamination. Cultivation of hyperaccumulator species such as *I. intermedia* and *B. laevigata* could produce a viable economic return while decontaminating the soils.

Caution should be exercised in extrapolating the results of these initial experiments to full-scale phytoextraction operations, as it is unknown what the effect of repeated cropping will have on thallium uptake by plants. There was a large variation in the BAC values (3 to 698) for both species, indicating that it may be possible to enhance thallium uptake by altering soil conditions or by selective breeding; a fertile area for future research. At both of these sites there is a range of heavy metal contamination but due to the high toxicity of Tl it should be considered the priority pollutant.

Conclusions

The thallium uptake of *I. intermedia* and *B. laevigata* is primarily dependent on the concentration of soluble thallium in the soil. As the percentage of total thallium that is soluble is relatively high compared to other metals, fewer problems should be encountered during a thallium phytoextraction operation, particularly when the total concentration of this element in the soil is low. Problems in other phytoextraction operations involve maintaining sufficient plant uptake as the soluble fraction of the target metal in the soil decreases. The current very high world price of thallium metal implies recouping much or all of the costs of phytoremediation by sale of the harvested biomass, or even giving an overall profit. Future work should focus on the enhancement of thallium uptake by these species, and on agricultural practices required for efficient crop production of them.

Chapter 4: Sequential Extraction of Thallium from *Iberis intermedia* and *Biscutella laevigata*

Abstract

Thallium accumulation is not well understood within plants relative to other plant-metal interactions. Two hyperaccumulators with high thallium concentrations were subjected to sequential extraction in order to identify specific locations of thallium distribution within the plant. *Iberis* and *Biscutella* species had similar thallium distributions with aqueous extracts having by far the highest concentrations, 54.3% and 36.7% respectively.

Introduction

In order to completely understand the process of plant uptake, it is useful to know where in the plant the metal is concentrated. This information is also helpful in determining the differences between hyperaccumulators, plants that are tolerant, and those that are inhibited by the metal. Because thallium is not an essential micronutrient, it is not as well studied as some of the other metals such as nickel. However, inferences that have been made from the study of various plants and metals, can been extended to this metal. When thallium concentrations are very low within the soil, the plant has the ability to maximise uptake by modifying the surrounding rhizosphere.

Welch (1995) suggests three mechanisms which could facilitate plant uptake; solubilisation of micronutrients from solid phases of the soil fractions, mobilisation of soluble ionic species to root surfaces and absorption of the micronutrient diffusion gradient for bulk soil solution to apoplastic spaces. Once the metal is within the plant root spaces its transport and subsequent storage are not well understood. Transport is from the symplasm into the xylem parenchyma and the lumen followed by the upward movement in the xylem sap and into storage within organelles. This section presents an extraction technique used by Bowen *et al.* (1962) to determine, in this instance, the distribution of thallium within plant tissue to better understand the storage mechanism of thallium in two plant species. Two species were elected for extraction; *Iberis intermedia* and *Biscutella laevigata* which are two known hyperaccumulators of thallium (Chapter 3).

Materials and Methods

The procedure was modified from Bowen's (1962) original work. Radioisotopes were not used because better analytical equipment was available and I also used wild plants grown on naturally contaminated soil. Samples were collected from plants grown in the same area of Les Malines in France. The leaf samples were obtained from the same aerial location on the plant. Samples were air dried and accurately weighed into a centrifuge tube.

The dried material was mixed with 10 mL of ethanol in a type of centrifuge tube and placed on a rotating electrical shaker. After 10 min on the shaker, the samples were transferred to the centrifuge for a ten- minute cycle at 1200 r.p.m. The supernatant was decanted and the residue was retained (e1). Two repeat extractions were performed with the same cycle of shaker and centrifuge (e2). Two further extractions were performed using distilled water. Following the aqueous extract the residue was extracted with hydrochloric acid (0.2 M). The supernatant was centrifuged and then an equal part volume of acetone was added. After this stage, the supernatant (e3) and the precipitate(p1) were retained. The residual material was further extracted using $HClO_4$ (e4)(0.5 M) at 90-95°C. The slurry was centrifuged and then reacted with acetone. This second precipitate (p2) was then retained. The following supernant was reacted for 10 minutes with 2M NaOH (e5). The material then extracted from the ethanol was ashed at 400°C overnight. The muffle furnace was set at a slightly lower temperature then recommended by Bowen *et al.* (1962), because of the volatile nature of thallium. Precipitates one and two (p1, p2) were dried on filter paper at 100°C. Thereafter the filter papers were ashed and the ash then dissolved in 1 mL of HCl.

All of the supernatants and other samples were analysed by FAAS. The samples that were in ethanol give a false high flame in the air/acetylene mixture, so standards were prepared in ethanol instead of an acid/water mix. The standards gave a good curve so this method was selected for quantification of thallium in the ethanol extract.

Table 5. Concentrations of thallium in sequential extracts according to the Bowen method.

Phase or Extractant	*Iberis intermedia*	*Biscutella laevigata*
Ethanol (e1)	0.69%	2.39%
Water (e2)	54.33%	36.72%
HCl (e3)	13.45%	28.84%
Precipitate 1 (p1)	6.38%	14.07%
$HclO_4$ (e4)	2.45%	7.33%
Precipitate 2 (p2)	1.44%	3.62%
Residue in NaOH (e5)	0.71%	1.89%
Final residue (p3)	0.58%	2.97%

Results and Discussion

Eight different phases were separated and retained for analysis. The initial phase was the ethanol extract involving small neutral molecules. The next phase was the aqueous phase containing low-molar-mass bodies. For both plant species the main concentration of metal was in the aqueous phase. This was expected as shown in other plants (Stockey 1980) who found up to 75% nickel in the aqueous phase, thus indicating complexing with low-molar-mass compounds such as citric and malic acids. Experiments by Kim and Hill (1993) showed no significant adsorption of thallium on borosilicate or polypropylene laboratory equipment so it is unlikely that thallium was lost during the sample preparation and analysis.

Further studies should also be made to determine the nature of the aqueous fractions (e2) and what specific complexes are formed with amino acids. This level of detail was not performed in this study but could be a focus for further research. The hydrochloric acid fraction (e3) contains the acid-soluble polar compounds. This was also a large percentage of the metal concentrate. Precipitate one (p1) is acetone-insoluble and contains the cellulose and lignin fraction, in which the percentage contained in the *Biscutella* was much higher that of the *Iberis*.. The perchloric acid residue (e4) included the thallium contained in the plant nucleic acids. The fraction contained in NaOH (e5) was relatively small with both plant species. The final residue was low in both species and indicated a low percentage in the insoluble lignin and the cell walls. Comparison of work presented in this paper with that reported by Bowen *et al.* (1962) shows some relation between thallium and potassium. Less then 10% remained after the hydrochloric acid extract of potassium from tomato plants which is similar to the 11% remaining for the *Iberis* after the same stage. This suggests that thallium also exists mainly as a free cation in cytoplasm, vacuoles and intercellular spaces. Upon entering the cytosol, phytochelatins are formed by the GSH reaction chelating the metal. The complexed metal is then transported into the vacuole. The acidic nature of the vacuole may release the PC and is then degraded (Zenk 1996)

Conclusions

This study is still rather preliminary compared with possible more in-depth surveys that could be accomplished to determine the potential for thallium enzyme activation and transport. Physiochemical studies could also be done to compare hyperaccumulator species with other species that are intolerant to thallium. An intolerant species may differ physiologically in its ability to sequester the free cation into vacuoles.

Chapter 5: Uptake of Thallium by Vegetables: its Significance for Human Health, Phytoremediation and Phytomining

Abstract

Eleven common vegetables (green bean, beetroot, green cabbage, lettuce, onion, pea, radish, spinach, tomato, turnip and watercress) as well as the thallium-hyperaccumulator *Iberis intermedia*, were grown in pot trials containing approximately 1.0 and 4.0 mg/kg thallium added to a silt loam soil. The aims of the experiments were threefold: to determine risks to human health of vegetables grown in thallium-rich soils, to investigate the potential of crops of these plants to remove thallium from polluted soils (phytoremediation), and finally to establish the degree to which part of the costs of remediation could be recouped by selling the extracted thallium (phytomining). Maximum thallium levels ranged from nearly 400 mg/kg (d.m.) in *Iberis* down to just over 1 mg/kg in green bean. The four vegetables with the highest thallium levels (watercress, radish, turnip and green cabbage) were all Brassicaceous plants, followed by the Chenopods beet and spinach. At a thallium concentration of 0.7 mg/kg in the soil only green bean, tomato, onion, pea and lettuce would be safe for human consumption. At 3.7 mg/kg thallium, only green bean and tomato could be eaten. The *Iberis* had by far the best potential for phytoremediation of thallium-contaminated land and would need 5 sequential croppings to reduce 1 mg/kg thallium to 0.1 mg/kg in the soil. By contrast rape would take 9 years and green cabbage over 30 years. Some of the costs of phytoremediation might be recouped by selling the thallium which currently has a world price of $US300/kg. It was concluded that phytoremediation of thallium-contaminated soils containing >1 mg/kg thallium will never be feasible by use of common vegetables. For soils containing 1 mg/kg thallium or less, use would have to be made of *Iberis intermedia* or *Brassica napus* (rape) rather than common vegetables.

Introduction

Tremel (1996), Tremel *et al.* 1997 and Tremel and Mench (1997) have found elevated concentrations of up to 20 mg/g (dry weight) thallium in French vegetables (particularly in green cabbage) and up to 40 mg/g in rape seed (*Brassica napus* L.).

Scientists at Hohenheim University, Stuttgart, Germany are investigating possible phytoremediation of soils polluted by heavy metals such as cadmium, zinc and thallium. Their work has shown that thallium is accumulated by food crops, particularly brassicaceous plants (Kurz *et al.*, 1995, 1997a, 1997b, 1998, 1999). They found up to 24 mg/kg thallium (d.m. = dry mass) in kale growing on contaminated soils containing only 1.4 mg/kg of this metal. However, it was discovered (Kurz *et al.* 1997a) that different cultivars of green cabbage has thallium levels (d.w.) ranging downwards from 24 mg/kg to as little as 1 mg/kg. Sequential cropping by use of cultivars with the highest thallium concentrations might be used to reduce the thallium burden of the contaminated soils to an acceptable level within 10 years.

Leblanc *et al.* (1999) and the study in Chapter 3 found inordinately high accumulation (*hyperaccumulation*) of thallium by two plants from Les Malines; *Iberis intermedia* and *Biscutella laevigata,* that could contain respectively 0.4% and 1.94% thallium (d.m. – Chapter 3). Either or both of these species could rapidly remove the thallium burden of contaminated soils. Because of the high value of thallium, there should be some potential to recoup some of the costs of phytoremediation by recovery of thallium from the biomass.

Thallium concentrations in plants have excited the most interest from scientists concerned with potential harmful effects on animals and humans. The Hohenheim work mentioned above, gives ample evidence of this interest and has been reinforced by observations in France of inordinately high uptake of thallium by plants of the Brassicaceae such as *Iberis intermedia, Biscutella laevigata* and *Brassica napus* (Leblanc *et al.* 1999; Chapter 3; Tremel 1996; Tremel and Mench, 1997; Tremel *et al.* 1997). This high uptake clearly poses a risk to human and animal health in specific regions of Europe and possibly elsewhere.

Thallium is a relatively obscure metal that, until recently, has received little attention for its potential toxicity in animal or human nutrition. It is difficult to find specific recommendations by health authorities concerning allowable levels in foods and animal feed. However an allowable range of 0.45-2.28 mg/kg thallium (d.m.) for animal feed has been proposed for Germany (Anon. 1998), and Crössmann (1984) has quoted a value of 0.25-0.5 mg/kg (f.m. = fresh mass) for human foods. Assuming a 12% conversion factor for f.m. to d.m., the recommended value is therefore about 2-4 mg/kg d.m. for humans. It is possible that this level is too high in comparison with levels set for animal feeds and that the former standard should also apply to human nutrition. It is clear from the published data that most crops grown over thallium-contaminated soils will have unacceptably high concentrations of this toxic metal. As shown in Table 1., few countries have established permissible levels, or even tested soil thallium concentrations. Cropland located near an industrial setting could easily contain high levels of thallium which is readily available for plant uptake.

The aim of the work presented below was to obtain independent data for thallium levels in common New Zealand vegetables intended solely for human consumption and to assess their risk for human consumption. Two further aims were to determine the possibility of using thallium-uptake by crops of these plants for phytoremediation (removal of pollutants by plants) and phytomining (growing a crop of a metal).

Since naturally occurring high-thallium soils were not available to us, mixtures of thallium added to a local silt loam were prepared. *Iberis intermedia* was also grown in these mixtures in order to serve as a control because wild plants of this species growing under natural conditions at Les Malines in southern France had already been studied (Chapter 3) and we could thereby obtain a factor to relate apparent concentrations of thallium in pot-grown plants to those to be expected in vegetables grown under similar wild conditions.

Materials and Methods

The names of plants used in the present study are shown in Table 6. They include *Iberis intermedia* and 11 vegetables commonly grown in New Zealand. Plants were grown in three different substrates based on the local *Manawatu Silt Loam* and to which was added approximately 1.0 and 5.0 mg/kg (ppm) thallium as the sulphate. The top 15 cm of soil had a pH of 5.7, total organic carbon 6.3%, and exchange capacity of 13.4 meq/100g. The phosphorus concentration was 30 mg/kg. A further set of soils was retained to act as a control. The soils contained 3-4 month Osmocote slow release fertiliser granules at a rate recommended by the manufacturer. Before planting, the soils were placed in 1 L plastic pots and allowed to stand on damp felt for two weeks with constant watering and reanalysed to give the equilibrium concentration of thallium left bound to the soil (largely in the humus) after the unbound thallium had been leached from these substrates.

Table 6. Species and cultivars used in experiments.

Common name	Species	Family	Cultivar or trade name
Green bean	*Phaseolus vulgaris*	Fabaceae	'Tendergreen'
Beetroot	*Beta vulgaris*	Chenopodaceae	'Derwent Globe'
Green cabbage	*Brassica oleracea*	Brassicaceae	'Early Ball'
Iberis	*Iberis intermedia*	Brassicaceae	-
Lettuce	*Lactuca sativa*	Asteraceae	'Great Lakes'
Onion	*Allium cepa*	Liliaceae	'Pukekohe Longkeeper'
Pea	*Pisum sativa*	Fabaceae	'Greenleaf'
Radish	Raphanus sativus	Brassicaceae	'Salad Crunch'
Spinach	Spinaceae oleracea	Chenopodaceae	'Winter Queen'
Tomato	Lycopersicon esculentum	Solanaceae	'Groslisse'
Turnip	Brassica rapa	Brassicaceae	'Purple Top'
Watercress	Nasturtium officinale	Brassicaceae	

Seedlings of all 12 species were raised separately in commercial seed-raising mixture and then transplanted into the large pots. The plants were allowed to grow for 12 weeks in a greenhouse maintained at 15-25°C with no humidity control. Pots were watered as required (usually every second day) and the pots switched around in a random manner to allow for equalisation of light intensity. Five replicates for each treatment and for each species (180 in total) were prepared. After the 12-week period, the plants were harvested and dried at 60°C before analysis. At harvest time, composite samples of the substrate were collected and dried before analysis.

Soil was dried at 60°C ground and sieved to <1mm with a nylon sieve. Vegetation and soil samples were digested with concentrated nitric acid on a hotplate (0.2g samples plant and 0.5g soil + 10 mL of acid) and heated to low volume(2-3 mL). The samples were then diluted to about 15 mL with distilled water and analysed for thallium either by flame atomic absorption spectrometry (FAAS) for samples with higher thallium concentrations, or by graphite furnace atomic absorption spectrometry (GFAAS) for lower concentrations of thallium. Care was taken to avoid the use of hydrochloric acid because the volatility of thallium in the presence of chloride would have precluded the use of GFAAS for analysis.

Results and Discussion

Figure 6 shows the absorption of thallium from solution by the soils used in these experiments. The results show that the over the range of concentrations tested, the soil absorbed 9.2 times the concentration of thallium that was in solution. This result will allow the results below to be compared others obtained on different soils, where the pH, organic matter and hence thallium absorption will be different.

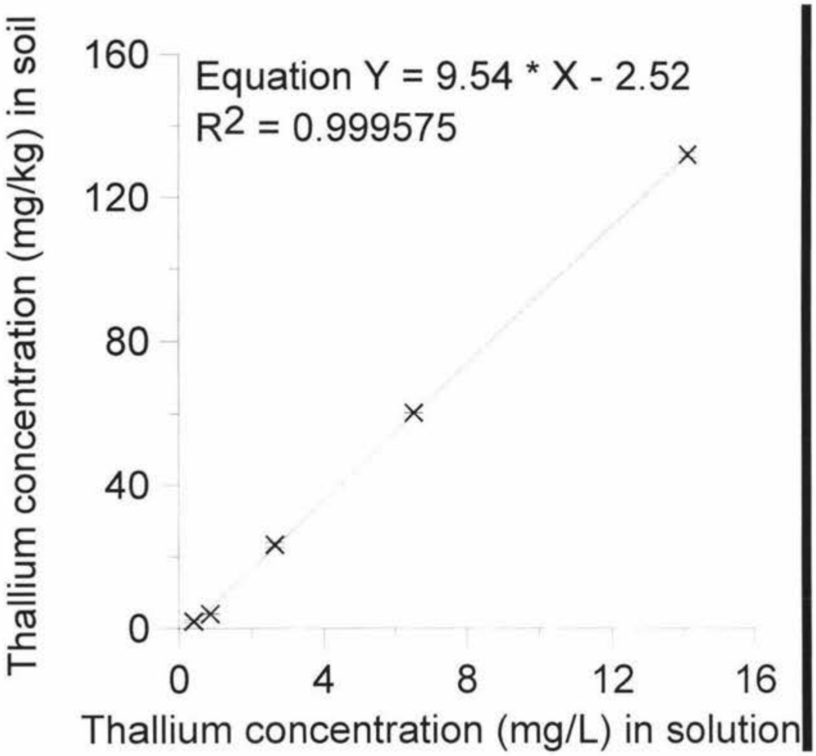

Figure 6. Concentration of soil absorbed thallium from solution.

Experimental data

Thallium concentrations in whole plants of *Iberis intermedia, Brassica juncea* and in edible parts (roots or tops depending on plant species) of 11 common vegetables are shown in Figure 7. The biological absorption coefficients (BAC = plant/substrate concentration quotient) for thallium in all the test plants are shown in Table 7 as their maximum values.

Table 7. Maximum values of biological absorption coefficients (BAC)* for thallium in plants.

	0.7 mg/kg thallium in substrate		3.7 mg/kg thallium in substrate	
Plant	Max. Tl**	BAC	Max. Tl**	BAC
Green bean	n.d.	n.d.	3.49	0.94
Beetroot	8.75	12.5	52.0	14.0
Green cabbage	7.85	11.2	129	34.9
Iberis intermedia	60.9	87.0	408	110
Lettuce	3.1	4.42	30.8	8.32
Onion	1.42	2.02	8.44	2.28
Pea	1.82	2.60	No survival	No survival
Radish	10.4	14.9	70.0	18.9
Spinach	15.9	22.7	48.7	13.2
Tomato	0.53	0.76	4.95	1.33
Turnip	7.8	11.1	139	37.6
Watercress	84.4	121	No survival	No survival

*Plant/soil thallium concentration quotient. **thallium concentration in plant (mg/kg dry mass)
n.d. - not determined.

It is noteworthy that whereas *Iberis intermedia* in its natural environment over thallium-rich tailings in southern France (Les Malines) had a mean of 1190 mg/kg thallium (d.m.), maximum of 3070 mg/kg, and mean BAC of 74 (Leblanc *et al.* 1999), the respective values for the pot-grown plants were 281, 408 and 76 for a potting medium with 3.7 mg/kg thallium. There is close agreement between the two BAC values. The higher means and maxima for the French plants result from the much higher thallium concentration (16 mg/kg) in the soil. The similarity in the BAC values indicates that wild plants from Les Malines were growing in a substrate with a similar plant-available thallium fraction as the media used in these pot trials and that these trials closely approximated to the natural situation.

It will be observed from Figure 7 that the four vegetables with the highest maximum or mean thallium levels, watercress, radish, turnip and green cabbage are all Brassicaceous. Kurz *et al.* (1997a) carried out analogous experiments with various vegetables including green cabbage for which they reported a mean of 13.7 mg/kg thallium when grown in a soil containing 1.4 mg/kg thallium. This study found 3 mg/kg thallium in green cabbage growing in 0.7 mg/kg thallium and 58 mg/kg in 3.7 mg/kg thallium. The results are therefore somewhat comparable.

Watercress when grown in a substrate with 0.7 mg/kg Tl, accumulated almost as much thallium (48 mg/kg) as the *Iberis* and did not survive at the higher metal concentration. A similar behaviour has been observed by Robinson *et al.* (1996) for uptake of arsenic by this plant. They found 300 mg/kg d.m. in plants growing near a geothermal source in the Waikato River, New Zealand.

The next highest thallium levels were found in the Chenopods beet and spinach. There were low levels of thallium in pea and green bean (Fabaceae) and in the tomato (Solanaceae). In the pea and tomato the edible parts are further removed physiologically from the rhizosphere than are leaves, so that these lower thallium levels may be a reflection of this greater isolation whereby translocation of the metal to the fruits was lower than to the leaves.

Fresh and dry weights were measured of the vegetable and remaining above ground portions of the plant. Total biomass of both treatments were compared with the control. A probability test was completed and summarised in Table 8. The root vegetables showed results different from the other vegetables. The radishes growing without thallium had a lower biomass then the two other treatments. Differences were noted as seen in photos, the difference in the development of the root. The control radishes showed normal bulb formation whereas the two thallium treatments showed a long, wide section larger then normal. The turnip showed no significant difference between 0 and 1.0 mg/kg thallium. However the higher concentration did have an impact on the biomass. Spinach and beetroot both of the Chenopod family were able to cope with the thallium burden and no differences were found in the weights. The peas showed no significant difference in the 0 to 1.0 mg/kg range, although the plants appeared to look different. Photos of plants are shown in Figures 8-20.

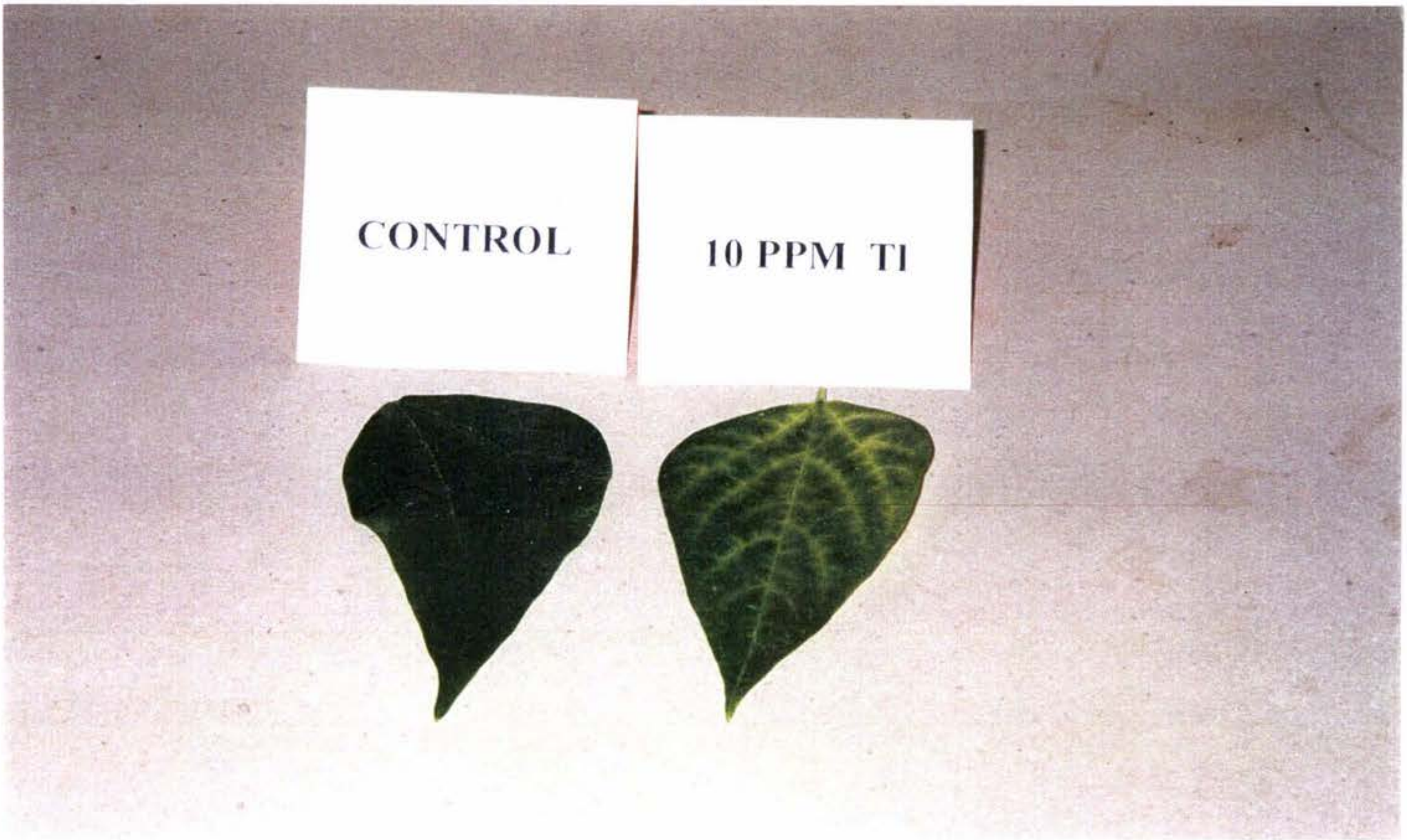

Figure 8. Bean leaf showing chlorosis along the veins typical of thallium toxicity.

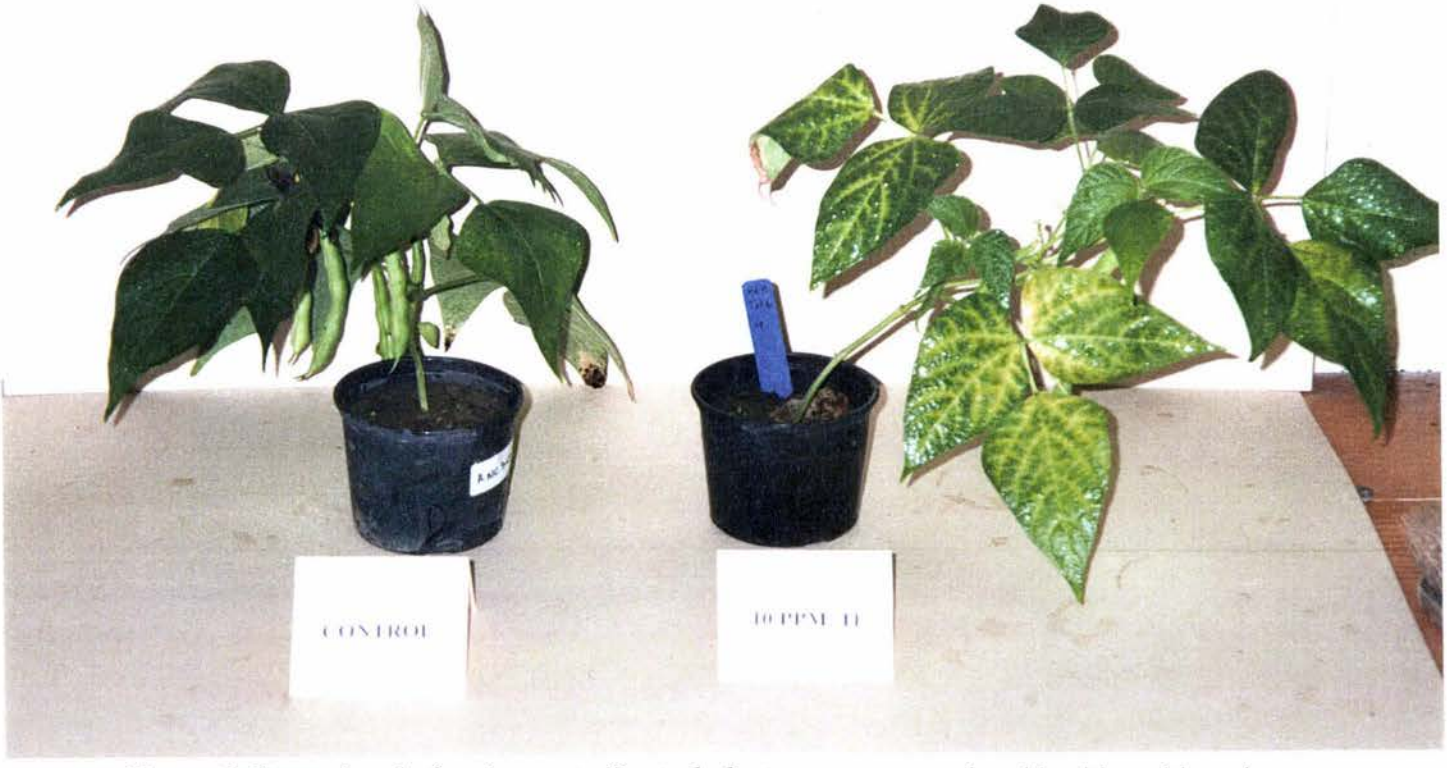

Figure 9. Bean plant before harvest. Control plant appears normal and healthy, chlorosis seen in the high treatment of 3.7mg/kg.

Figure 10. All three beetroot plants look normal and healthy with a slight decrease in biomass with increasing Tl concentration.

Figure 11. Control and low treatment of cabbage are normal and healthy. Highest treatment caused slight yellowing but difference in height is observed.

Figure 12. Iberis plant in bloom in 3.7mg/kg of thallium which has no effect on germination or growth.

Figure 13. Lettuce growth appeared normal even when treated with thallium.

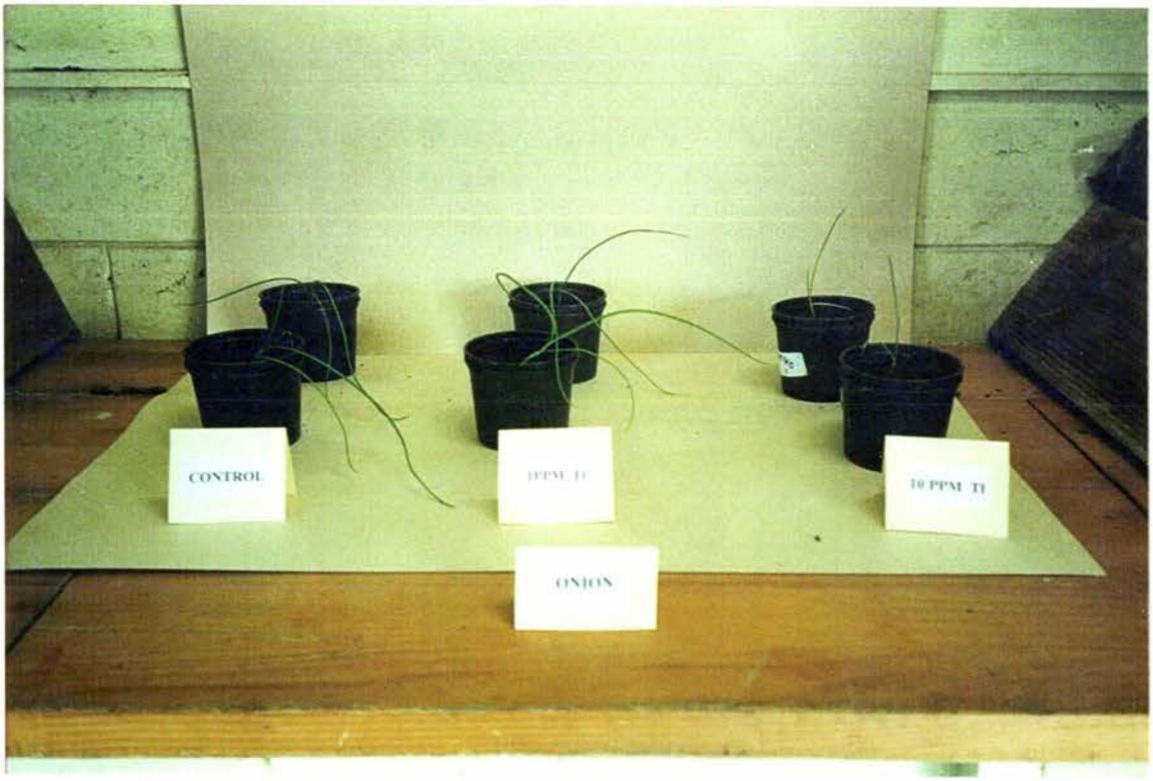

Figure 14. Onion shown one week prior to harvest. Thallium treatments caused slower germination and decreased biomass.

Figure 15. Pea plants shown; control and treatment one exhibited normal growth. Treatment two plants were yellow at the bottom and the tendrils were not responsive.

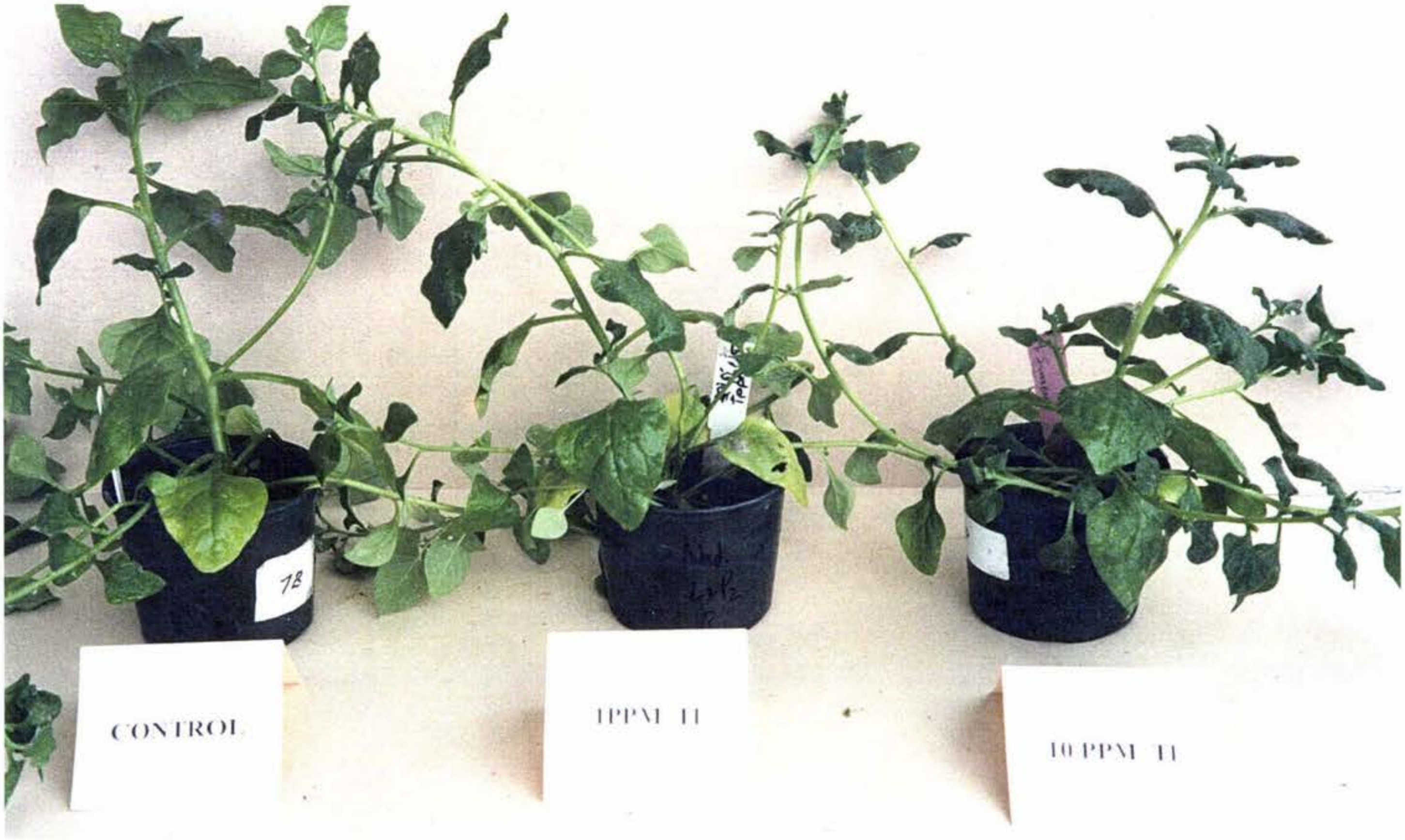

Figure 16. Spinach plants treated with 0.7ppm Tl and the control were normal and healthy. The highest treatment showed no effects other then less new shoot growth.

Figure 17a. Radish control and two thallium treatments shown. Leaf growth appears normal.

Figure 17 b,c,d (from left to right). Close up of radish root. The control is a normally developed pear shape root. Radish grown in 0.7mg/kg (b) and 3.7mg/kg (c) exhibits a long tube formation possibly indicating hormonal disruption due to the thallium.

Figure 18. Tomato plants with no visible difference between treatments.

Figure 19. Turnip plants; highest treatment poor growth of leaves and root and chlorosis.

Figure 20. Watercress growth slightly inhibited at the first Tl level, no survival at 3.7mg/kg.

This difference was probably due to the mass of the pea pods which were not affected by the thallium and which is relatively heavier then the rest of the plant mass. The whole-plant mass of 5 mg/kg-treated peas was significantly decreased and their was no production of pods. Cabbage and lettuce were also unaffected by the treatments and no chlorosis was detected in the leaves. Onion and bean biomass were reduced in the 5mg/kg thallium range although no 1mg/kg-thallium beans were planted.

Table 8. Effect on biomass of vegetables after various thallium-addition treatments.

	Pea	Onion	Spinach	Bean	Cabbage	Tomato	Radish	Turnip	Lettuce	Beet
%DM	14.69	8.26	6.27	14.33	16.90	6.79	7.25	8.71	5.80	14.24
0 Tl	3.51	1.25	3.96	9.29	5.82	22.69	1.85	3.7	5.86	6.99
1 Tl	4.56	1.59	3.98		5.25	18.21	2.96	4.94	6.06	6.13
	(PS)	(NS)	(NS)		(NS)	(PS)	(S**)	(NS)	(NS)	(NS)
5 Tl	0.22	0.28	3.88	0.034	0.114	18.41	2.76	1.72	5.91	6.12
	(S**)	(S*)	(NS)	(S)	(NS)	(NS)	(S)	(PS)	(NS)	(NS)

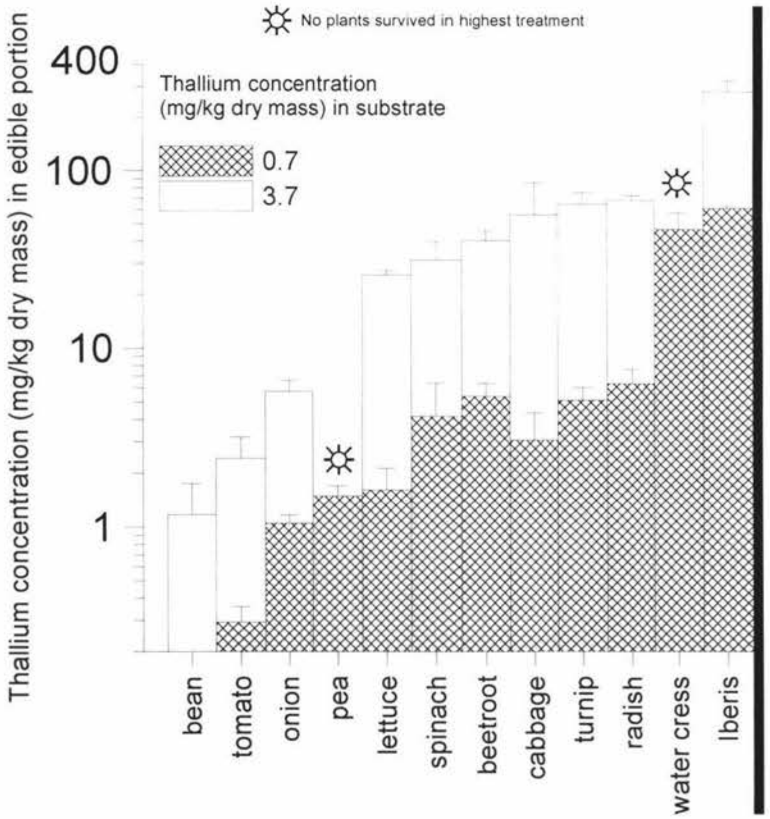

Figure 7. Thallium concentration of vegetables and Iberis when grown in loam treated with 0.7mg/kg and 3.7mg/kg.

Health hazards of ingestion of thallium-rich vegetables

The health hazards inherent in consuming vegetables growing over thallium-rich soils are difficult to establish because of the lack of data on long-term effects of thallium ingestion by humans. There are many reports on the clinical effects of poisoning of humans by exposure to, or ingestion of, thallium (Prick 1979). There are however few sources that pinpoint the acceptable daily intake (ADI) of thallium by humans, a toxic metal described by Crössmann (1984) as a new environmental contaminant. The toxic effects of thallium on humans are linked partly to the readiness whereby thallium (I) substitutes for potassium (I) as activators of some reactions catalysed by enzymes. The monovalent thallium ion has an especial affinity for the sulfahydryl grouping that can cause disorders of metabolic or energy processes in cells of animals.

In Europe (Anon. 1998) a maximum thallium level of 0.46 to 2.24 mg/kg d.m. for animal forage has been proposed. If it is assumed that the same maximum level of about 2 mg/kg d.m. Thallium in human food as being acceptable for human health, it is clear from Figure 7 that only green beans, tomato, onion, pea and lettuce would be safe to eat if grown in soils containing 0.7 mg/kg thallium. However, Kurz *et al.* (1999) have shown that under field conditions, levels of thallium in vegetables were usually only one third of those found in pot-grown plants due to a lower root density in the field. Therefore, if the thallium levels in Fig.7 are reduced to a third to make the conversion from pot-grown to field-grown plants, all of the vegetables except for radish and watercress could be added to the list that are safe to eat. For highly contaminated soils with 3.7 mg/kg thallium, only green bean, and tomato would have permissible concentrations of thallium. Although there are no values for vegetables grown at various thallium concentrations between the limits of 0.7 and 3.7 mg/kg in the soil, extrapolation of the data indicates that even at a thallium concentration of 1 mg/kg, most of the vegetables and certainly spinach, beetroot, green cabbage and turnip would be unfit for human consumption.

Phytoremediation of thallium-contaminated soils

The use of vegetables and other Brassicaceous plants as a means of phytoremediation of thallium-contaminated soils has already been proposed by Anderson *et al.* (1999), Brooks

(1998), Kurz *et al.* (1997, 1998, 1999), Chapter 3 and Leblanc *et al.* (1999). Kurz *et al.* (1997) showed that cropping of rape (*Brassica napus*) and green cabbage in one growing season together, removed 6 to 8% of the thallium burden from the top 30 cm of contaminated soil. Using the high biomass *Iberis intermedia* growing over thallium-contaminated mine tailings at Les Malines in southern France, Leblanc *et al* (1999) showed that this plant with its very high thallium concentration (800 mg/kg d.m.) could remove most of the thallium from the substrate in only three croppings. This does however presuppose that thallium yields were the same after each cropping. Kurz *et al.* (1997) did however show that during three sequential croppings with green cabbage, thallium removal decreased from 50 mg/kg of soil in the first year to 20 mg/kg in the third.

From the data presented in Table 9 it was possible to calculate annual removal of thallium from field crops of the plants used in these experiments. These experiments had been carried out as pot trials and Kurz *et al.* (1997) had shown that field crops usually gave only one third of the annual removal of thallium compared with pot trials with their greater density of root development. The calculated value of 81 g/ha of thallium removed by green cabbage (i.e. one third of the mean for the two sets of experiments) is within the range of 59-133 g/ha reported for the same plant by Kurz *et al.* (1997). Among the plants studied by us, only green cabbage, turnip, red beet and spinach would have potential for phytoremediation of thallium-contaminated soils because of their combination of high biomass and higher thallium concentrations. The *Iberis* has by far the best potential for phytoremediation as already indicated by Leblanc *et al.* (1999). The calculated value of 600 g/ha is over double the 298 g/ha reported by Kurz *et al.* (1997) for *Brassica napus* (rape).

A hectare of soil with a depth of 15 cm and density of 1.3 contains about 2000 tonnes of material. If the concentration of thallium is 1 mg/kg, the soil would contain 2000 g of thallium. A crop of *Iberis* would remove 1800 g of thallium over three years: leaving only 0.1 mg/kg thallium. A crop of green cabbage would take 22 years to achieve the same degree of remediation and a crop of spring rape (Kurz *et al.* 1997) would achieve the same goal in six years. These calculations have assumed that there is no loss of thallium yield with successive crops. Allowing a further 50% of croppings to allow for such a loss, the *Iberis* would require up to 5 years, the rape 9 years and the green cabbage over 30 years. The following conclusions can be made from these calculations:

1 - phytoremediation of thallium-contaminated soil is impracticable by use of common vegetables.

2 - phytoremediation will only be economically feasible by use of plants such as *Iberis* or possibly rape.

3 - for soils with thallium levels significantly above 1 mg/kg d.m., phytoremediation will never be a viable option.

Phytomining for thallium

The world price of thallium is currently around $US300/kg with world production almost entirely from one mine in Macedonia. Leblanc *et al.* (1999) proposed that it should be possible to produce 8 kg of thallium (worth $US2400) from a hectare of land contained 10 mg/kg thallium and planted with *Iberis intermedia.* If phytomining were to be combined with phytoremediation, it is obvious that much lower thallium concentrations would be treated: i.e. of the order of 1 mg/kg rather than 10 mg/kg. Using this scenario the hectare of *Iberis* yielding 600 g/ha thallium (Table 3) would produce a crop worth about $US180 (about half the value of a crop of wheat), whereas green cabbage would yield only $US40. The operation would obviously be uneconomic *per se* but it would serve to recoup some of the costs of phytoremediation.

Table 9. The potential of plants for phytoremediation of thallium-contaminated soils.

	3.7 mg/kg thallium in substrate			0.7 mg/kg thallium in substrate			
Species	A	B	C	D	E	F	G
Iberis	10.0	300	3000	60	600	1800	600
Radish	0.9	68	61	6.0	5.4	33	11
Turnip	3.6	64	230	5.3	19	125	42
Green cabbage	8.4	55	462	3.0	25	244	81*
Beet	6.7	42	281	5.5	37	159	53
Spinach	5.4	33	178	4.2	23	100	33
Lettuce	6.0	25	150	1.8	11	80	27
Onion	5.8	33	1.2	1.2	7	20	7
Tomato	9.0	2.5	22	0.1	0.9	12	4
Green bean	1.6	1.2	2.0	-	-	2.0	0.7
Pea	1.1	-	-	1.6	1.8	1.8	0.6

*Value obtained by *Kurz et al.* (1997) was about 60 g/ha.

A - biomass (tonnes/ha - Clarke *et al.* (1986), B - mean thallium concentration in mg/kg d.m., C - thallium removed (A x B) in g/ha, D - as B, E - as C, F - mean of C + D, G - F divided by 3 to make allowance for lower yields under field conditions (*Kurz et al.* 1997).

Conclusions

From this experiment it demonstrated the range of thallium uptake between different vegetables. These recommendations are useful for farmers growing crops on weekly contaminated soils.

Chapter 6: Summarising conclusions

In this thesis, experiments were presented to understand plant thallium relationships, analytical techniques and basic toxicity data. These experiments demonstrate a technique for thallium quantification with a sensitivity to the parts per billion range and with concomitant adequate accuracy. Analyses were carried out on plants that hyperaccumulate high levels of thallium. *Iberis intermedia* and *Biscutella laevigata* both have potential for phytoremediation and phytomining. No full-scale field trials have been conducted and currently no technique has been devised for the recovery of the bio-ore. These are two possible areas where further research would be necessary to take phytomining into the next stage of industrial feasibility.

These species are native in parts of Central and Southern Europe, are tolerant of extremes of temperature and will grow under arid conditions. These properties make them ideal for use in other contaminated sites throughout the world. Nevertheless, care should always be taken in introducing any exotic species into a new country. Another approach would be to identify the genes responsible for thallium hyperaccumulation and incorporate them into high biomass yielding native species. Little is understood about the specific mechanism of uptake and storage within the plant. Comparisons could be made for extraction data presented in this paper using the two hyperaccumulators and other less tolerant species. The mobility of thallium was high in the soil column but the specific interactions within the soil structure are unknown. This thesis provides a basic understanding of plant thallium relations but because of its relative rarity, research has not made thallium a priority.

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